A REFUTATION OF VIROLOGY

FORWARD

“If I could live my life over again, I’d devote it to proving that germs seek their natural habitat, disease tissue, rather than being the cause of disease tissue.” -Dr. Rudolph Virchow, renowned scientist, considered the ‘Father of Pathology’

"....disease investigations are greatly biased against toxicology; witness the usual omission and avoidance of toxicology. Virology stands on relatively weak ground, having created its own standards of proof for causation that would never hold in any other science.

Money influences the theater of medical science and viruses have few dollars to spend on lawyers and laboratories, whereas chemical poisons are defended by the world's largest corporations. Mere association seems sufficient to identify and declare a virus the culprit."

https://www.bmj.com/rapid-response/2011/10/29/ddt-and-polio?fbclid=IwAR1abs77wnl4XmwMH6AE6FHIcI7sv7EKMiN-t5grWoR5UECxbNKpORUOfK4

In order to deal with the topic of “virology” and the existence of “viruses” we first need to answer the simple question, how do we prove the existence of a “virus”?

If people in the same area come down with the same set of symptoms is this proof of a “virus”? No, if that were how we determine if a “virus” exists or not then what happened at Hiroshima and Nagasaki during the 1940’s could be considered proof of a “virus”. Scurvy, Beriberi and Pellagra are other good examples of people coming down with the same set of symptoms in the same area which in reality was a result of nutrient

deficiency.

If something seems to spread from place to place with the same set of symptoms, is this proof of a “virus”? No, if that were how we determine if a “virus” exists or not then what happened at Chernobyl

So how do we do it then, how do we prove the existence of a “virus”? Well how do we prove that a specific animal exists? We first have to find the animal in nature and then isolate it (meaning separating the animal from everything else) where we can then characterize it, only then can we ensure that we know its length, height, width and its genetic makeup. Unless we do this basic step we are simply working under an assumption that the animal exists and not scientific evidence. When it comes to a “virus” then we would have to take a sample directly from a person and isolate it(separate it from everything else) BEFORE any experiment with cell cultures or animals.

Now is this how “virologists” prove that a “virus” exists? No it isn’t, the way they do it is by taking a sample from a human or animal where they assume that the “virus” is in the sample without actually proving it where they then put the sample in a cell culture that contains monkey kidney cells(Vero cells) along with fetal bovine serum and other foreign genetic material. They then add antibiotics and antifungal to the cell culture in order to ensure that it does not contain any bacteria or fungus where they also starve the cells of nutrients.

After all this is done they’ll wait for a few days for the cytopathic effect to occur which is essentially when the cells starts dying, when that happens they use that as “evidence” of a “virus”. This process is then falsely defined by “virologists” as isolation which clearly is the opposite of the meaning of the word isolation and this is where the confusion comes in when discussing the issue of isolation of a “virus”.

Official Information Act Requests: Isolation of viruses on the New Zealand immunisation schedule, SARS-CoV-1 and vaccines

FOIA request to the US CDC regarding documentation of isolation of SARS-CoV-2

Now the problems here are two fold. The first problem is that they always assume that a “virus” is present in the sample taken from a patient without ever verifying it before the cell culture experiment takes place. The second problem is that they never conduct proper control experiments where they do the exact same procedure where they also take a sample from a person they deem not infected and see if they get the same results or not. This simple control experiment has never been done for any alleged “virus” by the “virologists” themselves which makes all claims of “viruses” unscientific since they are working entirely with assumptions and not objective scientific evidence.

In 2016 a German molecular & marine biologist named Dr Stefan Lanka won in the supreme court in Germany in regards to the lack of scientific evidence for the measles “virus”. What is important to note here is that he did not just prove that there is a lack of evidence for the measles “virus” due to the lack of proper control experiments but he also managed to remove the entire basis of modern “virology” which was set in 1954 by a microbiologist named John Franklin Enders. Enders was the one that established the method of using cell cultures with various foreign genetic material, toxic agents where they starve the cells in order to prove that a “virus” exists despite the fact that Enders himself admitted because of his limited control experiment that the result of the study did not prove the existence of a “virus”. Why I bring up the Enders study from 1954 is because it was one of the six studies that were used against Dr Lanka during the trials where the supreme court rejected it as evidence for the measles “virus”.

Propagation in Tissue Cultures of Cytopathogenic Agents from Patients with Measles.

During the measles trials that took place between 2012-2017 Dr Lanka also contacted 2 independent laboratories and asked them to do the proper control experiments that should have been done in the 1950’s in regards to the measles “virus”. What the proper control experiments showed was that the use of antibiotics in combination with the starvation of cells is the real cause of the cytopathic effect where the cells die and not some alleged measles “virus”.

Cytopathic effect in monkey kidney cells is not specific for measles virus

As we speak another set of control experiments are being made that will prove the same thing which is that the experiment itself is the cause of the cytopathic effect and the death of cells and not any alleged “virus”. This time Dr Lanka will go even further and also attempt to prove that the alleged genome of the alleged SARS-CoV-2, HIV & Ebola “virus” can be manufactured from the same cell culture and mixture without any “infected” material. The last control experiment will also challenge the electron microscope pictures of various alleged “viruses”. These results will later be published in various medical journals once all control experiments have been completed.

So the idea of “viruses” is entirely based on misconceptions due to a lack of proper control experiments and false assumptions where the “virologists” deceive themselves unknowingly. If this just affected the profession of “virology” this wouldn’t be so bad but when almost the entire population of the world is being imprisoned by various policies and pandemic laws where people are being killed and violated as a result of this, then this is an extremely serious issue that needs to be discussed openly scientifically and legally and brought up in various lawsuits in courts.

So what are the ramifications of non-existent “viruses”?

There are no deaths or illnesses attributed to “viruses”

There are no mutations, variants or strains of “viruses”

There are no “spike proteins”

There are no valid tests for “viruses”

There are no valid statistics about “infection rates” and mortality rates due to a “virus”

There is no such thing as “herd immunity” or “immunity”

The real purpose of “antibodies” is something else entirely

There are no reasons to fear your friends, family, neighbors and fellow men and women

There are people getting killed because of misdiagnosis and mistreatment in hospitals and care homes

There are no justifications for injections that are experimental or otherwise

There are no justifications for the creation of policies, recommendations and laws based on non-existent “viruses”

There are no justifications for the wearing of masks, social distancing, self isolation, forced isolation and restriction of travel

There are no justifications for shutting down businesses and the economy

There are no justifications for a “health pass” or “vaccine passport”

The real reasons behind these symptoms wrongfully attributed to “viruses” have never been seriously investigated and addressed where no real prevention can occur

So what we need now are more brave souls that are open minded enough to dive into this subject both scientifically and legally and expose the lack of scientific foundation behind “virology” so we can get rid of the inhumane restrictions put upon humanity and the antihuman behavior that makes our brothers and sisters turn against each other. If we do not address this now then this can and will be used against us over and over again as an excuse to gain more control over human activity and freedom where human rights are being violated.

https://truthseeker.se/the-case-against-virology-and-the-idea-of-viruses-dawn-of-peace/

Scientists

must question everything and especially what they love the most, i.e. their own discoveries and ideas. This basic rule of scientific research helps avoid erroneous developments and reveals the ones that already exist. Also, we must all be allowed to question the status quo, otherwise we would live in a dictatorship. Moreover, science cannot be limited to a selected number of institutions and experts. Science can and must be conducted by anyone who has the necessary knowledge and the appropriate methods.

Science can be considered science only if its claims are verifiable, reproducible and if they allow predictions. Science also needs external control, because, as we will see, a part of the medical sciences has lost touch with reality for quite some time. Anyone who has knowledge of biology and the genesis of life, of the development and functions of the tissue, of the body and of the brain, will automatically question the assumptions about viruses.

In the reality of the body and of its mechanisms, there is no place for hypothetical malignant processes. All biological processes, including those that can end in suffering, pain and death, are originally meant to be useful.

A different approach to the virus phenomenon is possible and necessary: any layman with some background knowledge reading scientific papers about pathogenic viruses can realize that such viruses do not exist and what is being described are only typical components and characteristics of cells. This background knowledge will be provided in this article.

The origins of the idea

The present notion of a virus is based on the ancient ideas that all diseases were caused by poisons ("toxins") and that people would regain their health by producing "antitoxins" as an "antidote". Indeed, a few diseases are caused by poisons. The subsequent idea, that the body can restore its health by producing or being given "antidotes", was born when it was observed that people survived bigger amounts of poison (such as alcohol) when their body was trained by consuming slowly increasing amounts of that poison. However, in reality there are no antidotes, instead the body produces enzymes, which neutralize and eliminate the poisons (alcohol).

In 1858, Rudof Virchow, the founder of modern medicine, plagiarized the findings of other scientists, suppressed their essential discoveries and thus a false view on the cause of diseases was born and imposed as a dogma, which is in fact still in effect to date. According to this dogma, all diseases supposedly originate inside the cells.1 Virchow's cellular pathology re-introduced into medicine the ancient and refuted the humoral doctrine and claimed that diseases develop from pathogenic poisons (in Latin: virus)

The search for these pathogenic poisons remains to date fruitless, however, when bacteria were discovered, it was assumed that they were producing the pathogenic poisons. This supposition, called "the germ theory", was immediately accepted and remains very successful up to the present time. This theory is so successful that the majority of the people are still not aware of the fact that the so-called bacterial toxins are actually normal enzymes, which either cannot appear in a human being, or, if they do, they never appear in such an amount as to make them dangerous.

Then it was discovered that, when they slowly begin to die, bacteria create tiny, apparently lifeless forms of survival, the so-called spores. It was then suspected that these spores were toxic and that they were the so-called pathogenic poisons. This was then refuted, since the spores are rapidly developing into bacteria when their vital resources are being restored. When scientists in the laboratory observed that the weak, highly inbred bacteria perished very quickly while turning into much smaller structures than the spores, it was first believed that the bacteria were being killed by the alleged pathogenic poisons, called viruses, and that the viruses were thereby replicating.

Due to the belief that these -at the time of their discovery still invisible- structures were killing the bacteria, they were called phages/bacteriophages, "eaters of bacteria". Only later it was determined that merely highly inbred and therefore almost non-viable bacteria can be made to turn into phages, or bacteria which are being destroyed so fast that they do not have time to form spores.

The introduction of the electron microscopy led to the discovery of the structures resulting from the transformation of bacteria when these were suddenly dying or when the metabolism of the highly inbred germs was overwhelmed by processes triggered by the adding of "phages". It was also discovered that there are hundreds of types of different-looking "phages". The discovery of phages, the so-called bacterial "viruses", reinforced the wrong assumption and the belief that there were human and animal viruses that looked the same and had the same structure. This is not and cannot be the case, for several different reasons.

After introducing chemical examination techniques in biology, it was discovered that there are thousands of types of phages and that phages of one type always have the same structure. They consist of a particular molecule, made of nucleic acid, which is covered in a shell of proteins of a given number and composition. It was only later discovered that merely the bacteria which had been highly inbred in the test tube could

turn into phages themselves, by contact with phages, but this never applied to natural bacteria or bacteria which had just been isolated from their natural environment. In this process, it was discovered that these “bacterial viruses” actually serve to provide other bacteria with important molecules and proteins, and that the bacteria themselves emerged from such structures.

Before it could be established that the “bacterial viruses” cannot kill natural bacteria, but they are instead helping them to live and that bacteria themselves emerge from such structures, these “phages” were already used as models for the alleged human and animal viruses. It was assumed that the human and animal viruses looked like the “phages”, were allegedly killing cells and thereby causing diseases, while at the same time producing new disease poisons and in this way transmitting the diseases. To date, many new or apparently new diseases have been attributed to viruses if their origin is unknown or not acknowledged. This reflex found an apparent confirmation in the discovery of the “bacterial viruses”.

It is important to note that the theories of fight and infection were accepted and highly praised by a majority of the specialists only if and when the countries or regions where they lived were also suffering from war and adversity. In times of peace, other concepts dominated the world of science.

It is very important to note that the theory of infection – starting from Germany – has only been globalized through the third Reich, when the Jewish researchers, most of which had opposed and refuted the politically exploited theories of infection, were removed from their positions.

From: "DISMANTLING THE VIRUS THEORY"

https://wissenschafftplus.de/uploads/article/Dismantling-the-Virus-Theory.pdf

INTRODUCTION

Contrary to what most people believe, pathogenic viruses do not exist. The claims about the existence of viruses and viral diseases are based on historic misinterpretations and not, as I thought in the past – on fraud or deliberate deception. We now have new, better, and in the positive meaning of the word “scientific” discoveries and explanations for the origin, therapy and prevention of many diseases, some of which are still called “viral” today.

The phenomenon of simultaneous or subsequent appearance of symptoms in different persons, which has been until now interpreted as contagion and was believed to be caused by the transmission of pathogens, is now also easy to understand through new discoveries. Thus, we now have a new view of life (which in reality is an old view) and of the cosmological integration of biological processes.

The “new”, but in reality only re-discovered perspective could only originate

outside of the official “science”; one of the reasons for this is that the people

involved in scientific institutions do not fulfil their first and most important scientific duty – to permanently doubt and question everything. Otherwise, they would have already discovered that the misinterpretation had been taking place for a long time already and had become a dogma only by means of unscientific activities in the years 1858, 1953 and 1954.

The transition to a new explanation of health, disease and healing will only succeed because all the concerned therapists and scientists can save face with it. From history and within the new perspective on biology and life, we now also have explanations of emotions, ignorance and all kinds of human behaviour. This is the second optimistic message. Turning around and forgiving the errors of the past can take place even more effectively, the more one understands what happened and learns for the future.

I am aware that for all the people directly involved, such as doctors, virologists, health care professionals, and above all for the people affected by the system, who suffer under misdiagnoses or who have even lost relatives on account of it, it may be difficult to intellectually accept the explanation of reality that I will offer in this article. In order that the germ theory doesn't develop a dangerous momentum, as was the case with AIDS, BSE, SARS, MERS, Corona and various other animal flu cases, or even lead to a public order breakdown, I am politely asking all the people who are discovering just now the facts about the "non-existence" of the alleged viruses to discuss the topic in an objective and unemotional manner.

The current situation

All claims about viruses as pathogens are wrong and are based on easily recognisable, understandable and verifiable misinterpretations. The real causes of diseases and phenonema which are ascribed to viruses have already been discovered and researched; this knowledge is now available. All scientists who think they are working with viruses in laboratories are actually working with typical particles of specific dying tissues or cells that were prepared in a special way. They believe that those tissues and cells are dying because they were infected by a virus.

In reality, those prepared tissues and cells are dying because they were starved and poisoned as a consequence of the experiments in the lab. Virologists primarily believe in the existence of viruses, beccause they add allegedly "infected" blood, saliva or other body fluids to the tissue and cell culture, and this, it must be stressed, after having withdrawn the nutrients from the respective cell culture and after having started poisoning it with toxic antibiotics. They believe that the cell culture is then killed by viruses. The key insight, however, is that the death of the tissue and cells takes place in the exact same manner when no "infected" genetic material is added at all. The virologists have apparently not noticed this fact! According to the most basic scientific logic and the rules of scientific conduct, control experiments should have been carried out. In order to confirm the newly discovered method of so-called "virus propagation", in order to see whether it was not the method itself causing or falsifying the result, the scientists would have had to perform additional experiments, called negative control experiments, in which they would add sterile substances or substances from healthy people and animals to the cell culture. This, of course, to check whether it is not the method itself that yields or falsifies the results.

These control experiments have never been carried out by the official “science” to this day. During the measles virus trial, I commissioned an independent laboratory to perform these control experiments and the result was that the tissues and cells die, due to the laboratory conditions, in the exact same way as when they come into contact with allegedly “infected”material.

The entire purpose of control experiments is to exclude the posibility that it is the applied method or technique which may cause the result. Control experiments, then, are the highest duty in science and also the exclusive basis of claiming that one’s conclusion is scientific. During the measles virus trial it was the legally appointed expert – Dr. Podbielski, see further in this article – who stated that the papers which are crucial for the entire science of virology contain no control experiments. From this we can conclude that the respective scientists have been working extremely unscientifically, and this without even noticing it.

This completely unscientific approach originated in June 1954, when an unscientific and refutable speculative article was published, according to which the death of tissue in a test tube was considered a possible evidence for the presence of a virus. Six months later, on 10 December 1954, the main author of this opinion was awarded the Nobel Prize for Medicine for another equally speculative theory. The speculation from June 1954 was then raised to a scientific fact due to this distinction (1) and became a dogma which has never been challenged to this date. Since June 1954, the death of tissue and cells in a test tube has been regarded as proof for the existence of a virus.

The apparent evidence for the existence of virusesThe death of tissues/cells is also regarded as the isolation of a virus, because they claim that something from the outside, from another organism, was presumably brought into the laboratory. The fact is and remains that a virus has never been, the fact is and remains that a virus has never been isolated according to the meaning of the word isolation – has never been isolated according to the meaning of the word isolation, and it has never been photographed and biochemically characterised as a whole unique structure. The electron micrographs of the alleged viruses, for example, really only show cellular particles from dying tissue and cells, and most photos show only a computer model (CGI – computer generated images). Because the involved parties BELIEVE that the dying tissue and cells transform themselves into viruses, their death is also regarded as propagation of the virus. The involved parties still believe this because the discoverer of this method was awarded the Nobel Prize and his papers remain the reference papers on “viruses”. More about this below.

It is important to mention that this unpurified mixture consisting of dying tissue and cells from monkeys, bovine foetuses and toxic antibiotics, is also being used as a “live” vaccine, because it is supposed to be composed of “attenuated” viruses. The death of tissue and cells – on account of starvation and poisoning and not because of an alleged infection – has continuously been misinterpreted as evidence for the existence of

viruses, as evidence for their isolation and as evidence of their propagation.

. Thus, the resulting toxic mixture full of foreign proteins, foreign nucleic acids (DNA/RNA), cytotoxic antibiotics, microbes and spores of all types is labelled as a “live vaccine”. It is implanted in children through vaccination mainly into the muscles, in a quantity which if it were injected into the veins would immediately lead to certain death. Only ignorant people who blindly trust in the state authorities who are “testing”and approving the vaccines can regard vaccination as a “small harmless prick”.

The verifiable facts demonstrate the danger and negligence of these scientists and politicians, who claim that vaccines are safe, have little or no side-effects and would protect us from a disease. None of these claims is true and scientific, on the contrary: upon precise scientific analysis, one finds that vaccines are useless and the respective literature admits to the lack of any evidence in their favour.

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Individual molecules are extracted from the components of dead tissue and cells, they are misinterpreted to be part of a virus and are theoretically put together into a virus model.

It must be stressed, that a real and complete virus does not appear anywhere in the entire “scientific” literature. This is because the process to come to such a description is not done by any scientific method, but purely by means of consensus, in which the participants traditionally argue for years on what pieces of genetic code “belong” to the “virus” and what pieces don’t. In the case of the measles virus, for example, this has taken several decades.

Surprisingly, in the case of the apparently new China Coronavirus 2019 (2019-nCoV, meanwhile re-named), this consensus-finding process has lasted only a few mouse clicks.

With only a few mouse clicks as well, a program can create any virus by putting together molecules of short parts of nucleic acids from dead tissue and cells with a determined biochemical composition, thus arranging them as desired into a longer genotype which is then declared to be the complete genome of the new virus. In reality, not even this manipulation, called "alignment" can result in the “complete” genetic material of a virus which could then be called its genome. In this process of theoretical construction of the so-called “viral DNA or viral RNA strands”, those sequences that don’t fit are “smoothed out” and missing ones are added. Thus, a RNA or DNA sequence is invented which doesn’t exist in reality and which was never discovered and scientifically demonstrated as a whole.

In a nutshell: From short fragments, theoretically and according to a model of a viral DNA or RNA strand, a bigger piece is also theoretically fabricated, which in reality doesn’t exist. For example, the “conceptual” construction of the “RNA strand” of the

measles virus with its short fragments of cellular particles lacks more than half of the genetic sequences which would represent a complete virus. These are in part artificially created by biochemical methods and the rest are simply invented.

The Chinese scientists, who now claim that the nucleic acids from which the genome of the new China-Coronavi-rus-2019 was theoretically constructed (4) probably originate from poisonous snakes, are just as much the victims of the global misconception regarding “viruses” as we all are.

The more viral genetic sequences are invented in the aforementioned way, the more they “discover” similarities with everything. As such, and quite ironically, there is method to the error. A large part of our academic science works like this: A theory is invented, it is always argued inside the the□ory, they call it science and claim that this represents the reality. In reality it just represents the postulated theory. (5)

The Virus Tests

Due to the lack of negative control experiments, it hasn’t yet occurred to the involved scientists that all tests for “viruses” will result in a certain number of “positives”, depending on the sensitivity of the calibration of the testing equipment. The templates that are used in the tests that supposedly find “viruses” don’t come from “viruses”, but rather from the tissue, cells and foetal serum (blood without specific components) coming from animals, mainly monkeys and calves. Because these animals are biochemically very similar to us humans, it is clear that such particles, which are misinterpreted as viral particles, can be found in all humans by means of “virus tests”. Some “viruses” and their vaccines – although not the measles “virus”– actually originate from aborted human foetuses. It is especially eye-opening here that all the tests detect molecules which exist in every human being and that vaccines can cause particularly dangerous allergic reactions, which have been named “auto-immune diseases”.

The use of foetal serum, considered to be “liquid” tissue, slows down the death of the cells and tissues under examination so much that, without it, most of these experiments could never be carried out in the first place. Only the employment of foetal serum is useful to these scientists, neither serum coming from adult living beings, nor any other synthetic product can be a substitute. One of the most contaminated and impure components of vaccines is the bovine foetal serum, without which the tissue and cells in the laboratory don’t grow at all or don’t grow quickly enough, and which is extracted in the most gruesome manner from foetuses without anaesthesia. It contains all kinds of known and unknown microbes, their spores and a huge number of unknown proteins. Besides the particles from monkey kidney tissue, it is also particles of this foetal serum that scientists are extracting and analysing when they believe that they are putting together a “virus”, which does not exist and was never proven in the entire “scientific” literature as a whole “virus”.

Because the vaccines are exclusively manufactured on the basis of these substances, this explains why it is especially the vaccinated people who test “positive” to all these imaginary “viruses” from which vaccines are manufactured. The tests only react to animal particles of the alleged viruses, animal proteins or nucleic acids which are often identical or very similar to human proteins and nucleic acids. The virus tests do not find anything specific, certainly nothing “viral” and on account of this they are worthless. The consequences, however, as we have seen with Ebola, HIV, Influenza etc., are that people become paralyzed with fear and they often die due to the very dangerous treatment.

It is noteworthy that no so-called “virus test” has a “yes” or “no” result, rather they are calibrated in a way that they can be interpreted as “positive” only after a particular concentration level has been reached. Thus, one can arbitrarily test “positive” just a few people, many people, none or all people and animals, according to the calibration of the test kit. The dimension of this entire scientific illusion becomes clear as soon as we understand that otherwise quite “normal” symptoms are only diagnosed as AIDS, BSE, flu, measles etc. if there is a “positive” test for it.

Crucial Details

Up to 1952, the virologists believed that a virus was a toxic protein or enzyme directly poisoning the body, and that it was somehow multiplied by the body itself and would spread in the body as well as between people and between animals. Medicine and science gave up on this idea in 1951, because the suspected virus had never been seen in an electron microscope and, above all, no control experiments had ever been carried out. It was acknowledged that even healthy animals, organs and tissue would release the same decay products during the decomposing process that had been previously misinterpreted as “viruses”. Virology had refuted itself. (6)

However, when the wife of the later Nobel prize winner Crick drew a double helix and this drawing was published in the famous scientific magazine Nature as an alleged scientifically developed model of the supposed DNA, a new and very successful hype began, the so-called molecular genetics. From that moment on, the causes of disease were thought to be in the genes. The idea of a virus changed and over night a virus was no longer a toxin, but rather a dangerous genetic sequence, a dangerous DNA, a dangerous viral strand etc. This new genetic virology was founded by young chemists who had no idea about biology and medicine, but they had unlimited research money. And most probably they didn’t know that the old virology had already refuted itself and given up."

FROM

The Virus Misconception part 1 -   Measles as an example
https://wissenschafftplus.de/uploads/article/wissenschafftplus-the-virus-misconception-part-1.pdf

Robert Koch was racing Pasteur to find the cause of a disease called anthrax, from which cattle in Europe were dying. Taking blood from the diseased cattle and isolating bacteria from it, Koch then injected mice with the bacteria. When the mice died, Koch then cultured blood from them and compared it to the original bacteria from the cattle. He formulated a set of RULES called Koch's postulates, to prove the germ theory was a LAW.

Koch made the first vaccine for tuberculosis, employing these same Postulates. He called the vaccine tuberculin. In Berlin alone, 2000 patients were inoculated with Tuberculin. Unfortunately they died at a higher rate than TB patients who hadn't been treated at all.

Tuberculin simply did not work. More distressing for Koch was the admission by the Prussian government that they'd made an exclusive agreement with Koch to sell the remedy and divide the profits. Not only was this a political disaster for the Prussian government and for Koch himself, but it was an embarrassment for the cause of scientific medicine when all the prestige of the scientific method suddenly suffered this blow. Koch never recovered his credibility and is remembered today only for his "Postulates." But Koch helped set the stage for the marriage of science and marketing, for which divorce does not appear likely any time soon, especially at present.

He developed procedures and his Postulates are still memorized by medical students the world over as the foundation of the Germ Theory:

1. the organism must be present in every case

2. must be isolated

3. must cause the disease in a healthy host

4. must be isolated again

Each postulate has been disproven, (meaning the germ theory has been disproven) then and now, but that has not cheated them of their place as basic tenets in the Germ Theory religion. Both Koch's and Pasteur's vaccines for anthrax were colossal failures, with thousands of sheep killed all over Europe as part of the "experiment," especially in Italy and Germany. It is also interesting to note that both Koch and Pasteur did everything possible to alter and cover up the results of these failures. (Hume) The Post-

Antibiotic Age: Germ Theory by Tim O'Shea

Virologists claim now that Koch's postulates do not fit with 'new discoveries' in 'virology'. Viruses were invented to plug the holes in the germ theory. They have NEVER been isolated and never proven to be pathogenic using the postulates of germ theory. They were first mentioned by Beijerinck when he failed to find microbes as the cause of disease in tobacco plants. He said there must be something else making them sick which we cannot see. He called it 'virus' meaning 'liquid poison'. The definition of the word 'virus' was changed in the 1930's to the definition we see today with no proof whatsoever of it's veracity. This was a marketing ploy again under orders from Rockefeller the 'father of modern medicine'.

So what are these 'new discoveries' that make Koch's postulates 'defunct'? Genetics. These people believe they can find viruses by finding their genetic codes but without proving they came from a virus nor proving said virus is a pathogenic invader. This new theory is also falling apart at the seams and the glue they use to hold it together are more theories and hypothesis called 'mutations' and 'strains'. MEANING the genes they are looking at can become different but they are still the same and that other bits of genes which match their home grown samples of genetic patterns from various species are crossing species somehow. At no point have they taken into account epigenetics. It has been known for some time that genes change with the environment, they adapt, it's a crucial survival mechanism and does not happen over generations but instantly. Bruce Lipton explains this well.

The NEW rules they are using to 'prove' contagion are called The Bradford Hill criteria. In 1965, the English statistician Sir Austin Bradford Hill proposed a set of nine criteria to provide epidemiologic evidence of a causal relationship between a presumed cause and an observed effect. This method uses epidemiology (that is observations of a population so anecdotal) It also uses correlation = causation. Doesn't science tell us constantly that

Correlation does NOT = causation...

If they have never isolated the virus in the first place AND subjected the purified matter to ALL of Koch's postulates then they cannot say that what they are looking at is the cause of any disease."

-N. TRACY

Why Koch's Postulates matter:

Explanation by David Crowe

"Koch's postulates are a STATEMENT OF FOUR LOGICAL RULES FOR

DETERMINING WHETHER A PATHOGEN EXISTS AND IS THE CAUSE OF A DISEASE (e.g. [Cann 1997]). THEY MUST BE SATISFIED before it can be accepted that a pathogen causes a disease. They statethat:1. The pathogen must be present in every case of the disease.14

2. It must be isolated from the host and grown in vitro (culture).3. The disease must be reproduced when a pure culture of the pathogen is inoculated into a healthy suscepϴble host.4. The same agent must be isolated once again from the experimentally infected hostKoch’s postulates are merely a STATEMENT OF THE MINIMAL EVIDENCE NECESSARY tohave confidence in the existence of a pathogen and its causal link to a disease. Itis important to note that THESE POSTULATES ARE NOT BASED ON EXPERIMENTALEVIDENCE, BUT ON SIMPLE LOGIC."There is no valid evidence of a purified/isolated "virus" that fulfills Koch's Postulates.   We have been lied to over and over again.

Chapter 1

ISOLATION

ISOLATION: the act of separating something from other things : the act of isolating something

https://www.merriam-webster.com/dictionary/isolation

This is the definition most people agree with and refer to when isolating something.

Not virologists. This is what virologists mean when using the word isolation:

“VIRUSES ARE BASICALLY INANIMATE OBJECTS WHICH NEED A CULTURE TO ACTIVATE IN. But the way they are phrasing the requests is that the sample must be COMPLETELY UNADULTERATED and not be grown in any culture – AND YOU CAN’T DO THAT,” she told AAP FactCheck in a phone interview.

“YOU CAN'T ISOLATE A VIRUS WITHOUT USING A CELL CULTURE, SO BY USING THEIR DEFINITION IT HASN'T BEEN ISOLATED. But it has been isolated and cultivated using a cell culture multiple times all around the world.”

https://www.aap.com.au/proof-the-virus-behind-covid-19-doesnt-exist-fails-basic-biology-test

The above quote is from a FB "factcheck."

In layman's terms, if using the agreed upon definition of isolation, they agree that they haven't isolated a "virus" from everything else as that is impossible. But that's ok because it's a "virus." It needs a host cell (which should be from the host they take the "virus" from but try not to think too hard about that logical inconsistency) in order to grow and replicate. But not just any host cell will do. In the case of "SARS-COV-2," it needs the kidney cells from an African Green Monkey.

But wait, there's more!

It also needs to be immediately placed in "Viral" Transport Media after being taken from a patient. This normally consists of animal DNA, antibiotics, and other chemicals/nutrients. In order to grow, it needs fetal bovine serum (blood taken from the hearts of baby cows). In order to be free of bacteria, it needs 2 or 3 cell toxic antibiotics. In order to "eat," it needs various unknown nutrients/chemicals in DMEM. All of this must be added to the unpurified sample from a patient, mixed together, and then incubated for days.

Once the expected Cytopathogenic Effect (i.e. cell death) is seen in the petri dish, then and only then has a "virus" been "isolated."

Virology subscribes to subtraction through ADDITION. Or, in other words, the EXACT OPPOSITE OF ISOLATION.

So once again, no "virus" has ever been properly purified nor ISOLATED.

Figure 1: Actual Isolation

Figure 2: Virology "Isolation"

Related Post and Collection on Cell Cultures:

https://www.facebook.com/502548575/posts/10158078047703576/

https://m.facebook.com/story.php?story_fbid=10158191240103576&id=502548575

All seven steps that virologists take to claim a virus, they refuse to adhere to the most important scientific duty, the verification of their methods.

7 ways to prove viruses don’t exist with Dr Stefan Lanka (BACKED UP BY INVESTIGATIONS INTO THE VERY PAPERS OF TOPIC)

In all seven steps that virologists take to claim a virus, they refuse to adhere to the most important scientific duty, the verification of their methods: They never document control experiments. For this reason alone, statements by virologists claiming that viruses

cause disease should never be considered scientific.

If you read the short methods and materials section of the supposedly scientific publications of the virologists, you will see that the virologists have refuted themselves with their explanations of the seven steps.

The ruling in the measles virus trial from 2012 to 2017 contains a meaning that goes beyond the measles compulsory vaccination: This jurisprudence removes the basis of the entire virology.

Dismantling the virus theory The “measles virus” as an example

https://wissenschafftplus.de/uploads/article/Dismantling-the-Virus-Theory.pdf

https://odysee.com/@oliverjanich:b/Interview-Lanka-englisch_kompr:c

EVIDENCE

Virologists interpret the death of cells in the laboratory as viral. Due to the lack of control

attempts (experiments), they overlook the fact that they kill the cells in the laboratory themselves and unintentionally by starving and poisoning the cells.

This misinterpretation is based on a single publication by John Franklin Enders and a colleague from June 1, 1954. This publication was ruled by the highest court in Germany in the measles virus trial that it contained no evidence of a virus. This publication became the exclusive basis not only for measles virology, but for all virology since 1954 and corona hysteria.

"This “gross misjudgment” by the Ravensburg Regional Court

was overturned on February 16, 2016 due to my successful

appeal by the Stuttgart Higher Regional Court. "

"The scientific counter-evidence to the allegations of the existence of the "measles virus" in the form of the fifth expert opinion. This report clearly refutes all existing allegations of the existence of a “measles virus.”

"Depending on the non-viral and non-infectious substances

added, changes in the cell morphology could be observed at

different times, which since 1954 has been equated with the

"isolation" of the "measles virus". Especially after the addition

of high concentrations of penicillin / streptomycin (20%) or

cultivation under deficient conditions (1% FCS), changes in

the cell morphology were found that were microscopically

identical to the syncytia formation described by the measles

virus (Figure 1).

The studies have clearly shown that syncytia formation is not

specific for measles infection. Thus, the forgotten

observations by both Enders & Peebles and Bech & von

Magnus were confirmed and the assumption that Enders &

Peebles and successors had proven the existence of a virus

with this technique was refuted."

https://blog.dawnofpeace.org/wp-content/uploads/2021/06/Measles-Control-Experiment-by-the-head-of-an-independent-laboratory-in-Germany.pdf

-paper on the trial

https://truthseeker.se/wp-content/uploads/2021/01/go-VIRUS-go-by-Dr-Stefan-Lanka.pdf

TO THE PAPERS FROM THE INVESITIGATION DURRING THE TRIAL -- WISSENSCHAFFTPLUS Ausgabe 4/2017 kaufen (wplus-verlag.ch)

Https://wplus-verlag.ch/de_DE/p/buy/wissenschafftplus-ausgabe-4-2017?fbclid=IwAR32IhaUzZVvkCNENA_uNKPZ7N6IPTWOFnC14n1IASEQdeIyCU2ECZEgkp8

ENDERS 1954 MEASLES PAPER:

John Franklin Enders 1954 paper "Propagation in Tissue Cultures of Cytopathogenic Agents from Patients with Measles" is considered the proof of the discovery of a Measles "virus." This was presented as the "isolation" of Measles and served the basis

for which the vaccine was based upon. Reading the paper and Enders conclusions, however, tells a completely different story than the isolation of a "virus."

"Materials and methods. Collection of specimens. Throat washings, venous blood and feces were obtained from 7 patients as early as possible after a clinical diagnosis of measles was established. In 5 instances the time at which specimens were collected in relation to the onset of exanthem is given in the case histories described below or in Table I. When capable, PATIENTS WERE ASKED TO GARGLE WITH 10-15 ML OF STERILE NEUTRALIZED FAT-FREE MILK. Certain specimens from the throats of younger children were OBTAINED BY COTTON SWAB PREVIOUSLY MOISTENED IN MILK. After swabbing the throat THE SWAB WAS IMMERSED IN 2 ML OF MILK. PENICILLIN, 100 u/ml, AND STREPTOMYCIN, 50 mg/ml. WERE ADDED TO ALL THROAT SPECIMENS which were then centrifuged at 5450 rpm for about one hour. SUPERNATANT FLUID AND SEDIMENT RESUSPENDED IN A SMALL VOLUME OF MILK were used as separate inocula in different experiments in amounts varying from 0.5 ml to 3.0 ml. About 10 ml of blood immediately after withdrawal were placed in tubes containing 2 ml of 0.05% SOLUTION OF HEPARIN. As inocula for tissue cultures amounts varying from 0.5 ml to 2.0 m of the whole blood were employed. AFTER ADDITION OF ANTIBIOTICS AS DESCRIBED ABOVE 10% FECAL SUSPENSIONS WERE PREPARED BY GRINDING THE MATERIAL IN BOVINE AMNIOTIC FLUID MEDIUM. The suspensions were then centrifuged at 5450 rpm for about one hour and the supernatant fluids used as inocula, in amounts varying from 0.1 ml to 3 ml. All specimens were refrigerated in water and ice or maintained in the cold at about 5°C from the time of collection until they were added to the cultures. The maximum time that lapsed between collection of specimens and inocula-tion was 35 hours."

Right off the bat you can see that the throat swabs were immediately placed in milk or were taken by cotton swabs doused with milk. Antibiotics were then added to these milk swab samples. Blood samples were placed in tubes containing heparin which is toxic to cells. Fecal samples were grinded up and added to bovine amniotic fluid serum. The addition of various chemicals/compounds to samples from sick patients is the exact opposite of isolation.

"Tissue culture technics. In the initial isolation attempts roller tube cultures (1 1 12) of human kidney, human embryonic lung, human embryonic intestine, human uterus and rhesus monkey testis were employed. Subsequent passages of the agents isolated were later attempted in human kidney, human embryonic skin and muscle, human foreskin, human uterus, rhesus monkey kidney and embryonic chick tissue. Stationary cultures prepared according to the technic of Youngner(13) WITH TRYPSINIZED HUMAN AND RHESUS MONKEY KIDNEY WERE LATER EMPLOYED FOR ISOLATION OF AGENTS and their passage. THE CULTURE MEDIUM CONSISTED OF

BOVINE AMNIOTIC FLUID (go%), BEEF EMBRYO EXTRACT (50/0), HORSE SERUM (5%), ANTIBIOTICS, AND PHENOL RED as an indicator of cell metabolism (1 2). SOYBEAN TRYPSIN INHIBITOR WAS ADDED TO THIS MEDIUM UNLESS IT WAS USED FOR THE CULTIVATION OF HUMAN AND MONKEYS KIDNEY (11). Fluids were usually changed at intervals of 4-5 days. For histological examination the cell growth after fixation in 10% formalin was embedded in collodion, dehydrated and stained with hematoxylin and eosin ."

You can see again that various chemicals/compounds were used during the "isolation" process. Trypsinized human or monkey kidneys cells were used. Trypsin has been shown to have negative effects on the kidneys and can be full of contaminants. Bovine amniotic fluid, beef embryo extract, horse serum, antibiotics, phenol red, and in some cases soybean trypsin inhibitors, were added to the culture. The addition of various cell-altering chemicals, compounds, animal DNA, etc is the exact opposite of isolation.

"In each of the 3 cultures that were inoculated cytopathic changes were observed on the 7th day. Since these changes presented a CHARACTERISTIC APPEARANCE NOT HERETOFORE ASSOCIATED DEFINITELY WITH A VIRUS they have provided the means for the further investigation of this agent as well as others that have been recently isolated."

Cytopathic changes were observed after culturing for 7 days. Any of the toxic compounds alone which were added to the sample to culture it could have caused these changes. There is no reason to assume a "virus" caused the cell death.

"In cultures consisting largely of monkey renal epithelial cells as prepared by Youngner’s modification of Dulbecco’s technic (13) cytopathic changes have been regularly observed which resemble closely those produced by these agents in human renal cells as seen in both fresh and stained preparations. These effects followed the addition of blood or throat washings from cases of measles as well as infected tissue culture fluids derived from previous passages. Monkey kidney cultures

may, therefore, be applied to the study of these agents in the same manner as cultures of human kidney. IN SO DOING, HOWEVER, IT MUST BE BORNE IN MIND THAT CYTOPATHIC EFFECTS WHICH SUPERFICIALLY RESEMBLE THOSE RESULTING FROM INFECTION BY THE MEASLES AGENTS MAY POSSIBLY BE INDUCED BY OTHER VIRAL AGENTS PRESENT IN THE MONKEY KIDNEY TISSUE (cf. last paragraph under G) OR BY UNKNOWN FACTORS."

Here Enders states that the cytopathic changes (CPE) seen in Monkey kidney cells resembles that of human kidney cells meaning that these cells can be used going forward as they regularly are today. However, even he states that the CPE observed may not be due to a Measles "virus" but by either other "viruses" or unknown factors.

"Other agents isolated during this study. TWO AGENTS HAVE BEEN ISOLATED WHILE THE PRESENT WORK WAS IN PROGRESS THAT APPEAR UNRELATED TO THOSE WE HAVE JUST DESCRIBED. The first was recovered from the throat washings of a typical case of measles occurring in the boys' school. Its wide cytopathogenic range, the character of the cytopathic changes induced and the fact that its infectivity for tissue cultures was neutralized by herpes simplex immune rabbit serum served to define its nature. A second agent was obtained from an uninoculated culture of monkey kidney cells.

THE CYTOPATHIC CHANGES IT INDUCED IN THE UNSTAINED PREPARATIONS COULD NOT BE DISTINGUISHED WITH CONFIDENCE FROM THE VIRUSES ISOLATED FROM MEASLES. But, when the cells from infected cultures were fixed and stained, their effect could be easily distinguished since the internuclear changes typical of the measles agents were not observed. Moreover, as we have already indicated, fluids from cultures infected with the agent failed to fix complement in the presence of convalescent measles serum. OBVIOUSLY THE POSSIBILITY OF ENCOUNTERING SUCH AGENTS IN STUDIES WITH MEASLES SHOULD BE CONSTANTLY KEPT IN MIND."

Here it shows other agents were "isolated" with measles and that they could not distinguish any difference between the CPE they claim was caused by Measles with one of the other agents. This is just further evidence that these were not purified samples and that they assume that whatever "virus" they believe is present must adhere to certain CPE changes even though there are other agents which produce the same effect.

"Discussion. Of the numerous experiments that have been reported in the past describing the successful isolation of the etiologic agent of measles ONLY THOSE IN WHICH MONKEYS WERE EMPLOYED AS THE EXPERIMENTAL ANIMAL have been consistently confirmed by other workers. GREAT CAUTION SHOULD THEREFORE BE EXERCISED IN THE INTERPRETATION OF ANY NEW CLAIMS THAT THE VIRUS HAS BEEN PROPAGATED IN OTHER HOSTS OR SYSTEMS. Accordingly, THE RESULTS THAT ARE SUMMARIZED HERE MUST BE SUBJECTED TO THE MOST CRITICAL ANALYSIS."

Enders seems to admit that Measles was only successfully isolated through monkeys in the past. Therefore, isolation of a "virus" from other hosts, such as HUMANS, should be treated with great caution and must undergo critical analysis. He undermines the

credibility of his own research and findings in his own paper.

"THE PATHOLOGIC CHANGES INDUCED BY THE AGENTS IN EPITHELIAL CELLS IN TISSUE CULTURE RESEMBLE, AT LEAST SUPERFICIALLY, THOSE FOUND IN CERTAIN TISSUES DURING THE ACUTE STAGES OF MEASLES. While there is NO GROUND FOR CONCLUDING THAT THE FACTORS IN VIVO ARE THE SAME AS THOSE which underlie the formation of giant cells and the nuclear disturbances IN VITRO, the appearance of these phenomena in cultured cells is consistent with the properties that a priori MIGHT BE ASSOCIATED WITH THE VIRUS OF MEASLES."

According to Enders, there is no grounds for stating what happens IN VITRO (in a lab) has any relation to what happens IN VIVO (within a living organism) thus once again throwing shade on his entire research and findings.

"Although we have thus already obtained CONSIDERABLE INDIRECT EVIDENCE supporting the etiologic role of this group of agents in measles, 2 EXPERIMENTS ESSENTIAL IN THE ESTABLISHMENT OF THIS RELATIONSHIP REMAIN TO BE CARRIED OUT. These will consist in the production of measles in the monkey and in man with tissue culture materials after a number of passages in vitro sufficient to eliminate any virus introduced in the original inoculum. THE RECOVERY OF THE VIRUS FROM THE EXPERIMENTAL DISEASE IN THESE HOSTS SHOULD THEN BE ACCOMPLISHED."

Enders admits that they only collected INDIRECT evidence supporting a role in the agents he studied yet further experiments needed to happen in order to prove this such as actually seeing if the agent can produce Measles in a human or monkey and whether the Measles "virus" can be recovered from them. Thus this paper is not proof of a Measles "virus" at all. If that wasn't enough to make this point absolutely clear, Enders conclusion definitely will:

"Conclusion. The findings just summarized support the PRESUMPTION THAT THIS GROUP OF AGENTS IS COMPOSED OF REPRESENTATIVES OF THE VIRAL SPECIES RESPONSIBLE FOR MEASLES."

https://journals.sagepub.com/.../10.3181/00379727-86-21073

The PRESUMPTION that the agents he worked with were Measles "virus."

In summary:

-there was no "isolation" of any "virus," just the usual toxic cell culture crap

-the samples were not purified and other agents other than the presumed Measles "virus" were detected

-Enders doubted the validity of his own work and stated more evidence was needed to establish a DIRECT link between the agent he PRESUMED was a Measles "virus" and the disease itself

This is the seminal Measles work and it does not offer any proof of the existence of a Measles "virus." Everything built upon this fraudulent paper is therefore fraudulent as well, which is the very nature of "Virology" and "Science" today.

https://docs.google.com/document/d/e/2PACX-1vS-ffMU9VlK0UBmWENeSrmEAFIu732T5G0GGFTb5swNWmYr8BC7ODOTbmkuDvzg6HnSvYA2DXjKO-_6/pub

Dismantling the virus theory ...

About the alleged proof of pathogenic viruses

The “bacteriophages”, correctly defined as incomplete mini spores and building blocks of the bacteria, have been scientifically isolated, while the supposed pathogenic viruses have never been observed in humans or animals or in their body

fluids and have never been isolated and subsequently biochemically analysed. To date, none of the researchers involved in this kind of work seems to have realised this. The use of the electron microscope and the biochemistry were very slowly returning to normal after 1945 and no one had realised that not one pathogenic virus had ever been isolated in humans or animals; thus, as of 1949 researchers started applying the same idea used for the (bacterio) phages, in order to replicate the human and animal “viruses”. John Franklin Enders, born in 1897 in the family of a rich financier, was active in various fraternities after having finished his studies, then he worked as a real estate agent and studied foreign languages for four years before turning to bacterial virology, which

fascinated him.

He then simply transferred the ideas and concepts that he learned in this area of research to the supposed pathogenic viruses in humans. With his unscientific experiments and interpretations that he had never confirmed through negative controls, Enders brought the entire “viral” infectious medicine to a dead end. It is important to note at this point that Enders, like many infectious diseases specialists, worked for the U.S. military, which had always been and remains to date a huge victim of the fear of contagion. It was mainly the U.S. military which spread its erroneous belief that besides chemical weapons there were also biological weapons in the form of bacteria and viruses.

In 1949, Enders announced that he had managed to cultivate and grow the alleged polio virus in vitro on various tissues. The American expert opinion believed everything immediately. What Enders did was to add fluids from patients with poliomyelitis to tissue cultures which he claimed to have had sterilized, then he alleged that the cells were dying because of the virus, that the virus was replicating in this way and that a vaccine could be harvested from the respective culture. At that time, summer polio epidemics (polio = flaccid paralysis) were very frequent during summer and they were believed to be caused by polio viruses. A vaccine was to help eradicate the alleged virus. After the polio vaccine was introduced, the symptoms were then re-diagnosed among other things as multiple sclerosis, flaccid acute paralysis, aseptic meningitis etc. and later polio was claimed to have been eradicated.   During his experiments, Enders et al. sterilised the tissue cultures in order to exclude the possibility of bacteria killing the cells. What he didn’t take into consideration was that the sterilisation and the The density gradient centrifugation is the scientifically required standard technique for the demonstration of the existence of a virus. Despite the fact that this method is described in all microbiology manuals as the “virus isolation technique”, it is never applied in experiments meant to demonstrate the existence of pathogenic viruses.

Centrifuge tube with silicone beads gradient layered with a suspension of viruses and cellular particles Centrifuge tube with“bands” of viruses and cellular particles after centrifugation By extracting the viral band with a pipette, the virus is thus isolated and purified.

treatment of the cell culture when preparing it for the alleged infection was exactly what was killing the cells. Instead, he interpreted the cytopathic effects as the existence and the action of polio viruses, without ever having isolated a single virus and described its biochemistry. The necessary negative control experiments, which would have shown that the sterilisation and the treatment of

the cells prior to the "infection" in the test tube was killing the cells, have never been performed.

However, for this "performance" Enders received the Nobel prize in 1954 1954 is also the year in which Enders applied and introduced the same technique in order to allegedly replicate the measles virus. As he had been awarded the Nobel prize for the alleged polio virus the same year, all researchers believed his technique to be scientifically valid. Thus, to date, the entire concept of measles has been based upon this technique. Thus, the measles vaccines do not contain viruses, but particles of dead monkey kidney tissue or human cancer cells.

To date, no negative control experiments have been done with respect to the so-called measles virus either, which would have shown that it is the laboratory procedures that lead to the cytopathic effects on the cells. Additionally, all claims and experiments made by Enders et al. and the subsequent researchers lead to the only objective conclusion that in fact they were observing and analyzing dying cellular particles and the activity thereof in the test tube, misinterpreting these as particles and characteristics of the alleged measles virus.

The measles virus as an example

The following explanations apply to all the socalled (human or animal) "pathogenic viruses".

The six papers provided by Dr Bardens in the course of the "measles trial" as proof for the existence of the measles virus describe in a did actically ideal way the various steps of the chain of misinterpretations up to the belief in the exist□ence of a measles virus. The first paper was published in 1954 by Enders et al.: "Propagation in tissue cultures of cytopathogenic agents from patients with measles" (Proc Soc Exp Biol Med. 1954 Jun; 86 (2): 277–286). This publication can be found on the internet, like all the other publications presented at the measles trial.In that experiment, Enders et al. cut down dramatically on the nutrient solution and added cell-destroying antibiotics to the cell culture before introducing the allegedly infected fluid. The subsequent dying of the cells was then misinterpreted as presence and also isolation of the measles virus. No control experiments were performed to exclude the possibility that it was the deprivation of nutrients as well as the antibiotics which led to the cytopathic effects. Enders' and his colleagues' blindness can be explained by the fact that he truly wanted to help people, while the virus hysteria was intensifying after the war and during the cold war. It can also be explained by the fact that Enders and many of his colleagues had no idea about medicine and they were competing

with the Soviet Union for the development of the first measles vaccine.

Such a pressure for success can also explain why Enders and his colleagues ignored their own reservations and cautions expressed in 1954, when they had observed and noted that many cells also died after being treated normally (i.e. without being “infected”), which they thought by unknown viruses and factors. All these facts and cautions were subsequently disregarded.

The second paper presented by the claimant in the measles trial was published in 1959 and, for the reasons presented above, the authors concluded that the technique introduced by Enders was not appropriate for the isolation of a virus.

This rebuttal is not only NOT being discussed by all the other researchers, but it is being ignored.

In the third paper5

, the authors photographed typical cellular particles inside the cells and misinterpreted these as measles virus. They did not isolate any virus. For unexplained reasons, they failed to determine and describe the biochemical structure of what they were presenting as a virus in a separate experiment. In the short description of the methods used, one can read that the authors did not apply the standard isolation technique for vi□ruses, i.e. the density gradient centrifugation. They simply centrifuged fragments of dead cells at the bottom of a test tube and then, without describing their biochemical structure, they misinterpreted the cellular debris as viruses. From the way the experiments were performed, one can only conclude that cellular particles were misinterpreted as viruses. We find the same situation in the fourth and

the sixth7

publication put forward by the claimant as proof of the existence of a measles virus.

The fifth publication is a review describing the consensus process as to which nucleic acid molecules from the dead cells would represent the so called genome of the measles virus. The result is that dozens of researchers teams work with short pieces of cell-specific molecules, after which -following a given model – they put all the pieces together on paper. However, this jigsaw puzzle made of so many pieces was never scientifically proven to exist as a whole and was never isolated from a virus, for a measles virus has never been seen, neither in humans nor in a test tube.

Referring to this publication, the court-appointed expert stated that it described the gold standard, i.e. the entire virus genome. It is obvious that the expert did not read this paper, whose authors stated that the exact molecular composition and functions of the measles virus genome will have to be the object of further research, which is why they had to rely on other virus models in order to achieve a consensus on the structure and functions of the measles virus genome.

The easiest thing for anyone to notice is that in all these publications, as well as in all other publications on the “measles virus” and other pathogenic viruses, no control experiments were ever performed. No researchers used the density gradient centrifugation technique; instead, they only centrifuged cellular debris at the bottom of a test tube. This technique, used to collect all the particles from a fluid, is called pelletising. From a logical and scientific perspective, it can be said that in all publications on so-called “pathogenic viruses”, the researchers demonstrated in fact only particles and characteristics of cells.

We would also like to point out another article, in which we described the so-called giant viruses i.e. an enwrapped nucleic acid that can be found everywhere in the sea and in basic organisms. Like all bacterial phages, not only they are harmless, but they have beneficial functions. They can be also isolated by using the density gradient centrifugation, which proves their existence (see the graphics above).

We also recommend Prof Lüdtke’s relevant review (1999).10 He noted that at the early beginnings of virology, the majority of virologists always concluded that the structures they had mistaken for viruses turned out to be components of the cells and thus, they were only the result of the experiment and not the cause of the changes observed. After the discovery and characterization of the phages and after introducing the dogma that the nucleic acid was the genome of all cells and viruses, the consensus was born, according to which such viruses must exist in humans and animals as well.

In 1992, the dogma stating that the nucleic acid is the genotype of all cells was retracted in the scientific community. In 2008, it was also retracted for a part of the German public community..

The dogma of pathogenic viruses, however, is still being promoted.

https://wissenschafftplus.de/uploads/article/Dismantling-the-Virus-Theory.pdf

Illustration:

"Cell culture

RELIES ON THE

ASSUMPTION that

the behavior of cells in vitro is fundamentally similar to their behavior as part of a tissue within an organ of a multicellular organism."

When Virologists claim they have isolated a "virus," they do not mean that they separated a particular particle they believe to be one from everything else. What they mean is that they took some fluid from a sick patient, added it to a cell culture typically consisting of African Green Monkey Kidney (VERO) Cells, Antibiotics, Fetal Bovine Serum, DMEM "nutrients," etc., and then left the toxic mixture to see if they observe what they call Cytopathic Effects, which is nothing more than cellular breakdown assumed to be caused by a "virus."

For example, this is the detailed method of "virus isolation" from one of the original "SARS-COV-2" papers "A pneumonia outbreak associated with a new coronavirus of probable bat origin:"

"Sample collection

Human samples, including oral swabs, anal swabs, blood and BALF samples were collected by Jinyintan hospital (Wuhan, China) with the consent of all patients and approved by the ethics committee of the designated hospital for emerging infectious diseases. Patients were sampled without gender or age preference unless indicated. For swabs, 1.5 ml DMEM CONTAINING 2% FBS WAS ADDED TO EACH TUBE. The supernatant was collected after centrifugation at 2,500 rpm, vortexing for 60 s and a standing period of 15–30 min. The supernatant from swabs or BALF (no pre-treatment) was added to either lysis buffer for RNA extraction OR TO VIRAL TRANSPORT MEDIUM for isolation of the virus. THE VIRAL TRANSPORT MEDIUM WAS COMPOSED OF HANK’S BALANCED SALT SOLUTION (pH 7.4) CONTAINING BSA (1%), AMPHOTERICIN (15 µg ml−1), PENICILLIN G (100 units ml−1) AND STREPTOMYCIN (50 µg ml−1). Serum was separated by centrifugation at 3,000g for 15 min within 24 h of collection, followed by inactivation at 56 °C for 1 h, and was then stored at 4 °C until use.

Virus isolation, cell infection, electron microscopy and neutralization assay

The following cell lines were used for virus isolation in this study: VERO E6 AND HUH7 CELLS, WHICH WERE CULTURED IN DMEM CONTAINING 10% FBS. All cell lines were tested and free of mycoplasma contamination, submitted for species identification and authenticated by morphological evaluation by microscopy. None of the cell lines was on the list of commonly misidentified cell lines (by ICLAC).

CULTURED CELL MONOLAYERS WERE MAINTAINED IN THEIR RESPECTIVE MEDIUM. The PCR-positive BALF sample from ICU-06 patient was spun at 8,000g for

15 min, filtered and diluted 1:2 with DMEM SUPPLEMENTED WITH 16 μg ml−1 TRYPSIN BEFORE IT WAS ADDED TO THE CELLS. After incubation at 37 °C for 1 h, THE INOCULUM WAS REMOVED AND REPLACED WITH FRESH CULTURE MEDIUM CONTAINING ANTIBIOTICS (see below) AND 16 μg ml−1 TRYPSIN. The cells were incubated at 37 °C and OBSERVED DAILY FOR CYTOPATHOGENIC EFFECTS. The culture supernatant was examined for the presence of virus by qRT–PCR methods developed in this study, and cells were examined by immunofluorescence microscopy using the anti-SARSr-CoV Rp3 N antibody that was generated in-house (1:1,000). PENICILLIN (100 units ml−1) AND STREPTOMYCIN (15 μg ml−1) WERE INCLUDED IN ALL TISSUE CULTURE MEDIA."

https://www.nature.com/articles/s41586-020-2012-7

As you can see, these cultures are a mixture of MANY different chemicals and nutrients and to believe that these have no effect on the cells causing the CPE claimed to be due to a "virus" is absurd. We know for a fact that these chemicals do alter the cell and potentially anything contained within the culture:

ANTIBIOTICS:

"However, we realized that the CELLS SHOWED A POOR GROWTH RATE AND ADHERENCE TO THE CULTURE DISH IN THE PRESENCE OF ANTIBIOTICS."

"The parallel test showed conclusively that CELLS TREATED WITH ANTIBIOTICS GROW SLOWER AND STOP PROLIFERATING EARLIER," says Ali."

"The above-mentioned findings strongly confirm PromoCell's recommendation of AVOIDING THE USE OF ANTIBIOTICS IN CELL CULTURE," concludes Ali. "Most primary or normal human cells show REDUCED GROWTH RATES IN THE PRESENCE OF ANTIBIOTICS. Keeping the cells free from microorganism contamination can be accomplished with proper knowledge of good laboratory practice. Following all the guidelines towards a sterile technique MAKES THESE COMPOUNDS UNNECESSARY."

https://www.promocell.com/.../antibiotics-in-cell.../

FETAL BOVINE SERUM:

"Here, using RNA sequencing, we demonstrate that FBS contains a diverse repertoire of protein-coding and regulatory RNA species, including mRNA, miRNA, rRNA and snoRNA. THE MAJORITY OF THEM (>70%) ARE RETAINED EVEN AFTER EXTENDED ULTRACENTRIFUGATION in the preparations of vesicle-depleted FBS (vdFBS) commonly utilized in the studies of extracellular vesicles (EV) and intercellular communication. FBS-ASSOCIATED RNA IS CO-ISOLATED WITH CELL-CULTURE DERIVED EXTRACELLULAR RNA (exRNA) AND INTERFERES WITH THE DOWNSTREAM RNA ANALYSIS. Many evolutionarily conserved FBS-derived RNA SPECIES CAN BE FALSELY ANNOTATED AS HUMAN OR MOUSE TRANSCRIPTS. Notably, specific miRNAs abundant in FBS, such as miR-122, miR-451a and miR-1246, have been previously reported as enriched in cell-culture derived EVs, POSSIBLY DUE TO THE CONFOUNDING EFFECTS OF THE FBS. Analysis of publically available exRNA datasets supports the notion of FBS contamination. Furthermore, FBS TRANSCRIPTS CAN BE TAKEN UP BY CULTURED CELLS AND AFFECT THE RESULTS OF HIGHLY SENSITIVE GENE EXPRESSION PROFILING TECHNOLOGIES. Therefore, precautions for experimental design are warranted to MINIMIZE THE INTERFERENCE AND MISINTERPRETATIONS CAUSED BY FBS-DERIVED RNA."

https://www.nature.com/articles/srep31175

DMEM (and other media):

"Notably, NONE OF THE EXAMINED MEDIA FULLY ADHERE TO PHYSIOLOGICAL VALUES OF ELECTROLYTES AND CARBOHYDRATES (Table 1). The most commonly used media, DMEM and RPMI 1640, DEVIATE THE MOST FROM PHYSIOLOGICAL VALUES. DMEM contains 25 mM glucose, FOUR TIMES MORE THAN NORMAL AND RARE EVEN IN HYPERGLYCEMIA (26). Sigma carries a low-glucose variant of DMEM; however, a Google Scholar search suggests that it was utilized in only 2,400 of 31,300 studies using DMEM in 2016. RPMI 1640 contains extremely low levels of calcium, magnesium, and sulfate, elevated glucose at 11 mM, and threefold higher than normal phosphate levels. Compared with the physiological levels in human plasma, DMEM and MEM HAVE HIGHER CALCIUM, MEM and M199 have higher chloride and sulfate, and M199 also has slightly increased sodium and potassium."

"The important question is WHETHER THE OBSERVED DEVIATIONS IN ELECTROLYTES AND GLUCOSE CAN IMPACT CELL BEHAVIOR."

"In conclusion, WE SUGGEST THAT THE NON PHYSIOLOGICAL ELECTROLYTE AND CARBOHYDRATE MICROENVIRONMENT OF THE MOST COMMONLY USED CELL CULTURE MEDIA MAY RESULT IN UNINTENDED AND UNCONTROLLED CHANGES IN CELL BEHAVIOR, THEREBY CONTRIBUTING TO THE DIFFICULTIES

IN REPRODUCIBILITY observed in modern publications."

https://journals.physiology.org/.../ajpcell.00336.2016

How can virologists claim that what is occuring in cell cultures is natural and not effected by all the added ingredients/chemicals/nutrients?

It is absurd to believe the original sample and the cell are unaltered.

These cell cultures are not PURIFIED unaltered particles that are separated from everything else. They are toxic concoctions for which a "virus" is ASSUMED to exist within.

2D or not, 3D? That is the question...

Besides the problem in cell cultures in regards to a lack of purification and proper isolation of a particle assumed to be a "virus" as well as the toxic nature of the chemicals used in "growing" them, there is another underlying issue:

2D Cell Cultures can not mimic or accurately represent the IN VIVO (associated with various biological processes that are made to occur WITHIN THE LIVING ORGANISM) physiology and complexity of a living organism.

In other words, what is taken from within a living organism and is then studied outside of it in controlled laboratory conditions can not accurately reflect what actually occurs when the studied material is within a living organism.

The fact that 2D Cell Culture can not accurately reflect these conditions should give pause to anyone reading any virology paper. When one then considers the numerous alterations to the starting material through the use of animal cells/chemicals/antibiotics/fetal bovine serum/DMEM etc., it is clear to see the results have absolutely no relation to reality whatsoever.

This has given rise to 3D Cell Cultures which aim to more accurately reflect what occurs within the living organism. These cultures, while claimed to be a better reflection, have their own problems but that is a whole different post. It is clear, when comparing and contrasting 2D/3D Cell Cultures, that the current crop of "evidence" for "SARS-COV-2" and any other "virus" should be immediately thrown out:

"Although the 2D culture system is simple and easily accessible, THE CULTURE ENVIRONMENT IS UNABLE TO REPRESENT IN VIVO EXTRACELLULAR MATRIX (ECM) MICROENVIRONMENT. Our study observed that 2D- culture derived EVs SHOWED SIGNIFICANTLY DIFFERENT PROFILES IN TERMS OF SECRETION DYNAMICS AND ESSENTIAL SIGNALING MOLECULAR CONTENTS (RNAs and DNAs), when compared to the three-dimensional (3D) culture derived EVs."

"For investigating cellular communications and behaviors EX VIVO, presently, the two-dimensional (2D) CELL CULTURE MODEL IS WIDELY USED AS THE “GOLD STANDARD”29. This 2D culture system serves as an essential model for investigating tissue physiology and complex biological activity, from cell differentiation to tissue morphogenesis30. Many 2D cell culture systems have also been widely employed for studying EV RNA expression profiles from tumor cells31, roles in promoting tumor growth17 and tumor biomarker discovery32,33,34. Although the 2D culture system provides simple cell attachment and nutrients supply, THE FLAT AND HARD SURFACE FROM PLASTIC OR GLASS SUBSTRATES ARE UNABLE TO REPRESENT THE IN VIVO EXTRACELLULAR MATRIX (ECM) MICROENVIRONMENT IN TISSUE OR ORGANS 35. The monolayer cells UNDER 2D CULTURE CONDITION COMPLETELY DIFFER FROM IN VIVO STATUS where cells grow in three dimensions (3D), IN TERMS OF CELL MORPHOLOGY, CELL-TO-CELL INTERACTIONS, GROWTH BEHAVIOR, AND INTERACTIONS WITH EXTRACELLULAR MATRIX 30. It has been well demonstrated that 2D cell monolayer is UNABLE TO REPRESENT THE PHYSIOLOGY OF IN VIVO 3D TISSUES OR ORGANS, due to the substantially different microenvironment (e.g., mechanical and biochemical properties) in tissue architecture 36,37,38,39,40,41"

"Unlike the 2D culture, 3D CELL CULTURE IS MORE RECOGNIZED FOR MIMICKING IN VIVO CELLULAR BEHAVIOR 41."

"More importantly, we observed that 3D cell-derived EV samples clustered together with two in vivo cervical cancer patient plasma sample derived EV miRNAs. It supports that 3D cell culture is necessary for reproducing the EV miRNA content sorted by in vivo cells and establishing an accurate disease model 57,58. On the other hand, the 2D-DERIVED SAMPLES WERE CLUSTERED AWAY FROM THE IN VIVO SAMPLES, INDICATING THAT 2D CELLS CULTURE WAS UNABLE TO REPRESENT IN VIVO BIOLOGICAL STATUS. This observation supports the statement from other studies 56,58 that 3D CULTURE SYSTEM WOULD BE MORE USEFUL AND ACCURATE FOR

MIMICKING IN VIVO PHYSIOLOGICAL ENVIRONMENT in studying EV functions."

https://www.nature.com/articles/s41598-019-49671-3#ref-CR9

"Despite its importance, 2D CELL CULTURE MODELS FAIL TO RECAPITULATE THE COMPLEXITY OF LIVING ORGANISMS AND OFTEN ACQUIRE PHENOTYPES THAT DIFFER SIGNIFICANTLY FROM NATIVE TISSUES, WHICH LEADS TO POOR PREDICTIONS OF RESULTS [7]. Therefore, the use of platforms that provide INCREASED SIMILARITY TO THE IN VIVO PHYSIOLOGY AND PATHOLOGY can contribute to advances in the treatments of COVID-19."

"Due to the seriousness of the pandemic situation caused by COVID-19, the rapid development of in vitro models to study SARS-CoV-2 infection was necessary in order to assist clinical approaches and treatments through the knowledge acquired in basic research, almost in real time. For these purposes, 2D MONOLAYERS HAVE BEEN EXTENSIVELY USED TO STUDY SARS-CoV-2 LIFE CYCLE AND PATHOGENESIS ANALYSIS, drug screening and preclinical evaluation of antiviral potential, and CYTOPATHIC EFFECT OF CANDIDATE MOLECULES [35].

Vero cells E6 cells, ISOLATED FROM AFRICAN GREEN MONKEYS KIDNEYS, are susceptible to many types of viruses, including the SARS-CoV [36] and SARS-CoV-2 [37]. They produce high viral titers, probably due to the expressive presence of ACE-2 in their apical region, and because these cells do not produce type I interferons (IFN) when infected by several viruses. This phenomenon is due to a deletion of ∼9 Mbp deletion on chromosome 12, which when in homozygous, results in a more permissive phenotype for viruses. Thus, the IFN deficiency allows SARS-CoV-2 to replicate sustainably in Vero cells [38]. THIS CELL LINE WAS USED IN SOME IMPORTANT STUDIES INVOLVING SARS-Cov-2, such as for identifying the ACE2 as the functional receptor of SARS-CoV, for demonstrating that anti-ACE2 acted as an inhibitor of viral replication in these cells [5], for identifying other potential routes of infection [31], and for testing the inhibition potential of antiviral candidates [6]. HOWEVER, THE HIGHLY PERMISSIVE PHENOTYPE VERO CELLS HAVE SOME LIMITATIONS, AS IT DOES NOT ACCURATELY REPRESENT THE PATHOGENESIS OF COVID-19, as its initial target organs are the air and pulmonary epithelia and the venous endothelium. Therefore, other cell types seem to serve as in vitro models THAT MAY BETTER RECAPITULATE THE REAL PHYSIOLOGY OF THE DISEASE.``

"Conventional 2D cell cultures have greatly contributed to the understanding of host cell–virus interactions, mechanisms of virus transmission, replication, and adaptation, as

well as screening of antiviral drugs [6,37]. However, THIS MODEL HAVE SOME LIMITATIONS THAT RELY ON THE DIFFICULTY OF RECONSTITUTING THE ACCURATE AND COMPLEXITY MICROENVIRONMENT FOUND IN LIVING ORGANISMS. Cell–cell junctions, apical-basal polarity, and cell communication through gradients of endogenous growth factors, chemokines, and nutrients MAY BE INADEQUATE TO GUARANTEE THE SIMILARITY WITH AN IN VIVO SYSTEM [7]. THESE LIMITATIONS EXEMPLIFY THE NECESSITY TO DEVELOP NEW PLATFORMS FOR IN VITRO MODELING [7,49]."

"Although 2D conventional cell culture has shown to be an important tool in virology studies, THIS MODEL FAILS IN REPLICATING THE CELLULAR MICROENVIRONMENT IN TERMS OF ARCHITECTURE, COMPOSITION, PHYSIOLOGICAL FUNCTIONS, AND MECHANICAL STIMULUS, WHICH MAY LEAD TO LOW PREDICTION OF RESULTS [7]. The establishment of 3D cell culture and the biofabrication of tissue-like structures can mimic the complex microenvironment found in the many organs affected by SARS-CoV-2 with higher accuracy, providing robust data to elucidate cellular and molecular mechanisms of virus infection, replication kinetics, and host–virus interaction."

https://www.sciencedirect.com/.../pii/S2319417020302079

In summary, 2D Cell Cultures:

-are unable to represent the in vivo extracellular matrix microenvironment

-differ completely in terms of cell morphology, cell-to-cell interactions, growth behavior, and interactions with the extracellular matrix

-are unable to represent in vivo physiology

-are unable to represent in vivo biological status

-often acquire phenotypes that differ significantly from native tissues

-lead to poor prediction of results

-can not replicate the cellular microenvironment in terms of architecture, composition, physiological functions, and mechanical stimulus

It's time to realize that the "evidence" we are presented regarding "viruses" is highly flawed, based on guesswork and assumptions, and does not reflect reality. It is time to

throw Virology out into the trash with the rest of the pseudosciences.

Related post on Cell Cultures:

https://m.facebook.com/story.php?story_fbid=10158065023878576&id=502548575

One of the most overlooked problems in regards to cell cultures and the reliability of the results obtained from them is the issue of misidentification resulting from contamination. It is a well-known issue and easily leads to false results built upon false results:

"Contaminants can affect all cell characteristics (e.g. growth, metabolism, and morphology) and CONTRIBUTE TO UNRELIABLE OR ERRONEOUS EXPERIMENTAL RESULTS."

"Data derived from undetected contaminated cultures CAN END UP PUBLISHED IN SCIENTIFIC JOURNALS, ALLOWING OTHERS TO BUILD HYPOTHESES FROM DUBIOUS RESULTS. The pervasiveness of cross-contaminated and misidentified cell lines is A DECADES-LONG ISSUE; in 1967, cell lines thought to be derived from various tissues were shown to be HeLa cells, a human cervical adenocarcinoma cell line.1 However, STUDIES INVOLVING THESE MISIDENTIFIED CELL LINES CONTINUED TO FEATURE IN HUNDREDS OF CITATIONS DURING THE EARLY 2000s.2"

"THIS PATTERN IS A WELL-ACKNOWLEDGED PROBLEM and threatens to undermine scientific integrity. The first published retraction in Nature Methods was due to cell line contamination3, and one conservative estimate of “contaminated” literature in 2017 FOUND 32,755 ARTICLES REPORTING ON RESEARCH WITH MISIDENTIFIED CELLS.4 While many scientists may have been blissfully ignorant in the past, awareness of misidentified cell lines is growing.

But what should be done about EXISTING CONTAMINATED LITERATURE? Mass retraction of affected articles may disproportionately punish the careers of a few scientists, and could be a waste of resources containing potentially valuable data. One recently proposed system of “self-retraction” recommends REPLACING BLAME WITH PRAISE IN ORDER TO ENCOURAGE SELF-CORRECTION.5 Post hoc labeling of published articles in the form of an “expression of concern” allows existing findings to remain accessible, WHILE GIVING READERS A CHANCE TO FORM THEIR OWN

JUDGEMENT."

https://www.technologynetworks.com/.../meet-the-culprits...

It is obvious cell misidentification and contamination has been a oft ignored problem even though there are numerous ways in which these cell lines and cultures can be corrupted:

"4.1. CELL LINE MISIDENTIFICATION

One of the most serious and persistent problems is cell line misidentification often RESULTING FROM CROSS-CONTAMINATION."

"4.2. MYCOPLASMA CONTAMINATION

Contamination of cell cultures with mycoplasma was first noted in the 1950s BUT IS STILL REGRETTABLY OFTEN DISREGARDED. The following important points should be noted:

1) Mycoplasma contamination is VERY FREQUENT, worldwide.

2) Using mycoplasma-contaminated cells can RESULT IN ERRONEOUS, MISLEADING OR FALSE EXPERIMENTAL RESULTS.

3) Owing to lack of visible signs mycoplasma-positive cell cultures CAN GO UNNOTICED."

"Mycoplasmas and the related Acholeplasmas (collectively referred to as ‘mollicutes’) are the smallest and simplest self-replicating bacteria and are significant in that THEY HAVE BECOME PROBABLY THE MOST PREVALENT AND SERIOUS MICROBIAL CONTAMINANT OF CELL CULTURE SYSTEMS USED IN RESEARCH AND INDUSTRY TODAY. Owing to the absence of any visible morphological changes or other symptoms mycoplasma infection of cell cultures OFTEN GOES UNDETECTED. However, it is the INVISIBLE EFFECTS OF THE CONTAMINATION ON THE INFECTED CELLS that makes it such a serious problem. It is therefore essential that

routine mycoplasma testing is performed regularly on all research cell lines to ensure the validity of study results before publication. Although >20 different species of mycoplasma have been isolated from cell cultures, >95% of infections are caused by six prevalent species, which are the following: M. arginini, M. fermentans, M. hominis, M.hyorhinis, M. orale and Acholeplasma laidlawii.

Although primary cell cultures and early passages are less frequently contaminated with reported incidences of between 1 and 5%; CONTINUOUS CELL LINES HAVE MUCH HIGHER INCIDENCES OF BETWEEN 15 to 35% (Drexler and Uphoff, 2002).

MYCOPLASMA ARE UNAFFECTED BY MANY OF THE ANTIBIOTICS COMMONLY USED IN CELL CULTURE, such as penicillin and can grow to extremely high titres (typically 1 × 107 to 1 × 108 organisms per ml) in mammalian cell cultures without producing any turbidity in the medium, or other obvious symptoms. In addition mycoplasma are extremely small (0.15–0.3 µm) and pleomorphic, AND WILL PASS THROUGH STANDARD 0.22-µm BACTERIOLOGICAL FILTERS (0.1-µm filters are required for sterilisation). The only assured way of detecting mycoplasma contamination is regular testing."

"4.2.1. EFFECTS

The effects of mycoplasma contamination on the host eukaryotic cell are quite variable but HAVE BEEN SHOWN TO ALTER MANY HOST CELL FUNCTIONS INCLUDING GROWTH, MORPHOLOGY, METABOLISM, THE GENOME AND ANTIGENICITY (Drexler and Uphoff, 2002). Using mycoplasma-contaminated cultures in experiments will therefore CLEARLY CALL INTO QUESTION THE VALIDITY AND SIGNIFICANCE OF ANY RESEARCH DATA GENERATED AND COULD RESULT IN THE PUBLICATION OF ERRONEOUS EXPERIMENTAL RESULTS. Research journals are NOW STARTING TO ASK FOR EVIDENCE THAT MYCOPLASMA-FREE CELL CULTURES ARE USED in studies before accepting papers for publication. In addition the time and cost involved in cleaning contaminated laboratories, obtaining new cell cultures and repeating experiments is significant as is the POTENTIAL REPUTATIONAL DAMAGE OF PUBLISHING ERRONEOUS RESULTS."

"4.2.2. SOURCES

Common sources of mycoplasma contamination in the laboratory include:

1) Cross-contamination from other mycoplasma-positive cell cultures.

2) Laboratory equipment and work surfaces.

3) Laboratory personnel (often via respiratory tract infections).

4) Cell culture media, sera and reagents.

5) The liquid phase of LN2 cryostorage vessels.

6) Feeder cell cultures.

7) Laboratory animals

4.3. CONTAMINATION BY OTHER MICROORGANISMS

With correct working practice IT SHOULD NOT BE NECESSARY TO USE ANTIBIOTICS TO CONTROL CONTAMINATION IN ESTABLISHED CELL LINES AND THEIR USE SHOULD BE DISCOURAGED. Microbial contamination may be obvious, indicating that the culture should be discarded, but, IF ANTIBIOTICS ARE USED, CONTAMINATION MAY BE REPRESSED BUT NOT ELIMINATED. Such cryptic contamination MAY COEXIST WITH THE CELL CULTURE and only appear when the culture conditions change or the organism develops antibiotic resistance. In addition as antibiotics and antifungal agents act by inhibiting biochemical functions of the organism, these activities may also affect animal cells PREJUDICING THE OUTCOME OF EXPERIMENTS. For example, amphotericin B is a membrane active agent and may therefore interfere with any mammalian cell experiments involving membrane trafficking or intercellular signalling."

"If a cell culture is contaminated with bacteria or fungi, then the best method of elimination is to discard the culture and initiate fresh cultures from frozen stock. In the case of irreplaceable stocks, it may be necessary to use antibiotics; the more antibiotics that are tested, the greater the chance of finding one that eliminates the infection. HOWEVER, IF THE CELLS HAVE BEEN ROUTINELY GROWN IN MEDIA SUPPLEMENTED WITH ANTIBIOTICS (WHICH IS NOT RECOMMENDED), IT IS ALMOST CERTAIN THAT THE CONTAMINATION WILL BE WITHIN ORGANISMS THAT ARE ALREADY RESISTANT TO THIS AND SOME OTHER ANTIBIOTICS."

"4.3.2. VIRUSES

As long as cell culture reagents of biological origin are used, such as serum to supplement media and natural trypsin for subculture, THERE WILL ALWAYS BE A RISK THAT ENDOGENOUS INFECTIONS IN THE SOURCE OF THE REAGENT WILL INFECT THE CULTURE. Any viral contaminant that grows in the cells will affect the cells' metabolism and could also present a safety hazard to lab workers. THE SOURCE OF VIRAL CONTAMINATION CAN BE FROM THE TISSUE FROM WHICH THE CELLS ARE DERIVED (e.g., HIV from Kaposi's sarcoma cells, EBV from lymphoma cells). Alternatively, contamination can be derived from other infected cultures or, as a more remote possibility, from laboratory personnel. Another route of infection can be CURING PASSAGE OF CELLS IN EXPERIMENTAL ANIMALS, important when considering the use of cell lines for or from implantation of xenograft tumours. Not only do the cells to be implanted need to be free from contamination by extraneous viruses but also the animals into which the transplant is to be made should not harbour viruses that could affect the growth and response to therapy of the cells under study."

Even more than with mycoplasma, ELIMINATION OF VIRAL CONTAMINATION IS DIFFICULT AND IS LIKELY TO BE IMPOSSIBLE. However, what is worse, THERE ARE NO SIMPLE UNIVERSAL DIAGNOSTIC TESTS TO IDENTIFY VIRAL CONTAMINATION. Next-generation sequencing techniques potentially offer such screening but are yet to be qualified for routine safety testing. Identifying viruses currently necessitates screening with a wide panel of immunological or molecular probes and may be best done by a specialist testing service. As yet, such testing is largely restricted to human pathogens such as EBV, HIV, HTLV I/II and Hepatitis B & C, and FEW LABORATORIES SCREEN FOR ANIMAL VIRUSES ON A ROUTINE BASIS, although some commercial suppliers and veterinary laboratories do."

"4.4. GENETIC INSTABILITY AND PHENOTYPIC DRIFT

Two other major problems that can affect the utility of cell lines are genetic instability and phenotypic drift, BOTH OF WHICH MAY PROGRESS THE LONGER THE CELL LINE IS CULTURED."

"4.4.1. GENETIC INSTABILITY

The chromosomal content of most continuous cell lines is both aneuploid (abnormal chromosome content) and heteroploid (variable chromosome content within the population). Many cancer cell lines have defects in p53 and other genes that monitor and repair DNA damage, resulting in an increased mutation frequency. Hence, THE GENOTYPE OF CONTINUOUS CELL LINES CAN CHANGE WITH TIME AND CELL LINES SHOULD NOT THEREFORE BE MAINTAINED FOR EXTENDED PERIODS OF

TIME IN CONTINUOUS CULTURE (Wenger et al, 2004; Saito et al, 2011)."

"4.4.2. PHENOTYPIC INSTABILITY

Lack of expression of the differentiated properties of the cells of origin is a major recurrent problem. This can be due to SELECTION OF THE WRONG CELL LINEAGE IN INAPPROPRIATE CULTURE CONDITIONS."

"It is important and probably ESSENTIAL FOR COMPARATIVE PURPOSES THAT DIFFERENT LABORATORIES USING THE SAME CELL LINE SHOULD MATCH THEIR CULTURE CONDITIONS AS CLOSELY AS POSSIBLE."

https://www.nature.com/articles/bjc2014166

Needless to say, there are numerous ways in which cell lines are misidentified and contaminated. It is impossible to say that the cell culture is free of all contaminants as many are unknown or are unable to be detected. The means by which cells are decontaminated with antibiotics can have disastrous effects on the cell culture.

There is no way to state that the results and evidence from cell cultures are reliable for various reasons (cells/chemicals/antibiotics used, lack of reproducibility, inability to recreate in vivo environment). High up on that list is the well-known and often ignored issue of misidentification/contamination.

Antibiotics such as Penicillin-streptomycin are commonly used in cell cultures even though they have various unwanted effects on the cells:

"Standard cell culture guidelines often use media supplemented with antibiotics to prevent cell contamination. However, RELATIVELY LITTLE IS KNOWN ABOUT THE EFFECT OF ANTIBIOTIC USE IN CELL CULTURE ON GENE EXPRESSION AND THE EXTENT TO WHICH THIS TREATMENT COULD CONFOUND RESULTS."

"Our results suggest that PenStrep treatment CAN SIGNIFICANTLY ALTER GENE EXPRESSION AND REGULATION in a common liver cell type such as HepG2, advocating that antibiotic treatment SHOULD BE TAKEN INTO ACCOUNT WHEN CARRYING OUT GENETIC, GENOMIC OR OTHER BIOLOGICAL ASSAYS

IN CULTURED CELLS."

"It is possible that antibiotics such as penicillin-streptomycin and gentamicin also induce a functional state that is significantly different from the basal state of these cell types. FURTHER EVALUATION OF THE BIOLOGICAL IMPACT OF ANTIBIOTIC TREATMENT ACROSS CELL LINES IS HIGHLY WARRANTED. However, we provide some evidence that using antibiotics in cell culture SHOULD BE AVOIDED- especially in studies focused on drug response as well as cell cycle regulation, differentiation, and growth. DATA FROM STUDIES IN WHICH ANTIBIOTICS ARE USED FOR CELL CULTURE SHOULD BE EXAMINED WITH CAUTION."

https://www.biorxiv.org/content/10.1101/130484v1.full.pdf

It has been clear that antibiotics should not be used in cell cultures as they can and will change the cells used in them. Various guidelines recommend against their use but alas, they continue to insist on using them.

Let's look at two of the most cited papers for the evidence of "SARS-COV-2" and see if they used antibiotics:

From "A Novel Coronavirus from Patients with Pneumonia in China, 2019:"

"The viral transport medium was composed of Hank's balanced salt solution (pH 7.4) containing BSA (1%), AMPHOTERICIN (15 µg ml−1), PENICILLIN G (100 units ml−1) and STREPTOMYCIN (50 µg ml−1)."

"PENICILLIN (100 units ml−1) AND STREPTOMYCIN (15 µg ml−1) WERE INCLUDED IN ALL TISSUE CULTURE MEDIA."

https://www.nejm.org/doi/full/10.1056/nejmoa2001017

From "Identification of Coronavirus Isolated from a Patient in Korea with COVID-19:"

"Oropharyngeal samples were diluted with viral transfer medium containing nasopharyngeal swabs and ANTIBIOTICS (NYSTADIN, PENICILLIN-STREPTOMYCIN 1:1 dilution) at 1:4 ratio and incubated for 1 hour at 4°C, before being inoculated onto Vero cells. Inoculated Vero cells were cultured at 37°C, 5% CO2 in 1× Dulbecco's modified Eagle's medium (DMEM) supplemented with 2% fetal bovine serum AND PENICILLIN-STREPTOMYCIN."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7045880/

Remember: "DATA FROM STUDIES IN WHICH ANTIBIOTICS ARE USED FOR CELL CULTURE SHOULD BE EXAMINED WITH CAUTION."

Both papers used antibiotics when culturing their "viruses."

I want to point out that the Zhu paper admitted to not fulfilling Koch's Postulates in order to prove pathogenicity and authors from both papers admitted to not purifying "virus" particles. The "evidence" within them is already faulty.

This is the "evidence" used to "confirm" to the world that "SARS-COV-2" exists and was used for the justification of lockdowns, quarantines, social distancing, masks, and vaccines.

It's time to wake up to the lies and the fraudulent methods used to justify them.

Cell Culture contamination is a serious and unavoidable problem and it is reason enough to question the evidence for "SARS-COV-2" and any other "virus" said to be "isolated" from this toxic process.

Some highlights from a guide on Cell Culture contamination:

"NO CELL CULTURE PROBLEM IS AS UNIVERSAL AS THAT OF CULTURE LOSS DUE TO CONTAMINATION. All cell culture laboratories and cell culture workers have experienced it. Culture contaminants may be biological or chemical, seen or unseen, destructive or seemingly benign, BUT IN ALL CASES THEY ADVERSELY AFFECT BOTH THE USE OF YOUR CELL CULTURES AND THE QUALITY OF YOUR RESEARCH. Contamination problems can be divided into three classes:

MINOR ANNOYANCES – when up to several plates or flasks are occasionally lost to contamination;

SERIOUS PROBLEMS – when contamination frequency increases or entire experiments

or cell cultures are lost;

MAJOR CATASTROPHES – contaminants (usually other cell lines or mycoplasma) are discovered THAT CALL INTO DOUBT THE VALIDITY OF YOUR PAST OR CURRENT WORK.

The most obvious consequence of cell culture contamination is the loss of your time, money (for cells, culture vessels, media, and sera), and effort spent developing cultures

and setting up experiments. However, the less obvious consequences are often more serious (Table 1).

First, there are the adverse effects on cultures suffering from undetected chemical or biological contaminants. These hidden (cryptic) contaminants can achieve high densities altering the growth and characteristics of the cultures. WORSE YET ARE THE POTENTIALLY INACCURATE OR ERRONEOUS RESULTS OBTAINED BY UNKNOWINGLY WORKING WITH THESE CRYPTICALLY CONTAMINATED CULTURES."

"PREVENTING ALL CELL CULTURE CONTAMINATION HAS LONG BEEN THE DREAM OF MANY RESEARCHERS, BUT IT IS FOR MOST, AN IMPRACTICAL, IF NOT IMPOSSIBLE, DREAM. CONTAMINATION CANNOT BE TOTALLY ELIMINATED, but it can be managed to reduce both its frequency of occurrence and the seriousness of its consequences."

"Since cytopathic viruses usually destroy the cultures they infect, they tend to be self-limiting. Thus, WHEN CULTURES SELF-DESTRUCT FOR NO APPARENT REASON AND NO EVIDENCE OF COMMON BIOLOGICAL CONTAMINANTS CAN BE FOUND, CRYPTIC VIRUSES ARE OFTEN BLAMED (Figures 3a and 3b). Viruses are perfect culprits, UNSEEN AND UNDETECTABLE; GUILTY WITHOUT DIRECT EVIDENCE. This is unfortunate, since the REAL CAUSE OF THIS CULTURE DESTRUCTION MAY BE SOMETHING ELSE, possibly mycoplasma or a chemical contaminant, and as a result will go undetected to become a more serious problem."

'EXPERIENCED CELL CULTURE USERS HAVE RECOMMENDED FOR MANY YEARS THAT ANTIBIOTICS NEVER BE USED ROUTINELY IN CULTURE MEDIA.3,7,12,17,18,26,27,49 In a major study, Barile found that 72% of cultures grown continuously in antibiotics were contaminated by mycoplasma, but only 7% grown without antibiotics were contaminated, a 10-fold difference.37 Similar results are common: WORKERS WHO ROUTINELY AND CONTINUALLY USE ANTIBIOTICS IN THEIR MEDIA TEND TO HAVE HIGHER CONTAMINATION PROBLEMS, INCLUDING MYCOPLASMA, THAN WORKERS WHO DO NOT."

"In 1966, Gartler used isoenzyme analysis to show that 20 commonly used human cell lines were intraspecies contaminated by HeLa cells.19,20 Contaminated is actually a misnomer since in fact 100% OF THE ORIGINAL CELLS HAD BEEN REPLACED BY THE HeLa CONTAMINANT. Unfortunately, the scientific community was slow to respond to this very serious problem. Tests done at one research center on 246 cell lines over an 18-month period prior to 1976 showed that nearly 30% WERE INCORRECTLY DESIGNATED: 14% were the wrong species, and 25% of the human cell lines were HeLa cells.21 A 1981 survey of cultures showed over 60 cell lines that were actually HeLa cells, 16 other human cell lines contaminated by non-HeLa human cell lines, and 12 cases of inter-species contamination (Table 4). Nor is the problem limited to

contamination by HeLa cells. The advent of DNA analysis HAS SHOWN THAT CALLS FROM A VARIETY OF SOURCES HAVE CONTAMINATED MANY OTHER CELL LINES.42 The Database of Cross-Contaminated or Misidentified Cell Lines (as of December 2016) maintained by the International Cell Line Authentication Committee (ICLAC) (iclac.org/databases/cross-contaminations) shows 488 cell lines that are cross-contaminated or misidentified.52"

https://www.google.com/url?sa=t&source=web&rct=j&url=https://safety.fsu.edu/safety_manual/supporting_docs/Understanding%2520and%2520Managing%2520Cell%2520Culture%2520Contamination.pdf&ved=2ahUKEwiVz7CHxPTuAhUKCM0KHXh0AD4QFjAAegQIARAC&usg=AOvVaw3M6StZvPbYyw30IqM9YhAY

There is too much information to copy/paste here but it is a highly recommended read if you want to understand the many faults associated with cell cultures and why any "virus" said to be "isolated" from one should raise an immediate red flag.

THE CYTOPATHIC EFFECT:

Cell cultures are the main method for "virus isolation" and are considered the gold standard even in the face of improving technology:

"With the recent advances in technology, CELL CULTURE IS CONSIDERED A GOLD STANDARD FOR VIRUS ISOLATION."

A typical cell culture consists of taking a sample from a sick person (called the isolate even though nothing is isolated), adding it to a culture containing either animal or cancer cells, mixing in toxic additives such as antibiotics, fetal bovine serum, DMEM "nutrients," etc., and then letting this concoction sit for a week or so while checking for what is called CYTOPATHIC EFFECTS.

"Changes in monolayer cells (e.g., swelling, shrinking, syncytium formation) indicate the presence of viruses. THESE CHANGES IN CELL CULTURE ARE DEFINED AS THE CYTOPATHIC EFFECT (CPE), WHICH IS DUE TO THE PRESENCE OF THE VIRUS [10]."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4850366/...

This effect is the goal of the culture. Without it, "infectious" or viable "virus" is not

considered to be within the sample.

"A PRACTICAL WAY OF "SEEING" AND INDIRECTLY MEASURING A VIRAL INFECTION IS BY LOOKING AT THE DAMAGE A VIRUS CAUSES TO A CELL. This suffering or damage is known as CYTOPATHIC EFFECT (CPE) and its measurement IS WIDELY USED IN VIROLOGY LABS ALL OVER THE WORLD."

https://www.cytosmart.com/.../virus-induced-cytopathic...

Looking at the definition of Cytopathic Effects (CPE), one can see that this measurement is only intended for the IDENTIFICATION OF "VIRUSES:"

"CYTOPATHIC EFFECT (CPE), structural changes in a host cell RESULTING FROM VIRAL INFECTION. CPE occurs WHEN THE INFECTING VIRUS CAUSES LYSIS (dissolution) OF THE HOST CELL or when the cell dies without lysis because of its inability to reproduce."

https://www.britannica.com/science/cytopathic-effect

So if CPE is the method used for identifying "viruses" in cell cultures and the only way to determine if the "viruses" are "viable" and "infectious," what does it mean if there are other possible causes of CPE in cell cultures that are not caused by "viruses?"

According to a manual on Cell Culture Contamination:

"Since cytopathic viruses usually destroy the cultures they infect, they tend to be self-limiting. Thus, WHEN CULTURES SELF-DESTRUCT FOR NO APPARENT REASON AND NO EVIDENCE OF COMMON BIOLOGICAL CONTAMINANTS CAN BE FOUND, CRYPTIC VIRUSES ARE OFTEN BLAMED (Figures 3a and 3b). Viruses are perfect culprits, UNSEEN AND UNDETECTABLE; GUILTY WITHOUT DIRECT EVIDENCE. This is unfortunate, since the REAL CAUSE OF THIS CULTURE DESTRUCTION MAY BE SOMETHING ELSE, POSSIBLY MYCOPLASMA OR A CHEMICAL CONTAMINANT, and as a result will go undetected to become a more serious problem."

https://www.google.com/url?sa=t&source=web&rct=j&url=https://safety.fsu.edu/safety_manual/supporting_docs/Understanding%2520and%2520Managing%2520Cell%2520Culture%2520Contamination.pdf&ved=2ahUKEwjPy_a7nPbuAhVPVs0KHbMbAoAQFjAAegQIARAC&usg=AOvVaw3M6StZvPbYyw30IqM9YhAY

It would appear as if it is known that there are other factors that can cause CPE in cell cultures such as mycoplasma or chemical contaminants yet unseen "viruses" are often

incorrectly blamed.

Let's see how many other CPE causing contaminants we can find:

BACTERIA:

Mycoplasmas

"SOME MYCOPLASMAS HAVE BEEN SHOWN TO PRODUCE SEVERE CYTOPATHIC EFFECTS (CPE) characterized by stunted, abnormal growth and rounded, degenerated cells, apparently due to the promotion or inhibition of apoptosis [56]."

"4.2. CYTOPATHIC EFFECTS

MYCOPLASMAL ATTACHMENT TO EUKARYOTIC CELLS MAY SOMETIMES LEAD TO A PRONOUNCED CYTOPATHIC EFFECT. Attachment permits the mycoplasma contaminant to release noxious enzymatic and cytolytic metabolites directly onto the tissue cell membrane. Some mycoplasmas selectively colonize defined areas of the cell culture. This results in microcolony formation producing microlesions and small foci of necrosis, e.g., M. pulmonis, or form plaques, e.g., M. gallisepticum, in an agar overlay system [5]. Micro Colonization suggests that mycoplasma-specific receptors are localized in defined areas of the cell monolayer. However, other fermenting mycoplasmas, e.g., M. hyorhinis, attach to every cell and destroy the entire monolayer, PRODUCING A GENERALIZED CYTOPATHIC EFFECT. With HeLa cells infected by the invasive M. penetrans, the most pronounced effect was the vacuolation of the host cells [22]."

"4.5. EFFECT ON VIRUS INFECTION

MYCOPLASMAS MAY ALTER THE PROGRESS OF VIRAL INFECTIONS IN CELL CULTURES [83, 84]. AS MYCOPLASMAS MAY ALSO CAUSE VIRUS-LIKE CPE, MANY INVESTIGATORS HAVE MISTAKEN CYTOLYTIC MYCOPLASMAS FOR VIRUSES. Like viruses, mycoplasmas are filterable, hemadsorption, hemagglutinin, resistant to certain antibiotics, able to induce chromosomal aberrations, and sensitive to detergents, ether and chloroform; thus the first established mycoplasma pathogens of humans (M. pneumoniae), animals (M. mycoides) or plants (Spiroplasma spp.) WERE BELIEVED TO BE VIRUSES."

https://www.google.com/url?sa=t&source=web&rct=j&url=https://cdn.intechopen.com/pdfs/40228.pdf&ved=2ahUKEwiusfed1fTuAhWCWc0KHRLNAbAQFjACegQIChAS&usg=AOvVaw1uaXT__

78vt9vVKxurQJdh

Isolated Dentilisin

"CYTOPATHIC EFFECTS OF ISOLATED DENTILISIN, including membrane blebbing, vacuolization, inhibition of motility, loss of epithelial cell contacts and release of a cytosolic enzyme HAVE BEEN DEMONSTRATED IN MULTI-LAYER EPITHELIAL CELL CULTURE SYSTEMS.94,95 "

https://www.sciencedirect.com/.../cytopathogenic-effect

E. Coli

"CYTOPATHIC EFFECTS INDUCED BY E. COLI LIVE BACTERIA OR PROTEIN EXTRACTS ON EPITHELIAL CELLS at three-day post-infection. For CNF and CDT, the cytopathic effect is only observable with bacterial lysates. In contrast, for colibactin and CIF, a contact between bacteria and host cells is required. Colibactin, CDT and CIF induced cytopathic effects as seen by enlarged nuclei and cell distension (megalocytosis), while CNF induced multinucleation and enlargement of HeLa cells."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3572998/

"BOTH PARASITIC AND FREE LIVING AMOEBA occasionally identified as cell culture contaminants.

THEY CAN CAUSE CPE RESEMBLING VIRAL

DAMAGE and completely destroy a culture within 10 days."

https://www.google.com/url?sa=t&source=web&rct=j&url=https://www.researchgate.net/profile/Yehya_Salih/post/How_can_I_remove_fungus_from_cells/attachment/59d644b279197b807799fea4/AS%253A449762882265088%2540148424323512 8/download/Cell%2Bculture%2Bcontamination%2B2.pdf&ved=2ahUKEwjGpurQ0_TuAhUHXM0KHaNlCmMQFjAVegQIIBAC&usg=AOvVaw3cvqiOn726Cpd6yWqD6awB

AMOEBA:

A. Castellani Trophozoites and/or Cysts

"In this study, WE OBSERVED THE CYTOPATHIC EFFECT, in vitro cytotoxicity, and

secretion pattern of cytokines in human corneal epithelial cells (HCECs) INDUCED BY A. CASTELLANI TROPHOZOITES AND/OR CYSTS. In vitro cytotoxicity assay revealed the highest cytotoxicity to HCECs in the co-culture system with amoeba cysts."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6616168/

Naegleria Fowleri

"NAEGLERIA FOWLERI, strain HB-1, CAUSED A DESTRUCTIVE CYTOPATHIC EFFECT (CPE) in secondary mouse-embryo (ME) cells. No evidence was found to suggest that cell-free cytotoxic factors secreted by the amoebae play a part in ME-cell destruction. In culture systems designed for the study of cytopathic factors, mammalian-cell damage SEEMED TO OCCUR ONLY AS A RESULT OF DIRECT CONTACT WITH ACTIVE AMOEBA."

https://pubmed.ncbi.nlm.nih.gov/682176/

PARASITES:

T. Vaginalis

"Thus, to establish the possible outcome from the interaction of T. vaginalis with lung cells, THE CYTOPATHIC EFFECTS OF THE PARASITES WERE EVALUATED USING MONOLAYER CULTURES of the human lung alveolar basal carcinoma epithelial cell line A549.

THESE OBSERVATIONS INDICATE THAT T. VAGINALIS CAUSES CYTOPATHIC EFFECTS ON A549 CELL. To date, this is the first report showing a possible interaction of T. vaginalis with the lung cells using A549 monolayer cultures."

https://pubmed.ncbi.nlm.nih.gov/25307688/

Cryptosporidium Parvum

"UNEXPECTEDLY, DIRECT CYTOPATHIC EFFECTS ARE NOTED IN INFECTED MONOLAYERS, with widespread programmed cell death (i.e., apoptosis) of biliary epithelial cells as assessed both morphologically and biochemically beginning within hours after exposure to the organism. THE NOVEL FINDING OF SPECIFIC CYTOPATHIC INVASION OF BILIARY EPITHELIAL BY C. PARVUM may be relevant to the pathogenesis and possible therapy of the secondary sclerosing cholangitis seen in AIDS patients with biliary cryptosporidiosis."

"The cell culture model for biliary cryptosporidiosis described in this report provides a useful method for INVESTIGATING THE CYTOPATHIC EFFECTS OF C. PARVUM, and

may yield information regarding the in vivo consequences of C. parvum infection."

https://www.google.com/url?sa=t&source=web&rct=j&url=https://aasldpubs.onlinelibrary.wiley.com/doi/pdfdirect/10.1002/hep.510280402&ved=2ahUKEwis09WR_fTuAhW8B50JHXYOBPkQFjALegQICRAC&usg=AOvVaw2x4tvgKx6Wt3jLfyIVVqZI

CHEMICAL CONTAMINANTS:

Antibiotics and Antifungals:

"The toxicity of drug mixtures has not been thoroughly studied. WE THEREFORE INVESTIGATED CYTOPATHIC EFFECTS ON PRIMARY CULTURES OF HUMAN CORNEAL CELLS OF SIX TOPICAL ANTIMICROBIALS SINGLY AND IN COMBINATIONS OF ANY TWO, to determine the combined toxicity ranking and the interaction between duration of exposure and concentration."

"AN IN VITRO SYSTEM WAS CHOSEN TO ISOLATE DRUG TOXICITY FROM CYTOPATHIC EFFECTS OF A MICROBIAL INFECTION. Human corneal epithelial cell cultures were exposed to antimicrobial drugs for a period commensurate with clinical use. They were exposed to fixed and to sequentially decreasing concentrations of test substance, the latter mimicking a tapering off regime. CYTOPATHIC EFFECTS DEPENDED ON THE DRUG(s), CONCENTRATION AND DURATION OF EXPOSURE. Rankings of single drugs and combinations were not correlated. Exposure to diminishing drug concentrations did not always improve the outcome."

https://www.google.com/url?sa=t&source=web&rct=j&url=https://www.nature.com/articles/eye199517.pdf%3Forigin%3Dppub&ved=2ahUKEwiDjLPun_buAhWFGM0KHSEYDnYQFjABegQICRAC&usg=AOvVaw2fEDOgV9MoMW3hP3SO11PE

Just off a cursory look, it would seem that there are various other factors besides "viruses" which cause CPE such as:

-Bacteria

-Parasites

-Amoebas

-Chemical Contaminants

Obviously, this means that the CPE observed in cell cultures is NOT SPECIFIC TO "VIRUSES" and can therefore not be used as indirect evidence of their existence nor as a measure of their viability or "infectious" potential.

Fetal Bovine Serum is the most commonly used sera in cell cultures. It is added to provide the necessary "nutrients" for the cell to grow. As shown here, FBS was used in 2 of the original "SARS-COV-2" papers:

"Inoculated Vero cells were cultured at 37°C, 5% CO_2 in 1× Dulbecco's modified Eagle's medium (DMEM) supplemented WITH 2% FETAL BOVINE SERUM and penicillin-streptomycin."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7045880/

"For swabs, 1.5 ml DMEM CONTAINING 2% FBS was added to each tube."

https://www.nature.com/articles/s41586-020-2012-7

While **FBS** is used in nearly every cell culture, there are many moral and scientific issues regarding its use. A few articles help to highlight these issues:

"FBS is used in a wide range of applications. One of the primary uses of FBS is in eukaryotic cell culture, WITH CONCENTRATIONS UP TO 20% [3] OR EVEN HIGHER, where it provides many essential nutrients and growth factors that facilitate cell survival and proliferation. However, IT IS IMPORTANT TO NOTE THAT FBS IN HUMAN CELL CULTURES MAY INTRODUCE RESEARCH ARTIFACTS; HUMAN CELLS CULTURED WITH HUMAN SERA BEHAVE DIFFERENTLY FROM THOSE CULTURED WITH FBS [4]."

"Cell culture media without any serum have been in use for many years. FETAL BOVINE SERUM MIGHT NOT BE THE BEST SUPPLEMENT FOR CELL CULTURE."

"Regular FBS contains A LARGE NUMBER OF EXTRACELLULAR VESICLES, SOME OF WHICH ARE EXOSOMES."

https://www.labome.com/method/Fetal-Bovine-Serum.html

From this article, we see FBS:

-is commonly used in very high concentrations

-can introduce research artifacts

-affects human cells differently than those which use human sera

-not considered the best supplement for cell culture even though it is widely used

-contains a large number of extracellular vesicles and exosomes

"Most academic labs culture cells by using fetal bovine serum (FBS), A LIQUID EXTRACTED FROM CLOTTED COW BLOOD AND COLLECTED FROM ABATTOIRS WHEN PREGNANT COWS ARE SLAUGHTERED. What ends up in the serum DEPENDS ON FACTORS such as diet, geographical location, time of year, whether the animals receive hormones or antibiotics and the gestational age of fetal calves. Substantial amounts of FBS are added as a supplement to the culture media in which cells grow; 5–15% of the volume of growth media is typical. FBS COMPOSITION CAN AFFECT HOW THICK AN ENGINEERED TISSUE BECOMES, CAUSE SPONTANEOUS ARTEFACTS THAT MIMIC CELL ACTIVITY AND EVEN INFLUENCE HOW SURFACE RECEPTORS RESPOND TO A GIVEN COMPOUND. “FBS is like a big dark cloud over our heads, NOT KNOWING WHAT'S REAL AND WHAT'S NOT,” says Khodabukus, now a postdoctoral researcher at Duke University in Durham, North Carolina."

"FBS IS NOT ONLY VARIABLE, IT ALSO DIFFERS FROM THE FLUID THAT CELLS ARE EXPOSED TO IN THEIR NATURAL ENVIRONMENT. Most cells are in contact not with blood directly but with the interstitial fluid that bathes organs, says Adam Elhofy, chief science officer at Essential Pharmaceuticals in Ewing, New Jersey, a company developing a serum replacement for multiple cell types. Hormones, growth factors and other signalling molecules are abundant in serum, but tightly regulated in organs, he says (see 'Bovine serum's wide range')."

“EVERYONE AGREES IT WOULD BE A GREAT THING IF WE CAN MOVE AWAY FROM FBS AND TO SOMETHING MORE DEFINED,” says Jon Lorsch, head of the US National Institute of General Medical Sciences in Bethesda, Maryland. “The question is how feasible it is, AND WE DON'T KNOW THE ANSWER TO THAT QUESTION.”

“Every year since 1980, PEOPLE HAVE BEEN SAYING THAT SERUM IS DEAD,” he says. “Serum is still very popular because people like the idea that they CAN GROW CELLS AND NOT HAVE FABULOUS TECHNIQUE.” CULTURE IS TOUGH ON CELLS: researchers pipette them from dish to dish, freeze and thaw them, add digestive enzymes to detach them from substrates and more. SERUM IS A BALM FOR SUCH ABUSES, says Price."

https://www.nature.com/articles/537433a

From this article, it is clear that FBS:

-is variable depending on many factors that go into its production

-can effect tissue thickness

-causes artefacts that mimic cellular activity

-can influence how surface receptors respond to a given compound

-differs from the fluid cells are exposed to in their natural environment

-is used for its ease of use without requiring fabulous technique

"However, the continued use of serum in cell culture features many drawbacks too. In particular, THE COMPOSITION OF SERUM IS POORLY DEFINED, and it is PRONE TO SIGNIFICANT BATCH-TO-BATCH VARIATION.(1,3,4) Further, serum MAY HARBOR A WIDE ARRAY OF CONTAMINANTS, such as bacteria, mycoplasma, viruses, endotoxins, and prions.(5−8)"

"The most commonly used type of serum in cell culture is fetal bovine serum (FBS) due to its strong growth-promoting capacity and relatively low immunoglobulin levels.(9) It has recently been reported THAT NEARLY 80% OF THE LATE CLINICAL STAGE CELL THERAPIES based on mesenchymal stem cells USE FBS."

"The use of 50 nm large-size virus retentive triple-layer hydrophilic PVDF (polyvinylidene difluoride)-based DV50 filters to filter 10% FBS in Dulbecco's modified eagle's medium (DMEM), has been reported, featuring robust clearance of large-size viruses BUT POOR CLEARANCE OF SMALL-SIZE 25 NM VIRAL PARTICLES.(17)"

https://pubs.acs.org/doi/10.1021/acsabm.0c01372

From this article, we see FBS:

-has a poorly defined composition

-is prone to significant batch-to-batch variations

-may harbor a wide array of contaminants

-filtration can not remove small "viral" particles

These 3 articles alone highlight many reasons why FBS should not be used in cell culture or at the very least cause concern about the validity of any culture using it. Once you read the description for how it is made, you will realize there are ethical issues as

well. Keep in mind that in most instances, the fetus is alive during this process:

"The general procedure of a cardiac puncture is the following (see diagram). AT THE TIME OF SLAUGHTER, THE COW IS FOUND TO BE PREGNANT DURING EVISCERATION (removal of the internal organs in the thorax and abdomen during processing of the slaughtered cow). The reproductive tract is removed from the carcass, and is dropped down a special stainless steel chute leading to the calf processing area, a room that is separated from the rest of the abattoir floor. The calf is removed quickly from the uterus and the umbilical cord is tied off, the fetus is cleaned from amniotic fluid, and is disinfected."

"A CARDIAC PUNCTURE IS PERFORMED BY INSERTING A NEEDLE BETWEEN THE RIBS DIRECTLY INTO THE HEART OF THE NONANAESTHETISED FETUS AND BLOOD IS EXTRACTED UNDER VACUUM INTO A STERILE BLOOD COLLECTION BAG VIA A TUBE. In the absence of a vacuum pump, fetal blood may be obtained by means of gravity or massage. In this case the blood collection bag is placed at a level below the fetus. Once the blood has been obtained, it is allowed to clot at low temperature, after which the clotted substance is separated from the serum by refrigerated centrifugation. The fetus is processed for animal feed and extraction of specific substances like fats and proteins, among other things. A much less common technique is umbilical cord puncture."

"SINCE THE FETUS IS EXPECTED TO BE ALIVE DURING BLOOD COLLECTION, its possible suffering is considered. The described procedure may cause pain in the fetus, thus raising ethical questions. First, literature on the resistance of fetuses to lack of oxygen is discussed. The bovine fetus experiences anoxia, acute lack of oxygen, since oxygen-rich blood supply to the placenta ceases upon death of the maternal animal. Lack of oxygen may interfere with neural processes such as transmission of stimuli, and eventually leads to death."

"SINCE FBS IS UNDEFINED, its application in culture media MAY ALTER THE OUTCOME OF SCIENTIFIC EXPERIMENTS INVOLVING CELL CULTURES and make it difficult to compare similar experiments performed with different batches of serum (6, 76). Hence, FBS may interfere with the advancement of biological science (92, 93). FBS should be replaced or its use reduced in cell culture both on scientific and moral grounds."

"The presence of many different growth and growth inhibition factors may lead to overgrowth of e.g. fibroblasts in mixed cultures. From a scientific point of view it may be questioned what the effects are of the absolute molecular composition of FBS relative to the serum of the species, gender and developmental stage the cultured cells are derived from (70). Proper cell growth does not necessarily coincide with proper cellular function (3). FETAL BOVINE SERUM CAN INTERFERE WITH GENOTYPIC AND PHENOTYPIC CELL STABILITY (71, 72), AND CAN INFLUENCE EXPERIMENTAL OUTCOME (5, 6, 71, 73-77). SERUM CAN SUPPRESS CELL SPREADING,

ATTACHMENT (78) AND EMBRYONAL TISSUE DIFFERENTIATION (76). Finally, serum CAN BE CONTAMINATED with viruses, bacteria, mycoplasmas, yeast, fungi, immunoglobulins, endotoxins, and possibly prions (11, 67, 72, 79-85). THESE UNDESIRED SUBSTANCES CAN AFFECT SCIENTIFIC EXPERIMENTS and bulk production of proteins. MANY SUBSTANCES PRESENT IN FBS HAVE NOT YET BEEN IDENTIFIED (67, 86, 87) AND OF MANY SUBSTANCES, WHICH HAVE BEEN IDENTIFIED, THE FUNCTION ON THE CULTURED CELLS IS UNCLEAR (79)."

"Fetal bovine serum is both a SCIENTIFICALLY AND A MORALLY PROBLEMATIC PRODUCT. Its application in cell culture experiments represents a scientific problem as FBS IS UNDEFINED AND MAY INTERFERE WITH THE OUTCOME OF EXPERIMENTS."

https://www.google.com/url?sa=t&source=web&rct=j&url=https://www.forskautandjurforsok.se/docs/Forskarrummet/Serum/the-use-of-fetal-bovine-serum-ethical-or-sceintific-problem.pdf&ved=2ahUKEwjd27PG0PbuAhWScc0KHXmJCFEQFjAKegQIChAC&usg=AOvVaw3C4fQwIdWNztcYFh2zIXuv

This last article really highlights the moral issues regarding the collection and use of FBS. It also highlights many of the scientific problems such as FBS:

-is undefined and may alter the outcome of cell cultures

-interferes with genotypic and phenotypic stability

-suppresses cell spreading, attachment, and differentiation

-can be contaminated

-has many unidentified substances and the ones that are known have an unknown effect on cell cultures

Needless to say, just like the use of antibiotics which have profound impacts on cell cultures, the same can be said of FBS, potentially even more so. On top of the scientific issues with its use, there are the moral issues to consider as well.

It is clear that any cell culture using FBS in the "isolation" of a "virus" is already of a highly questionable quality.

Another area of concern with Cell Cultures is **environmental contamination**. This can occur from the plastic dishes used during cultures, from the purity of the water used, or even unintentional effects from certain light sources:

"THE CELLS' PHYSICAL ENVIRONMENT IS A PROFOUND INFLUENCE. Researchers at the Wyss Institute in Boston, Massachusetts, found that mechanical peristalsis-like deformations and fluid flow changes alone could, without any alterations to the growth media, induce functional villi from cells that otherwise grow flat (6).

LAB DISHES OF DIFFERENT BRANDS LEACH DIFFERENT CHEMICALS INTO CELL-CULTURE MEDIA, and can confound studies of cell metabolites. DELIBERATE ADDITIVES CAN CHANGE CELL METABOLISM in unappreciated ways: ANTIBIOTICS in particular frequently impair mitochondrial activity. EVEN A GLASS DOOR ON A LAB REFRIGERATOR CAN RUIN EXPERIMENTS, because some chemicals in growth media are sensitive to light. JUST CHANGING THE LABORATORY PLATES, and thus the height of media in which cells are sitting, CAN ALTER HOW CELLS BEHAVE. What's more, CELLS GROWING IN A GIVEN CULTURE ARE NOT IDENTICAL, and the subset of cells that thrives the most can quickly dominate a population. That means cells may not revert back to former behaviour if a researcher decides to restore previous experimental conditions."

https://www.nature.com/articles/537433a

Creating the Cytopathic Effect

“Cytopathic effect (CPE), structural changes in a host cell resulting from viral infection. CPE occurs when the infecting virus causes lysis (dissolution) of the host cell or when the cell dies without lysis because of its inability to reproduce.”

https://www.britannica.com/science/cytopathic-effect

I’ve already gone through some of the various factors (bacteria, amoeba, parasites, chemical additives) which can be a cause of the CPE said to be specific to “viral” invasion.

I’ve also outlined many of the numerous problems regarding the use of cell cultures as proof of “virus” isolation in previous posts. However, this syllabus on cell culture techniques that I came across was full of additional insight on causes of CPE as well as some interesting admissions regarding cell cultures so I felt the need to share it. Below are some highlights along with a summary at the end. I recommend reading the whole syllabus sometime as it is a long but very worthwhile read.

A few things to think about while reading:

Note the various forms of contamination in cell culture

Look at the many admittances to CPE being caused by factors other than a “virus”

Notice that kidney cells are considered sanctuaries of “viruses”

Look for the different ways they try to bring about CPE with cell-altering tricks (longer culturing times, blind cultures, or changing cell lines)

Think about the numerous assumptions which are made during the “isolation” process

Hopefully, this information may shed some new light for some of you as well:

Cell Culture Techniques

“To paraphrase recent statements by a colleague, “All cells cultured in vitro are angry; they are outside of their normal environment and maintained under artificial conditions, surrounded by physiologically incorrect concentrations of all things important to their well-being. No wonder it is so difficult to have well-behaved cell cultures”! Without adequate training and preparation, cell culture as an art and science becomes sloppy, and data generated by such practices are questionable. We have often not been able to repeat the results of others, and they have not been able to repeat ours, due to a difference in the cells used in our experiments. In some cases, the cells are not what they should be, in other cases, the cells are contaminated with adventitious agents that confound the results, and sometimes, the cells have changed, either through differentiation or genetic instability.”

COMPLICATIONS ARISING FROM THE USE OF PRIMARY CELLS FOR VIRUS

ISOLATION

“Primary cells (Table 1), which are non-immortalized cells taken directly from a living organism, are often used in clinical laboratories for the isolation of various viruses. For example, primary monkey kidney cells, which in the USA are obtained from rhesus or other macaques or from various African “green” monkey species, are used for the isolation of echo and other picorna viruses, and human parainfluenza and other paramyxoviruses. Primary cells are especially useful for diagnostic virology because some viruses are easier to isolate (or can only be isolated) in them. However, primary cells often harbor latent viruses that become reactivated once the cells are separated from kidneys and propagated in vitro, or, contain viruses that produce a persistent but subclinical infection of the host. The latter viruses may not cause significant (if any) pathology in vivo, where the cells exist in an environment with a functional immune system. But outside of the host and away from the immune system, the cells may be fully permissive and the same virus cause highly cytopathic effects (CPE). Unfortunately, some primary cells may also harbor viruses that can replicate in the host cells without causing easily recognized CPE, and also in the indicator cells used for their isolation (or detection) in vitro. Unwanted viruses in primary cells cause various complications relevant to the isolation of a target virus, including:

They might quickly overtake a cell culture, reducing the chances of isolating the target virus.

They may cause CPE identical to those of the target virus, thus causing a false positive preliminary assessment.

They are obvious sources of contamination that complicate the isolation of the target virus in “PURE” form.

They may pose a biosafety risk to laboratory workers.

Noteworthy, primary cells can harbor contaminating agents other than viruses. For example, mycoplasma species are present in most animals, and are prevalent on the

surfaces of the respiratory tract. Moreover, mycoplasma species exist as intracellular and extracellular varieties. For reasons not yet entirely clear, kidneys are “sanctuaries” for viruses. For this reason, we often hunt for new viruses in kidney cells sourced from exotic species (J. Lednicky, unpublished).”

“Primary cells also have a finite lifespan, and should be used with minimal passages in vitro. Otherwise, senescence of the cells can be mistaken for CPE caused by viruses.”

“A common mistake is to assume that primary cells obtained from the suppliers are certified to be virus free. In reality, this is not the case. For example, the donors of primary human cells sold in the USA are examined (by serology) for antibodies to Hepatitis B and C viruses, and to HIV, following United States Food and Drug Administration (USFDA) guidelines, and if that information is not available, the cells are checked by PCR or other methods for the same viruses. [The USFDA is an agency of the United States Department of Health and Human Services responsible for protecting and promoting public health through the regulation and supervision of biopharmaceuticals, blood transfusions, cosmetics, dietary supplements, electromagnetic radiation emitting devices (ERED), food safety, medical devices, over-the-counterpharmaceutical drugs (medications), tobacco products, prescription, vaccines and veterinary products]. However, additional tests for other adventitious agents have not been mandated by the USFDA, and it may be impractical to check for the presence of many other agents with regard to cost and representative sampling reasons. Thus, commercially supplied human primary cells are sold with an advisory statement indicating the cells should be considered as potentially infected, and that biosafety practices be used when working with the cells.”

“Cell deterioration in primary cells due to improper cell growth media formulation can also be confused for CPE caused by viruses.”

“We have noted cell deterioration due to l-glutamine deficiency, and to improper dosage of antifungal agents in the growth media, among a few batches of commercially bought primary cells. Similarly, commercial media formulations for primary human cells often include additives such as epinephrine, human recombinant epidermal growth factor, hydrocortisone, insulin, transferrin, and others; a mistake in the amounts of some of these biomolecules added to the cell growth media can adversely affect cell viability.”

“Thus, primary cells are useful for the isolation of some viruses, but should be used with

caution because: (a) they can contain adventitious agents, and (b) cell deterioration due to one of many different reasons can be mistaken for virus-induced CPE."

ADVENTITIOUS VIRUSES IN CELL-LINES

"It is not uncommon to receive virus-contaminated cell lines from suppliers, and this is especially true for cells obtained through inter-laboratory transfer. One problem is that the cells may have become infected with bovine viruses (from serum) that replicate relatively slowly (i.e., the time it takes for them to complete a replication cycle and form progeny virions is higher than that of the cell population doubling time). These contaminating viruses are referred to as "adventitious" viruses (i.e., they are viruses that should not be present).

Many times, the adventitious viruses go unnoticed, and the deterioration of the cells is attributable to some type of "folklore" prevalent among cell culture practitioners."

"Apart from sera, contaminating viruses can also be traced to laboratory workers, to animal-sourced enzymes used for cell culture (such as porcine trypsin), and to other biological used for cell culture. A recent compilation of bovine and porcine viruses that may contaminate bovine serum and porcine trypsin is available in ref. 59. As new viruses are discovered, awareness of their possible presence in biologicals like sera and trypsin draws more interest and attention."

"We recently traced a filtered amino acid supplement as the source of a contaminating reovirus, and learned from some industry colleagues they had made the same finding. However, as typical of these cases, the findings are not published and thus the information not widely disseminated."

"In some cases, unusual bacteria, and even some single-celled eukaryotic microorganisms cause cell contamination problems that are attributed to viruses. This is because many people engaged in cell culture have little experience with the detection and identification of these types of organisms."

“It is also distressing when one performs electron microscopy and discovers that more than one virus is present in the specimen being viewed (or worse, only the wrong virus is visualized). Contaminated cell lines are a main reason gene expression studies can vary significantly between laboratories.”

PARADIGMS FOR VIRUS ISOLATION

“Cultures should not be considered negative for virus isolation if CPE are not detected. A second measure should be considered and well-thought criteria should be developed before rejecting a “negative” culture. For example, would CPE form if the cultures were held for a longer period of time?“

“Electron microscopy should be performed using material from spent media to detect liberated virions, and also, on a sample of the infected cells (often, the number of liberated virions is too low to be easily visualized through electron microscopy, and virus infection is determined only by examining the infected cells themselves).”

“Some viruses require “adaptation” prior to adequate replication in cultured cells and the formation of CPE. In the past, the process referred to as “blind culture” was performed when virus was suspected but CPE inapparent. A popular version of this method is to periodically remove samples from a culture of presumably infected cells, and to inoculate that into a new batch of cells. This process is repeated four times. An adjunct to former process is to split the infected cells (if confluent) into a larger flask or into several flasks and allow the cells to replicate. This may make CPE apparent if actively replicating cells are optimal for the detection of the CPE caused by a particular virus.”

“During the primary isolation of virus from clinical or environmental specimens, many laboratories routinely filter specimens though a 0.45μm filter prior to inoculation of cell cultures. This filtration step is performed to remove bacteria, fungi, and other potential microbial contaminants, and non-living particulates. A problem with this filtration step is that many viruses are pleomorphic and some have long, filamentous forms that may exceed 0.45 μm. This includes some influenza viruses, and morbilliviruses. Also, in clinical specimens, many viruses are attached to cellular and other debris, and are trapped by the

filter. We recommend the inoculation of two batches of cells; one with a filtered aliquot, the other unfiltered, of the virus specimen. Unfortunately, it is not uncommon these days for bacteria in clinical specimens (such as normal flora that are contaminants of naopharyngeal swabs) to be resistant to penicillin and streptomycin; we prefer to use an antibiotic mixture that includes neomycin in addition to penicillin and streptomycin."

CLOSING REMARKS

"Due to the complexities of cell culture, and the nature of the biomaterials used, it is not possible to consistently attain the same end results at all times. Moreover, viruses constantly mutate, and so the "rules of the game" can change. Therefore, the practice of cell culture for virus isolation is part art, part science, and part luck."

https://www.alexandriarepository.org/syllabus/cell-culture-techniques/

In Summary:

"All cells cultured in vitro are angry; they are outside of their normal environment and maintained under artificial conditions, surrounded by physiologically incorrect concentrations of all things important to their well-being"

The author states that his lab has been unable to reproduce the cell culture experiments of others and vice versa

Primary cells, which are non-immortalized cells taken directly from a living organism, are often used in clinical laboratories for the isolation of various "viruses"

These cells are harbors of "viruses"

The latent "viruses" may not cause CPE and go unnoticed

They might quickly overtake a cell culture, reducing the chances of isolating the target "virus"

They may create identical CPE to the target "virus"

They may make isolating the target "virus" in a "PURE" (his quotations, not mine) form difficult (i.e. impossible)

These cells also harbor contaminates other than "viruses"

Kidney cells (ex. Vero cells) are sanctuaries of “viruses” and are used to hunt for new “viruses”

Senescence of the cells (deterioration caused by aging) can be mistaken for CPE

A common mistake is assuming primary cells bought from suppliers are “virus-free”

Additional tests for other adventitious agents in primary cells have not been mandated by the USFDA

Commercially supplied human primary cells are sold with an advisory statement indicating the cells should be considered as potentially infected (in other words, they admit these come contaminated)

Cell deterioration in primary cells due to improper cell growth media formulation can also be confused for CPE caused by “viruses”

Mistakes in cell growth media formulations can impact cell viability

Primary cells should be used with caution due to:

Contamination from adventitious agents

Cell deterioration can be mistaken for CPE

It is not uncommon to receive “virus-contaminated” cell lines from suppliers

He claims cells may have become infected with bovine “viruses” from serum

These contaminating “viruses” are referred to as “adventitious viruses” (i.e., “viruses” that should not be present)

Adventitious “viruses” go unnoticed, and the deterioration of the cells is attributable to some type of “folklore”

In other words, they see CPE that does not line up with the target “virus” so they assume there must be some other “virus” present which caused it

Contaminating “viruses” can also be traced to laboratory workers, to animal-sourced enzymes used for cell culture (such as porcine trypsin), and to other biological used for cell culture

As new “viruses” are discovered, awareness of their possible presence in biologicals like sera and trypsin draws more interest and attention

In other words, they have no clue whether or not there are undiscovered “viruses” in their sera, culture, media, etc.

Unusual bacteria, and even some single-celled eukaryotic microorganisms cause cell

contamination problems that are attributed to “viruses”

The author admits that it is distressing when one performs electron microscopy and discovers that more than one “virus” is present in the specimen being viewed (or worse, only the wrong “virus” is visualized) which obviously implies that the samples were neither purified nor isolated

Contaminated cell lines are a main reason gene expression studies can vary significantly between laboratories

Cultures should not be considered negative for “virus isolation” if CPE are not detected thus destroying the importance of CPE as an identifier of “viruses”

He asks the question “Could CPE form if the cultures were held for a longer period of time?” thus demonstrating that the culture process is the cause of the CPE, not a “virus”

Often the number of liberated “virions” is too low to be easily visualized through electron microscopy, and “virus” infection is determined only by examining the infected cells themselves

In other words, they often can not find any “virus” visually even if there is CPE indicative of one

Some “viruses” require “adaptation” prior to adequate replication in cultured cells and the formation of CPE once again confirming that the cell culture process itself causes this effect, not “viruses”

In the past, the process referred to as “blind culture” was performed when “virus” was suspected but CPE inapparent

In other words, they sub-culture until the cell starts to die and they get the effect they want in order to claim “virus”

A problem with the filtration step is that many “viruses” are pleomorphic and some have long, filamentous forms that may exceed 0.45 μm

It is not uncommon these days for bacteria in clinical specimens (such as normal flora that are contaminants of naopharyngeal swabs) to be resistant to penicillin and streptomycin

Due to the complexities of cell culture, and the nature of the biomaterials used, it is not possible to consistently attain the same end results at all times

It is clear to see based on the numerous statements in this syllabus that the culture conditions have a profound impact on the results of the cell culture experiments. This stretches far beyond the various forms of contamination that are sure to affect the cells. The deterioration of the cells due to aging and/or to the nature of the biochemicals used causes the exact same cytopathic effect (CPE) said to be caused by “viruses.” Yet though

they know this, it is often the invisible "viruses" getting the blame even when they can not be found or seen. The researchers assume other "viruses" are present and causing the effect.

Even worse is when no CPE is observed. Instead of stating no "viruses" are present in the sample, the author states that this should not necessarily be the conclusion. He ponders whether further culturing will bring about the CPE if it is done over a longer period of time. This leads to a process known as blind passaging which is where the cells are divided and put into new petri dishes with fresh cell altering media/chemicals. This process is repeated multiple times until they finally achieve the CPE result that they are looking for. It is well known that cells react negatively to environmental/physical stressors and the process of blind passaging is highly stressful on them. This leads to cell deterioration. Virologists are literally creating the effect they want to see through their culturing process. Once they get their CPE, virologists get to claim a "virus" is present even if they are unable to obtain an EM image of the suspected "virus."

This is scientific fraud being perpetrated on a massive scale. They know it and they admit it in their work. They hope no one notices.

It's far past time we all take notice and start to hold them accountable.

https://viroliegy.com/2021/09/04/creating-the-cytopathic-effect/

Let's look a little more in-depth on some of these

contaminants:

Environmental Contamination

PLASTIC WARE:

"Control of the cellular environment is a principal attribute of in vitro cell cultures. UNINTENTIONAL EXPOSURE TO ENVIRONMENTAL COMPOUNDS CAN ADVERSELY AFFECT CULTURES AND, THEREFORE, EXPERIMENTAL RESULTS. Estrogenic compounds arising from common plastic ware have been found during cell culture."

https://pubmed.ncbi.nlm.nih.gov/17316062/

"One might think so initially, BUT CELL CULTURES PLASTIC WARE MAY MAKE A DIFFERENCE IN YOUR CELL CULTURE ESPECIALLY IN YOUR ASSAYS. Depending on the manufacturer, the starting material does not differ (all cell culture flasks, dishes and multiwell plates to date are manufactured from polystyrene) BUT THE SURFACE MODIFICATIONS, THE DESIGN, AIR FLOW, EVAPORATION AND OTHER FEATURES MAY DIFFER GREATLY."

"The base material polystyrol without further treatment is not sufficient for cell adhesion in most cases. Therefore, ALL SUPPLIERS OF CELL CULTURE PLASTICWARE TREAT THE SURFACES OF FLASKS, DISHES AND MULTIWELL PLATES WITH IONIZED GAS (corona-treatment) TO PRODUCE SURFACE MODIFICATIONS THAT MAKE THE SURFACE MORE POLAR OR CHARGED. THE MIXTURE OF PLASMA GASES IS SECRET and therefore, EACH SUPPLIER HAS A DIFFERENT SURFACE MODIFICATION.

The following surface modifications are used:

positive charges negative charges positive and negative charges mixed polar groups additional hydrophobic modifications (for e.g. suspension cells)"

"Depending on the quality and quality control of the plasma treatment, the surfaces may be activated for adhesion either homogeneous or ARTEFACTS MAY BE INTRODUCED SUCH AS REGIONS LIKE EDGES ARE NOT TREATED OR PARTS IN THE MIDDLE AREA OR OVER OR UNDER ACTIVATED as shown in the images below."

"Another crucial point when choosing your cell culture consumable supplier is that the CONSTRUCTION DESIGN OF ESPECIALLY DISHES AND MULTIWELL PLATES STRONGLY INFLUENCES THE ADHESION (general and areas), THE DISTRIBUTION OF CELLS AND THE EVAPORATION FROM THE PLATE OR DISH. In multiwell plates,

this can prove problematic as the outer rows and columns USUALLY GIVE RESULTS TOTALLY DIFFERENT FROM THE OTHER WELLS AND THEREBY INCREASE THE STANDARD DEVIATION OF THE RESULTS."

"Plastic may contain certain chemicals that are used in the process of production or convey certain attributes to the plastic (e.g. release aid or softeners). THESE MAY LEAK OUT OR MAY BE EXTRACTED FROM THE PLASTIC DURING THE CULTURE PERIOD. Then, CELLS OR TISSUES MAY TAKE UP THESE COMPOUNDS and eventually they are transplanted with the ATMP / TEP into the patient."

https://incelligence.de/.../cel.../cell-culture-plastic-ware

WATER:

"CONTAMINATED CULTURES AND CELL DEATH ARE A MAJOR PROBLEM THAT CAN NEGATIVELY IMPACT ON DOWNSTREAM EXPERIMENTS. Water is used in many steps of the cell culture process – IT IS THE MAIN COMPONENT OF BUFFERS AND MEDIA, and may be used for dissolution of additives and drugs. The use of purified water is essential for successful experimental outcomes, AS CULTURES ARE ADVERSELY AFFECTED BY CONTAMINATING MICROORGANISMS, BIOLOGICALLY ACTIVE CELL DEBRIS AND BY-PRODUCTS, AND ORGANIC AND INORGANIC COMPOUNDS."

"What Types Of Contaminants In Water Can Affect Cell Culture Results?

The main types of impurity that affect the performance of cell culture techniques are bacteria, endotoxins, organic compounds and ionic contaminants.

1. Bacteria

BACTERIA THRIVE IN TYPICAL CELL CULTURING CONDITIONS, and can quickly outgrow the cells of interest, causing nutrient levels to fall and toxic by-products to increase. Bacterial contamination can also LEAD TO SUDDEN CHANGES IN MEDIA pH AND THE CONTAMINATION OF PREVIOUSLY PURE CULTURES.

2. Endotoxins

Endotoxins are released by most Gram-negative bacteria. These endotoxins affect various cell types, even those lacking CD14 endotoxin receptors, and stimulate macrophages and mononuclear phagocytes to release a variety of pro-inflammatory

cytokines. THE RESULTING ADVERSE EFFECTS INCLUDE CHANGES IN CELL GROWTH AND FUNCTION, THE PRODUCTION OF RECOMBINANT PROTEINS AND A REDUCTION IN THE EFFICIENCY OF CLONING.

3. Organic Compounds

SMALL ORGANIC COMPOUNDS COMMONLY FOUND IN WATER – such as humic acids, tannins, pesticides and endocrine disruptors – CAN AFFECT CELL DEVELOPMENT. They provide an uncontrolled source of nutrients for bacterial growth, and should be removed from water used for preparation of materials for cell culture.

4. Ions

Ionic contaminants, particularly multivalent ions and heavy metals, must be kept low. Heavy metals – for example, mercury and lead – ARE KNOWN TO BE CYTOTOXIC TO A RANGE OF CELL TYPES.

https://www.elgalabwater.com/cell-tissue-cultures

LIGHT:

"However, photo-sensitive molecules inside cells and in standard cell culture media GENERATE TOXIC BY-PRODUCTS THAT INTERFERE WITH CELLULAR FUNCTIONS AND CELL VIABILITY WHEN EXPOSED TO LIGHT."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5429800/

"THE SHARP DECLINE IN GROWTH-SUPPORTING CAPACITY OF DMEM EXPOSED TO FLUORESCENT LIGHT has been attributed to two mechanisms: the photoactivation of riboflavin leading to tryptophan free radical production accompanied by peroxide formation (5, 6) and the formation of photoadducts of riboflavin and tryptophan (7). OUR RESULTS SHOW THAT CELL YIELDS IN DMEM THAT HAD BEEN EXPOSED TO LIGHT WERE 10% TO 40% OF THOSE IN NON-EXPOSED DMEM. The loss of growth capacity was dose-dependent, WITH GREATER DECLINES OBSERVED WITH EITHER HIGHER LIGHT INTENSITIES OR LONGER EXPOSURE PERIODS.

Furthermore, we examined the effect of exposure to laboratory light on FBS. The data indicate that FBS as compared with DMEM is relatively stable to light exposure and partially stabilizes cell culture medium against the light-induced loss of growth capacity. Some of the protective effect of serum can be attributed to the catalase activity in serum

(5), which could interrupt free radical-mediated reactions. BOTH LIGHT EXPOSURE AND PROLONGED STORAGE AT ROOM TEMPERATURE RESULTED IN DECLINES IN CELL CULTURE PERFORMANCE OF FBS when performance was measured by cloning efficiency. In fact, the decline attributable to exposure to light for 28 days was nearly equal to the decline attributable to storage at room temperature (22°C) rather than at refrigerator temperature (4°C) for 28 days. BOTH CONDITIONS RESULTED IN APPROXIMATELY 40% REDUCTIONS IN RELATIVE CLONING EFFICIENCY.

As demonstrated here, the DELETERIOUS EFFECTS OF LABORATORY LIGHTS ON CELL CULTURE MEDIUM PERFORMANCE can be reduced by keeping medium in the dark or in protective yellow bags. Further precautions may include covering fluorescent lights in storage areas and cell culture hoods with yellow plastic films. These same procedures are recommended AS WAYS TO LIMIT THE HANDFUL EFFECTS OF LIGHT ON SERUM where serum can be exposed for an extended time or to repeat short exposures."

https://www.google.com/url?sa=t&source=web&rct=j&url=https://www.cytivalifesciences.co.kr/wp-content/uploads/2020/04/Effect-of-laboratory-lights-on-cell-culture-media.pdf&ved=2ahUKEwiRzZ_6h_nuAhUbXM0KHeXaAxcQFjADegQIGBAC&usg=AOvVaw1_F5uRUJD-XZUkGaArjU8J

Plastic, water, and light are all environmental factors which can impact cell cultures in negative ways. This is just the tip of the iceberg as we haven't even touched on how room temperature, atmospheric conditions, and pH levels affect cells. There are so many sources of contamination with cell cultures as well as many variables which affect the cell growth, performance, genomic stability, viability, etc. that it seems one would need a 100% completely sterile environment in order to be able to trust any end product derived from the culture. Contamination isn't the exception, it is the norm.

“Evidence suggests that up to one-third of tumor cell lines being used in scientific research are affected by inter- or intra species cross-contamination or have been wrongly identified, THEREBY RENDERING MANY OF THE CONCLUSIONS DOUBTFUL IF NOT COMPLETELY INVALID.” — Lancet Oncology, vol. 2, July 2001, p. 393

Cell line misidentification is a huge problem in cell cultures. It has been known for over half a century and instead of the problem getting better, it has only become worse over time. This has led to many false and erroneous papers being published and their findings built upon by other researchers which has created a spiraling problem that has yet to be resolved. The two articles presented below highlight this troublesome issue and provide ample evidence for why all cell culture studies should be questioned.

"Cell lines are used extensively in research and drug development as models of normal

and cancer tissues. However, A SUBSTANTIAL PROPORTION OF CELL LINES IS MISLABELED OR REPLACED BY CELLS DERIVED FROM A DIFFERENT INDIVIDUAL, TISSUE OR SPECIES. The scientific community has failed to tackle this problem and CONSISTENTLY THOUSANDS OF MISLEADING AND POTENTIALLY ERRONEOUS PAPERS HAVE BEEN PUBLISHED USING CELL LINES THAT ARE INCORRECTLY IDENTIFIED."

https://www.nature.com/articles/nrc2852

"WHILE PROBLEMS WITH CELL LINE MISIDENTIFICATION HAVE BEEN KNOWN FOR DECADES, AN UNKNOWN NUMBER OF PUBLISHED PAPERS REMAINS IN CIRCULATION REPORTING ON THE WRONG CELLS WITHOUT WARNING OR CORRECTION. Here we attempt to make a conservative estimate of this 'contaminated' literature. WE FOUND 32,755 ARTICLES REPORTING ON RESEARCH WITH MISIDENTIFIED CELLS, IN TURN CITED BY AN ESTIMATED HALF A MILLION OTHER PAPERS. The contamination of the literature IS NOT DECREASING OVER TIME and is anything but restricted to countries in the periphery of global science. The decades-old and often contentious attempts to stop misidentification of cell lines have proven to be insufficient."

"The misidentification of cell lines is a stubborn problem in the biomedical sciences, CONTRIBUTING TO THE GROWING CONCERNS ABOUT ERRORS, FALSE CONCLUSIONS AND IRREPRODUCIBLE EXPERIMENTS [1, 2]. As a result of mislabelled samples, cross-contaminations, or inadequate protocols, some research papers report results for lung cancer cells that turn out to be liver carcinoma, OR HUMAN CELL LINES THAT TURN OUT TO BE RAT [3, 4]. In some cases, these errors may only marginally affect results; in others THEY RENDER RESULTS MEANINGLESS [4]."

"Although no exact numbers are known, THE EXTENT OF CELL LINE MISIDENTIFICATION IS ESTIMATED BETWEEN ONE FIFTH AND ONE THIRD OF ALL CELL LINES [4, 14]. (Although currently only 488 or 0.6% of over 80,000 known cell lines have been reported as misidentified, most cell lines are used infrequently [15].) In addition, MISIDENTIFIED CELL LINES KEEP BEING USED UNDER THEIR FALSE IDENTITIES LONG AFTER THEY HAVE BEEN UNMASKED [16], WHILE OTHER RESEARCHERS CONTINUE TO BUILD ON THEIR RESULTS. Considering the biomedical nature of research conducted on these cell lines, CONSEQUENCES OF FALSE FINDINGS ARE POTENTIALLY SEVERE and costly [17], with grants, patents and even drug trials based on misidentified cells [18]."

"Before any action can be taken, it is essential that we get a sense of the size and nature of the problem of contaminated literature. This raises several questions. First, HOW MANY RESEARCH ARTICLES HAVE BEEN BASED ON MISIDENTIFIED OR CONTAMINATED CELL LINES? HOW WIDE IS THEIR INFLUENCE ON THE SCIENTIFIC LITERATURE? Second, what can we say about origins and trends in the

contaminated literature? Is the problem getting better, or restricted to peripheral regions of the world's research, where perhaps protocols are less strict? Third, what could be appropriate ways to deal with the contaminated literature?"

"Using complementary search strategies (see methods), WE WERE ABLE TO IDENTIFY 32,755 ARTICLES (on August 4th, 2017) BASED ON CELL LINES THAT ARE CURRENTLY KNOWN TO BE DIFFERENT FROM THE CELL LINES REPORTED IN THESE PUBLICATIONS. As we only searched for cell lines known to be misidentified, THIS CONSTITUTES A CONSERVATIVE ESTIMATE OF THE SCALE OF CONTAMINATION IN THE PRIMARY LITERATURE."

"In addition, RESEARCH BASED ON MISIDENTIFIED CELL LINES HAS A WIDE IMPACT ON THE SCIENTIFIC LITERATURE, AS IT APPEARS THAT THESE RESEARCH PAPERS ARE COMPARATIVELY HIGHLY CITED. WoS does not allow for precise total numbers, but we can give indications of this 'secondary contamination' of the literature. Analysing citations to primary contaminated articles, WE FOUND 46 PAPERS WITH MORE THAN A THOUSAND CITATIONS AND OVER 2600 CONTAMINATED ARTICLES WITH OVER A HUNDRED CITATIONS. Furthermore, OVER 92% OF THE CONTAMINATED PAPERS ARE CITED AT LEAST ONCE, which is more than average for biomedical literature [34]. In total, we can CONSERVATIVELY ESTIMATE THE CITATIONS TO THE PRIMARY CONTAMINATED PRIMARY LITERATURE AT OVER 500,000, excluding self-citations, thereby leaving traces in a substantial share of the biomedical literature. Even though it is clear that articles may receive citations for many reasons, including negative or even ritual citations, and hence not all citing articles contain (critical) errors, THE AMOUNT OF RESEARCH POTENTIALLY BUILDING ON FALSE GROUNDS REMAINS WORRISOME."

"One might wonder whether the contamination of the research literature is mainly a problem of the past, given that the FIRST CONCERNS ABOUT MISIDENTIFIED CELL LINES WERE EXPRESSED HALF A CENTURY AGO [9, 10] and that numerous initiatives have tried to alleviate the problem since.

Based on the set of 32,755 records of primary contaminated literature, we analysed the publication dates of the articles. THE MAJORITY OF THE ARTICLES, 57%, WERE WRITTEN SINCE 2000 AND THE NUMBER OF ARTICLES USING MISIDENTIFIED CELL LINES IS STILL GROWING (see Fig 2). Clearly, the problem is definitely not one of the past, but is very relevant to contemporary science, with 58 new articles based on contaminated literature appearing even as recently as February 2017."

"Fig 2 indicates three moments in history when cell line contamination became evident. First, through the work of Stanley Gartler it became possible to detect intraspecies cell contamination, AFTER WHICH SEVERAL OF SUCH CONTAMINATIONS INVOLVING

HeLa CELLS WERE REPORTED IN NATURE IN 1968 [9, 10]. Second, cell culture contamination was put on the global research agenda by the work of Walter Nelson-Rees et al. in the 1970s [7, 8], CULMINATING IN A LIST OF CONTAMINATED CELL CULTURES IN SCIENCE IN 1981 THAT DEMONSTRATED LARGE-SCALE CONTAMINATION OF CELL CULTURES BY HeLa CELLS [44]. From this point on, it could be expected that most scientists working in those areas of research frequently employing cell cultures, were aware of the potential issues with their research material. HOWEVER, THE VAST MAJORITY OF RESEARCH PAPERS BASED ON MISIDENTIFIED CELL LINES WAS PUBLISHED AFTER THIS POINT IN TIME. Even after the introduction of STR in 2001 [45], THE ANNUAL NUMBER DOES NOT DECREASE."

"Similar to the primary literature, the number of articles in the secondary literature is also still growing. IN 2016, OVER 40,000 PAPERS WERE PUBLISHED THAT REFERRED TO PRIMARY CONTAMINATED LITERATURE. In addition, from the information in the Supplementary Material (S2 File), we conclude that THE MAJORITY OF MISIDENTIFIED CELL LINES CONTINUE TO CONTAMINATE THE SECONDARY LITERATURE IN 2017."

"For example, several recent publications indicate levels of CELL LINE CONTAMINATION FOR CHINA BETWEEN 25% [13] AND 46% [46] AND DEMONSTRATE THAT OF ALL 'NEW' CELL LINES DEVELOPED IN CHINA 85% ACTUALLY TURNED OUT TO BE HeLa CELLS [13].

However, THE MAJORITY OF THE ARTICLES USING MISIDENTIFIED CELL LINES ORIGINATE FROM COUNTRIES HOLDING WELL-ESTABLISHED RESEARCH TRADITIONS (e.g. US, Japan, Germany). Relative to their share of total research output, authors from these countries OFTEN PERFORM RESEARCH ON MISIDENTIFIED CELL LINES. In fact, mainly due to their enormous share of total literature on cell lines, OVER 36% OF ALL CONTAMINATED PRIMARY LITERATURE STEMS FROM THE US."

"Our results seem to present worrying problems for the biomedical sciences. ALTHOUGH THE ISSUE OF MISIDENTIFIED CELL LINES HAS LONG BEEN KNOWN, ITS EFFECT ON THE SCIENTIFIC LITERATURE HAS NOT BEEN PROPERLY RECOGNIZED, LET ALONE PROPERLY TREATED [47, 48]."

"Despite measures to authenticate new and existing cell lines [27], RESEARCH BASED ON THE WRONG CELLS IS STILL PRESENT IN THE LITERATURE AND IN FACT CONTINUES TO BE PUBLISHED."

https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0186281

It is clear that cell line misidentification is a problem that is not going away and has grown out of control over time. Taken into consideration with the evidence of the vast problem of cell line contamination from biological, chemical, and environmental factors, the toxic effects of the antibiotics/fetal bovine serum/media used, the lack of proper replication of the in vivo environment, and the inability to reproduce results, it is a wonder why any cell culture study should be considered valid.

It is fraud built upon fraud.

https://viroliegy.com/2021/09/02/environmental-contamination/

The case against cell culture

"MANY CLINICALLY RELEVANT VIRUSES ARE SIMPLY DIFFICULT TO GROW OR CANNOT BE GROWN AT ALL IN CULTURED CELLS, while other viruses require specialized culture systems that are either not available or too complicated for routine use in diagnostic laboratories. Traditional tube cultures, although viewed as being comprehensive in growing a wide range of viruses and capable of detecting unsuspected new viruses or more common viruses in new places, FAIL TO ISOLATE VIRUSES IN MANY INSTANCES and can take days to weeks to provide a final result. While centrifugation-assisted cultures using individual, mixed, or genetically engineered cell lines are designed to be faster and more user-friendly than tube cultures, THEY ARE NOT ALWAYS AS SENSITIVE AND ARE NORMALLY LIMITED BY THE QUALITY AND AVAILABILITY OF REAGENTS AND THE NUMBER AND TYPES OF CELL LINES THAT CAN BE USED TO GROW A VARIETY OF DIFFERENT VIRUSES."

"VIRAL CULTURE SYSTEMS REALLY HAVE NOT BEEN STANDARDIZED OR SCRUTINIZED TO THE SAME EXTENT AS MOLECULAR TESTING AND CAN VARY CONSIDERABLY, depending upon the selection of appropriate cell lines; the adequate collection, transport, and handling of specimens to ensure virus viability; and the maintenance of viable and healthy inoculated cells."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3536207/

The above two paragraphs offer a decent summary on the state of cell culturing and the

inherent difficulties as well as the inability to grow or produce a "virus." These systems have not been standardized nor scrutinized as well as they should be and the multitude of variables that need to be met in order to produce the intended results are vast. I took a deep dive into cell cultures over the past week and I am sharing the various posts here for easy reference as well as to present the case against the use of cell cultures as proof of any "virus."

For a brief overview on the cell culture process as well as a few of the toxic ingredients used, start here:

https://m.facebook.com/story.php?story_fbid=10158065023878576&id=502548575

When Virologists claim to isolate a "virus," they are referring to the end result of a cell culture experiment. They never actually separate a particle they assume to be a "virus" directly from the sample obtained from a sick human first, they simply assume there is a "virus" already within the patient sample and go from there. There are a few problems with this.

1. There are billions of different micro and nanoparticles within the patient sample, including cellular debris, extracellular vesicles, and exosomes which are indistinguishable from "viruses."

2. The sample from a sick patient is immediately placed in what is called Viral Transport Media which contain chemicals that are toxic to cells.

VIRAL TRANSPORT MEDIA:

These chemicals are added as a way to "safely" transfer the patient sample to the lab for testing, culturing, and other molecular biological techniques. They often are composed of some sort of salt solution, fetal bovine serum, antibiotics, and can be contaminated by disinfectants such as ethanol. These are all substances which are toxic to cells and can change the sample before the culturing process even begins.

https://m.facebook.com/story.php?story_fbid=10158076366038576&id=502548575

After this, they may do some centrifugation (spin the sample really fast) to separate out larger particles (leaving behind many EV's, exosomes, "viruses," etc. that are too small to be filtered out) and then will take what is called the supernatant, which is what is

collected after letting the sample settle, and add it to a cell to be cultured.

There are many different types of cells that can be chosen from to culture a "virus" in and they typically come from either human cancer cells or from animals such as monkeys and rabbits:

"Examples of well-known cell types that are standard for most virology laboratories are primary rhesus monkey kidney (RhMK) cells, primary rabbit kidney cells, human lung fibroblasts (MRC-5), human foreskin fibroblasts, human epidermoid carcinoma cells (HEp-2), human lung carcinoma cells (A549), and others."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1797634/

The cells that are used are critical to producing a "virus" which makes choosing the right one extremely important. There are problems with this step which can lead to contamination, errors, and faulty research.

CELL LINE MISIDENTIFICATION:

There is a crisis in cell cultures which threatens to throw out the results of many studies. Cell lines have been continuously mislabeled or replaced by cells from different individuals, tissues, or species. This problem has been known since the 1960's and instead of being corrected over the years, it has only grown worse. Over half of all studies using misidentified cell lines have come since the year 2000, well after the problem was discovered. The results of experiments from studies using misidentified cell lines are cited and built upon by other researchers confounding the problem and throwing uncertainty over the results over a vast amount of scientific literature.

https://m.facebook.com/story.php?story_fbid=10158074436313576&id=502548575

CELL CULTURE MEDIA:

The chosen cell is contained within cell culture media and there are many types that they choose from including both natural and artificial varieties. The actual composition and make-up of these media is unknown in most cases and can vary batch-to-batch but, like the VTM, they typically contain a salt solution, antibiotics, and fetal bovine serum as well as added amino acids, glucose, vitamins, and nutrients. The compounds that make up these media individually have been shown to be detrimental to cells and the

combined effects are relatively unknown and understudied.

https://m.facebook.com/story.php?story_fbid=10158073300108576&id=502548575

A quick look at a few of the compounds making up VTM and Cell Culture Media show how they can impact the results of the cell culture and why they should not be used.

ANTIBIOTICS:

Antibiotics are regularly used in cell cultures in order to prevent bacterial contamination. However, it is well known that they are toxic to cells and their use impairs cell growth and differentiation. They have an effect on the metabolism of cultured cells, cell proliferation, differentiation and gene expression. They can also attack non-bacterial structures within the cell.

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FETAL BOVINE SERUM:

Fetal bovine serum is derived from the blood of the unborn fetus of slaughtered pregnant cows. It's use is questionable not only on a moral ground as the fetus is normally alive as the blood is drained from its heart but also due to the fact that the RNA from the serum is nearly impossible to separate in the cell culture and can influence the results. It has also been shown to affect the genotypic and phenotypic response. The batches vary in composition and many of the components are unknown.

https://m.facebook.com/story.php?story_fbid=10158071825078576&id=502548575

Once the cell and the cell culture media are chosen, the supernatant from the patient sample is added to the cell culture. Media/Antibiotics are added and changed throughout this process. An example from the Zhou study, one of the original "SARS-COV-2" papers:

"Cultured cell monolayers were maintained IN THEIR RESPECTIVE MEDIUM. The PCR-positive BALF sample from ICU-06 patient was spun at 8,000g for 15 min, filtered and DILUTED 1:2 WITH DMEM SUPPLEMENTED WITH 16 μg ml−1 TRYPSIN BEFORE IT WAS ADDED TO THE CELLS. After incubation at 37 °C for 1 h, the inoculum was removed and REPLACED WITH FRESH CULTURE MEDIUM

CONTAINING ANTIBIOTICS (see below) AND 16 μg ml−1 TRYPSIN. The cells were incubated at 37 °C and OBSERVED DAILY FOR CYTOPATHOGENIC EFFECTS."

https://www.nature.com/articles/s41586-020-2012-7

As can be seen from the above example, after the supernatant and the media are added, the cell culture is incubated and observed daily for what is called cytopathic or cytopathogenic effects.

THE CYTOPATHIC EFFECT:

Cytopathic Effects are the holy grail of the cell culture experiment. These are structural changes to the host cell said to be caused by an invading "virus." As "viruses" are unable to be seen without the use of an Electron Microscope, they look for this effect as INDIRECT evidence that a "virus" is present in the cell culture. If they observe CPE, they ASSUME a "virus" is present as this effect is supposedly specific to "viruses." However, this is not the case at all as there are many other factors which can cause the very same effect such as: bacteria, parasites, amoebas, and chemical contaminants such as antibiotics and antifungals.

https://m.facebook.com/story.php?story_fbid=10158071286553576&id=502548575

It's clear to see that if CPE, the end result of a cell culture experiment used as proof of a "virus," can be caused by other organisms and chemicals, CPE is not specific to "viruses." Antibiotics and antifungals are pretty much a given to be in cell cultures at this point and alone are enough to cause CPE. However, It is also well known that these various other factors (bacteria, parasites, amoebas) are most likely in the culture as well.

CONTAMINATION:

Cell culture contamination is not the exception but the norm. They try to mitigate and control the effects of contamination yet admit there is no way that they can eliminate it. Contamination can come in many forms such as bacteria like mycoplasmas, other "viruses," parasites, yeast, fungus, etc.

Contamination can also come from the environment in various ways such as plastic ware chemicals leaching into the cell culture from petri dishes, organic/inorganic compounds in the water used invading the culture, and even effects from the types of lights used altering the cells. There are other factors such as temperature, atmospheric

conditions, and pH levels to consider.

REPRODUCIBILITY CRISIS:

Taking into consideration the numerous sources of contamination, the huge problem of misidentification of cell lines, the various chemicals/antibiotics/serum/animal cells etc. used in the cultures and the toxic, cell-altering effects they have, is it any wonder why there is a reproducibility crisis is science, especially regarding cell cultures? Experiments are rarely able to be reproduced which leads to the nearly 500,000 "variants" we are currently in the midst of seeing with a new one seemingly popping up every day.

One other important factor to consider with cell cultures is their inability to recreate the environment that cells normally function in vivo (within the living organism). None of the added chemicals/antibiotics/serums/nutrients would be added to or come into contact with the cells in their natural environment as they do in culture experiments. 2D cell cultures are unable to provide the extracellular microenvironment necessary for the cell to thrive as it normally would. They have tried to combat this problem with 3D cell cultures but they have their own issues as well.

Once the supposed "viral" CPE is observed in the toxic cell culture, the unpurified supernatant is once again collected for EM imaging, genome sequencing, and animal testing. It should be clear from the various reasons listed above why this is not adequate proof of any "virus."

1. Without proper purification/isolation, there is absolutely no way to tell that the particle they pick out to be the representation of their "novel virus" in the EM image actually is a "virus" at all.

2. Without purification and due to the numerous toxic ingredients added to the original sample, there is no way to confirm that the RNA/DNA used for the genome actually comes from one unaltered source.

3. Without purification/isolation, there is no way to definitively say that there was a "virus" contained within the cell culture goo which is unnaturally shoved intranasally down the noses of test animals. If the animals do get sick, it could be due to the antibiotics, the FBS, the media, the nutrients, the contaminants, the stress of the experiments, or a combination of any of these factors.

It should be obvious that the end result of a cell culture experiment in no way reflects what was originally taken from the patient sample. The results in no way reflect reality. Cell cultures are nothing more than a recipe to create cell death which is blamed on invisible "viruses" never proven to exist.

For this, we locked down, quarantined, social-distanced, shut down economies and small businesses, shunned the elderly, mask-upped, and vaccinated with rushed experimental gene therapies.

All based on "scientific" fraud.

https://docs.google.com/document/d/e/2PACX-1vQzmhs4R7NkbxToUR1GyeLdF5iLZhgDkYkooiNFrg4HzielJu231losXzaWIPAxZFGmX-GJRnujhH0x/pub

Cell Culture Media

Cell Culture Media is arguably the most important component in the culture beyond the type of cell used. This is a liquid or gel that is said to contain all the various vitamins and micronutrients needed for the cell to survive. They are typically listed in studies under the names Dulbecco's Modified Eagle's Medium (DMEM) or Minimal Essential Medium (MEM). There are many different kinds of media such as natural, artificial, chemical, serum, serum-free, protein-free, etc. so selecting the right type of media is considered crucial.

So what exactly does the cell culture media typically consist of?

"Cell culture media are comprised of a combination of compounds and nutrients designed to support cellular growth. Common components of cell culture media include:

Amino acids: Every cell culture media contains a mixture of amino acids, the building blocks of protein. Both essential and nonessential amino acids may be used to boost cell viability and growth.

Vitamins: Vitamins are included to facilitate cellular growth and proliferation. Serum is used as the source of many vitamins in serum-containing media, but vitamins must be added to serum-free media.

Carbohydrates: Carbohydrates provide an energy source for living cells. Glucose is commonly used, but other carbohydrates, such as galactose, fructose, or maltose, are available.

Inorganic salts: Inorganic salts are needed to regulate membrane potential and osmolality.

Basic and trace elements: Cells need elements like iron, potassium, magnesium, and zinc to grow.

Serum: Serum contains growth factors and inhibitors, hormones, protease inhibitors, chelators, amino acids, carbohydrates, lipids, vitamins, trace elements, minerals, and more that are needed for cellular growth. Bovine serum is commonly used.

Hormones: Certain hormones may be added to influence cell function, growth, and proliferation.

Buffering systems: Buffering systems regulate pH.

Supplements: Supplements like hormones, enzyme inhibitors, and trace elements are sometimes added to cell culture media that cater to the cell-type and research goal.

Antibiotics: Antibiotics are added to cell culture media to inhibit fungal and bacterial growth. Antibiotics are best suited for serum media thanks to proteins that bind some of the antibiotic load. In contrast, cells in serum-free media are at a greater chance of antibiotic toxicity."

https://www.google.com/amp/s/www.technologynetworks.com/cell-science/articles/amp/which-cell-culture-media-is-right-for-you-331552

It is clear that the media consists of many different compounds said to keep the cell alive and aid in the growth of any "viruses" present. However, there are many issues related to the cell culture media that can influence the outcome of the culture and the results obtained. These include: the unknown and variable composition of the vitamins/micronutrients within the media, the unknown interactions between the various components in the media and the stability of the cell, the inclusion of antibiotics and fetal bovine serum in many media, etc.

For instance, from the same source quoted above regarding the makeup of cell culture media, come these admittances regarding serum media, the most commonly used being Fetal Bovine Serum:

“But there are downsides to serum media. When it comes to its drawbacks, serum media:

Lack compositional uniformity, making it poorly suited for large-scale experiments.

Contain a mixture of compounds, some of which can be harmful to or inhibit the growth of certain cell types.

Have a greater risk of contamination when compared to artificial media.

May complicate the isolation of cell culture products.”

These are but a few of the issues and more are outlined in the articles below:

“Commercially available cell culture media contains buffers, inorganic salts, glucose, amino acids,vitamins and numerous bioactive compounds but the levels of each may differ substantially. For instance, the concentration of glucose in the media varies between 5.5 mM and 25 mM (Minimum Essential Medium (MEM) vs. Dulbecco’s Modified Eagle’s Medium (DMEM)), and the presence of ferric nitrate in DMEM and copper, iron and zinc sulphate in F12 medium, might interact with specific metal dependent enzymes of importance in cellular reactions.”

“Immortalized cells are a commonly used model system in biomedical research due to its simplicity, availability and high throughput characteristics. However, the composition of cell culture medium

used varies extensively and may affect the results from biochemical, toxicological and pharmacological studies substantially depending on the experimental conditions.”

“However, the differences demonstrated in cell proliferation may not only depend on the different levels of glucose and cysteine since the media differ greatly in other components as demonstrated by iron, for example.”

“A change in phenotype due to medium composition is a serious effect that could profoundly change the outcome of any pharmacological and mechanistic studies. These observations must therefore be carefully taken into consideration when interpreting any

data from cell experiments."

https://www.google.com/url?sa=t&source=web&rct=j&url=https://www.mdpi.com/2076-3921/8/5/130/pdf&ved=2ahUKEwiL0P2SvPfuAhVDZM0KHYdfBTUQFjAOegQIJRAC&usg=AOvVaw2dHpmdFQSYx7Nj6rf0OIIl

From this article, it is clear that the media:

contains substantially differing levels of micronutrients

may effect the results of experimental conditions due to varying composition

These next two sources also provide evidence that the makeup of the various compounds in the media can have an affect on the results obtained from the cell culture experiment:

15 things about cell culture you might not know

"Components of culture medium can interact with each other and influence cultured cells

When you are trying to optimize your culture medium, consider this: Individual components of the medium do not act alone. Components can interact, and their effects on cells are not always predictable. This is particularly important when replacing animal sera in culture media. You might need to use mathematical algorithms to optimize the combination of multiple compounds and to establish the best conditions for cellular growth (Yao and Asayama, 2017; Kim and Audet, 2019)."

15 things about cell culture you might not know

The Influence of Micronutrients in Cell Culture: A Reflection on Viability and Genomic

Stability

“Cell-culture media try to mimic the in vivo environment, providing in vitro models used to infer cells’ responses to different stimuli. This review summarizes and discusses studies of cell-culture supplementation with micronutrients that can increase cell viability and genomic stability, with a particular focus on previous in vitro experiments. In these studies, the cell-culture media include certain vitamins and minerals at concentrations not equal to the physiological levels. In many common culture media, the sole source of micronutrients is fetal bovine serum (FBS), which contributes to only 5–10% of the media composition. Minimal attention has been dedicated to FBS composition, micronutrients in cell cultures as a whole, or the influence of micronutrients on the viability and genetics of cultured cells. Further studies better evaluating micronutrients’ roles at a molecular level and influence on the genomic stability of cells are still needed.”

“The composition of these media includes certain vitamins and minerals, but unfortunately, in many common culture media, the only source of micronutrients is fetal bovine serum (FBS), which contributes to only 5–10% of the media composition. Moreover, the appropriate proportion of micronutrients is not always provided because the precise composition of each batch of FBS is in fact extremely variable [34].”

“Certain micronutrients, such as calcium, folate, magnesium, and iron, have been reported as key elements in cellular processes, including the proliferation, survival, and even differentiation of cell cultures [35–38]. However, the particular concentration of micronutrients in a culture as well as the cell type may trigger different responses. Further studies of micronutrients’ roles at a molecular level and influence on genomic stability are still required.”

“Table 2 presents interesting data regarding the micronutrients that may interfere with genomic stability and the micronutrient concentration values found in typical cell-culture media, FBS, and human serum. Unfortunately, data are not available for all of the micronutrients in the media, and even the proportions of micronutrients in FBS, as an organic product, are not all well characterized. Additionally, as demonstrated by Bryan et al. [34], the concentration of many micronutrients in FBS can vary significantly between batches.

Although cell-culture media attempt to provide an environment similar to the in vivo milieu of cell development, there is an evident imbalance of micronutrients between the

media and human serum. Certain micronutrients are present in these media at concentrations higher than those found in human serum (e.g., vitamins B7 and B12), whereas other nutrients are present at significantly lower concentrations than in human serum (e.g., iron and zinc)."

"Cells are typically maintained at an appropriate temperature and CO2 concentration (usually 37°C and 5% CO2 for mammalian cells) in an incubator. Beyond these parameters, the most commonly varied factor in culture systems is the growth medium. The recipes for growth medium can vary in pH, glucose concentration, growth factors and the presence of other nutrients and micronutrients."

"Bryan et al. [34] stated that one of the major obstacles to obtaining human cells of a defined and reproducible standard, and thus suitable for use in medical therapies, is the routine necessity of supplementing cell-culture media with FBS. In this study, FBS variants were evaluated, in terms of both elemental (micronutrient) composition and the variants' effects on the expression of a group of proteins associated with the antigenicity of primary human umbilical vein endothelial cells (HUVECs). A combination of inductively coupled plasma mass spectrometry (ICPMS) and flow cytometry was used to achieve these experimental objectives. Statistically significant differences in antigenic expression during cell culture were demonstrated for a set of trace elements in FBS (e.g., lithium, boron, magnesium, phosphorus, sulfur, potassium, titanium, vanadium, chromium, manganese, iron, copper, zinc, gallium, and selenium). The lack of reproducibility and the variation in protein expression in the primary human cells was attributed to the FBS supplementation.

Culture conditions for cell lines are known to affect gene expression [154–156], while stem cells grown in different types of serum exhibit variable differentiation and proliferation characteristics [157, 158] the same cell line, if cultivated in different conditions, can present different phenotypes. Nevertheless, the cellular requirement for a specific micronutrient is directly correlated with the cell type, the rate of cell grow, and the stage of cell differentiation. In light of this, it is important to observe that minimal attention has been dedicated to the composition of FBS and the micronutrient supplementation of media in cell cultures or the fact that micronutrients can influence the viability and genomic stability of cultured cells."

"Even though there are some highly enriched media available as basal media for serum-free cell culture, like Medium 199 or Ham F-12 nutrient mixture, the most common source of micronutrients currently used in cell cultures is still FBS. The limitations of FBS

in providing adequate micronutrient concentrations have been analyzed and described in the literature [34]. Given that cell- and tissue-culture models are generally important in scientific research, the development of standards in vitro methods is mandatory. “

“At the very least, an evaluation of FBS composition, in terms of micronutrients and possibly other factors, should be strongly considered in the laboratories that focus on in vitro studies. Knowledge of the micronutrient composition of FBS may help to minimize the bias in experimental results. However, maintaining both successful and consistent cell cultures can be difficult, as FBS is a complex natural product and may vary between batches, even if obtained from a single manufacturer. More specifically, the quality and concentration of both bulk and specific proteins in cell cultures can affect cell growth [210]. Adjusting the in vitro micronutrient levels to physiological values will guarantee a better environment for cell development, mimicking the in vivo milieu.

Further studies on the effects of micronutrients on cell viability, proliferation, and stability, as well as gene expression and integrity are still required, but the information already available is a sufficient call to action. As mentioned by Ferguson and Fenech [141], most investigations have been limited to studying the effects of single micronutrients and have not considered genetic consequences. Thus, there is an important need for studies that also examine nutrient-nutrient and nutrient-gene interactions. Determining the physiological range of such significant micronutrients as iron and then adjusting the concentrations currently found in cell-culture media may be beneficial for in vitro assays. More specifically, the viability and genomic stability of cell lines and primary cultures may be improved. Depending on the cell type (primary, immortalized, tumor, or normal) and origin (lung, hepatic, neural, or other), the requirement for a micronutrient may vary widely, so this subject should be carefully evaluated. Finally, the form of the micronutrient used in supplementation media may also influence experimental results.

https://www.hindawi.com/journals/bmri/2013/597282/

From these two articles, it is shown that:

media contains components that interact with each other and can influence the cells

the effects are not always predictable

media lacks compositional uniformity

FBS is a main component of many media

minimal attention has been paid to the composition of the media as well as the interaction on viability/genetics of cells

media can influence and interfere with genomic stability

each batch of media is variable

concentration of micronutrients may trigger different cellular responses

data is not available for all the micronutrients in media nor how they interact/affect the cells

there is an imbalance in micronutrients and human serum

media recipes vary in Ph, glucose concentrations, growth factors, and the presence of other nutrients/micronutrients

culture conditions affect gene expression

further studies on the effects of media on cell cultures are needed

the form of the micronutrients may influence experimental results

It is clear that there are numerous issues with cell culture media, even in the absence of antibiotics and FBS which by themselves can have profound impacts on the cells and the outcome of a culture. There are too many unknowns about the makeup of the media and the interactions between the various components. With so many known and unknown variables/issues, there is no way to be confident that cell culture media has no impact on the end result of any culture/experiment. In fact, it is a guarantee for media to have an impact. The problem is that the extent of this impact is unknown.

https://viroliegy.com/2021/08/30/cell-culture-media/

The problem of reproducibility in cell cultures:

Along with all the issues already outlined about the problems of relying on cell culture for "viral" identification (cells/antibiotics/chemicals/nutrients used, inability to replicate

microenvironment, contamination altering results) comes the problem of reproducibility. Being able to reproduce the results of others is essential but has become a huge problem in science and is prevalent in cell cultures. A few articles highlight this glaring problem:

"Advances in life science research build upon the reproducibility of previously published data and findings, YET IRREPRODUCIBILITY IN BASIC AND PRECLINICAL BIOLOGICAL RESEARCH IS A PERVASIVE, EXPENSIVE AND INCREASINGLY WELL-RECOGNIZED PROBLEM1,2. Also called replication, validation, verification or reanalysis3, in simplest terms, REPRODUCIBILITY MEANS THAT AN EXPERIMENT SHOULD BE ABLE TO BE CONFIRMED IN AN INDEPENDENT LABORATORY WITH RESULTS THAT BROADLY SUPPORT THE CONCLUSIONS OF THE ORIGINAL SCIENTISTS. Excluding deliberate scientific misconduct4, irreproducibility typically results from errors or flaws in one or more of the following areas of the research process: reference materials, study design, laboratory protocols, and data analysis and reporting5,6. Irreproducible preclinical research contributes to both delays and increased costs in drug discovery."

https://cellariainc.com/wp-content/uploads/2017/02/White-Paper_Nature_Freedman_reproducibility_cell_lines_June-2015.pdf

"WHILE MUCH OF BIOLOGY RESEARCH SUFFERS FROM A LACK OF REPRODUCIBILITY, no single factor has emerged as the driver of this problem. In a multi-lab study published this week in Cell Systems, researchers have ATTEMPTED TO REPRODUCE THE RESULTS of an assay in which cultured cells were treated with cancer drugs. THEIR LACK OF SUCCESS HIGHLIGHTS THE ROLE THAT TECHNICAL VARIABLES PLAY IN THE ABILITY TO REPEAT EXPERIMENTS."

https://www.the-scientist.com/news-opinion/potential-causes-of-irreproducibility-revealed-66146

"However, the explosion in research utilizing cell culture in this way HAS NOT BEEN MATCHED BY EFFORTS TO ENSURE QUALITY CONTROL OF THE CELLS IN QUESTION. This poses even further risk to the successful translation of research, WITH A LACK OF QUALITY CONTROL RESULTING IN A LACK OF REPRODUCIBILITY."

https://www.biotechniques.com/cell-and-tissue-biology/quality-control-the-dark-side-of-cell-culture

"CONCERNS REGARDING THE REPRODUCIBILITY OF OBSERVATIONS IN LIFE SCIENCES research has emerged in recent years, particularly in view of unfavorable experiences with preclinical in vivo research."

"Detailed guidance documenting the appropriate handling of cells has been authored, BUT WAS RECEIVED WITH QUITE DISPARATE PERCEPTIONS BY DIFFERENT BRANCHES IN BIOMEDICAL RESEARCH. In that regard, we intend to RAISE AWARENESS OF THE REPRODUCIBILITY ISSUE among scientists in all branches of contemporary life science research and their individual responsibility in this matter."

"A survey published in Nature in 2016 (Baker, 2016) evaluating questionnaires on reproducibility in life science research disclosed NOT ONLY THE DIFFICULTIES RESEARCHERS HAVE REPRODUCING EXPERIMENTS FROM OTHER LABORATORIES, BUT ALSO FROM THEIR OWN. Even more surprising was the fact that AWARENESS OF THIS PROBLEM WAS WIDESPREAD WITHIN THE SCIENTIFIC COMMUNITY. The inability to reproduce study results, often inherent in observations from academic laboratories, are usually uncovered not without relevant delay, e.g. when potential therapies that are based on these findings transition from preclinical testing to the far more stringent conditions of clinical trials (Collins and Tabak, 2014)."

"In toxicology, which may better reflect the background of most readers of this journal, awareness of this problem has emerged only gradually IN ASSOCIATION WITH INSUFFICIENT IN VIVO REPRODUCIBILITY (Kilkenny et al., 2009; Voelkl et al., 2018). Such disclosures, in concert with studies indicating that in vivo data from rats and mice combined can only predict human clinical toxicology of less than 50% of candidate pharmaceuticals (Olson et al., 2000), promoted a revision of several toxicologists' opinions towards mechanistic in vitro assays from the traditional reliance on pharmacological and toxicological in vivo animal testing."

"A major concern raised by researchers in different fields of biomedicine was HOW A CELL CULTURE MODEL, OFTEN NOT EVEN ORIGINATING FROM THE ORGAN OF INTEREST, COULD PROVIDE INFORMATION ABOUT MULTILAYER PROCESSES AND PATHOLOGICAL OUTCOMES IN HUMANS."

"A defined assay performed with a defined in vitro model NEEDS TO YIELD IDENTICAL RESULTS— NO MATTER WHEN OR WHERE IT IS PERFORMED. As trivial as this

statement may appear, its implementation is QUITE DIFFICULT IN REALITY. The Nature survey of 2016 (Baker, 2016) highlighted the degree of inadequate reproducibility in biomedical research and underlined the widespread awareness of the problem within the scientific community. It is, thus, all the more astonishing that SYSTEMATIC COMPARISONS OF EXPERIMENTAL MODELS APPLIED IN DIFFERENT LABORATORIES ARE RATHER RARE, particularly in the field of in vitro research."

"An exemplary illustration of this transparency is a publication by Elliott and colleagues (Elliott et al., 2017) assessing the reproducibility of MTS-tetrazolium reduction assay results as indicators of cell viability in an international inter-laboratory comparison study with five independent laboratories. Strict standard operating procedures (SOP) were employed using a sophisticated 96-well plate design that allowed detection of up to seven parameters of assay performance, including accuracy of multi-channel pipetting, cell handling/cell growth, and instrument performance (i.e. plate reader issues) (Rösslein et al., 2015). A549 cells were purchased from two independent, credible, accepted commercial sources and both, seemingly identical, cell cultures were used in all labs. EVEN UNDER SUCH STRICT CONDITIONS, EC50 values of the two A549 cultures upon CdSO4 treatment DIFFERED BY A FACTOR OF TWO IN ALL LABORATORIES. In the course of these investigations, CELL LINE AUTHENTICATION WAS DISCOVERED TO BE ONE OF THE MAIN FACTORS INFLUENCING ASSAY RESULTS. Short tandem repeat sequencing revealed a partial chromosome deletion in one of the cell cultures. Technical aspects also contributed to result variability. For example, SIMPLE CELL HANDLING STEPS, SUCH AS PBS WASHING, WERE IDENTIFIED TO SIGNIFICANTLY CHANGE ASSAY OUTCOMES. This example provides a vivid illustration of the IMPACT OF SEEMINGLY TRIVIAL DETAILS AND THE NECESSITY TO DRAW ATTENTION TO ALL ASPECTS OF IN VITRO EXPERIMENTATION."

"A recent evocative study of the mammary epithelial cell line MCF10A and growth rate inhibition by anti-cancer drugs systematically addressed inter- and intra-study center variations and identified factors contributing to insufficient reproducibility (Niepel et al., 2019). ALTHOUGH THE FIVE RESEARCH CENTERS APPLIED CELLS AND CHEMICALS OF THE SAME STOCK, ASTONISHING CENTER-TO-CENTER VARIATIONS UP TO 200-fold WERE OBSERVED IN GROWTH INHIBITION RATES. Cell seeding, i.e. slight variations in initial cell numbers, was identified as one key source of these variations (for more details see Recommendation 5) (Cell density and medium change). Overall, THE SUBTLE INTERPLAY BETWEEN EXPERIMENTAL METHODS AND A VAST ARRAY OF POORLY DEFINED SOURCES OF BIOLOGICAL VARIABILITY WAS FOUND TO BE THE MAIN CAUSE OF THE OBSERVED IRREPRODUCIBILITY."

"As a consequence of the huge number of individual biological factors involved, Niepel and colleagues came to the rather discouraging conclusion that “MOST EXAMPLES OF IRREPRODUCIBILITY ARE THEMSELVES IRREPRODUCIBLE” (Niepel et al., 2019)."

"This spectrum of biological factors further depends on the complexity of the cell model applied. The introduction of 2D co-culture models and 3D cell models was motivated by the ambition to recapitulate the natural in vivo environment of cells in a cell culture dish. In fact, CELLS IN A 3D CULTURE DIFFER MORPHOLOGICALLY AND PHYSIOLOGICALLY FROM THEIR COUNTERPARTS IN A 2D SETUP (Baharvand et al., 2006; Edmondson et al., 2014). Introduction of the third dimension in a cell culture model results in additional parameters THAT COULD POTENTIALLY AFFECT REPRODUCIBILITY, including spheroid size and consequently the oxygen and nutrient supplies to cells in different layers within the structure; spatial organization of surface receptors involved in interactions with neighboring cells; activation of signal transduction pathways; and induction of gene expression profiles (Vinci et al., 2012). ALL OF THESE CHANGES ULTIMATELY HAVE THE POTENTIAL TO INFLUENCE CELL BIOLOGY AND CELLULAR RESPONSE TOWARDS EXOGENOUS STRESSORS."

"Even with the best of intentions, IT MUST BE CONCLUDED THAT THE LIMITS OF REPRODUCIBILITY IN CELL CULTURE WORK IS REACHED WHEN CONFRONTED WITH THE QUESTION OF REFERENCE STANDARDS, PARTICULARLY FOR ESTABLISHED AND WIDELY DISTRIBUTED CELL LINES. Simply put, which of the currently available and characterized stocks of common cell lines, like HeLa cells, should be considered as the gold standard? EVEN IF A CONSENSUS COULD BE REACHED FOR INDIVIDUAL CELL LINES, STORAGE CAPACITY LIMITATIONS FORCE EVEN LARGE CELL BANKS TO PASSAGE THEIR CELLS, WHICH NECESSARILY INFLUENCES THE CELLS IN ONE WAY OR ANOTHER OVER TIME."

https://www.frontiersin.org/articles/10.3389/fphar.2019.01484/full

As can be seen, reproducibility is cell culture (and in all of science) is a huge problem. There are many factors which contribute to this inability to recreate the same results independently. In order for the results to be considered accurate and valid, all of these various factors would need to be taken into consideration and do exactly what was intended without any unforeseen problems arising. Even if things do seemingly go correctly, reproducibility is hard to come by.

It's clear that cell cultures are not an accurate representation of reality and can not be considered as proof of a "virus." These toxic concoctions are akin to witches brew in that what comes from them is not what was presented at the start but a creation stemming from the interactions of various cells, antibiotics, chemicals, serums, "nutrients," etc.

https://docs.google.com/document/d/e/2PACX-1vSKzeVhg8xU5UaPZYD2KcisOSNkyKgylG3vfdD1fCqQ36knuLDVlwkAZ8u4j3FeJLZgpRRktUmqQaYk/pub

SUB-CULTURING and CELL CULTURE ADAPTATIONS

"Barbara McClintock might be surprised to learn how well recent discoveries support her hypotheses. Her experiments of 60 years ago led her to propose that CELLS UNDER ENVIRONMENTAL STRESS ACTIVATE TRANSPOSABLE ELEMENTS IN ORDER TO RESTRUCTURE THE CELL GENOME (McClintock, 1984)."

https://www.sciencedirect.com/science/article/pii/S1097276507004510

It is well known that cells under stress change and adapt due the environmental and physical stresses placed upon them. This was discovered by Barbara McClintock in the 1960's who won a Nobel Prize for her work. The changes that occur from stressors alter the genetic expression of the cells as they adapt to survive. The toxic antibiotics, the foreign human and animal DNA, the chemicals and "nutrients added, etc. can all stress the cell yet there is another more physical stressors that can cause these changes on top of all of that. This is a process called Sub-Culturing, also known as Passaging:

"SUBCULTURING, also referred to as PASSAGING, is the REMOVAL OF THE MEDIUM AND TRANSFER OF CELLS FROM A PREVIOUS CULTURE INTO FRESH GROWTH MEDIUM, a procedure that enables the further propagation of the cell line or cell strain.

The growth of cells in culture proceeds from the lag phase following seeding to the log phase, where the cells proliferate exponentially. When the cells in adherent cultures occupy all the available substrate and have no room left for expansion, or when the cells

in suspension cultures exceed the capacity of the medium to support further growth,

CELL PROLIFERATION IS GREATLY REDUCED OR CEASES ENTIRELY (see Figure 4.1 below). To keep them at an optimal density for continued growth and to stimulate further proliferation, THE CULTURE HAS TO DIVIDED AND FRESH MEDIUM SUPPLIED."

https://www.google.com/url?sa=t&source=web&rct=j&url=https://www.vanderbilt.edu/viibre/CellCultureBasicsEU.pdf&ved=2ahUKEwiyzt6Lk4DvAhXjdc0KHRwECGUQFjAPegQIBRAC&usg=AOvVaw0Uts4tzzBDi8AMtyXXiwSQ

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https://arthritis-research.biomedcentral.com/.../ar3010

As can be seen, this process is a problem that has profound effects on the culture. Some further information on the problems associated with passaging cells:

"This subculture is also known as a “passage.” A passage number is the number of times a cell culture has been subcultured, and KNOWING THE PASSAGE NUMBER CAN MAKE OR BREAK AN EXPERIMENT.

All cell cultures start somewhere; this “somewhere” is the reference strain, or reference culture. These are fresh cells that come from a reliable source, like the ATCC. While many labs may passage cells dozens, even hundreds of times, THIS MANY PASSAGES PROBABLY RESULTS IN CELLS THAT HAVE LITTLE IN COMMON WITH THE ORIGINAL REFERENCE STRAIN. These “working cultures,” if passaged enough times, CAN SHOW EVIDENCE OF GENETIC DRIFT—CHANGES IN GENOTYPE FROM THE ORIGINAL REFERENCE STRAIN which may or may not result in observable changes in phenotype. OTHER GENOTYPE CHANGES MAY NOT SHOW ANY PHENOTYPIC VARIATION IMMEDIATELY, BUT COULD RESULT IN CHANGES AFTER FURTHER SUBCULTURING. In addition, genetic changes caused by subculturing COULD CREATE EPIGENETIC CHANGES that could affect how genes are regulated. MORE PASSAGES ALSO INCREASE THE RISK OF CONTAMINATION. Not good."

"Good cell practice calls for starting any experiment WITH LOW-PASSAGE CELL CULTURE, AND LIMIT THE NUMBER OF PASSAGES YOU’LL ACCEPT IN YOUR EXPERIMENT. But what is a good passage number (BESIDES “ZERO,” that is)? The numbers have differed over the years. Some standards recommend three stock subcultures and three “working culture” subcultures—those add up to seven passages, including the original passage from the reference. Meanwhile, some cell culture producers charge more for cultures of two passages or less. However, the ATCC warns researchers to ASSUME THAT A CELL CULTURE FROM A COMMERCIAL SOURCE

MAY BE ALREADY ONE OR TWO PASSAGES AWAY FROM THE REFERENCE STRAIN. Generally, the ATCC recommends that cell culture SHOULD BE LIMITED TO FIVE PASSAGES, at least for use in medical and biopharmaceutical applications."

https://bitesizebio.com/13685/cell-culture-passage-number-explained

It's clear that sub-culturing cells before and during cell culture experiments can alter the cell. The stress from the change in environment and the added media can and will change gene expression, alter morphology, effect growth rate, hinder stimulus response, change protein expression, increase contamination, etc. These cell culture adaptations are attributed to "natural" mutations and variations and are accepted as new variants of the same "virus" even though there are numerous other explanations for why these changes occur and why they can never get the same exact sequence twice.

https://docs.google.com/document/d/e/2PACX-1vSmTM9raiDxVAp64o3yTdILEtWCuwmrhG7nb25YQbJKtuh2d2P9PMKOUD7Fv72Po-Ev3CJgKikiKEEr/pub

VIRAL TRANSPORT MEDIA:

Viral Transport Media is used to "preserve" a "virus" after the sample has been taken from the sick person. It is intended to keep the "virus" in a stable condition while it is transported to laboratories in order to be tested, cultured, sequenced, etc. Looking at two of the original "SARS-COV-2" papers for what was used as a VTM sheds quite a bit of light on this hoax:

From the **Zhou** paper:

"For swabs, 1.5 ml DMEM CONTAINING 2% FBS was added to each tube."

"THE VIRAL TRANSPORT MEDIUM was composed of Hank's balanced salt solution (pH 7.4) containing BSA (1%), AMPHOTERICIN (15 μg ml−1), PENICILLIN G (100 units ml−1) AND STREPTOMYCIN (50 μg ml−1)."

https://www.nature.com/articles/s41586-020-2012-7

"Oropharyngeal samples were diluted with VIRAL TRANSFER MEDIUM CONTAINING nasopharyngeal swabs and ANTIBIOTICS (NYSTADIN, PENICILLIN-STREPTOMYCIN 1:1 dilution)"

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7045880/

Knowing what we already know about the disastrous effects of DMEM, Fetal Bovine Serum, and Antibiotics on cell cultures should be enough to question the results of these studies. Keep in mind that adding VTM is done to the sample BEFORE the Cell Culture. It is already contaminated by toxic chemicals and then it is added to African Green Monkey Kidney cells which is further bombarded by cell altering chemicals/contaminants.

It is clear that VTM is a toxic mixture currently being used in the transport of patient samples. Are there other solutions that are less toxic to the samples and cells that can be used as VTM?

PHOSPHATE BUFFERED SALINE:

Other media has been used to transport specimens during this "pandemic" such as Phosphate Buffered Saline, which is already used for other aspects of Cell Culture such as washing and dilution:

"PBS (phosphate buffered saline) is a balanced salt solution used for a variety of cell culture applications, such as washing cells before dissociation, transporting cells or tissue, diluting cells for counting, and preparing reagents."

https://www.thermofisher.com/.../catalog/product/10010023...

PBS is considered a relatively safe and non-toxic salt solution for most cells. However, even this relatively "non-toxic" solution can alter the cells during the culturing process so it's use as a transport media should be questioned as well:

"THE EFFECT OF TIME AND DILUENTS ON CELL CULTURE IS NOT WELL UNDERSTOOD."

"Furthermore, LENGTH OF TIME samples were incubated in phosphate buffered saline also CONTRIBUTED TO THE OBSERVED DROP IN CELL VIABILITY."

"THIS REPORT PRESENTS THE ADVERSE EFFECTS OF, and alternatives for, CELL

CULTURE SAMPLES DILUTED IN PHOSPHATE BUFFERED SALINE (PBS). LOWER VIABILITY AND GREATER VARIABILITY WAS OBSERVED WITH PBS DILUTED SAMPLES. Furthermore, the viability of PBS diluted samples CONTINUOUSLY DECREASED OVER TIME AND AT A FASTER RATE THAN THE OTHER CONDITIONS. This phenomenon was observed with multiple cell lines and different culture systems."

"Therefore, care needs to be taken when preparing viability samples with diluents TO ENSURE THE RESULTS ARE ACCURATE AND REPRESENTATIVE OF THE CULTURE."

"Cell culture sample dilution is sometimes necessary to preserve the working volume and/or to extend assay measurement range. Therefore, IT IS IMPERATIVE TO IDENTIFY ALTERNATIVE DILUENTS THAT DO NOT INADVERTENTLY DECREASE VIABILITY."

"A systematic investigation has revealed that CELL CULTURE SAMPLES DILUTED IN PBS COULD INADVERTENTLY LOWER THE VIABILITY WHEN MEASURED USING AUTOMATED CELL COUNTERS. The effect of PBS on viability can be consistently reproduced, and is independent of process scale, cell line, operator and automated cell counter used. In addition, THE NEGATIVE IMPACT OF PBS ON THE VIABILITY IS PROPORTIONAL TO THE SAMPLE INCUBATION TIME, AND INVERSELY PROPORTIONAL TO THE TCC."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5338812/

From a Cell Counting Manual:

"However resuspension in PBS BUFFER RESULTS IN A DRASTIC LOSS OF VIABILITY AND SIGNIFICANT REDUCTION IN AVERAGE CELL DIAMETER. There is also some minor reduction in cell concentration which may be expected from losses during resuspension. Spinning and resuspending a second time in PBS HAS A FURTHER NEGATIVE IMPACT ON CELL VIABILITY AND CELL SIZE."

"It is recommended that prior to running a cell culture on a complete plate that some initial evaluation is performed to determine the robustness of the cells involved AND THEIR ABILITY TO TOLERATE THE SAMPLE PREPARATION METHODS AND PROLONGED EXPOSURE OUTSIDE THE INCUBATOR ENVIRONMENT. Even engineered cells such as CHO cells or immortal HELA cells, which are generally selected to be quite durable, CAN BE EFFECT BY STRESS OR PARTICULAR

SAMPLE PREPARATION CONDITIONS."

https://www.google.com/url?sa=t&source=web&rct=j&url=https://www.beckman.com/gated-media%3FmediaId%3D%257B06D69A51-A996-456C-9E06-6E8A23E2D79C%257D&ved=2ahUKEwiOwIqvqvvuAhXUHc0KHe25A5sQFjADegQIBBAG&usg=AOvVaw0fqNRJAJ-I6vIFhB3g1eXk

It appears even the relatively "harmless" PBS has an adverse effect on cell cultures including lower viability, greater variability, and a decrease in cell size. It is admitted that the length of time and effect of PBS on cell cultures is not well known, which seems to be a common theme regarding the interactions of all of the various chemicals, antibiotics, nutrients, serums, etc. on the samples and cell cultures.

Trust the conclusions of these "studies" at your own risk.

MORE VTM

Relating to the earlier post on Viral Transport Media, these are the guidelines for VTM for "SARS-COV-2" put forth by the CDC. Note, this is to provide a standard which means the VTM is not standardized across all laboratories. They even allow alterations as long as it is documented:

"Purpose

TO PROVIDE A STANDARD OPERATING PROCEDURE (SOP) for producing viral transport medium (VTM) for specimens for viral culture or other means of viral detection.

This SOP PROVIDES AN ALTERNATIVE TO COMMERCIALLY AVAILABLE PRODUCTS. THERE ARE MANY ACCEPTABLE FORMULATIONS OF THIS MEDIUM THAT MAY BE SUITABLE FOR THE UNIQUE CONDITIONS OF INDIVIDUAL LABORATORIES. The specific needs of the shipping and receiving laboratories should be considered when choosing a VTM formulation."

"Responsibility

It is the responsibility of personnel preparing VTM for CDC's COVID-19 outbreak response to follow this SOP accurately. IF THERE ARE VARIATIONS from this SOP,

THE VARIATIONS SHOULD BE DOCUMENTED, AND DATA GENERATED TO DEMONSTRATE EQUIVALENT PERFORMANCE OF THE VTM."

Here is the VTM formula recommended by the CDC:

"Reagents

6.11 Sterile Hanks Balanced Salt Solution (HBSS) 1X with calcium and magnesium ions, no phenol red, 500mL bottle (or HBSS containing phenol red as a pH indicator)

6.12 Sterile, heat-inactivated FETAL BOVINE SERUM (FBS)

6.13 GENTAMICIN SULFATE (50mg/mL) (OR SIMILAR ANTIBIOTIC at an appropriate concentration to prevent bacterial contamination and growth)

6.14 AMPHOTERICIN B (250µg/mL) (Fungizone) (or similar antifungal at an appropriate concentration to prevent fungal contamination and growth)

6.15 Blood agar plate

6.16 Disinfectant, such as 70% ETHANOL"

https://www.google.com/url?sa=t&source=web&rct=j&url=http://www.cdc.gov/coronavirus/2019-ncov/downloads/Viral-Transport-Medium.pdf&ved=2ahUKEwigj-r3x_vuAhUMV80KHYMbAFAQFjAIegQIDxAC&usg=AOvVaw3pTsWq8rGHibhocLI1EVl0

A quick note on the use of Gentamicin and Amphotericin B.

THEY ARE TOXIC TO CELLS:

GENTAMICIN:

"THERE ARE MANY REPORTS OF ANTIBIOTICS CAUSING MITOCHONDRIAL DAMAGE. In this study, we tested the effect of gentamicin in culture media on human mammary epithelial MCF-12A and breast cancer MCF-7 and MDA-MB-231 cell lines by real time PCR, immunofluorescent microscopy, lactate assay, DNA damage assay. WE FOUND THAT THE ADDITION OF GENTAMICIN IN MEDIA UNREGULATED THE GENE EXPRESSION OF HYPOXIA INDUCER FACTOR 1 ALPHA (HIF1a), GLYCOLYTIC ENZYMES AND GLUCOSE TRANSPORTERS, compared to the cells cultured in gentamicin free media. GENTAMICIN ALSO INCREASED THE LACTATE PRODUCTION AND INHIBITED MITOCHONDRIAL MEMBRANE POTENTIAL OF THE CELL LINES. Furthermore, THE ANTIBIOTICS IN MEDIA INDUCED MITOCHONDRIAL REACTIVE OXYGEN SPECIES CAUSING DNA DAMAGE. We found an increase of 8-hydroxy-2'-deoxyguanosine a product of DNA oxidative damage in the media of MCF-12A, MCF-7 and MDA-MB-231 cell lines."

"THE METABOLIC CHANGES IN ALL CELL LINES WERE DRAMATICALLY DIFFERENT BETWEEN THOSE IN ANTIBIOTIC FREE MEDIA VERSUS ANTIBIOTIC CONTAINING MEDIA. There was a MARKED DIFFERENCE IN GENE EXPRESSION OF GLYCOLYTIC ENZYMES, REACTIVE OXYGEN SPECIES PRODUCTION AND EFFECTS ON MEMBRANE POTENTIAL. Ironically, our first studies were done in media containing gentamicin, and repeated studies were done in gentamicin free media. THE RESULTS WERE VERY DIFFERENT. The purpose of this report is to EMPHASIZE THAT METABOLIC CELL CULTURE DATA MAY BE INACCURATE BECAUSE EXPERIMENTS WERE PERFORMED IN CELL CULTURE MEDIA CONTAINING ANTIBIOTICS."

"Based on our study of the effect of gentamicin in three mammary cell lines, WE ARE CONVINCED THAT ANTIBIOTICS DO CAUSE MITOCHONDRIAL DYSFUNCTION, AND THIS IS TRUE FOR BACTERICIDAL AND BACTERIOSTATIC ANTIBIOTICS REGARDLESS OF PARADOXICAL REPORTS IN THE LITERATURE. We have emphasized the importance of CELL CULTURE STUDIES BEING DONE IN ANTIBIOTIC FREE CULTURE MEDIA; especially when studying cellular metabolism. We have stressed that MANY REPORTS MAY HAVE INACCURATE DATA, as the study was done in antibiotic containing cell culture media. When studying mitochondrial function, we must remember that mitochondria are evolutionary bacteria; AND ANTIBIOTICS HAVE DAMAGING EFFECTS ON THEM AND BACTERIA."

https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0214586

AMPHOTERICIN B:

"The results of the cell viability assays CONFIRM HIGH TOXICITY OF AMPHOTERICIN B TOWARDS HUMAN CELLS."

"According to a current knowledge, biomembranes of human and fungi cells are a primary target of the drug and both the therapeutic and toxic side effects of AmB are BASED UPON IMPAIRING OF PHYSIOLOGICAL PROCESSES TAKING PLACE IN MEMBRANES."

"Two human cell lines, CCD 841 CoTr and HT-29, were cultured in the presence of AmB in a concentration range of 0.05 to 25 μg/ml in the growth medium. AS EXPECTED, HIGHER CONCENTRATIONS OF THE ANTIBIOTIC ARE TOXIC TO HUMAN CELLS (above 5 μg/ml, see Fig. 1)."

https://www.nature.com/articles/s41598-018-32301-9

Oh yeah...we can't forget the 70% Ethanol.

ETHANOL:

"ALL CELLS WERE KILLED BY A 15-s EXPOSURE TO 30–40% ETHANOL WHILE A CONCENTRATION AS LOW AS 15–20% GAVE A TOTAL RESPONSE AFTER 5–10-min EXPOSURES. After a one-hour exposure of F9 carcinoma cells and hepatocytes, a total or nearly total response was achieved with 10% ethanol. THE CYTOTOXIC EFFECT WAS THUS DEPENDENT BOTH ON THE EXPOSURE TIME AND ON THE CONCENTRATION OF ETHANOL ."

"ETHANOL SEEMED TO KILL CELLS IN THE CELL CULTURE EFFECTIVELY IN MUCH LOWER CONCENTRATIONS than those currently used in tumour ablation."

https://www.tandfonline.com/doi/abs/10.3109/02841859609175470?fbclid=IwAR1OsWu-Gbdisq2MzKyIDYvOmBN2IwJiEP9arp-6QSDHwi67N4BR-A6V4P8&

And this is primarily about Ethanol being a CONTAMINANT when sprayed as a disinfectant in laboratories:

"Here we want to put a spotlight on the importance of being careful on the quantity sprayed and where to spray it, and particularly to avoid its contact with experimental

cells, SINCE THIS WILL LEAD TO RADICAL INFLUENCE ON CELLS PATHOPHYSIOLOGICAL CONDITION."

"In conclusion, ethanol is largely utilized as antiseptic in cell experiment environment, and at the same time IT HAS A HUGE NUMBER OF POSSIBLE IMPLICATIONS IN DIFFERENT CELLULAR MECHANISMS"

https://medcraveonline.com/JMEN/ethanol-in-cell-culture-disinfectant-or-contaminant.html?fbclid=IwAR0ooUZ36568HWhBnVPS11MRCOHPyM_TcKAW32U4w8sjREGW-qneyr1ogU8

Remember: samples from sick patients are immediately placed in Viral Transport Media.

Why are these obviously toxic Antibiotics (along with the damaging effects of Fetal Bovine Serum and the potential contamination by Ethanol) added to the samples before testing, culturing, sequencing, etc.? Why is it assumed they will have no effect on the sample when the evidence clearly shows otherwise?

There is nothing pure about the cell culture process even from the very first step.

https://docs.google.com/document/d/e/2PACX-1vRIV5oXy0ZIFvSry6gJ09maJTqz1dJz_HTtzlZNfJYPB3k7vvrPrM2q3hT5F8r_zfv27rxqdTU2CiwZ/pub

SUB-CULTURING and CELL CULTURE ADAPTATIONS:

"Barbara McClintock might be surprised to learn how well recent discoveries support her hypotheses. Her experiments of 60 years ago led her to propose that CELLS UNDER ENVIRONMENTAL STRESS ACTIVATE TRANSPOSABLE ELEMENTS IN ORDER TO

RESTRUCTURE THE CELL GENOME (McClintock, 1984)."

https://www.sciencedirect.com/science/article/pii/S1097276507004510

It is well known that cells under stress change and adapt due the environmental and physical stresses placed upon them. This was discovered by Barbara McClintock in the 1960's who won a Nobel Prize for her work. The changes that occur from stressors alter the genetic expression of the cells as they adapt to survive. The toxic antibiotics, the foreign human and animal DNA, the chemicals and "nutrients added, etc. can all stress the cell yet there is another more physical stressors that can cause these changes on top of all of that. This is a process called Sub-Culturing, also known as Passaging:

"SUBCULTURING, also referred to as PASSAGING, is the REMOVAL OF THE MEDIUM AND TRANSFER OF CELLS FROM A PREVIOUS CULTURE INTO FRESH GROWTH MEDIUM, a procedure that enables the further propagation of the cell line or cell strain.

The growth of cells in culture proceeds from the lag phase following seeding to the log phase, where the cells proliferate exponentially. When the cells in adherent cultures occupy all the available substrate and have no room left for expansion, or when the cells in suspension cultures exceed the capacity of the medium to support further growth, CELL PROLIFERATION IS GREATLY REDUCED OR CEASES ENTIRELY (see Figure 4.1 below). To keep them at an optimal density for continued growth and to stimulate further proliferation, THE CULTURE HAS TO DIVIDED AND FRESH MEDIUM SUPPLIED."

https://www.google.com/url?sa=t&source=web&rct=j&url=https://www.vanderbilt.edu/viibre/CellCultureBasicsEU.pdf&ved=2ahUKEwiyzt6Lk4DvAhXjdc0KHRwECGUQFjAPegQIBRAC&usg=AOvVaw0Uts4tzzBDi8AMtyXXiwSQ

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https://arthritis-research.biomedcentral.com/.../ar3010

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"This subculture is also known as a “passage.” A passage number is the number of times a cell culture has been subcultured, and KNOWING THE PASSAGE NUMBER CAN MAKE OR BREAK AN EXPERIMENT.

All cell cultures start somewhere; this “somewhere” is the reference strain, or reference culture. These are fresh cells that come from a reliable source, like the ATCC. While many labs may passage cells dozens, even hundreds of times, THIS MANY PASSAGES PROBABLY RESULTS IN CELLS THAT HAVE LITTLE IN COMMON WITH THE ORIGINAL REFERENCE STRAIN. These “working cultures,” if passaged enough times, CAN SHOW EVIDENCE OF GENETIC DRIFT—CHANGES IN GENOTYPE FROM THE ORIGINAL REFERENCE STRAIN which may or may not result in observable changes in phenotype. OTHER GENOTYPE CHANGES MAY NOT SHOW ANY PHENOTYPIC VARIATION IMMEDIATELY, BUT COULD RESULT IN CHANGES AFTER FURTHER SUBCULTURING. In addition, genetic changes caused by subculturing COULD CREATE EPIGENETIC CHANGES that could affect how genes are regulated. MORE PASSAGES ALSO INCREASE THE RISK OF CONTAMINATION. Not good."

"Good cell practice calls for starting any experiment WITH LOW-PASSAGE CELL CULTURE, AND LIMIT THE NUMBER OF PASSAGES YOU’LL ACCEPT IN YOUR EXPERIMENT. But what is a good passage number (BESIDES “ZERO,” that is)? The numbers have differed over the years. Some standards recommend three stock subcultures and three “working culture” subcultures—those add up to seven passages, including the original passage from the reference. Meanwhile, some cell culture producers charge more for cultures of two passages or less. However, the ATCC warns researchers to ASSUME THAT A CELL CULTURE FROM A COMMERCIAL SOURCE MAY BE ALREADY ONE OR TWO PASSAGES AWAY FROM THE REFERENCE STRAIN. Generally, the ATCC recommends that cell culture SHOULD BE LIMITED TO FIVE PASSAGES, at least for use in medical and biopharmaceutical applications."

https://bitesizebio.com/13685/cell-culture-passage-number-explained

It's clear that sub-culturing cells before and during cell culture experiments can alter the

cell. The stress from the change in environment and the added media can and will change gene expression, alter morphology, effect growth rate, hinder stimulus response, change protein expression, increase contamination, etc. These cell culture adaptations are attributed to "natural" mutations and variations and are accepted as new variants of the same "virus" even though there are numerous other explanations for why these changes occur and why they can never get the same exact sequence twice.

Passage in Vero Cells: The Variant Game

"In addition, passage in cell culture can result in artificial mutations in the sequences, which were not present in the original clinical sample. This can have major implications for subsequent analyses. Using cell culture solely for the purpose of amplifying virus genetic material for SARS-CoV-2 sequencing should therefore be avoided"

https://www.google.com/url?sa=t&source=web&rct=j&url=https://apps.who.int/iris/rest/bitstreams/1326052/retrieve&ved=2ahUKEwi9i5ql-M7xAhUJnGoFHQvEBaIQFjADegQICBAC&usg=AOvVaw0gjHudaoThq3mU1JVxALpd

The above statement comes from a "SARS-COV-2" Genome Sequencing manual from the WHO. They seemingly admit that the cell culture process results in the mutations and variations seen in the over 3 million "SARS-COV-2" variants currently running around at the time of this writing. They claim that this is due to passaging of cell cultures. What exactly is the WHO referring to here?

"Subculturing, also referred to as passaging cells, is the removal of the medium and transfer of cells from a previous culture into fresh growth medium, a procedure that

enables the further propagation of the cell line or cell strain."

https://www.thermofisher.com/us/en/home/references/gibco-cell-culture-basics/cell-culture-protocols/maintaining-cultured-cells.html

This process of transferring cells from one culture to another while changing up the chemicals/medium used is common practice. They do this numerous times in order to keep the "virus" within the cell culture soup "alive" indefinitely. However, this process of removing the cells from one petri dish to another is very stressful on the cells and the addition of fresh cell-altering and DNA-damaging chemicals only heightens this stress. This practice inevitably leads to more damage/cell death and changes the morphology of the cell culture soup the longer this process carries on.

Passage Number Effects in Cell Lines

"Cell lines at high passage numbers experience alterations in morphology, response to stimuli, growth rates, protein expression and transfection efficiency, compared to lower passage cells.

The scientific community is taking notice that cell line quality is crucial to successful experimentation and that avoiding the use of cell lines that have been in culture too long is an important step to ensure reliable and reproducible results. But while the evidence for passage number-related effects on cell lines is compelling, much less is understood about the mechanisms underlying passage-dependent changes and about actions researchers can take to avoid passage number effects in their experiments."

How many passages are too many?

"A straightforward method for determining the passage number of a cell line does not exist. A review of the literature on passage-related effects in cell lines demonstrates that the effects are complex and heavily dependent on a host of factors such as the type of cell line, the tissue and species of origin, the culture conditions and the application for which the cells are used."

https://www.google.com/url?sa=t&source=web&rct=j&url=https://www.atcc.org/-/media/resources/technical-

documents/passage-number-effects-in-cell-lines.pdf%3Frev%3De598802603464319ada04494c9112fee&ved=2ahUKEwiIgeD35c7yAhWrlWoFHQsWAPMQFnoECAQQBg&usg=AOvVaw1b6hKbbqbdubO68acv8ek5

It is clear that the culture conditions are a major factor in the data generated from the cell culture supernatant. Virologists are essentially creating new sequences/mutations every time they whip up their witches brew. In the case of "SARS-COV-2," this is highlighted by passages (pun somewhat intended) from two recent studies:

A cautionary perspective regarding the isolation and serial propagation of SARS-CoV-2 in Vero cells

"An array of SARS-CoV-2 virus variants have been isolated, propagated and used in in vitro assays, in vivo animal studies and human clinical trials. Ensuring the genetic stability of SARS-CoV-2 during in vitro propagation is essential but has been too frequently overlooked. Our observations of working stocks of SARS-CoV-2 suggest that sequential propagation in Vero cells leads to critical changes in the region of the furin cleavage site (FCS), which significantly reduce the value of the working stock for critical research studies, vaccine development, production, evaluation and use."

"The authors of this paper, members of the WHO working group on SARS-CoV-2 virus propagation in cell lines, have pooled the results of carefully analysed genetic data generated from sequencing multiple isolates of serially propagated SARS-CoV-2 in different cell types. Serially propagating SARS-CoV-2 in Vero E6 cells leads to rapid increases in genetic variants, particularly those located in the sequence coding for the FCS of the spike protein."

"Early findings

In the early phase of the global response to SARS-CoV-2, quality assurance measures taken by Public Health England (PHE) to check the England 02 isolate provided to the Biodefense and Emerging Infections Resources (BEI Resources) included deep sequencing of the second Vero E6 cell line passage of this isolate. This analysis indicated that, although the first passage (P1) stock had no detectable changes (<1%, EPI_ISL_40703) (Table 1), over 90% of the virus content of the P2 stock and 100% of

the P3 stock (multiplicity of infection (MOI) ranging from 1.0E−02 to 1.0E−03) contained a 24-nucleotide in-frame deletion in the spike region resulting in loss of 8 amino acids including the FCS (see details in Supplementary Table 1). This observation raised concern among virologists that SARS-CoV-2 isolates being propagated and studied around the globe were not accurately representing the virus circulating in humans.”

“In addition, we noted that a P2 stock propagated in Calu-1 cells did not lose these variants when grown in Vero/SLAM cells but seemed to retain low levels during propagation, whereas the levels of FCS variants rose rapidly in Vero E6 cells irrespective of their source (Table 1).”

“Discussion

Studies conducted at PHE, NIBSC, University of Wisconsin-Madison and BEI Resources all conclude that, when SARS-CoV-2 is propagated in Vero E6 cells, there is a risk that during the sequential passage of this virus for working stock generation, deletions may arise in critical virulence components of the virus, including the FCS. Such deletions appear to result in the stock virus being less virulent in animal models (as measured by clinical observations and/or viral titration in mucosal secretions).”

“On the basis of this preliminary data, we encourage researchers producing stocks of SARS-CoV-2 to consider:

limitation of the number of passages in cell culture, using low MOI, in an effort to maintain wild-type properties;

evaluation and selection of a cell line that supports viral isolation and working stock production with acceptable (<1%) variant thresholds for downstream use;

evaluation of both the consensus sequence and inclusion of analysis of minor variants of each virus preparation;

incorporation of LoFreq4 (or equivalent) sequencing analysis for low-frequency variant calling.

“Spontaneous mutations due to virus adaptation to both Vero and Vero E6 cells have also been reported for viruses such as Ebola virus and Zika virus.8,9 However, deletions and mutations in the SARS-CoV-2 FCS became so frequently observed in passages 4 and 5 that they dominated the reads taken from workings stocks by up to 99% (Tables 1 and 3). The results at passage 4 were, however, variable such that the FCS region of

two different passage 4 stock contained mutations at a frequency of ≈16% in one stock but <1% for another (Table 3). This latter stock when taken to passage 5 did, however, yield a stock with >10% FCS variants. These data suggest that even the same passage level of virus can exhibit entirely different genetic characteristics, further emphasizing that investigators need to confirm the genetic sequence after propagation rather than relying on the sequence of the seed stock.

The findings of this group in this publication support the observations of other groups2,10,11 that FCS changes occur during serial propagation of SARS-CoV-2 in some cell lines. Despite the publication of these articles, there is continued production and dissemination of stocks of virus that are compromised in this manner, especially as there is a perceived need to rapidly isolate and distribute new variants with a combination of changes in the spike protein."

https://www.nature.com/articles/s41541-021-00346-z

IN SUMMARY (PART 1):

–ensuring the genetic stability of "SARS-CoV-2" during in vitro propagation is essential but has been too frequently overlooked

-sequential propagation in Vero cells leads to critical changes in the region of the furin cleavage site (FCS)

-serially propagating "SARS-CoV-2" in Vero E6 cells leads to rapid increases in genetic variants

–over 90% of the "virus" content of the P2 stock and 100% of the P3 stock (multiplicity of infection (MOI) ranging from 1.0E−02 to 1.0E−03) contained a 24-nucleotide in-frame deletion in the spike region resulting in loss of 8 amino acids including the FCS

-virologists have become concerned that "SARS-CoV-2" isolates being propagated and studied around the globe were not accurately representing the "virus" circulating in humans

-the levels of FCS variants rose rapidly in Vero E6 cells irrespective of their source

–there is a risk that during the sequential passage of this “virus” for working stock generation, deletions may arise in critical virulence components of the “virus”

-they ultimately reccomend limiting of the number of passages in cell culture

-there are acceptable (<1%) variant thresholds and they recommend the inclusion of evaluations of minor variations based on consensus sequence (seemingly confirming there are always variations present)

-deletions and mutations in the “SARS-CoV-2” FCS became so frequently observed in passages 4 and 5 that they dominated the reads taken from workings stocks by up to 99%

-their data suggests that even the same passage level of “virus” can exhibit entirely different genetic characteristics

-they state that that investigators need to confirm the genetic sequence after propagation rather than relying on the sequence of the seed stock

-however. despite the publication of these articles, there is continued production and dissemination of stocks of “virus” that are compromised in this manner

As can be seen from the highlights from this first study, the culture conditions greatly influence the end results of the cell culture experiments. Serial propagation leads to dramatic increases in “variants” to the point that Virologists are concerned these stocks no longer resemble the circulating “virus.” They even advise researchers to always sequence their stock after propagation rather than relying on the original sequence due to the mutations which occur during the culturing process. However, not a single genome from any of these cultures ever matches 100%. They even seem to suggest

that there is an acceptable level of variation. Even though the issues outlined above are known, the researchers admit that they are frequently overlooked and that there is continued production of “viral” stocks in this manner.

This second article sheds even more light on this problem:

SARS-CoV-2 variants with mutations at the S1/S2 cleavage site are generated in vitro during propagation in TMPRSS2-deficient cells

“Notably, viruses with S gene mutations emerged rapidly and became the dominant SARS-CoV-2 variants in TMPRSS2-deficient cells including Vero cells. Our study demonstrated that the S protein polybasic cleavage motif is a critical factor underlying SARS-CoV-2 entry and cell tropism. As such, researchers should be alert to the possibility of de novo S gene mutations emerging in tissue-culture propagated virus strains.”

“SARS-CoV-2 uses its spike (S) protein to enter target cells. Unlike other similar coronaviruses, the nascent S protein has a polybasic cleavage motif and is cleaved by the host protease. We have identified SARS-CoV-2 variants with mutations at the cleavage motif of S protein (S gene mutants) which undergo inefficient proteolytic cleavage, generate smaller plaques, and infect fewer cell lines. Notably, S gene mutants emerged rapidly through SARS-CoV-2 propagation in Vero cells. Since Vero cells are commonly used for SARS-CoV-2 propagation, it is a very real possibility that researchers have performed experiments, screened antivirals, and developed vaccines using SARS-CoV-2 S gene mutants without realizing.”

“In this study, we isolated S gene mutants from SARS-CoV-2 WK-521, a strain isolated from a clinical case in Japan [17], via serial passage in Vero cells. Other studies have reported viruses with S gene mutations, including amino acid deletions and substitution at the S1/S2 cleavage site from clinical isolates in Australia [21], China [20,22], England [23], and the USA [24] that emerged during cultivation in Vero cells or in its derivative, Vero/hSLAM, which are cells that do not express TMPRSS2. Although these studies demonstrated the spontaneous mutations in the S1/S2 cleavage site during the in vitro propagation of SARS-CoV-2, the virological properties of these mutants requires further investigation. This study showed a difference in cell tropism and entry pathway between

SARS-CoV-2 WT and S gene mutants. We also demonstrated that the SARS-CoV-2 S gene mutants emerged within a few passages and became the dominant SARS-CoV-2 variants in TMPRSS2-deficient cells."

"The spontaneous mutations in the S gene that lead to a loss of sensitivity to protease have been identified during the passage of cultured cells and this phenomenon is considered an adaptation of coronaviruses, such as human coronavirus OC43 and feline coronavirus UCD, to cell culture [18,38]. Our deep sequencing analysis revealed that S gene mutants emerged at P1 and rapidly became the dominant variant within the virus populations that emerged from Vero cell passage. Our findings indicate that replication of SARS-CoV-2 in TMPRSS2-deficient Vero cells results in the selection of S gene mutants; as such, passage in this cell line is technically inappropriate, as it becomes difficult to maintain SARS-CoV-2 with the S1/S2 cleavage site in its intact form."

"At this time, many studies are conducted using SARS-CoV-2 propagated in Vero cells. Considering the very real possibility that these virus stocks will accumulate S gene mutations, researchers must pay careful attention to the passage history of any working stocks of SARS-CoV-2. Moreover, we must be very objective when interpreting the results from studies using Vero-passaged virus, especially those focused on S protein cleavage, virus entry and on cell tropism of SARS-CoV-2."

https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1009233

IN SUMMARY (PART 2):

-researchers should be alert to the possibility of de novo S gene mutations emerging in tissue-culture propagated "virus" strains

–S gene mutants emerged rapidly through "SARS-CoV-2" propagation in Vero cells

-since Vero cells are commonly used for "SARS-CoV-2" propagation, it is a very real possibility that researchers have performed experiments, screened antivirals, and developed vaccines using "SARS-CoV-2" S gene mutants without realizing it

-the researchers state that they demonstrated that the “SARS-CoV-2” S gene mutants emerged within a few passages and became the dominant “SARS-CoV-2” variants in TMPRSS2-deficient cells

-they state that their findings indicate that replication of “SARS-CoV-2” in TMPRSS2-deficient Vero cells results in the selection of S gene mutants; as such, passage in this cell line is technically inappropriate, as it becomes difficult to maintain “SARS-CoV-2” with the S1/S2 cleavage site in its intact form

-they conclude that researchers must be very objective when interpreting the results from studies using Vero-passaged “virus“

From this study, the researchers claim that not only does passaging in Vero cells lead to mutations, it does so in the infamous spike (S) protein. And just as the previous paper stated, they claim that this is so common that researchers are most likely using these mutants in their research without ever realizing it. They also warn that results from Vero-passaged studies must be interpreted very objectively.

If one looks at this critically and logically, it is clear that the cell culture conditions greatly influence the results of the experiments and data generated from them. Cell cultures are unnatural mixtures of human/animal DNA, numerous chemicals/antibiotics, nutrients, etc. that are carried out in laboratory conditions that have no relation to reality whatsoever. The variations and mutations will remain as no two culture conditions are ever the exact same and thus the results will always be different. There can be no claim that what ultimately results from the cell culture process is the same as what went in at the beginning. In fact, the evidence points to the fact that this is never the case.

Is complete purification/isolation of a "virus" even possible?

When getting down to the size of nanoparticles and the expected billions of identical particles at that level, it would be logical to assume that completely separating the exact particle a Virologist is looking for from everything else in the sample is downright impossible. Thus, asking Virologists to completely purify and isolate the suspected "viral" particle from an unaltered sample from a sick patient and prove its pathogenicity in a realistic way may seem like a Herculean task and an unfair demand.

However, this is the corner Virology and Germ Theory has backed itself into. In order to claim a particular particle is a "virus" and can cause the symptoms of disease associated with it, logic dictates that it must be completely separated from all other potential variables/factors in order to prove that particular particle is indeed the cause. This is the only logical way to show that no other particles in the sample could have been the cause and in the case of genomics, that the DNA/RNA sequences belong to only that particular particle which is believed to be a "virus."

We can find out if complete purification/isolation is possible by looking at exosome research and the methods used. "Viruses" are considered exosomes in every sense of the word as they are identical in size, shape, and appearance. The methods used to purify/isolate exosomes are the same ones which are supposed to be used for "viruses" but which are never done, especially in the absence of toxic cell culture processes. These methods are considered the best available purification/isolation methods today.

Let's look at a few of them briefly:

ULTRACENTRIFUGATION:

"THE CURRENT GOLDEN STANDARD FOR EXOSOME ISOLATION IS ULTRACENTRIFUGATION 58. As known, this technique exploits the particle movement principle due to gravitational acceleration in an inertial field 59. Differential and density gradient ultracentrifugation are among the most commonly used ultracentrifugation methods for exosome isolation 60."

"During differential ultracentrifugation, exosomes are separated based on their density and size. Thus, CONTAMINATION FROM OTHER VESICLES, MOLECULES OR PARTICLES THAT OVERLAP IN THESE PARAMETERS IS EXPECTED. To REDUCE the presence of cell debris and large vesicles, cleaning steps are needed before pelleting the exosomes."

"It should be noted that THE g-FORCE USED during ultracentrifugation protocols HAS A SIGNIFICANT EFFECT ON THE PURITY AND YIELD OF EXOSOMES."

"However, as it has been mentioned, THIS METHOD IS NOT SPECIFIC AND CONTAMINATION WITH OTHER EXTRACELLULAR VESICLES IS UNAVOIDABLE. If the protocol is not well standardized and adapted (in terms of time and gravitational force) to the characteristics of the equipment being used, EXOSOME ISOLATION WILL NOT BE CONSISTENT, AND LOSSES WILL OCCUR INEVITABLY."

ULTRAFILTRATION:

"In this process, extracellular vesicles suspended in a solution can be separated by size or molecular weight. Usually, different forces are applied to make them pass through (or be retained on) a selective membrane. Centrifugal force, pressure or vacuum are usually applied for ultrafiltration through a membrane that is commonly built from low protein affinity materials."

"Nevertheless, ultrafiltration, a simple protocol, IS INCAPABLE OF ISOLATING ONLY EXOSOMES, AS MICROVESICLES AND ACOUSTIC BODIES WILL ALSO BE PRESENT IN THE RESULTING PRODUCT. Moreover, LARGE AMOUNTS OF HIGHLY ABUNDANT PROTEINS, THAT MIMIC EXOSOME SIZE OR MOLECULAR WEIGHT, WILL ALSO BE FOUND IN THE RESULTING SOLUTION. Such contamination arises from the physical limits of the procedure and the overlapping properties of the particles in the matrix being processed. Furthermore, THE EFFECTS OF THE APPLIED FORCE AND THE CONTACT WITH THE MEMBRANE ON THE EXOSOMES NEED TO BE

FURTHER STUDIED. Potential deformation and exosome losses due to extrusion and membrane binding are expected 104."

PRECIPITATION:

"Most precipitation methods consist on mixing the sample, which can be either a biological fluid or cell culture medium, with a hydrophilic polymer. After mixing, the sample is incubated overnight at 4ºC and afterwards low speed centrifugation is used to precipitate the exosomes which are later resuspended in the preferred buffer for further analysis 116. Protamine, sodium acetate, and organic solvents can also be used for precipitation procedures."

"Nonetheless, LOW PURITY IS A KEY DISADVANTAGE. CONSOLATION OF NON-VESICULAR CONTAMINANTS such as lipoproteins and ribonucleoprotein complexes, albumin, immunoglobulins and other soluble proteins IS UNAVOIDABLE 126. CONTAMINATION WITH OTHER VESICLES IS ALSO EXPECTED. Unfortunately, THIS CONTAMINATION MAY INTERFERE WITH FURTHER BIOCHEMICAL AND IMMUNOLOGICAL ANALYSIS."

CONCLUSIONS:

"EXOSOME ISOLATION REMAINS A CHALLENGE FOR BIOMEDICAL RESEARCH. There is still NO CONSENSUS over which purification technique produces the best results and there is intense competition within the field. Moreover, an accurate comparison between methods cannot be easily made BECAUSE OF THE INHERENT EXOSOME COMPLEXITY."

"Moreover, CONSOLATION OF CONTAMINANTS SHOULD BE MINIMUM SINCE CONTAMINATION IS THE MOST COMMON COMPLICATION OF CURRENT ISOLATION TECHNIQUES 58. Almost invariably, CONSOLATION OF OTHER VESICLES AND NON-VEHICULAR MOLECULES OCCURS, interfering with data comparison between research laboratories."

"Although, ultracentrifugation is currently the gold standard for exosome isolation. THERE IS NO IDEAL METHOD THAT FITS ALL PURPOSES. The selection of the procedure usually depends on the capabilities and resources of each research team and sacrifices must be made in terms of recovery, purity or work load. Moreover, DOWNSTREAM ANALYSIS MAY BE COMPROMISED BY THE ISOLATION TECHNIQUE THAT IS CHOSEN 90. In this sense, the final selection of the most suitable technique for exosome isolation and purification needs to CONSIDER THE EFFECTS THAT THE METHODOLOGY MAY EXERT OVER THE SAMPLE INTEGRITY particularly for the intended final use. For instance, recovery techniques such as

ultracentrifugation and filtration TEND TO RENDER A POPULATION OF “SAUCER-LIKE” OR “DEFLATED-FOOTBALL” SHAPED VESICLES THAT MIGHT NO LONGER BE USEFUL 157. Furthermore, the integrity of the exosomal cargo to unravel exosome-specific functions and biomarkers should also be considered even when no apparent degradation is present 158. This is especially true for microfluidic techniques or after isolation when exosomes are stored under freezing OR OTHER HARSH CONDITIONS 159."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6972601/...

The three methods discussed above all inevitably suffer from contamination as well as potential damages to the particles in the sample. The forces and methods used on these particles are unlike anything they encounter in reality. There is absolutely no way to say that the resulting particles are in the same form as they were originally in at the start of the purification/isolation process.

Purification/isolation of these particles is an impossible task. It may even be an unfair demand to ask for this. However, logic does not deal with fairness. In order to prove a "virus" exists and causes a particular disease, it must be completely purified/isolated from an unaltered sample first.

Unfortunately for Virology/Germ Theory, they have the unenviable responsibility to show that complete purification/isolation can be done. No conclusions about any particle as a "virus" can be made until this logical step occurs. To date, they have failed to do so every time.

https://docs.google.com/document/d/e/2PACX-1vTx9BIQAwWZm7gi6v1_w9T_tWyknOEuRx8kCJVJ7tN_dcYUc8EwsF92AShh3HwmDOxLDVDd417jWjzO/pub

IF VIRUS'

DO NOT EXIST

BUT ONLY IN THE

VIROLOGIST MIND,

THEN....

Purification of a "virus" IS impossible

It is becoming increasingly clear that purification of a "virus" is impossible. There are too many contaminants, variables, unknowns, and nanoparticles of similar shape/size to be able to say with certainty that the particles assumed to be a "virus" in a cell culture are the same ones imaged by TEM or for which the genome sequence is said to be based upon.

"EACH VIRUS POSES AN INDIVIDUAL PURIFICATION PROBLEM that is related to the properties of the virus, the nature of the host, and the CULTURE CONDITIONS. Consequently, IT IS NOT POSSIBLE TO OUTLINE A PURIFICATION PROCEDURE THAT WILL WORK WITH EQUAL EFFECTIVENESS FOR ALL VIRUSES."

"In these terms, PURITY MEANS THE DEGREE OF FREEDOM OF VIRAL PARTICLES FROM NONVIRAL COMPONENTS, or, conversely, the extent to which viral particles show gross physicochemical homogeneity. NO SINGLE TEST IS SUFFICIENT TO ESTABLISH THIS TYPE OF PURITY, but a consistent answer from each of several tests establishes the degree of homogeneity of the preparation in question and hence the reliance to be placed on analytical data and other results obtained with such a preparation."

"THE LOWER LIMIT OF CONTAMINANT DETECTABLE by either sedimentation analysis or electrophoresis IS VARIABLE, and is dependent upon the nature of the material and the circumstances of the test. As usually applied in testing virus preparations,

THESE METHODS CANNOT BE EXPECTED TO DETECT LESS THAN A FEW

PERCENT OF CONTAMINANT (Sharp 1953).

For many purposes, it is satisfactory to measure purity to this degree, but as the tools for chemical and biological analyses become sharper and sharper, it will be increasingly necessary to remember the limitations of sedimentation and electrophoresis measurements."

"The electron microscope can be used to examine directly the physical homogeneity of a virus preparation. Under favorable conditions it is possible to detect an impurity present in a concentration of as little as 1 percent of the virus (Williams 1954). IT IS OBVIOUS, OF COURSE, THAT IMPURITIES WILL ESCAPE DETECTION IF THEY HAVE THE SAME SIZE AND SHAPE AS THE VIRUS PARTICLES, OR IF THEY ARE BELOW THE SIZE RESOLVED BY THE MICROSCOPE. Also, particles present in small number but large in mass ARE EASILY OVERLOOKED, owing to sampling difficulties (Lauffer 1951)."

"In summary, NO SINGLE CRITERION OF PURITY IS SUFFICIENT TO ESTABLISH THE HOMOGENEITY OF A PREPARATION OF VIRUS. This must be done by applying critically as many tests as possible (see Knight 1974)."

https://link.springer.com/content/pdf/10.1007/978-3-642-85899-4_2.pdf

https://docs.google.com/document/d/e/2PACX-1vTjyUqE8tA2J7hdxTrmdgsa5o8EOR2DjAI08i52V6X_gAyHA6vMNas3dRwgm10FmYM12JKWeTrmyXUs/pub

"...the greater framework

of the misguided development of biology and medicine, the untenable dogma of the so-called cell theory, which claimed that the body develops from cells and not from tissues.

The cell theory of life, the “cellular pathology”, invented by Rudolf Virchow in 1858, which to date is the exclusive basis for biology and medicine, claims that all disease (as well as all life) originates from a single cell, which is somehow hijacked by a virus, starts to deteriorate and then propagates that virus. Two crucial aspects served as precondition and basis for the current global acceptance of cellular pathology, from which the infectious theory, the genetic, immune and cancer theories have developed, was only possible because of two crucial aspects.

A. The cell theory was only implemented because Rudolf Virchow suppressed crucial discoveries about tissues.

The findings and insights with respect to the structure, function and central importance of tissues in the creation and development of life, which were already known in 1858, comprehensively refute the cell theory and the subsequently derived genetic, immune and cancer theories.16

B. The infection theories were only established as a global dogma through the concrete politics and eugenics of the Third Reich. Before 1933, scientists dared to contradict this theory; after 1933, these critical scientists were silenced.17

In order to work with “viruses” and carry out so-called infectious experiments, before the concept of virology was abandoned in 1952, the “virologists” were forced to dissolve and filtrate “diseased” and putrescent tissue. The concentrated filtrate, so they believed, contained a pathogen, a toxin, which they thought would be constantly produced by the infected cells. Until 1952, a “virus” was defined as a pathogenic poison in the form of a protein, which as an enzyme caused damage in an unknown manner, would cause disease and be transmissible. After 1953, the year in which the alleged DNA in the form an alleged alpha helix was publicly announced, the idea of a virus became a malignant genotype wrapped in proteins. Thus, a paradigm shift took place between 1952 to 1954 regarding the image of a virus."

From: The Misconception of Virus Theory

Virologists mentally assemble the shortest **pieces of so-called genetic information** from dying cells to form a very long genetic strand, which they output as the genetic strand of a virus. This conceptual / computational process is called alignment. In doing so, they did not make the control attempts, the attempt to conceptually / computationally construct the desired genetic strand even from short pieces of so-called genetic information from non-infected sources.

Illustration:

"Purification clearly means separating the virus from all other organic materials."

-David Crowe

Purification of a "virus" is absolutely necessary to prove it exists. However this step is either skipped over entirely or not done properly. They sequence genomes of "viruses" from unpurified particles which may or may not belong to the "virus" they are attempting to sequence.

"Since for the established viruses their genomes have already been known, virus identification is possible EVEN IN A MIXED STATE."

They claim that since there are previous genomes for which they can compare current "viruses" to, they don't need to purify the isolate. This is based on the assumption that the previous genomes were isolated and purified properly.

This is what they are supposed to do for purification:

"When extracting virus genome using the classical method, THE VIRUS PARTICLES MUST FIRST BE PURIFIED. Then the virus genome extracted from the particles is examined. ULTRACENTRIFUGATION PLAYS AN IMPORTANT ROLE IN THE PROCESS. Purifying the virus particles makes it possible from the

beginning to ensure that we are dealing with the rotavirus genomes in the virus particles. CURRENTLY SUCH ANALYSIS IS PERFORMED A MOST ALL THE TIME AFTER HASTILY EXTRACTING THE GENOME WITHOUT ACTUALLY PURIFYING THE SPECIMENS. This practice is common since the genome of rotavirus is well established and it is a common knowledge that if the genome (Fig. 1) characteristic of rotavirus is present, there is no doubt that the genome is present in rotavirus particles as well."

However, suppose, for example, that we are dealing with the problem of determining what kind of host cell organelles or virus proteins and genomes are aggregated in an infected cell, ULTRACENTRIFUGATION BECOMES INDISPENSABLE.

Moreover, while studying new viruses, it becomes increasingly NECESSARY TO INVESTIGATE WHETHER OR NOT THE GENOME IS PRESENT IN THE PARTICLE. In such cases, PURIFICATION WITH AN ULTRACENTRIFUGE BECOMES A NECESSITY. Information on the buoyant density, size and

sedimentation coefficient (Svedberg value, S value), all of which are taken into consideration in ultracentrifugation, is in fact the fundamental aspect of virology which taken together are called the physiochemical properties of viruses."

Yet here they shoot their purification of past "viruses" right in the foot:

"In particular, recently, the detection sensitivity of real-time PCR has increased, making it NECCESSARY TO CONSIDER LEVELS OF CONTAMINATION THAT COULD HAVE PREVIOUSLY BEEN IGNORED."

What's interesting here is the admittance that even while purifying, it seems they had an acceptable level of contamination they would ignore. This would mean that the particles were not completely purified.

"At first, TO ELIMINATE CELLULAR DEBRIS, 1 liter of ICF is divided into 6 bottles which is centrifuged for 10 minutes at 15,000 xg using a JA-14 fixed-angle rotor. THIS STEP CAN BE OMITTED, BUT IF DONE SO, THERE WILL BE MORE IMPURITIES during ultracentrifugation with the cushion method in the next step. The more the pretreatment, the easier and cleaner will be the subsequent operations. MOREOVER, IF THERE IS A LARGE QUANTITY OF IMPURITIES, THE VIRUS GETS ENMESHED IN THEM, WHICH MIGHT DECREASE THE YIELD."

https://www.google.com/url?sa=t&source=web&rct=j&url=https://ls.beckmancoulter.co.jp/files/cases/Fundamentals_of_Ultracentrifugal_Virus_Purification.pdf&ved=2ahUKEwjalYaHj7_rAhVHZM0KHUcwAu0QFjAHegQICBAB&usg=AOvVaw3cKCC79yUHTTF6sLRZe-Yw

Again, it seems they provide a step for purifying away cellular debris but then in the same paragraph state it can be skipped with the consequence of more impurities. So once again, not a completely purified particle.

You need more than one purified particle to sequence the genome. From my understanding, the steps necessary to get to this point are either not done or missing crucial parts.

They then sequence the genome from an unpurified "virus." The problem here is they need a reference genome from previous "viruses." In the case of "SARS-COV-2" that came in the form of the original SARS genome (78% match which isn't that close of a relation at all) and Betacoronaviruses from bats. The problem again: none of these genomes came from purified particles either. It is faulty science built upon faulty science.

Other drawbacks of genome sequencing for "virus" identification from unpurified particles:

"Perhaps one of the biggest drawbacks IS THE NEED FOR A REFERENCE GENOME FOR COMPARISON with your sequence. If you don't have one of these to compare your results to, YOU HAVE NO REAL WAY OF DETERMINING WHAT IS NORMAL AND WHAT IS UNIQUE ABOUT YOUR SAMPLE. Good luck identifying an insertion mutation without an unaltered genome to compare to! While de novo sequencing for when a reference is not available is possible, IT CAN LEAD TO MORE ERRORS SINCE

YOU HAVE NOTHING TO COMPARE TO."

https://bitesizebio.com/.../good-tbad-expensive-whole.../

"If the depth coverage is not sufficient, IT IS POSSIBLE THAT A BASE WILL BE IDENTIFIED THAT IS NOT ACTUALLY PRESENT IN A PERSON'S GENOME. For example, IF A MUTATION THAT LEADS TO A DISEASE IS MISTAKEN FOR A NORMAL GENETIC (A FALSE NEGATIVE), the person could think they have been successfully tested for a condition and found to be "negative" for it, whereas that might not be the case. Conversely, a gene could be misread as a mutation that is expected to lead to an adverse condition, whereas in reality, the person is not harbouring such a mutation in their genome (A FALSE POSITIVE). A SMALL FRACTION OF THE GENOME MIGHT NOT BE SEQUENCED IF IT READS BELOW THE MINIMUM COVERAGE DEPTH."

"It is therefore important to remember that information obtained from genome sequencing IS NOT TO BE USED FOR MEDICAL INTERPRETATION UNLESS IT IS VALIDATED BY ADDITIONAL MEANS."

"Another often cited limitation is the lack of clinical validity and utility for systematic mass scale use of genomic sequencing technology for public's benefit, and is only being currently investigated at clinical research institutions around the world. Simply put, CURRENTLY THERE IS NO GOLD STANDARD AGAINST WHICH THE PERFORMANCE OF POPULATION GENOMIC SCREENING CAN BE JUDGED."

"Factors outside the control of the service provider TASKED WITH ISOLATION AND SEQUENCING OF DNA CAN NEGATIVELY INFLUENCE THE QUALITY OF THE GENOME SEQUENCE AND THEREFORE ITS INTERPRETATION. This can include the QUALITY OF THE DNA SAMPLE provided for analysis, such as LOW QUANTITY, HIGH BACTERIAL CONTAMINATION, OR SAMPLE DEGRADATION. Such factors can even prevent the procedure from being undertaken. In such a circumstance, the client might be obliged to deliver a new sample."

https://merogenomics.ca/.../advantages-and-limitations.../

Drawbacks of using PCR for whole genome sequencing:

"The PCR amplification method, compared to the others, is particularly relevant for samples containing very low viral genetic material, it presents several disadvantages, though. THE SEQUENCE OF THE VIRUS OF INTEREST HAS TO BE KNOWN AND NOT TOO VARIABLE TO BE CORRECTLY AMPLIFIED BY THE SET OF DESIGNED PRIMERS. A second pitfall is due to the fact that the PCR CYCLES CAN INTRODUCE SOME AMPLIFICATION ERRORS ALONG THE SEQUENCE WHICH MAKES THE ASSEMBLY STEP MORE PRONE TO MISTAKES. Finally, this method can only be used for small genomes because of the number of PCR reactions which has to be limited."

https://www.sciencedirect.com/.../pii/S0042682219300728

Why purification of a "virus" matters:

"The second Koch's postulate and textbooks state and leading virologists such as Luc Montagnier as well that complete cleaning of particles ("PURIFICATION") IS AN INDISPENSABLE PRE-REQUISITE FOR THE DETECTION OF A VIRUS (see quotes in the section "Lousy, More Lousy, Corona PCR Test" later in this chapter). However, the authors two significant papers, which are mentioned in connection with the detection of SARS-CoV-2, CONCEDE ON REQUEST THAT THE ELECTRON MICROSCOPE IMAGES SHOWN IN THEIR WORK DO NOT DEPICT COMPLETELY PURIFIED PARTICLES. But how can one then conclude with certainty that the RNA gene sequences "pulled" from the tissue samples prepared in these studies and to which the PCR tests are then "calibrated" belong to a very specific virus—in this case SARS-CoV-2? Especially since studies also show that the very substances (including antibiotics) used in the test tube experiments (in vitro) can "stress" the cell culture in such a way that new gene sequences can be formed that were previously undetectable?"

-Virus Mania

All of this is to say that before a genome can be sequenced and before a "virus" can be proven to exist, it must be properly isolated and purified first.

Without this absolutely essential step, there is no proof a novel "virus" exists let alone causes disease.

This is exciting news for humanity. The end of virology & vaccines is coming soon.

The complete fraudulent virology will soon be unmasked on the example of the computer conversion of parts of the alleged HIV into alleged Sars-cov-2.

An example of creating the genome of the alleged sars-cov-2 from the sequences of the alleged HIV will destroy the complete fraudulent virology.

The point is that parts of alleged HIV cannot be parts of alleged Coronavirus at the same time. But that is exactly the case the virologist sells.

You all know that virologists do not isolate an alleged "viral" particle because it does not exist. Here's another piece of evidence:

Virologists are rapidly moving to bizarre collections of genetic material after a procedure of mixing or contamination with animal RNA. The total RNA is extracted not from the alleged viral particle, which would be logical, but from BALF or CC - supernatant. Now let's look at what the scientific publication that is the basis of the corona scandal says:

"Bronchoalveolar-lavage fluid samples were collected in sterile cups to which virus transport medium was added."

"VIRAL GENOME SEQUENCING

RNA extracted from bronchoalveolar-lavage fluid and culture supernatants was used as a template to clone and sequence the genome."

https://www.nejm.org/doi/full/10.1056/NEJMoa2001017

"When asked by the court whether it is possible to prove the causality of infection with a pathogen in this latter form exclusively on the basis of the genome sequence: NO, IT IS NOT POSSIBLE exclusively in this form. Here, one can only achieve computer models with correspondingly high probability values, but cannot bring about direct proof."

"Following dictation up to this point, the expert adds of his own accord: We cannot, I must clarify, comprehensively infer the characteristics or behavior of an organism from the genome sequence alone. We can only determine structural features and predict with a greater or lesser degree of probability how the organism will behave in a certain way; even the genome sequence does not yet permit a comprehensive statement on overall behavior."

https://nateserg808.wixsite.com/my-site/post/the-controls

"For over 2000 years we have the saying: “Forgive them, for they know not what they do”. Since 1995, since we asked the questions about the evidence and published the answers, we can add: “ For they can’t admit that what they have learned and practiced isn’t true and, and stronger even, that it is dangerous and even lethal”. Because nobody until now understood the entire context and had the courage to say the truth, we now have even more “evil spirits” (quoting Goethe) and subsidiary hypotheses, such as the “immune system” or “epigenetics”, merely in order to maintain the fictitious theories.

In origin, the idea of a virus arose from the forced logic of the dogma of cellular theory . Then came the idea of the pathogenic bacteria, the bacterial toxins, then the viral toxins, until this idea was finally given up in 1952. Starting with 1953, Virchow’s idea of a

disease poison (Latin for: “poison”) became the genetic virus, which in turn gave birth to the idea of the cancer genes. Then we had the “war against cancer” of the Nixon era, and later the idea of genes for everything appeared.

In the year 2000, however, the entire genetic theory was refuted as well, after the contradictory data of the so-called human genome project was published together with the embarrassing claim that the entire human genome had been mapped, even though more than half of it was completely invented.7

People are not aware that it is very difficult for the respective academics to admit that they were involved in such misconceptions.

The so-called bacteria-eaters

The source for the idea of a genetic virus in humans, animals and plants, which started to develop from 1953 onwards, were the so-called bacteria-eaters, called (bacterio) phages, which had drawn the attention of scientists since 1915. From 1938 on, when commercially available electron microscopes were applied in research, these phages could be photographed, isolated as whole particles and all their components could be biochemically determined and characterised. This is real, and cannot be contested. To isolate them, i.e. concentrate the particles and separate them from all other components (=isolation), to photograph them immediately in the isolated state and to biochemically characterise them all in one. This, however, has never happened with the alleged viruses of humans, animals and plants because these do not exist. The scientists researching bacteria and phages, who worked with actual existing structures, provided a model as to what human, animal and plant viruses could look like. However, the “phage experts” have overlooked by their misinterpretation of phages as bacteria eaters that the phenomenon of the formation of these particles is caused by the extreme inbreeding of bacteria. This effect, i.e. the formation and release of phages (bacteria eaters, aka bacteria viruses), doesn’t happen amongst pure bacteria, freshly extracted from an organism or the environment. When their nutrients are withdrawn slowly or their living conditions become impossible, normal bacteria – that is: bacteria which are not grown in the lab – create the known survival forms, the spores, which can survive for a long time or even “eternally”.

From spores, new bacteria appear as soon as the living conditions improve. However, isolated bacteria, when grown in the lab, lose all characteristics and abilities. Many of them do not perish automatically through this in-breeding, but rather turn suddenly and completely into small particles, which in the “good versus evil” theory perspective have been misinterpreted as bacteria-eaters. In reality, bacteria originate from these exact “phages” and they turn back again into these life forms when the living conditions are no longer available. Günther Enderlein (1827–1968) described exactly these processes more than a century ago: how bacteria appear from invisible structures, their development into more complex forms and back again. That is why Enderlein did not

agree with the cell theory, according to which life appears from cells and is organised at cellular level.8

As a young student, I myself isolated such a “phage” structure from a sea algae. and believed at that time to have discovered the first harmless virus, the first stable “virus host system”.9

The idea, furthermore, that bacteria exist as single viable organisms, which can exist alone without any other life forms, is incorrect. In isolated form, they automatically die off after some time. This never occurred to the scientists, because after a successful “isolation” of a bacterium, a part of it is frozen and can be worked with in the lab decades later. The idea of bacteria being living independent structures which can survive by themselves is a laboratory artefact, a misinterpretation.

. Thus, the claim that is made on the basis of that myth, that bacteria are immortal, is therefore untrue. Bacteria are immortal only in symbiosis with a huge number of other bacteria, fungi and probably many more unknown life forms which are difficult to characterise, such as for example the amoeba. Amoebae, bacteria and fungi form spores as soon as their living environment disappears and re-emerge once the liv□ing conditions return. If one compares that with humans, we have the same perspective: without a living environment, from and with which we live, nothing can exist.

However, these discoveries go much deeper. Not only the entire species concept is dissolving, but also the idea and the claim about the alleged existence of dead matter. Observations and conclusions about a living “active matter” (as physicists call it) are dismissed as unscientific vitalism. There is considerable evidence, however, that all those elements which the “dominant opinion” in “science” does not consider as being alive, actually originate and develop from the membrane of water, i.e. the “Ursubstanz”10, or primordial source of life. These elements then create the nucleic acids, and around the nucleic acids they create the biological life in the form of amoebae, bacteria, tardigrades and ever-more complex life forms.

THE CASE AGAINST "VIRAL" GENOMES:

I've come across quite a few people who seem to believe that the existence of "viral"

genomes and "viral' RNA sequences somehow proves the existence of "viruses." One person even believed that the genome was a representation of purified/isolated "viruses." Disregarding the fact that random A,C,T,G's that exist only inside a computer database is at best INDIRECT evidence, there is no DIRECT evidence of a PHYSICAL entity called a "virus." There are no particles ever purified/isolated from the samples taken from a sick person nor are they ever proven pathogenic.

Remember the definitions for purification/isolation:

PURIFICATION:

1. to make pure; free from anything that debases, pollutes, adulterates, or CONTAMINATES:

2. to FREE FROM FOREIGN, EXTRANEOUS, or OBJECTIONABLE ELEMENTS:

https://www.dictionary.com/browse/purification

ISOLATION:

the act of SEPARATING SOMETHING FROM OTHER THINGS : the act of isolating something

https://www.merriam-webster.com/dictionary/isolation

When you break down the steps for sequencing a genome, you will realize that there is absolutely no way that this process can be said to fulfill these two basic criteria, starting with the very cell culture process used to get the sample for sequencing. There are different ways to sequence a genome so I'm following the blueprint set forth by the CDC in the image below:

"VIRAL" CELL CULTURE:

In order to get enough RNA needed to sequence a "virus," the sample taken from a sick person is usually cultured first. This process consists of taking the unpurified sample, subjecting it to "Viral" Transport Media, and adding the mixture to either human cancer or animal cells. This concoction has further substances added to it such as Fetal Bovine Serum, Antibiotics/Antifungals, DMEM, "nutrients," etc. There are numerous sources of contamination which they admit can only be mitigated at best, not eliminated. The added chemicals can lead to changes in gene expression, characterization, and genomic

instability. The stress from the culture process itself can alter the cells and the final product. There are also problems with cell-line misidentification and reproducibility.

For a breakdown of the numerous problems inherent with this initial cell culture process, read the below post:

STEP 1. EXTRACTING RNA:

This initial step after cell culturing utilizes various methods to attempt to "purify" RNA from the cell culture supernatant. The methods consist of chemical and physical means. They are:

1. The phenol/chloroform method
2. The spin column/column chromatography method
3. The magnetic beads method

All of these methods have drawbacks and contamination is a guarantee as is the possibility of RNA degradation. Keep in mind that "purifying" RNA is not "purifying" particles said to be "virus." They are breaking down various substances within the culture into a pool of RNA to be used for sequencing.

THE CHALLENGES RELATED TO RNA EXTRACTION FOR GENOME SEQUENCING:

https://docs.google.com/document/d/e/2PACX-1vRhekRLZlJufN-f3YdlvSFjy8gNyI1ez8K4gAhU6ZB4GWf-fjYhtxdJJNyDtVItqWp8My6n5wk41rgJ/pub

STEP 2. DNA/RNA FRAGMENTATION:

The next step consists of breaking the RNA into fragments. This can be done either before or after converting the RNA into cDNA for library preparation. The main methods used for RNA fragmentation include using metal ions or using RNase III. These two methods can introduce bias, RNA degradation, and contamination. After conversion to

cDNA, methods used for fragmenting include:

1. Enzyme-based treatments
2. Acoustic shearing
3. Sonication
4. Centrifugal shearing
5. Point-sink shearing
6. Needle shearing

As with the previous RNA shearing methods, contamination, sequencing errors, bias, degradation, and loss of sample can and do occur with these DNA shearing practices.

DNA/RNA SHEARING:

https://docs.google.com/document/d/e/2PACX-1vQNOdYLD63yx3bOC13MWI3HOUWb1nrSeZakPgiFFh02RTrUBu6yEMYe5LOWi5nBPGuCYpl4Ema7gAXu/pub

STEP 3. RNA INTO cDNA:

As RNA is considered highly unstable and the PCR used to create the sequencing library only uses DNA, there is an added step of converting the RNA into cDNA. This is done through a process called Reverse Transcription (RT) PCR. The problem, once again, is that PCR is prone to contamination and bias. Seeing a theme yet?

CONVERTING RNA INTO cDNA:

https://docs.google.com/document/d/e/2PACX-1vSwjxcYenfE2zeZ1Pgn7UTA9amnO7zP2D2viLjcuemiM8zoKw-WH-6TIoVZJFDG6hcHEBq1CDqwvDQC/pub

PCR LIMITATIONS AND CONTAMINATION:

https://docs.google.com/document/u/1/d/e/2PACX-1vScAYf21V3Me1nwoPcju5RJo4Tqe

zl5epR8PceergOQk02dNSTWARZLHc9ui6vYXYZlIXPgnydX_63C/pub

STEP 4. LIBRARY CONSTRUCTION:

After extracting the RNA, fragmenting it, and converting it into cDNA, the sequencing library must be prepared. The library is essentially just a set of DNA fragments used for sequencing. The steps listed previously are used to prepare the library and once this is done, the library is loaded into a DNA sequencer to create "reads." As stated before, the process of generating this library is rampant with contamination, batch errors, sequencing errors, biases, etc. They admit that both bias and batch errors are inevitable and can not be eliminated.

CHALLENGES IN GENOME LIBRARY CONSTRUCTION:

https://docs.google.com/document/d/e/2PACX-1vT-0pv03brnfYCXbmsWyrOOxKY0dmci6370SpOsULhuzp84MRA2fYJiP5Yflk5XOeDh5ia7DE8sbNU-/pub

STEP 5. DNA SEQUENCE ANALYSIS:

Now that the library has been prepped and loaded into the sequencer, the sequencer produces millions of DNA reads and specialized computer programs put them together. They commonly refer to this part of the process as putting together a jigsaw puzzle. Granted, it is done by automated algorithms. However, if you have incorrect pieces due to contamination, bias, batch errors, degraded RNA, low quality DNA, sequencing errors, etc., how accurate will the picture truly be?

Keep in mind that the technology used to sequence the data has limitations/drawbacks as well. The popular Illumina sequencer has issues with GC content bias, substitution errors, low sequence diversity, read length limitations, and technical problems related to reproducibility.

LIMITATIONS IN SEQUENCING

TECHNOLOGY:

This is a broad area with too many different sequencing platforms with too many alternative methods each with their own set of advantages/disadvantages. It would take a book rather than a post to break down the various technologies and processes used but I wanted to provide an idea about the limitations some of these sequencers have, focusing mostly on Illumina. Highlights from two sources and a summary below:

BIAS IN RNA-seq LIBRARY PREPARATION: CURRENT CHALLENGES AND SOLUTIONS

Sequencing and Imaging

"IT IS VERY IMPORTANT FOR THE SELECTION OF SEQUENCING PLATFORM IN RNA-seq experiment. Currently, commercially available NGS platforms include Illumina/Solexa Genome Analyser, Life Technologies/ABI SOLiD System, and Roche/454 Genome Sequencer FLX [61]. THESE PLATFORMS USE A SEQUENCING-BY-SYNTHESIS APPROACH TO SORT TENS OF MILLIONS OF SEQUENCE CLUSTERS IN PARALLEL.

Generally, the NGS platform can be classified as either ensemble-based (sequencing multiple identical copies of a DNA molecule) or monomolecular (sequencing a single DNA molecule). Nevertheless, STUDIES HAVE FOUND THAT SEQUENCING TECHNOLOGIES OFTEN HAVE SYSTEMATIC DEFECTS. For example, WHEN THE WRONG BASES ARE INTRODUCED in the process of template cloning and amplification, SUBSTITUTION BIAS MAY APPEAR in platforms such as Illumina and SOLiD®, WHICH LIMITS THE UTILITY OF DATA. In addition, STUDIES POINTED OUT THAT SEQUENCE-SPECIFIC BIAS MAY BE CAUSED BY SINGLE-STRAND DNA FOLDING OR SEQUENCE-SPECIFIC CHANGES IN ENZYME PREFERENCE [62]. Pacific Biosciences SMRT platform produces long single molecular sequences that are VULNERABLE TO MISINSERTION from non fluorescent nucleotides [63, 64]. Besides, THE SEQUENCING PLATFORM CAN PRODUCE REPRESENTATIVE BIASES, that is, some base composition regions (especially those with very high or very low GC composition) are not fully represented, THUS LEADING TO BIAS IN THE RESULTS [65]. Consequently, we will briefly discuss the bias of sequencing platforms, mainly including the Illumina and single-molecule-based platforms. A sum up of suggestions for improvement is presented in Table 4.

"Currently, THE ILLUMINA HiSeq PLATFORM IS THE MOST WIDELY USED NEXT-

GENERATION RNA SEQUENCING TECHNOLOGY AND HAS BECOME THE STANDARD OF NGS SEQUENCING. The platform has two flowcells, each of which provides eight separate channels for sequencing reactions. The sequencing reaction takes 1.5 to 12 days to complete, depending on the total read length of the library. Minoche et al. 's [66] study DISCOVERED THAT THE HiSeq PLATFORM EXISTS ERROR TYPES OF GC CONTENT BIAS.

In addition, Illumina released the MiSeq, which integrates NGS instruments and provides end-to-end sequencing solutions using reversible terminator sequencing-by-synthesis technology. The MiSeq instrument is a desktop classifier with low throughput but faster turnaround (generating about 30 million paired-end reads in 24 h). Simultaneously, it can perform on-board cluster generation, amplification, and data analysis in a single run, including base calls, alignment, and variant calling. At the present, MiSeq HAS BECOME A DOMINANT PLATFORM FOR GENE AMPLIFICATION AND SEQUENCING IN MICROBIAL ECOLOGY. Nevertheless, VARIOUS TECHNICAL PROBLEMS STILL REMAIN, SUCH AS REPRODUCIBILITY, HENCE HAMPERED HARNESSING ITS IT'S TRUE POTENTIAL TO SEQUENCE. Furthermore, Fadrosh et al.'s [67] study found that MiSeq 16S rRNA gene amplicon sequencing MAY ARISE "LOW SEQUENCE DIVERSITY" PROBLEMS IN THE FIRST SEVERAL CYCLES.

Furthermore, the emergence of single-molecule sequencing platforms such as PacBio makes single-molecule real-time (SMRT) sequencing possible [68]. In this method, DNA polymerase and fluorescent-labeled nucleoside were used for uninterrupted template-directed synthesis. One advantage of SMRT is that it does not include the PCR amplification step, as a consequence avoiding amplification bias. At the same time, this sequencing approach can produce extraordinarily long reads with average lengths of 4200 to 8500 bp, which greatly improves the detection of new transcriptional structures [69, 70], in addition, due to the relatively low cost per run of PacBio, which can reduce the cost of RNA-seq. However, PacBio CAN USUALLY INTRODUCE HIGH ERROR RATES (~5%) compared to Illumina and 454 sequencing platform [71]. Due to the fact that IT IS DIFFICULT TO THE MATCHING ERRONEOUS READS TO THE REFERENCE GENOME, THUS THE HIGH ERROR RATE MAY BE LEAD TO MISALIGNMENT AND LOSS OF SEQUENCING READS. Furthermore, Fichot and Norman's [72] study showed that PacBio's sequencing platform CAN SHUN ENRICHMENT BIAS OF EXTREMELY GC/AT."

"At the present, RNA-seq has been widely used in biological, medical, clinical, and pharmaceutical research. However, ALL THESE SEQUENCING STUDIES ARE LIMITED BY THE ACCURACY OF UNDERLYING SEQUENCING EXPERIMENTS, BECAUSE RNA-seq TECHNOLOGY MAY INTRODUCE VARIOUS ERRORS AND BIASES IN SAMPLE PREPARATION, LIBRARY CONSTRUCTION, SEQUENCING

AND IMAGING, etc."

https://www.hindawi.com/journals/bmri/2021/6647597/

This next article from 2017 was 27 pages long and goes into great detail on the different techniques. I focused briefly on Illumina as it seems to be the most commonly used:

NEXT-GENERATION SEQUENCING: ADVANTAGES, DISADVANTAGES, AND FUTURE

"Instead, SUBSTITUTION ERRORS ARE MORE COMMONLY OBSERVED IN ILLUMINA SYSTEMS DUE TO NOISE BACKGROUND GROWING EACH SEQUENCING CYCLE (Hutchison 2007). Also, after cleavage of blocking group, SCARS REMAINED ON NUCLEOTIDE STRUCTURE WHICH EVENTUALLY CAUSED INTERACTION WITH PROTEINS AND DECREASED EFFICIENCY OF SEQUENCING REACTIONS (Chen et al. 2013). Another problem about Illumina systems was GC BIAS INTRODUCED IN BRIDGE AMPLIFICATION STEP (Mardis 2013). These limitations originated from the nature of the method have been reduced with enhancements in its chemistry. Although engineering of DNA polymerase and rearrangement of flow cell channels has provided better accuracy and cluster densities, READ LENGTH LIMITATION STILL STAYS AS THE MAIN ISSUE FOR REVERSIBLE TERMINATOR CHEMISTRY-BASED SEQUENCING WHICH PRESENTS NOTICEABLE OBSTACLES ESPECIALLY IN DE NOVO SEQUENCING (Chen et al. 2013)."

"On the other hand, ACCURACY PROBLEMS STAND AS THE MOST IMPORTANT ISSUE FOR ALL NEWLY DEVELOPED TECHNOLOGIES and a revolutionary advancement is required to make a significant change in this regard also."

https://doi.org/10.1007/978-3-319-31703-8_5

In Summary:

-it is very important for the selection of sequencing platform in RNA-seq experiment

-these platforms use a SEQUENCING-BY-SYNTHESIS approach to sort tens of millions of sequence clusters in parallel

-nevertheless, studies have found that SEQUENCING TECHNOLOGIES OFTEN HAVE

SYSTEMATIC DEFECTS

-when the wrong bases are introduced in the process of template cloning and amplification, substitution bias may appear in platforms such as Illumina and SOLiD®, WHICH LIMITS THE UTILITY OF DATA

-studies pointed out that SEQUENCE-SPECIFIC BIAS may be caused by single-strand DNA folding or sequence-specific changes in enzyme preference

-Pacific Biosciences SMRT platform produces long single molecular sequences that are VULNERABLE TO MISINSERTION from non fluorescent nucleotides

-the sequencing platform can produce REPRESENTATIVE BIASES, that is, some base composition regions (especially those with very high or very low GC composition) are not fully represented, THUS LEADING TO BIAS IN THE RESULTS

-the Illumina HiSeq platform is the most widely used next-generation RNA sequencing technology and has become the standard of NGS sequencing

-Minoche et al.'s [66] study DISCOVERED THAT THE HiSeq PLATFORM EXISTS ERROR TYPES OF GC CONTENT BIAS

-Illumina MiSeq has become a dominant platform for gene amplification and sequencing in microbial ecology

-various technical problems still remain, such as REPRODUCIBILITY, hence hindering harnessing its true potential to sequence. -Fadrosh et al.'s study found that MiSeq 16S rRNA gene amplicon sequencing MAY ARISE "LOW SEQUENCE DIVERSITY" PROBLEMS in the first several cycles

-PacBio can usually introduce HIGH ERROR RATES (~5%) compared to Illumina and 454 sequencing platform

-due to the fact that it is difficult to the matching erroneous reads to the reference genome, thus the HIGH ERROR RATE MAY BE LEAD TO MISALIGNMENT AND LOSS OF SEQUENCING READS

-Fichot and Norman's study showed that PacBio's sequencing platform CAN SHUN ENRICHMENT BIAS of extremely GC/AT

-SUBSTITUTION ERRORS are more commonly observed in Illumina systems due to noise background growing each sequencing cycle

-after cleavage of blocking group, SCARS REMAINED on nucleotide structure which eventually caused interaction with proteins and DECREASED EFFICIENCY OF

SEQUENCING REACTIONS

-another problem about Illumina systems was GC BIAS INTRODUCED IN BRIDGE AMPLIFICATION STEP

-READ LENGTH LIMITATION still stays as the main issue for reversible terminator chemistry-based sequencing WHICH PRESENTS NOTICEABLE OBSTACLES especially in de novo sequencing

-ACCURACY PROBLEMS STAND AS THE MOST IMPORTANT ISSUE for all newly developed technologies and a revolutionary advancement is required to make a significant change in this regard also

The sequenced genome is only as good as the technology used for it. There are many to choose from each with advantages and disadvantages and they all have some systematic defects. For Illumina, this seems to be GC content bias, substitution errors, low sequence diversity, read length limitations, and technical problems related to reproducibility. Even if Illumina and other sequencing technologies were 100% accurate, the contamination, bias, batch errors, etc. inherent in the processes leading up to the sequencing analysis would be enough to question anything assembled from the data. Adding in the technological challenges just makes it even more apparent that there are too many different technologies, too many different processes, and far too many different variables to be able to say that the end product is a reliable and accurate representation of the nonexistent entity it is supposed to represent.

https://docs.google.com/document/d/e/2PACX-1vR6hDiRmY3UaDu3Z4yoJ8ySWJbm0b-ibZVi69vgW90SpkTfQW8IK99JUoDR94_BuS7a9ijn1GeIAneo/pub

It should be apparent that the existence of a genome is not proof of a purified/isolated "virus." There are far too many issues throughout the sequencing process such as:

1. The contamination/reproducibility issues with the initial cell culturing process

2. The contamination/biases/errors which occur during the extraction/fragmentation/conversion of RNA to cDNA

3. The OVERALL contamination/batch errors which are inevitable in the library preparation

4. The technological and reproducibility issues with the sequencing technology

There is no area of the genome sequencing process that is untouched by serious problems which affect the end result. This laundry list of issues should make anyone question the overall outcome. Take into account that there are no physical particles ever free from contamination/foreign material nor separated from everything else that are being sequenced. The particles said to be "viruses" are only ASSUMED to be there. The "viral" RNA is only ASSUMED to be 'viral" based on sequences from reference genomes previously created without purified/isolated "viruses" using older technology with even more issues.

Next time you hear about a more dangerous variant, just remember the various issues outlined above as well as the fact that the WHO admits that cell culturing leads to mutations and variants in genomes:

"PASSAGE IN CELL CULTURE CAN RESULT IN ARTIFICIAL MUTATIONS IN THE SEQUENCES, WHICH WERE NOT PRESENT IN THE ORIGINAL CLINICAL SAMPLE."

"Levels high following culture, BUT CULTURE MAY INDUCE ARTIFICIAL VARIANTS"

https://docs.google.com/document/d/e/2PACX-1vRi5OJP-hIifqDSz57IWOLImZecd9C35HGVsaBqyRo6j1f7qQqpD9ZX1rI9IrXNeqIaLIgkU0uYn1RL/pub

It's obvious if you look at the genome sequencing process from start to finish critically and logically, not only can a genome not be considered DIRECT evidence for a purified/isolated "virus," it can not count as INDIRECT evidence either.

Related:

HOW RELIABLE AND ACCURATE ARE GENOMES?:

One argument people try to make as proof of "viruses" is the existence of "viral" genomes. They believe that if a genome can be sequenced from unpurified cell culture soup where a "virus" is assumed to exist, that this is proof enough that a "virus" actually physically exists. Looking beyond the irony of claiming random A,C,G,T's in a computer database can somehow be used as evidence for the physical existence of an unseen entity, there are numerous reasons to question the reliability and accuracy of genomes. These include, but are not limited to, the reliance on inaccurate reference genomes, the inability to replicate results, the numerous technological hurdles based on the tech that is used, the introduction of biases, errors, and artefacts, the uncurated databases, etc. It is utterly ridiculous to believe that these non-reproducible and error-prone sequences from unpurified cell culture soup can be used as INDIRECT proof of a "virus" when the DIRECT proof, i.e. purified/isolated particles taken directly from sick humans which are proven pathogenic in a natural way, have yet to be scientifically proven first.

Below are highlights from one article and one review showcasing many of these faults:

ACCURACY OF HUMAN DNA SEQUENCING

"But just HOW ACCURATE IS DNA SEQUENCING AND ITS DATA STORAGE TECHNIQUES? What effect do these INACCURACIES HAVE ON GENOMICS and their use in pharmacogenetics?

Throughout the course of the Human Genome Project, there have been VARYING LEVELS OF TARGET ACCURACIES that the research institutes have aimed for. In 2000, the first draft was released with an ERROR RATE OF ONE ERROR PER EVERY 1,000 BASE PAIRS. In 2003, the official results were cited to have an ERROR RATE OF ONE PER EVERY 10,000 BASE PAIRS1. Currently, this requires going through and sequencing the DNA a total of ten times to achieve that level of accuracy3. Known as the Bermuda Standards, the international standard for accuracy is currently held at one error per 10,000 base pairs for the entire contiguous sequence – THE DNA IS SEQUENCED IN PARTS, AND OFTEN TIMES, GAPS EXIST BETWEEN THESE DIFFERENT PARTS. Regardless of how accurate this process of sequencing MAY SEEM, through the sequencimg of the entire human genome, THIS YIELDS A TOTAL OF APPROXIMATELY 300,000 BASE PAIR ERRORS.

But how significant is a 00.0001% error rate? The Human Genome Project has brought attention to the significance of single nucleotide polymorphisms (SNPs). SNPs are NATURAL DNA SEQUENCING VARIATIONS of a single nucleotide (A, T, C or G) that occur every 100 to 300 base pairs5. THE VARIATIONS CAUSED BY SNP CAN

DRAMATICALLY AFFECT HOW HUMANS REACT DIFFERENTLY TO THINGS SUCH AS DRUGS, VACCINES, OR DISEASES. However, BECAUSE OF THE INHERENT AND ALLOWABLE ERRORS for companies such as 23andMe that sequence DNA, THEIR RESULTS WILL CERTAINLY SEQUENCE SOME SNPs INACCURATELY. The problem is that companies like 23andMe expect to use their DNA sequencing results to provide medical advice for the participants and their doctors so that they can better prescribe more accurate drug dosages. However, WITH OVER 300,000 BASE PAIR ERRORS, HOW ACCURATE CAN THIS MEDICAL ADVICE BE? If the capabilities and limitations of the human body are sensitive down to the individual nucleotide (as with SNP), CAN HUMAN GENOME SEQUENCING BE RELIABLE ENOUGH TO SERVE ITS PURPOSE AS A SOURCE FOR PERSONALIZED MEDICAL INFORMATION COMPLETELY DEPENDENT ON HUMAN DNA?

https://cs.stanford.edu/.../2010-11/Genomics/accuracy.html

In Summary (Part 1):

-there have been VARYING LEVELS OF TARGET ACCURACIES that the research institutes have aimed for

-in 2000, the first draft was released with an error rate of ONE ERROR PER EVERY 1,000 BASE PAIRS

-in 2003, the official results were cited to have an ERROR RATE OF ONE PER EVERY 10,000 BASE PAIRS

-the DNA is sequenced in parts, and often times, GAPS EXIST BETWEEN THESE DIFFERENT PARTS

-this yields a total of approximately 300,000 BASE PAIR ERRORS

-SNPs are NATURAL DNA SEQUENCING VARIATIONS of a single nucleotide (A, T, C or G) that occur every 100 to 300 base pairs

-the VARIATIONS caused by SNP can DRAMATICALLY AFFECT how humans react differently to things such as drugs, vaccines, or diseases

-because of the INHERENT AND ALLOWABLE ERRORS for companies such as 23andMe that sequence DNA, their results will certainly sequence some SNPs INACCURATELY

-with over 300,000 base pair errors, HOW ACCURATE can this medical advice be?

-can human genome sequencing BE RELIABLE ENOUGH to serve its purpose as a source for personalized medical information completely dependent on human DNA?

From a 2019 Review:

IS RELIANCE ON AN INACCURATE GENOME SEQUENCE SABOTAGING YOUR EXPERIMENTS?

"However, new technologies and algorithmic advances DO NOT GUARANTEE FLAWLESS GENOMICS SEQUENCES OR ANNOTATION. BIAS, ERRORS, AND ARTIFACTS can enter at any stage of the process from library preparation to annotation."

"ALL GENOME SEQUENCES HAVE “ISSUES”

There are MANY FACTORS that can affect the ultimate genome sequence and annotation that are produced, and both SHOULD BE CONSIDERED “WORKS IN PROGRESS.”

"What is the origin of the sample used to generate the genome sequence?

THE ORIGIN MATTERS. Did the sample originate from a clone, a mixed population (common with microbes), or possibly a hybrid? Differences between individuals can be single nucleotide polymorphisms (SNPs), but often they involve INSERTIONS OR DELETIONS (indels) OF VARIOUS SIZES, COPY NUMBER VARIATIONS (CNV), AND EVEN SMALL REARRANGEMENTS. Hybrids can have dramatic differences between orthologous chromosomes [1]. Genome sequences derived from a heterogenous population, especially when CNVs exist, COMPLICATE GENOME ASSEMBLY, and often THE SEQUENCE PRODUCED IS A COMPOSITE of the major alleles present in the sequenced sample. Genome sequences derived from clonal laboratory strains are often easier to assemble, BUT THEY MAY NOT BE TRULY REPRESENTATIVE OF CIRCULATING WILD TYPE STRAINS because they are adapted to culture and, if propagated for a long time, MAY HAVE LOST GENES OR ACCUMULATED MUTATIONS [2]."

"Does the genome have troublesome characteristics?

Some genome sequences are physically difficult to sequence BECAUSE OF EXTREME NUCLEOTIDE BIAS."

"Long homopolymeric runs of any base are PARTICULARLY TROUBLESOME for some sequencing technologies [4] and MAY LEAD TO AN INCORRECT NUMBER OF

NUCLEOTIDES, resulting in frame-shifts if the sequence is coding."

"If the genome sequence CONTAINS NUMEROUS REPETITIVE SEQUENCES, retrotransposons or mobile elements, or large, highly similar gene families, THE GENOME ASSEMBLY WILL BE AFFECTED (Fig 1), especially if only short-read sequences were used."

"Repetitive sequences are a HUGE CHALLENGE for most assembly algorithms."

"Low-coverage, LESS ACCURATE, long-molecule reads can be used as a framework upon which shorter-read sequences can be mapped"

"There is an easy way to assess the quality of your organism's genome assembly. Map the reads from the sequencing project back to the ASSEMBLED GENOME SEQUENCE and have a look."

"The reference genome assembly for the apicomplexan parasite Toxoplasma gondii ME49 contains several collapsed regions that vary by strain (Fig 1C) [8]. DESPITE THE HIGH QUALITY OF THIS GENOME SEQUENCE AND ITS CORRESPONDENCE TO GENETIC MAPS, ISSUES RELATED TO THE NUMBER OF CHROMOSOMES STILL EXIST [13, 14]."

"GENOME SEQUENCES THAT RELIED ON CLONING AND BIOLOGICAL REPLICATION HAVE ADDITIONAL ISSUES THAT NEED TO BE CONSIDERED. SOME SEQUENCES SIMPLY CANNOT BE CLONED; they are toxic to the organism used for cloning and replication and thus, WILL BE MISSING IN THE GENOME SEQUENCE PRODUCED. Unclonable sequences often contain a few select genes and heterochromatin. The inverse is also true; A DNA SEQUENCE FROM THE CLONING VECTOR OR ORGANISM USED TO CONSTRUCT THE LIBRARY CAN END UP IN THE ASSEMBLED TARGET GENOME SEQUENCE."

"HIGH-THROUGHPUT NGS LIBRARY PREPARATION PLAYS A CRITICAL ROLE WITH RESPECT TO THE QUALITY OF THE GENOME SEQUENCE PRODUCED. Many protocols contain amplification steps, WHICH CAN INTRODUCE BIAS. For example, single cells can be used for genome sequencing but via the application of whole genome amplification (WGA). The approach is powerful when material is limited, but the amplification process is biased, and several different WGA reactions (on different cells or populations of like cells) are necessary to fully identify and remove the amplification bias [15, 16]. IT SHOULD BE NOTED THAT BIAS IS RARELY REMOVED FROM THE READS SUBMITTED TO ARCHIVES, so it is imperative to know if WGA was utilized."

"What sequencing platform was used?

DIFFERENT SEQUENCING PLATFORMS HAVE DIFFERENT STRENGTHS AND WEAKNESSES [9], and they continue to evolve rapidly and often complement each other if several different approaches are applied. Genome sequences assembled with Sanger chemistry will have good quality sequence, BUT THE ASSEMBLED GENOME SEQUENCE WILL BE AFFECTED BY THE LIBRARY ISSUES MENTIONED PREVIOUSLY. Genome sequences generated with legacy systems, e.g., 454 and Ion Torrent, WILL HAVE HOMOPOLYMER MISCOUNT ISSUES. Newer genome sequences will consist of highly accurate Illumina short-read technology, BUT THE ASSEMBLED SEQUENCE, especially if repeats are present, WILL BE INCOMPLETE AND CONTAIN GAPS AND MIS-ASSEMBLIES unless a hybrid assembly using long-read technologies like PacBio or Oxford Nanopore are utilized.

How was the genome assembled?

Sequence assemblies are of two types: de novo, assembled from scratch, and reference-based. THE LATTER IS NORMALLY USED WHEN AN ESTABLISHED ORGANISMAL REFERENCE GENOME ALREADY EXISTS AND THE EXPERIMENTAL GOAL IS TO DETERMINE VARIATION WITH RESPECT TO IT. IT IS NOT A GOOD APPROACH TO DETECT REARRANGEMENTS OR SYNTENIC BREAKS, but it is ideal to detect SNPs, some indels, and CNV. REFERENCE-BASED APPROACHES WILL NOT REVEAL GENOME FEATURES NOT PRESENT IN THE REFERENCE, A SIGNIFICANT DRAWBACK. Due to the large volume of population studies focused on SNPs, MOST GENOMES SEQUENCE DATA, SADLY, REMAINS AS UNASSEMBLED FILES OF READS.

De NOVO ASSEMBLIES ARE THE ONLY OPTION FOR AN ORGANISM'S FIRST GENOME SEQUENCE, and when possible, they should be performed as a matter of practice to permit discovery of new features. In the case of eukaryotic genome sequences, especially when the karyotype is UNKNOWN AND PHYSICAL MAPS DO NOT EXIST, READS CAN ONLY BE PARTIALLY ASSEMBLED into contiguous reads, "contigs," or scaffolds of contigs, CONTAINING GAPS. Contigs often contain sequences that are fairly unique because REPETITIVE SEQUENCES ARE OFTEN "MASKED" in a de novo assembly because of the issues they cause. As a result, contigs often end at, or are separated by, MISSING REPETITIVE REGIONS THAT WERE NOT UTILIZED (e.g., masked) OR COULD NOT BE RESOLVED DURING THE ASSEMBLY. VARIATION FOUND AT THE ENDS OF CONFIGS SHOULD BE TREATED WITH CAUTION. Gaps between contigs that have been ordered and oriented into scaffolds are often indicated by exactly 100 "N's" to indicate a gap of unknown size. In some cases, scaffolds representative of whole chromosomes are assembled, but these, too, often contain numerous gaps or ambiguous bases (Table 1). SOME ASSEMBLERS ALSO CREATE A SCAFFOLD THAT LINKS TOGETHER ALL "LEFTOVER" CONFIGS. BEWARE OF

THIS SCAFFOLD, often named “scaffold 0,” AS THE ORDER AND ORIENTATION OF THESE CONFIGS BEARS NO RESEMBLANCE TO THEIR BIOLOGICAL LOCATION; it is simply a convenient mechanism to make sure all contigs are available to those using or searching the genome sequence.

"If a reference genome sequence is already available, you can use unassembled reads to detect sequence variants and CNVs much faster without assembly."

"EACH TYPE OF SEQUENCE ASSEMBLY COMES WITH A SET OF INHERENT ISSUES, and most genome sequence projects produce an assortment of leftover reads and contigs THAT DO NOT ASSEMBLE. In some cases, THESE READS CAN BE IDENTIFIED AS CONTAMINATION, AN UNEXPECTED SYMBIONT, OR ORGANELLAR GENOME SEQUENCE. In other cases, THE LEFTOVER BITS ARE A TELL-TALE SIGN OF PARTICULAR TYPES OF ASSEMBLY ERRORS OR UNEXPECTED GENOME SEQUENCE VARIATION, e.g., CNV (Fig 1) OR HIGH LEVELS OF HETEROZYGOSITY BETWEEN ALLELES (especially if a population was sequenced, rather than an individual)."

"Was the genome sequence “corrected,” and if so, how?

ERROR-PRONE LONG-SEQUENCE READS can be corrected prior to assembly using proovread [21]. CORRECTION PRIOR TO ASSEMBLY CAN FACILITATE ASSEMBLY WHEN THE ERROR RATE IS HIGH, e.g., in low-coverage PacBio reads. ASSEMBLED GENOME SEQUENCES CAN ALSO BE “POLISHED.” Polishing involves base call correction, and ICORN2 [22] is a popular tool. Polishing is performed using highly accurate Illumina reads mapped back against the final genome assembly. Read correction and polishing are useful and recommended steps, but THEY ARE HIGHLY DEPENDENT ON THE PERFORMANCE OF THE ALIGNER, and the end user must be aware that the CORRECTED AND POLISHED SEQUENCES WILL REPRESENT THE MOST ABUNDANT ALLELES PRESENT IN THE READS. In other words, ISOFORMS AND RARE VARIANTS OF REPETITIVE SEQUENCES WILL BE “CORRECTED,” i.e., OVERWRITTEN, IN THE FINAL ASSEMBLY BY MORE ABUNDANT SEQUENCE VARIANTS."

"GENE PREDICTIONS ARE GENOME-ASSEMBLY DEPENDENT, WHICH MEANS IF A REGION IS MISSING, IT CANNOT BE ANNOTATED. Likewise, IF THE REGION IS POORLY ASSEMBLED OR MISSING IN A REFERENCE GENOME SEQUENCE USED FOR ORTHOLOGS, IT MAY END UP MISSING IN THE GENOME SEQUENCE THAT IS BEING ANNOTATED. A good example is Cryptosporidium. The genome sequence for C. parvum was released in 2004, with a state-of-the-art assembly and annotation for the time [27]. This genome sequence was used as the reference sequence for several additional Cryptosporidium strains and species [28, 29]. This practice can be dangerous,

as one of the genome features that facilitates speciation is genome rearrangement, which affects chromosome pairing during reproduction. AS THERE ARE NO GENETIC SYSTEMS FOR MANY PATHOGENS THAT CAN BE USED TO GENERATE A PHYSICAL MAP, reference mapping is useful, BUT IT IS EASY TO FORGET THE ORIGINS OF GENOME SEQUENCE ASSEMBLIES AND ANNOTATION CREATED OR PROPAGATED IN THIS WAY, SO CARE MUST BE EXERCISED WHEN USING REFERENCE-MAPPED GENOME ASSEMBLIES AS THE BASIS FOR EXPERIMENTS."

"The gene is annotated as single copy, is it?

Additional copies of genes can thwart experiments designed to target, clone, delete, or modify a particular gene. The annotation may indicate a single-copy gene, but DEPENDING ON THE TECHNOLOGY USED TO GENERATE YOUR GENOME SEQUENCE, NEARLY IDENTICAL COPIES OF GENES CAN BECOME ASSEMBLED AS ONE GENE (short-read only assemblies are most prone to this issue), and slightly divergent gene family members, especially if they are in tandem repeats, OFTEN DON'T ASSEMBLE AND CAN BE FOUND IN THE LEFTOVER READS OR SMALL UNASSEMBLED CONTIGS (Fig 1)."

"The annotation doesn’t describe your gene. Is it really missing from the genome?

IT IS EASY TO BE MISLED ON THE BASIS OF EXISTING ANNOTATION that a gene is missing. Genes can be lost, and they do decay or evolve beyond recognition, BUT THEY MAY ALSO BE MISSING BECAUSE OF A SEQUENCE ASSEMBLY GAP."

"Alternatively, the region may be missing from the genome assembly, i.e., a gap relative to the comparator sequence. MISASSEMBLIES AND GAPS CAN PROVIDE THE ILLUSION OF MISSING GENES, WHEN IN REALITY, THEY ARE MISSING FROM THE ASSEMBLY, HAVE EVOLVED INTO PSEUDO GENES, OR, IN SOME CASES, HAVE BEEN REPLACED BY A HORIZONTAL GENE TRANSFER LOCATED ELSEWHERE IN THE GENOME.

GENOME SEQUENCE GAPS HAVE MANY DOWNSTREAM CONSEQUENCES. The number of genes MAY BE REDUCED relative to the actual number, and ironically, the number of genes CAN ALSO BE INFLATED because a portion of the same gene can be found on each side of the gap, RESULTING IN TWO PARTIAL PREDICTIONS. Small assembly gaps often lead to frameshifts in coding sequences, which, in turn, LEAD TO AN ARTIFICIAL INCREASE IN THE NUMBER OF PSEUDOGENES, when, in reality, the culprit is an assembly gap. Gaps can also indicate the location of a missing tandem array of genes or repeat sequences that COULD NOT BE PROPERLY ASSEMBLED

(Fig 1C)."

"Can I trust the annotation?

Some organismal genome sequences are continuously curated by the community or experts and have a good, recent genome annotation (Table 1). However, ANNOTATORS CANNOT ANNOTATE WHAT DOES NOT EXIST (e.g., GAPS). Eukaryotic genome sequences, especially from animal, vector, or plant hosts, are complex, and even with continuous curation, there is much more to be fixed and discovered as new sequence technology, assembly algorithms, and experimental evidence appear. For example, UNTRANSLATED REGIONS AND NONCODING RNAs AREN'T ROUTINELY ANNOTATED. ALL GENOME SEQUENCES AND THEIR ANNOTATION ARE "WORKS IN PROGRESS" AND ARE STATIC REPRESENTATIVES OF ONE POINT IN TIME FOR A CONTINUOUSLY EVOLVING MOLECULE WITHIN A GENETICALLY DIVERSE POPULATION."

"Does the annotation affect pathway analyses?

Yes. Studies aimed at drug target discovery often look for a gene that appears to be essential to a pathway. Once discovered, the gene is knocked out, and to everyone's dismay, it was not essential, and the organism survives in the presence of drug. There are many reasons this may have happened, which range from the ability of the drug to reach the target to the possibility that the assessment of essentiality is flawed. ERRORS IN THE ANNOTATION OR THE ASSEMBLY CAN ALSO LEAD TO THIS RESULT. For example, the gene may not be single copy, or the knockout construct behaved oddly and targeted a related or additional gene copy of the target, producing unusual or hard to interpret results. Alternatively, THE LARGE PROPORTION OF GENES OF UNKNOWN FUNCTION (AS HIGH AS 40% IN SOME ORGANISMS) ENCODE FUNCTIONS THAT ALLOW THE ORGANISM TO CIRCUMVENT THE KNOCKOUT. Much work is still needed on this important class of genes."

"Some genome sequences will require additional approaches beyond long reads, such as Hi-C (chromatin conformation capture) [35], Chicago library methodologies [36], or optical mapping [37]. Truly difficult genome sequences can be hexaploid (like wheat), have enormous numbers of scaffolds (like Ixodes scapularis, which has >350,000), be littered with highly similar repeat elements (like T. vaginalis), or suffer from extreme heterogeneity and length differences between sister chromosomes (as in the hybrid T. cruzi). SOME GENOME SEQUENCES HAVE ALREADY BEEN "FIXED" WITH THESE NEW TECHNOLOGIES, BUT THERE IS STILL SIGNIFICANT WORK REQUIRED TO MAKE THEM AS GOOD AS THEY CAN BE."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6742220/

In Summary (Part 2):

-new technologies and algorithmic advances DO NOT GUARANTEE flawless genomic sequences or annotation

-BIAS, ERRORS, and ARTEFACTS can enter at any stage of the process from library preparation to annotation

-ALL genome sequences have “ISSUES"

-there are MANY FACTORS that can affect the ultimate genome sequence and annotation that are produced, and both SHOULD BE CONSIDERED “WORKS IN PROGRESS”

-the ORIGIN of the genome MATTERS -whether it originates from a clone, a mixed population (common with microbes), or possibly a hybrid

-differences between individuals can be single nucleotide polymorphisms (SNPs), but often they involve INSERTIONS OR DELETIONS (indels) OF VARIOUS SIZES, COPY NUMBER VARIATIONS (CNV), AND EVEN SMALL REARRANGEMENTS

-hybrids can have DRAMATIC DIFFERENCES between orthologous chromosomes

-genome sequences derived from a heterogenous (diverse in content) population, especially when CNVs exist, COMPLICATE GENOME ASSEMBLY, and often THE SEQUENCE PRODUCED IS A COMPOSITE of the major alleles present in the sequenced sample

-genome sequences derived from CLONAL laboratory strains are often easier to assemble, BUT THEY MAY NOT BE TRULY REPRESENTATIVE OF CIRCULATING WILD TYPE STRAINS because they are adapted to culture and, if propagated for a long time, MAY HAVE LOST GENES OR ACCUMULATED MUTATIONS

-some genome sequences are physically difficult to sequence BECAUSE OF EXTREME NUCLEOTIDE BIAS

-long homopolymeric runs of any base are PARTICULARLY TROUBLESOME for some sequencing technologies and MAY LEAD TO AN INCORRECT NUMBER OF NUCLEOTIDES, resulting in frame-shifts if the sequence is coding

-if the genome sequence CONTAINS NUMEROUS REPETITIVE SEQUENCES, retrotransposons or mobile elements, or large, highly similar gene families, THE GENOME ASSEMBLY WILL BE AFFECTED, especially if only short-read sequences

were used"

-repetitive sequences are a HUGE CHALLENGE for most assembly algorithms

-low-coverage, LESS ACCURATE, long-molecule reads can be USED AS A FRAMEWORK upon which shorter-read sequences can be mapped

-they state that there is an easy way to assess the quality of the organism's genome assembly which is to map the reads from the sequencing project BACK TO THE ASSEMBLED GENOME SEQUENCE and have a look (**however, if the reference genome is inaccurate...)**

-case in point: the REFERENCE GENOME ASSEMBLY for the apicomplexan parasite Toxoplasma gondii ME49 contains several collapsed regions that VARY BY STRAIN and DESPITE THE HIGH QUALITY of this genome sequence and its correspondence to genetic maps, ISSUES RELATED TO THE NUMBER OF CHROMOSOMES STILL EXIST

-genome sequences that relied on CLONING AND BIOLOGICAL REPLICATION HAVE ADDITIONAL ISSUES that need to be considered

-some sequences SIMPLY CANNOT BE CLONED; they are TOXIC to the organism used for cloning and replication and thus, WILL BE MISSING IN THE GENOME SEQUENCE PRODUCED

-a DNA sequence from the cloning vector or organism used to construct the library CAN END UP IN THE ASSEMBLED TARGET GENOME SEQUENCE

-in other words, unwanted DNA sequences from other organisms used for cloning find their way into the new genome

-high-throughput NGS library preparation plays a CRITICAL ROLE WITH RESPECT TO THE QUALITY OF THE GENOME SEQUENCE produced and many protocols contain amplification steps, WHICH CAN INTRODUCE BIAS

-it should be noted that BIAS IS RARELY REMOVED FROM THE READS SUBMITTED TO ARCHIVES

-different sequencing platforms have different strengths and weaknesses

-genome sequences assembled with Sanger chemistry will have good quality sequence, BUT THE ASSEMBLED GENOME SEQUENCE WILL BE AFFECTED BY THE LIBRARY ISSUES MENTIONED PREVIOUSLY

-genome sequences generated with legacy systems, e.g., 454 and Ion Torrent, WILL HAVE HOMOPOLYMER MISCOUNT ISSUES

-newer genome sequences will consist of highly accurate Illumina short-read technology,

BUT THE ASSEMBLED SEQUENCE, especially if repeats are present, WILL BE INCOMPLETE AND CONTAIN GAPS AND MIS-ASSEMBLIES

-REFERENCE GENOMES are normally used when an ESTABLISHED ORGANISMAL REFERENCE GENOME ALREADY EXISTS and the experimental goal is to determine variation with respect to it

-Drawbacks to Reference Genomes:

1. It is not a good approach to detect rearrangements or syntenic breaks

2. Reference-based approaches WILL NOT REVEAL GENOME FEATURES NOT PRESENT IN THE REFERENCE, a significant drawback

-due to the large volume of population studies focused on SNPs, MOST GENOME SEQUENCE DATA, SADLY, REMAIN AS UNASSEMBLED FILES OF READS

-De novo assemblies are the ONLY OPTION FOR AN ORGANISM'S FIRST GENOME SEQUENCE

-in the case of eukaryotic genome sequences, especially when the karyotype is UNKNOWN AND PHYSICAL MAPS DO NOT EXIST, READS CAN ONLY BE PARTIALLY ASSEMBLED into contiguous reads, "contigs," or scaffolds of contigs, CONTAINING GAPS

-contigs often contain sequences that are fairly unique because REPETITIVE SEQUENCES ARE OFTEN "MASKED" in a de novo assembly BECAUSE OF THE ISSUES THEY CAUSE

-as a result, contigs often end at, or are separated by, MISSING REPETITIVE REGIONS THAT WERE NOT UTILIZED (e.g., masked) OR COULD NOT BE RESOLVED DURING THE ASSEMBLY

-variation found at the ends of contigs should be treated with caution

-some assemblers also create a scaffold that links together all “leftover” contigs, often named “scaffold 0,” but the order and orientation of these contigs BEARS NO RESEMBLANCE TO THEIR BIOLOGICAL LOCATION

-each type of sequence assembly COMES WITH A SET OF INHERENT ISSUES, and most genome sequence projects produce an assortment of leftover reads and contigs THAT DO NOT ASSEMBLE

-in some cases, these reads can be identified as:

1. CONTAMINATION

2. UNEXPECTED SYMBIONT

3. ORGANELLAR GENOME SEQUENCE

4. TELL-TALE SIGN OF PARTICULAR TYPES OF ASSEMBLY ERRORS

5. UNEXPECTED GENOME SEQUENCE VARIATION, e.g., CNV (Fig 1) or HIGH LEVELS OF HETEROZYGOSITY between alleles (especially if a population was sequenced, rather than an individual)

-assembled genome sequences can also be “polished"

-however, "polishing" is HIGHLY DEPENDENT ON THE PERFORMANCE OF THE ALIGNER, and the end user must be aware that the corrected and polished sequences will represent the most abundant alleles present in the reads

-in other words, isoforms and rare variants of repetitive sequences will be “CORRECTED,” i.e., OVERWRITTEN, in the final assembly by more abundant sequence variants

-gene predictions are GENOME-ASSEMBLY DEPENDENT, which means if a region is missing, it cannot be annotated

-IF THE REGION IS POORLY ASSEMBLED OR MISSING IN A REFERENCE GENOME sequence used for orthology, IT MAY END UP MISSING IN THE GENOME SEQUENCE THAT IS BEING ANNOTATED

-as there are NO GENETIC SYSTEMS FOR MANY PATHOGENS THAT CAN BE USED TO GENERATE A PHYSICAL MAP, reference mapping is useful, BUT IT IS EASY TO FORGET THE ORIGINS OF GENOME SEQUENCE ASSEMBLIES AND ANNOTATION CREATED OR PROPAGATED IN THIS WAY, so care must be exercised when using reference-mapped genome assemblies as the basis for experiments

-depending on the technology used to generate the genome sequence, NEARLY IDENTICAL COPIES OF GENES CAN BECOME ASSEMBLED AS ONE GENE (short-read only assemblies are most prone to this issue)

-slightly divergent gene family members, especially if they are in tandem repeats, OFTEN DON'T ASSEMBLE AND CAN BE FOUND IN THE LEFTOVER READS OR SMALL UNASSEMBLED CONTIGS

-IT IS EASY TO BE MISLED ON THE BASIS OF EXISTING ANNOTATION that a gene is missing

-genes can be lost, and they do decay or evolve beyond recognition, BUT THEY MAY ALSO BE MISSING BECAUSE OF A SEQUENCE ASSEMBLY GAP

-MISASSEMBLIES AND GAPS CAN PROVIDE THE ILLUSION OF MISSING GENES, when in reality, THEY ARE MISSING FROM THE ASSEMBLY, have evolved into pseudogenes, or, in some cases, have been replaced by a horizontal gene transfer located elsewhere in the genome

-genome sequence gaps have many downstream consequences:

1. The number of genes MAY BE REDUCED relative to the actual number, and ironically, the number of genes CAN ALSO BE INFLATED because a portion of the same gene can be found on each side of the gap, RESULTING IN TWO PARTIAL PREDICTIONS

2. Small assembly gaps often lead to frameshifts in coding sequences, which, in turn, LEAD TO AN ARTIFICIAL INCREASE IN THE NUMBER OF PSEUDOGENES, when, in reality, the culprit is an assembly gap

3. Gaps can also indicate the location of a missing tandem array of genes or repeat sequences that COULD NOT BE PROPERLY ASSEMBLED

-annotators cannot annotate what does not exist (e.g., GAPS)

-untranslated regions and noncoding RNAs aren’t routinely annotated

-highlighting important statement:

"ALL GENOME SEQUENCES AND THEIR ANNOTATION ARE “WORKS IN PROGRESS” AND ARE STATIC REPRESENTATIVES OF ONE POINT IN TIME FOR A CONTINUOUSLY EVOLVING MOLECULE WITHIN A GENETICALLY DIVERSE POPULATION."

-errors in the annotation or the assembly can also affect pathway analyses

-the large proportion of genes OF UNKNOWN FUNCTION (as high as 40% in some organisms) encode functions that allow the organism to circumvent the knockout

-in other words, if a drug doesn't perform as expected and they don't want to blame genome assembly, they can blame the unknown functions of certain genes

-some genome sequences have already been “FIXED” with these new technologies, BUT THERE IS STILL SIGNIFICANT WORK REQUIRED TO MAKE THEM AS GOOD AS THEY CAN BE

After reading the laundry list of problems associated with the creation of genomes, how reliable and accurate do you believe these "WORKS IN PROGRESS" truly are?

https://docs.google.com/document/u/1/d/e/2PACX-1vROZPyhjCQjajIhQknPYIPqgWJWAudCqxgY61-WevArw61PDTECsX9oBywKdyEhL4_mZjp-STcEX7Na/pub

THE EPISTEMOLOGICAL CRISIS IN GENOMICS:

EPISTEMOLOGICAL: relating to the theory of knowledge, especially with regard to its

methods, validity, and scope, and the distinction between justified belief and opinion

How do we know what we take as "fact" in science? Is it really based on observable phenomena that we can see with our own eyes or do most of the results we claim as knowledge come from computational data analysis prone to biases and errors that are often not discussed and commonly ignored?

The following highlights come from an article by Edward R Dougherty:

"Edward R. Dougherty is an American mathematician, electrical engineer, Robert M. Kennedy '26 Chair, and Distinguished Professor of Electrical Engineering at Texas A&M University. He is also the Scientific Director of the Center for Bioinformatics and Genomic Systems Engineering"

https://engineering.tamu.edu/electrical/profiles/edougherty.html

In it, he focuses on the crisis of what constitutes scientific knowledge in genomics. He touches on the problems associated with relying on an abundance of data and analysis to act as science when in fact it fails as valid scientific knowledge. It is a long article and I'm sure I left out some useful information so I recommend giving the whole article a read sometime:

ON THE EPISTEMOLOGICAL CRISIS IN GENOMICS

Edward R Dougherty

"THERE IS AN EPISTEMOLOGICAL CRISIS IN GENOMICS. AT ISSUE IS WHAT CONSTITUTES SCIENTIFIC KNOWLEDGE IN GENOMICS SCIENCE, or systems biology in general. Does this crisis require a new perspective on knowledge heretofore absent from science or is it merely a matter of interpreting new scientific developments in an existing epistemological framework?"

"There is an epistemological crisis in genomics. THE RULES OF THE SCIENTIFIC GAME ARE NOT BEING FOLLOWED. Given the historical empirical emphasis of biology and the large number of ingenious experiments that have moved the field, one might suspect that the major epistemological problems would lie with mathematics, but this is not the case. While there certainly needs to be more care paid to mathematical modeling, THE MAJOR PROBLEM LIES ON THE EXPERIMENTAL SIDE OF THE MATHEMATICAL-EXPERIMENTAL SCIENTIFIC DUALITY. High-throughput technologies such as gene-expression microarrays have lead to the ACCUMULATION OF MASSIVE AMOUNTS OF DATA, orders of magnitude in excess to what has heretofore been conceivable. BUT THE ACCUMULATION OF DATA DOES NOT CONSTITUTE SCIENCE, NOR DOES THE A POSTIORI RATIONAL ANALYSIS OF DATA."

"A good deal of the crisis in genomics turns on a return to “groping in the dark.”

In previous papers, we have considered how the model-experiment duality leads to a contemporary epistemology for computational biology [3], treated the validation of computational methods in genomics [4], and characterized inference validity for gene regulatory networks in the framework of distances between networks [5]. Here we focus on how the experimental method leads to a general scientific epistemology and how CONTEMPORARY GENOMIC RESEARCH OFTEN FAILS TO SATISFY THE BASIC REQUIREMENTS OF THAT EPISTEMOLOGY, THEREBY FAILING TO PRODUCE VALID SCIENTIFIC KNOWLEDGE."

"It is not that we are without any understanding whatsoever; as previously noted, we understand the mathematical model. OUR KNOWLEDGE OF PHENOMENA RESIDES IN THE MATHEMATICAL MODEL, INSOFAR AS THAT KNOWLEDGE IS CONCEPTUAL. BUT HERE WE MUST AVOID THE DANGER OF SLIPPING INTO RATIONALISM, MISTAKING THE CONCEPTUAL SYSTEM FOR NATURE HERSELF. Scientific knowledge does not stop with reasoning about possibilities and creating a model. It goes further to include a predictive validation methodology and then actual validation. Reichenbach notes that “the very mistake which made rationalism incompatible with science” is “THE MISTAKE OF IDENTIFYING [SCIENTIFIC] KNOWLEDGE WITH MATHEMATICAL KNOWLEDGE” [22]. It is here that we see a great danger lying in Gould’s formulation. Without operational definitions and concomitant experimental protocols for validation, as well as the validation itself, the development of “theories that coordinate and explain” facts quickly drifts into rationalism. REASONING, EITHER IN THE FORM OF CONCEPTUAL CATEGORIES SUCH AS

CAUSALITY OR VIA A MATHEMATICAL SYSTEMS, IS APPLIED TO DATA ABSENT ANY PROBABILISTIC QUANTIFICATION RELATING TO THE OUTCOME OF FUTURE OBSERVATION."

"THE CURRENT SITUATION IN GENOMICS

Almost from the onset of the high-throughput microarray era, papers reporting classifiers based on gene-expression features have appeared. THERE HAVE ALSO BEEN CAUTIONARY WARNINGS ABOUT THE DANGERS OF MISAPPLICATION OF CLASSIFICATION METHODS DESIGNED FOR USE WITH AT MOST HUNDREDS OF FEATURES AND MANY THOUSANDS OF SAMPLE POINTS TO DATA SETS WITH THOUSANDS OR TENS OF THOUSANDS OF FEATURES (GENES) AND LESS THAN ONE HUNDRED SAMPLE POINTS (MICROARRAYS) [31-32]. Keeping in mind the thousands of gene expressions on a microarray, consider a sampling of sample sizes for cancer classification: acute leukemia, 38 [33]; leukemia, 37 [34]; breast cancer, 38 [35]; breast cancer, 22 [36]; follicular lymphoma, 24 [37]; glioma, 50 (but only 21 classic tumors used for class prediction) [38]; and uveal melanoma, 20 [39]. This is a tiny sampling of the host of microarray classification papers based on very small samples and selecting feature sets from among thousands of genes.

Since the foundation of scientific knowledge is prediction, the scientific worth of a classifier depends on the accuracy of the error estimate. If a classifier is trained from sample data and its error estimate, then classifier validity relates to the accuracy of the error estimate, since this estimate quantifies the predictive capability of the classifier. An inability to evaluate predictive power would constitute an epistemological barrier to being able to claim that a classifier model is scientifically sound. CERTAINLY, THERE ARE MATHEMATICAL ISSUES AT EACH STEP IN APPLYING CLASSIFICATION TO MICROARRAY DATA. CAN ONE DESIGN A GOOD CLASSIFIER GIVEN THE SMALL SAMPLES COMMONPLACE IN GENOMICS? [40] CAN ONE EXPECT A FEATURE-SELECTION ALGORITHM TO FIND GOOD FEATURES UNDER THESE LIMITATIONS? [41] These concerns, while important for obtaining useful classifiers, are epistemologically overridden by the concern that the predictive capability, and therefore the scientific meaning, of a designed classifier lies with the accuracy of the error estimate. Except in trivial cases, THERE HAS BEEN NO EVIDENCE PROVIDED THAT ACCEPTABLE ERROR ESTIMATION IS POSSIBLE WITH SOME MANY FEATURES AND SUCH SMALL SAMPLES. EVEN WORSE, IN MANY CASES STUDIES IT HAS BEEN SHOWN TO BE IMPOSSIBLE [42-45]. HENCE, NOT ONLY HAVE THE VAST MAJORITY OF THE PAPERS NOT BEEN SHOWN TO POSSESS SCIENTIFIC CONTENT, LARGE NUMBERS OF THEM HAVE BEEN SHOWN NOT TO POSSESS SCIENTIFIC CONTENT. Braga-Neto writes, “Here, we are facing the careless, unsound

application of classification methods to small-sample microarray data, WHICH HAS GENERATED A LARGE NUMBER OF PUBLICATIONS AND AN EQUALLY LARGE AMOUNT OF UNSUBSTANTIATED SCIENTIFIC HYPOTHESES” [40]. The failure of the research community to demand solid mathematical demonstrations of the validity of the classification methods used with the type of data available HAS RESULTED IN A LARGE NUMBER OF PAPERS LACKING SCIENTIFIC CONTENT. MANY EPISTEMOLOGICAL ISSUES IN GENOMICS RELATE TO STATISTICS. Mehta et al. write, “Many papers aimed at the high-dimensional biology community describe the development or application of statistical techniques. THE VALIDITY OF MANY OF THESE IS QUESTIONABLE, AND A SHARED UNDERSTANDING ABOUT THE EPISTEMOLOGICAL FOUNDATIONS OF THE STATISTICAL METHODS THEMSELVES SEEMS TO BE LACKING” [46]. They are calling attention to a lack of sound statistical epistemology, which renders the results meaningless. The point is further emphasized by Dupuy and Simon, who write, “BOTH THE VALIDITY AND THE REPRODUCIBILITY OF MICROARRAY-BASED CLINICAL RESEARCH HAVE BEEN CHALLENGED” [47]. To examine the issue, they have reviewed 90 studies, 76% of which were published in journals having impact factor larger than 6. Based on a detailed analysis of the 42 studies published in 2004, they report:

Twenty-one (50%) of them contained at least one of the following three basic flaws: (1) in outcome-related gene finding, an unstated, unclear, or inadequate control for multiple testing; (2) in class discovery, a spurious claim of correlation between clusters and clinical outcome, made after clustering samples using a selection of outcome-related differentially expressed genes; or (3) in supervised prediction, a biased estimation of the prediction accuracy through an incorrect cross-validation procedure [47].

The situation is actually much worse than stated here, since in high-dimensional, small-sample settings, CROSS-VALIDATION ERROR ESTIMATION, WHICH IS UBIQUITOUS IN MICROARRAY STUDIES, DOES NOT PROVIDE ACCEPTABLE ERROR ESTIMATION (as will be illustrated in the following paragraph) [42-45]. Thus, USING CROSS-VALIDATION IN SUPERVISED PREDICTION UNDERMINES SCIENTIFIC VALIDITY."

"Experimental design is a key element in drawing statistical conclusions. A properly designed experiment can substantially increase the power of the conclusions, whereas a poorly designed experiment can make it impossible to draw meaningful conclusions. Potter has drawn attention to this issue in the context of high-throughput biological data by distinguishing between mere observation and experimental design, the fundamental distinction between pre-modern and modern science:

MAKING THE OBSERVATIONS WITH NEW AND POWERFUL TECHNOLOGY SEEMS TO INDUCE AMNESIA AS TO THE ORIGINAL NATURE OF THE STUDY DESIGN. It is though astronomers were to ignore everything they knew both about how to classify stars and about sampling methods, and instead were to point spectroscopes haphazardly at stars and note how different and interesting the pattern of spectral absorption lines were. Nonetheless, I doubt the astronomers would claim to be doing an experiment. This dilettante's approach to either astronomy or biology has not been in vogue for at least half a century [32].

In fact, it has not been in vogue since Galileo and Torricelli. Are we to return to "groping in the dark?"

IN THIS VEIN, THE UBIQUITY OF DATA MINING TECHNIQUES IS PARTICULARLY WORRISOME. THESE TEND TO SEARCH FOR PATTERNS IN EXISTING DATA WITHOUT REGARD TO EXPERIMENTAL DESIGN OR PREDICTIVE CAPABILITY. Keller points out the danger of trying to draw grand inferences from patterns found in data. Referring to William Feller's classic text [52] on probability theory, she writes,

By 1971, the attempt to fit empirical phenomena to such distributions was already so widespread that Feller felt obliged to warn his readers against their overuse....Feller's emphasis on the logistic curve as 'an explicit example of how misleading a mere goodness of fit can be' was motivated precisely by the persistence of such 'naïve reasoning' [53].

Data mining is often erroneously identified with pattern recognition when, in fact, they are very different subjects. Pattern recognition can be used as a basis for science because it is based on a rigorous probabilistic framework [54]. On the other hand, all too often, DATA MINING TECHNIQUES CONSIST OF A COLLECTION OF COMPUTATIONAL TECHNIQUES BACKED BY HEURISTICS AND LACKING ANY MATHEMATICAL THEORY OF ERROR, AND THEREFORE LACKING THE POTENTIAL TO CONSTITUTE SCIENTIFIC KNOWLEDGE.

WHILE INATTENTION TO EPISTEMOLOGY IN GENOMIC CLASSIFICATION IS TROUBLING, THE SITUATION WITH CLUSTERING IS TRULY ASTOUNDING. As generally practiced, THERE IS NO PREDICTIVE ASPECT AND HENCE NO

SCIENTIFIC CONTENT WHATSOEVER. Indeed, Jain et al. state that "clustering is a subjective process," [55] so that it lacks the basic scientific requirement of inter-subjectivity. In the context of genomics, Kerr and Churchill have asked the epistemological question: "How does one make statistical inferences based on clustering" [56]. Inferences are possible when clustering is put on a sound probabilistic (predictive) footing by recognizing that, whereas the epistemology of classification lies in the domain of random variables, [54] the epistemology of clustering must lie within the framework of random sets [57]. A great deal of study needs to be done in this direction before clustering can practically provide scientific knowledge. In the meantime, SO-CALLED "VALIDATION INDICES" ARE SOMETIMES USED TO SUPPORT A CLUSTERING RESULT, BUT THESE ARE OFTEN POORLY CORRELATED TO THE CLUSTERING ERROR AND THEREFORE DO NOT PROVIDE SCIENTIFIC VALIDATION [58]."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2674806/

In Summary:

-there is an EPISTEMOLOGICAL CRISIS in genomics

-at issue is WHAT CONSTITUTES SCIENTIFIC KNOWLEDGE in genomic science

-the rules of the scientific game are not being followed

-while there certainly needs to be more care paid to mathematical modeling, THE MAJOR PROBLEM LIES ON THE EXPERIMENTAL SIDE OF THE MATHEMATICAL-EXPERIMENTAL SCIENTIFIC DUALITY

-high-throughput technologies such as gene-expression MICROARRAYS have lead to the ACCUMULATION OF MASSIVE AMOUNTS OF DATA, orders of magnitude in excess to what has heretofore been conceivable

-the ACCUMULATION OF DATA DOES NOT CONSTITUTE SCIENCE, nor does the a posteriori rational analysis of data

(Quick Detour on MICROARRAYS:

"A microarray is a laboratory tool USED TO DETECT THE EXPRESSION OF THOUSANDS OF GENES AT THE SAME TIME. DNA microarrays are microscope slides that are printed with thousands of tiny spots in defined positions, with each spot containing a known DNA sequence or gene. Often, these slides are referred to as gene chips or DNA chips. The DNA molecules attached to each slide act as probes to detect

gene expression, which is also known as the transcriptome or the set of messenger RNA (mRNA) transcripts expressed by a group of genes."

https://www.nature.com/scitable/definition/microarray-202/

What are Microarrays used for?

"Scientists use DNA microarrays to measure the expression levels of large numbers of genes simultaneously OR TO GENOTYPE MULTIPLE REGIONS OF A GENOME."

https://en.m.wikipedia.org/wiki/DNA_microarray

What is GENOTYPING?

"Genotyping is the technology that DETECTS SMALL GENETIC DIFFERENCES that can lead to major changes in phenotype, including both physical differences that make us unique AND PATHOLOGICAL CHANGES UNDERLYING DISEASE."

https://www.thermofisher.com/us/en/home/life-science/pcr/real-time-pcr/real-time-pcr-learning-center/genotyping-analysis-real-time-pcr-information/what-is-genotyping.html

In other words, microarrays are used to compare the genomic diversity of genomes in order to determine differences and pathological changes leading to disease.

How accurate are microarrays?

MICROARRAY EXPERIMENTS AND FACTORS WHICH AFFECT THEIR RELIABILITY

"Microarray analysis offers a variety of methods allowing, among other, identification of genes WHICH MIGHT BE SIGNIFICANT in a specific cellular response mechanism or a particular gene expression pattern that characterizes a particular disease. To obtain significant results, MICROARRAY DATA NEED TO UNDERGO STATISTICAL PROCESSING to differentiate between signal changes caused by direct experimental factors and arising from the indirect experimental factors such as specific methods used, AS WELL AS FROM INACCURACIES OF THE MEASUREMENTS. This level of processing challenges led to studies of the compatibility of different microarray platforms [23–28] which usually is achieved by standardizing protocols and data analysis pipelines [29, 30]. Selection of an appropriate statistical method for microarray processing is a significant subject of scientific discussion and although microarrays have been in use for

more than fifteen years, MANY ISSUES RELATED TO DATA ANALYSIS REMAIN UNRESOLVED.

THE MOST DISCUSSED ISSUES CONCERN THE ALGORITHMS USED FOR THE DATA NORMALIZATION [31, 32], whose goal is to eliminate differences between samples that originate from technical aspects of the microarray handling WHICH MAY CONFOUND THE BIOLOGICAL DIFFERENCES IN A GIVEN EXPERIMENTAL SETUP. A similar goal underlies methods used for batch-effect removal, a step which is crucial when comparing datasets that originate from different times and laboratories [33]. Other frequently-discussed issues CONCERN THE IDENTIFICATION OF SAMPLE DIFFERENTIATING GENES [34, 35] AND EVALUATION OF NOISE LEVEL IN THE SAMPLE [36], AS WELL AS METHODS TO EVALUATE CONTAMINATION OR DAMAGE ON THE MICROARRAYS SURFACE [37, 38]. The most commonly used microarrays, produced by Affymetrix, ARE KNOWN FOR ADDITIONAL ISSUES RELATED TO THEIR PARTICULAR DESIGN WHICH INFLUENCE THE FINAL RESULTS. These include problems resulting from several measurements of expression level for a single gene [39, 40], INCORRECT ALIGNMENTS OF PROBES TO GENES [41, 42], INCORRECT EVALUATION OF THE BACKGROUND LEVEL AND NON-SPECIFIC PROBE HYBRIDIZATION SIGNALS [43], AND THE EFFECTS OF DISTINCT PROBE FEATURES ON DATA PROCESSING ALGORITHMS [44].

THE MOST SIGNIFICANT DISADVANTAGES of microarrays include the high cost of a single experiment, the large number of probe designs BASED ON SEQUENCES OF LOW-SPECIFICITY, AS WELL AS THE LACK OF CONTROL OVER THE POOL OF ANALYZED TRANSCRIPTS since most of the commonly used microarray platforms utilize only one set of probes designed by the manufacturer. OTHER WEAKNESSES OF MICROARRAYS ARE THEIR RELATIVELY LOW ACCURACY, PRECISION AND SPECIFICITY [45] AS WELL AS THE HIGH SENSITIVITY OF THE EXPERIMENTAL SETUP TO VARIATIONS IN HYBRIDIZATION TEMPERATURE [46], THE PURITY AND DEGRADATION RATE OF GENETIC MATERIAL [47], AND THE AMPLIFICATION PROCESS [48] which, together with other factors, MAY IMPACT THE ESTIMATES OF GENE EXPRESSION."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4559324/

"LIMITATIONS OF MICROARRAYS

Hybridisation-based approaches are high throughput and relatively inexpensive, but have several limitations which include (6):

RELIANCE UPON EXISTING KNOWLEDGE about the genome sequence

HIGH BACKGROUND LEVELS owing to cross-hybridisation

LIMITED DYNAMIC RANGE OF DETECTION owing to both background and saturation signals

COMPARING EXPRESSION LEVELS ACROSS DIFFERENT EXPERIMENTS IS OFTEN DIFFICULT and can require complicated normalisation methods

https://www.ebi.ac.uk/training/online/courses/functional-genomics-ii-common-technologies-and-data-analysis-methods/microarrays

Beyond all of those weaknesses and limitations, I'm sure the microarray results are "accurate" and "reliable." 🤦)

-contemporary genomic research often FAILS TO SATISFY THE BASIC REQUIREMENTS of that epistemology, thereby FAILING TO PRODUCE VALID SCIENTIFIC KNOWLEDGE

-Dougherty states that our knowledge of phenomena resides in the mathematical model, insofar as THAT KNOWLEDGE IS CONCEPTUAL

-he advises that we must avoid the danger of slipping into rationalism, MISTAKING THE CONCEPTUAL SYSTEM FOR NATURE HERSELF

-Reichenbach notes that “the very mistake which made rationalism incompatible with science” is “THE MISTAKE OF IDENTIFYING [SCIENTIFIC] KNOWLEDGE WITH MATHEMATICAL KNOWLEDGE”

-Reasoning, either in the form of conceptual categories such as causality or via a mathematical system, is APPLIED TO DATA ABSENT ANY PROBABILISTIC QUANTIFICATION relating to the outcome of future observation

-there have also been CAUTIONARY WARNINGS ABOUT THE DANGERS OF MISAPPLICATION OF CLASSIFICATION METHODS designed for use with at most hundreds of features and many thousands of sample points to data sets with thousands or tens of thousands of features (genes) and less than one hundred sample points (microarrays)

-there are MATHEMATICAL ISSUES at each step in applying classification to microarray data:

1. Can one design a good classifier given the small samples commonplace in genomics?

2. Can one expect a feature-selection algorithm to find good features under these limitations?

-there has been NO EVIDENCE provided that acceptable error estimation is possible with so many features and such small samples and in many cases studied IT HAS BEEN SHOWN TO BE IMPOSSIBLE

-he states that not only have the VAST MAJORITY OF THE PAPERS NOT BEEN SHOWN TO POSSESS SCIENTIFIC CONTENT, large numbers of them have been shown not to possess scientific content

-Braga-Neto writes, “Here, we are facing the careless, unsound application of classification methods to small-sample microarray data, WHICH HAS GENERATED A LARGE NUMBER OF PUBLICATIONS AND AN EQUALLY LARGE AMOUNT OF UNSUBSTANTIATED SCIENTIFIC HYPOTHESES”

-the failure of the research community to demand solid mathematical demonstrations of the validity of the classification methods used with the type of data available HAS RESULTED IN A LARGE NUMBER OF PAPERS LACKING SCIENTIFIC CONTENT

-many epistemological issues in genomics relate to STATISTICS

-Mehta et al. write, “Many papers aimed at the high-dimensional biology community describe the development or application of statistical techniques. THE VALIDITY OF MANY OF THESE IS QUESTIONABLE, and a shared understanding about the epistemological foundations of the statistical methods themselves seems to be lacking”

-Dupuy and Simon, who write, “Both the VALIDITY and the REPRODUCIBILITY of microarray-based clinical research HAVE BEEN CHALLENGED”

-cross-validation error estimation, which is ubiquitous in microarray studies, DOES NOT PROVIDE ACCEPTABLE ERROR ESTIMATION

-using cross-validation in supervised prediction UNDERMINES SCIENTIFIC VALIDITY

-making the observations with new and powerful technology seems to induce amnesia as to the original nature of the study design

-in this vein, the ubiquity of DATA MINING techniques is particularly worrisome as these

tend to search for patterns in existing data WITHOUT REGARD TO EXPERIMENTAL DESIGN OR PREDICTIVE CAPABILITY

(Quick Detour on DATA MINING:

"Data mining is the process of finding anomalies, patterns and correlations within large data sets to predict outcomes."

https://www.sas.com/en_us/insights/analytics/data-mining.html

Data Mining in Genomics

"Challenge 1: Multiple comparisons issue

Analysis of high-throughput genomic data requires handling an astronomical number of candidate targets, MOST OF WHICH ARE FALSE POSITIVES."

"Challenge 2: High dimensional biological data

The second challenge is the high dimensional nature of biological data in many genomic studies [3]. In genomic data analysis, many gene targets are investigated simultaneously, yielding dramatically sparse data points in the corresponding high-dimensional data space. IT IS WELL KNOWN THAT MATHEMATICAL AND COMPUTATIONAL APPROACHES OFTEN FAIL TO CAPTURE SUCH HIGH DIMENSIONAL PHENOMENA ACCURATELY."

"Challenge 3: Small n and large p problem

The third challenge is the so-called “small n and large p” problem [2]. Desired performance of conventional statistical methods is achieved when the sample size of the data, namely “n”—the number of independent observations and subjects—is much larger than the number of candidate prediction parameters and targets, namely “p”. IN MANY GENOMIC DATA ANALYSES THIS SITUATION IS OFTEN COMPLETELY REVERSED."

"Challenge 4: Computational limitation

We also note that no matter how powerful a computer system becomes, IT IS OFTEN PROHIBITIVE TO SOLVE MANY GENOMIC DATA MINING PROBLEMS BY EXHAUSTIVE COMBINATORIAL SEARCH AND COMPARISONS [4]. In fact, many current problems in genomic data analysis have been theoretically proven to be of NP

(non-polynomial)-hard complexity, IMPLYING THAT NO COMPUTATIONAL ALGORITHM CAN SEARCH FOR ALL POSSIBLE CANDIDATE SOLUTIONS. Thus, heuristic—most frequently statistical—algorithms that effectively search and investigate a very small portion of all possible solutions are often sought for genomic data mining problems."

"Challenge 5: Noisy high-throughput biological data

The next challenge derives from the fact that high-throughput biotechnical data and large biological databases are inevitably noisy because BIOLOGICAL INFORMATION AND SIGNAL OF INTEREST ARE OFTEN OBSERVED WITH MANY OTHER RANDOM OR CONFOUNDING FACTORS. Furthermore, a one-size-fit-all experimental design for high-throughput biotechniques CAN INTRODUCE BIAS AND ERROR FOR MANY CANDIDATE TARGETS."

"Challenge 6: Integration of multiple, heterogeneous biological data for translational bioinformatics research

The last challenge is the integration of genomic data with heterogeneous biological data and associated metadata, such as gene function, biological subjects' phenotypes, and patient clinical parameters."

"EFFECTIVE COMBINATION AND UTILIZATION OF THE INFORMATION FROM SUCH HETEROGENEOUS GENOMIC, CLINICAL AND OTHER DATA RESOURCES REMAINS A SIGNIFICANT CHALLENGE."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2253491/

These are just a few problems associated with data mining in relation to genomics that hinder accuracy and reliability.)

-data mining techniques consist of a collection of computational techniques backed by heuristics and lacking any mathematical theory of error, and therefore LACKING THE POTENTIAL TO CONSTITUTE SCIENTIFIC KNOWLEDGE

-Dougherty states that while inattention to epistemology in genomic classification is troubling, the situation with CLUSTERING is truly astounding

(Quick Detour on CLUSTERING:

"Clustering is the task of DIVIDING THE POPULATION OR DATA POINTS INTO A NUMBER OF GROUPS such that data points in the same groups are more similar to other data points in the same group than those in other groups. In simple words, THE AIM IS TO SEGREGATE GROUPS WITH SIMILAR TRAITS AND ASSIGN THEM INTO CLUSTERS."

In Genomics, there are numerous clustering algorithms that can be used. There are too many approaches to list here but this paper goes through many of the main ones used and gives an overview of each:

A Comprehensive Survey of Clustering Algorithms

"On the other hand, EACH CLUSTERING ALGORITHMS HAS IT'S OWN STRENGTHS AND WEAKNESSES, due to the complexity of information."

"The complete definition for clustering, however, ISN'T COME TO AN AGREEMENT"

https://link.springer.com/article/10.1007/s40745-015-0040-1

New algorithms are always created leading to an overabundance of methods with their own strengths/weaknesses.)

-there is NO PREDICTIVE ASPECT AND HENCE NO SCIENTIFIC CONTENT WHATSOEVER REGARDING CLUSTERING

-so-called “validation indices” are sometimes used to support a clustering result, but these are often poorly correlated to the clustering error and THEREFORE DO NOT PROVIDE SCIENTIFIC VALIDATION

Should we be relying on computer algorithms, modeling, mining/clustering of massive amounts of data, etc. each with various drawbacks/limitations/weaknesses to shape what we are supposed to take as "scientific knowledge?"

Seeing that so many different computational methods with various limitations are needed in order to interpret the results to paint what is thought to be an "accurate" representation of the data, is it any wonder why there is a REPRODUCIBILITY CRISIS in the world of Science and Genomics?

https://docs.google.com/document/u/1/d/e/2PACX-1vQxap9AIzhnAG5J-Na4I7j_Yyg82NRF9E1oVwkYFhks0j9bnyUBbrAi0zohJMKa0xzxCmk45RkXx8JW/pub

There are many problems when using "Viral" Genomics to find and determine a "virus."

There are issues of cost, biases, contamination, technical errors, reproducibility, etc. On top of these issues is the fact that a genome is only as good as its reference genome.

What is a reference genome?

"A reference genome (also known as a reference assembly) is a DIGITAL NUCLEIC ACID SEQUENCE DATABASE, assembled by scientists AS A REPRESENTATIVE EXAMPLE OF THE SET OF GENES IN ONE IDEALIZED INDIVIDUAL ORGANISM OF A SPECIES."

https://en.m.wikipedia.org/wiki/Reference_genome

The problem when relying on reference genomes to sequence a novel "virus," as in the case of "SARS-COV-2," is the limitations of the technologies and the many errors that can and do occur. If these errors are in the reference genome, they will transfer over into the new genome. You end up building a genome on top of a house of cards.

Highlighted below are some of the various issues with the technology and why we cannot rely on strings of letters in a database to serve as proof of a "virus."

First, we see the amount of data is crucial for accuracy yet the issue of determining the

optimal amount necessary is unresolved:

"The use of next-generation sequencing has become an established method for virus detection. Efficient study design for ACCURATE DETECTION RELIES ON THE OPTIMAL AMOUNT OF DATA REPRESENTING A SIGNIFICANT PORTION OF A VIRUS GENOME."

"Next-generation sequencing (NGS) has proven to be a valuable tool for virus detection, discovery or diversity studies and has increased in popularity, while decreasing in cost. THE PERCENTAGE GENOME-WIDE COVERAGE OBTAINED, EITHER THROUGH THE MAPPING OF READS OR CONFIGS (assembled reads) ONTO A REFERENCE GENOME, CAN SERVE AS A FORM OF VIRUS DETECTION. The confidence in a positive identification increases with greater coverage. DUE TO THE VARIATION IN THE NUMBER OF READS ASSOCIATED WITH DIFFERENT GENOMIC REGIONS, AND UNEVEN COVERAGE OF THE VIRAL GENOME IS OFTEN OBSERVED in RNA-Seq data. Variation in sequencing depth will, consequently, influence the percentage of genome coverage that can be obtained. IT IS THEREFORE NECESSARY TO FIND THE OPTIMAL AMOUNT OF DATA NEEDED TO COVER THE COMPLETE OR ALMOST COMPLETE GENOME WITHOUT GENERATING AN EXCESS OF SEQUENCE DATA."

"In spite of decreases in the cost of NGS, THERE IS STILL A NEED TO DETERMINE THE OPTIMAL AMOUNT OF DATA NEEDED FOR ACCURATE AND RELIABLE VIRUS DETECTION. The number of reads required for detection is INFLUENCED BY THE NUMBER OF VIRUS-DERIVED READS IN THE DATA, THE SIZE OF THE VIRUS GENOME AND THE COMPLEXITY OF THE VIROME (not assessed here). SOME VIRUSES OR VIROIDS MAY BE MORE REPRESENTED IN THE DATA depending on (i) the degree of infection, which may be host and/or virus dependent, (ii) the plant response to infection in the case of sRNAs and (iii) THE NATURE OF THE GENOME IN THE CASE OF DIFFERENT LIBRARY TYPES (i.e., DNA, poly(A)-selected RNA, ribo-depleted RNA libraries)."

https://virologyj.biomedcentral.com/articles/10.1186/s12985-016-0539-x

Second, we see that analysis of the NGS data is complex and that a range of internal biases and errors can lead to false results and misdiagnosis:

"THE ANALYSIS OF NEXT-GENERATION SEQUENCING (NGS) DATA IS COMPLEX, owing to the breadth of sequences tested AND THE RANGE OF INTERNAL BIASES

AND ERRORS. In a clinical context, THIS CAN LEAD TO FALSE POSITIVES AND FALSE NEGATIVES, AND THE POTENTIAL FOR MISDIAGNOSIS."

"Next-generation sequencing (NGS) provides a broad investigation of the genome, and it is being readily applied for the diagnosis of disease-associated genetic features. However, the INTERPRETATION OF NGS DATA REMAINS CHALLENGING OWING TO THE SIZE AND COMPLEXITY OF THE GENOME AND THE TECHNICAL ERRORS THAT ARE INTRODUCED DURING SAMPLE PREPARATION, SEQUENCING AND ANALYSIS."

https://www.nature.com/articles/nrg.2017.44

Finally, here we see many issues regarding the use of NGS to obtain accurate genome assembly and the many errors that occur such as: mix-ups, contamination, mutations, labeling and sample issues, considerable nucleotide variations/mutations in lines thought to be isogenic, dramatic variations from reference genomes, a reproducibility crisis, ill-suited technology for microbe sequencing, unavailable or incomplete reference genomes, short reads hampering the ability to resolve repetitive regions, etc.

"Laboratory strains, cell lines, and other genetic materials CHANGE HANDS FREQUENTLY IN THE LIFE SCIENCES. DESPITE EVIDENCE THAT SUCH MATERIALS ARE SUBJECT TO MIX-UPS, CONTAMINATION, AND ACCUMULATION OF SECONDARY MUTATIONS, VERIFICATION OF STRAINS AND SAMPLES IS NOT AN ESTABLISHED PART OF MANY EXPERIMENTAL WORKFLOWS."

"The frequent transfer of genetic materials between life science organizations INTRODUCES OPPORTUNITIES FOR QUALITY CONTROL ISSUES. Genetic mutations accumulate naturally over time, and HUMAN ERRORS IN LABELING AND SAMPLE PREPARATION ARE UNAVOIDABLE. Anecdotally, it is NOT UNCOMMON FOR RESEARCHERS TO COMPLAIN OF SAMPLES EXHIBITING UNEXPECTED BEHAVIORS, ONLY TO LATER DISCOVER THAT THE GENETIC MATERIAL THEY'RE WORKING WITH IS NOT AS EXPECTED.

Laboratory strains, cell lines, and mutant collections EXHIBIT CONSIDERABLE NUCLEOTIDE VARIATION AND BACKGROUND MUTATIONS EVEN AMONG LINES THOUGHT TO BE ISOGENIC 1,2,3,4. Despite a growing awareness, CELL-LINE CONTAMINATION AND MISIDENTIFICATION ARE PERSISTENT PROBLEMS,

particularly in mammalian cell research 5,6,7,8,9. Comparably, much less attention has been paid to the potential for similar issues in non-mammalian samples. Yet EVEN COMMONLY USED PLASMIDS HAVE BEEN SHOWN TO VARY DRAMATICALLY FROM THEIR PUBLISHED SEQUENCE 10."

"THE METHODS CURRENTLY USED TO VERIFY SAMPLES/STRAINS ARE BIASED TOWARDS A PARTICULAR GOAL. For instance, diagnostic techniques such as PCR, targeted sequencing, or restriction enzyme-based methods are often used to identify whether or not a marker gene OR KNOWN SEQUENCE variant is present, or for analysis of variable repeat regions such as in 16 s rRNA profiling12,13. THESE APPROACHES ARE LIMITED TO PARTICULAR REGIONS OF THE GENOME OR ARE INSUFFICIENTLY SENSITIVE FOR CAPTURING MANY TYPES OF SEQUENCE VARIATIONS 2,14,15."

"GIVEN REPORTS THAT THE LIFE SCIENCES ARE FACING A REPRODUCIBILITY CRISIS, it is more important than ever for researchers to verify the samples and strains they work with."

"Many tools have been developed for assembling sequenced genomes and detecting variants by aligning sequencing reads to a reference genome18, but these tools have been largely developed and validated using human sequencing data. THE SAME TOOLS MAY NOT PERFORM WELL WHEN ANALYZING MICROBIAL SEQUENCING DATA DUE TO DIFFERING PLOIDY, GENOME SIZE, AND MUTATION RATES. The applicability of assembly and, especially, variant calling tools to microbial sample and strain verification HAS NOT BEEN THOROUGHLY EXPLORED."

"An ideal approach to sample verification by WGS would be to sequence the sample, assemble the genome, and then compare the assembled genome to the exact reference genome (the genetic background plus any known variations). Unfortunately, THERE IS NOT A FINISHED REFERENCE GENOME AVAILABLE FOR THE GENETIC BACKGROUND USED IN OUR ANALYSIS (BY4741), DESPITE THE FACT THAT IT IS A COMMONLY USED LABORATORY STRAIN. We thus conducted reference-based assemblies USING THE CLOSELY RELATED S288C GENOME."

"ALTHOUGH COMPARABLE to the previously published BY4741 draft genome27, THE QUALITY METRICS OF ALL ASSEMBLIES VARIED MARKEDLY from the S288C reference in number of contigs and N50 values."

"A MEANINGFUL DIRECT COMPARISON OF OUR ASSEMBLED GENOMES WITH

THE REFERENCE WOULD REQUIRE A MORE COMPLETE ASSEMBLY THAN WE WERE ABLE TO ACCOMPLISH USING SHORT READS ALONE. As such, we conducted the remainder of our analysis by aligning trimmed reads to the reference genome."

"While this approach was successful in identifying the mutation that was likely the cause for an unexpected phenotype, THERE MAY BE MORE CHANGES TO THE GENOME THAT WERE MISSED. Clearly, UNEXPECTED MUTATIONS IN COMMON LABORATORY CELL LINES CANNOT BE IGNORED, BUT THE TECHNOLOGY NEEDED TO GET A CLEAR VISION OF THE MAGNITUDE OF THE PROBLEM IS UNDERDEVELOPED.

THE VARIANT-FINDING TOOLS USED IN THIS ANALYSIS WERE NOT IDEALLY SUITED TO VERIFICATION WORKFLOWS. Most data analysis pipelines, including those described here, rely on ad-hoc or heuristic decision points that require an advanced understanding of the software tools used for analysis19. Analyzing the results required MANUALLY VALIDATING THE CALLS by visualizing the reads, as well as looking up the function of each individual gene – processes that are tedious, time consuming, AND POTENTIALLY ERROR-PRONE. Additionally, the SNPs/INDELs called differed dramatically depending on the tools and parameters used. NONE OF THE TOOLS AND PARAMETERS TESTED SUCCESSFULLY IDENTIFIED ALL OF THE KNOWN INDELs (Table 3). It was only when we adjusted the parameters to find the known INDELS, that we identified a large transposon insertion in an important gene. In conclusion, COMMONLY USED SOFTWARE TOOLS COULD NOT RELIABLY RETURN EXPECTED OUTCOMES, WERE INDIVIDUALLY TOO NARROW IN FOCUS, AND COLLECTIVELY TOO SENSITIVE TO PARAMETERS TO BE INTEGRATED INTO A CONSISTENT PIPELINE FOR VERIFICATION BY WGS."

"Before WGS can be used for routine sample and strain validation, genome finishing also needs to be streamlined and made more affordable, SUCH THAT REFERENCE GENOMES ARE AVAILABLE FOR ALL COMMONLY USED LABORATORY STRAINS. THE SHORTCOMINGS OF USING SHORT READ SEQUENCING IN GENOME ASSEMBLY HAVE BEEN WELL REPORTED. In the described use case, THE USE OF SHORT READS SIGNIFICANTLY HAMPERED OUR ABILITY TO RESOLVE REPETITIVE REGIONS OF THE GENOME."

"Variability and repetitive sequences (such as at telomeres, transposons, and ribosomal RNA genes), on the one hand, complicate analysis by WGS, but, on the other hand,

EMPHASIZE THE IMPORTANCE OF FREQUENTLY VERIFYING STRAINS, BECAUSE THE GENOME IS A LIVING DYNAMIC STRUCTURE, NOT A RIGID SET OF PERMANENT INSTRUCTIONS."

https://www.nature.com/articles/s41598-020-62364-6

Keep in mind that when attempting to sequence the genome of a "virus," they do not PURIFY/ISOLATE a "virus" from everything else first. They assume that they can accurately sequence the genome from cell cultures which contain animal cells (usually African Green Monkey Kidneys), antibiotics, fetal bovine serum, and various other "nutrients" mixed together along with the human sample. It is well known that the antibiotics alone will alter the materials in the culture, as is the case with the fetal bovine serum as well.

They also do not account for exosomes or other extracellular vesicles that are guaranteed to be within the sample.

Taking everything into consideration, do you feel confident that "Viral" Genomics can accurately identify a "novel virus?"

Problems with "Viral" Genomics:

Virologist Charles Calisher once spoke up about the problems of relying on the use of genomics in order to determine and study "viruses:"

"Although all that is terrific, says Calisher, A STRING OF DNA LETTERS IN A DATA BANK TELLS LITTLE OR NOTHING about how a virus multiplies, which animals carry it, how it makes people sick, or whether antibodies to other viruses might protect against it. JUST STUDYING SEQUENCES, Calisher says, IS "LIKE TRYING TO SAY WHETHER SOMEBODY HAS BAD BREATH BY LOOKING AT HIS FINGERPRINTS."

https://fdocuments.in/amp/document/virology-old-guard-urges-virologists-to-go-back-to-

basics.html

There are many issues relying solely on genomics for "viruses," especially since no "virus" has ever been properly purified/isolated nor proven pathogenic. This is absolutely necessary in order to know whether these strings of letters in a database actually exist in reality and have any meaning.

These are a few of the many flaws when using genomics for "viral" identification/characterization:

"Notable technical challenges have impeded progress; for example, FRAGMENTS OF VIRAL GENOMES ARE TYPICALLY ORDERS OF MAGNITUDE LESS ABUNDANT than those of host, bacteria, and/or other organisms in clinical and environmental metagenomes; OBSERVED VIRAL GENOMES OFTEN DEVIATE CONSIDERABLY FROM REFERENCE GENOMES DEMANDING USE OF EXHAUSTIVE ALIGNMENT APPROACHES; HIGH INTRAPOPULATION VIRAL DIVERSITY CAN LEAD TO AMBIGUOUS SEQUENCE RECONSTRUCTION; and finally, the RELATIVELY FEW DOCUMENTED VIRAL REFERENCE GENOMES COMPARED TO THE ESTIMATED NUMBER OF DISTINCT VIRAL TAXA RENDERS CLASSIFICATION PROBLEMATIC. Various software tools have been developed to accommodate the unique challenges and use cases associated with characterizing viral sequences; however, THE QUALITY OF THESE TOOLS VARIES, and their use often necessitates computing expertise or access to powerful computers, THUS LIMITING THEIR USEFULNESS TO MANY RESEARCHERS."

"For example, A FAST SEQUENCE CLASSIFIER MIGHT FAIL ENTIRELY TO DETECT A NOVEL STRAIN OF A WELL-CHARACTERIZED VIRUS, AND EQUALLY MIGHT PERFORM WELL WITH ILLUMINA SEQUENCES YET DELIVER POOR RESULTS FOR DATA GENERATED WITH THE Ion Torrent PLATFORM. Furthermore, results arising from these analyses SHOULD BE REPLICABLE, intelligible, and useful to the end user, with provision for quality control and error management."

"METHODOLOGICALLY, MOST GENOMIC SEQUENCE ANALYSIS SOFTWARE IS NOT WELL SUITED FOR VIRAL GENOMES. Generic tools that are able to address the challenges posed by viral sequences ARE OFTEN APPLICABLE ONLY IN LIMITED CIRCUMSTANCES. Choosing between approaches is made difficult due to an abundance of disparate yet functionally equivalent methodologies and IN GENERAL A

LACK OF RIGOROUS BENCHMARKS FOR VIRAL DATASETS. While there is much ongoing research in this area, BOTH THE SENSITIVE DETECTION OF PREVIOUSLY CHARACTERIZED VIRUSES AND VIRAL DISCOVERY REMAIN KEY CHALLENGES OPEN FOR INNOVATION."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5822887/

From this article, we see:

-"viral" fragments are not abundant

-observed genomes deviate from reference genomes

-there are relatively few reference genomes

-quality of the tools vary

-most analysis software is not well-suited for "viral" genomes

-tools used to overcome challenges have limited usefulness

-lack of rigorous benchmarks for "viral" datasets

-detection of previously characterized "viruses" and "viral" discovery remain key challenges

There are further issues when relying on genomics for "viruses:"

"OBTAINING VIRUS GENOME SEQUENCE directly from clinical samples IS STILL A CHALLENGING TASK DUE TO THE LOW LOAD OF VIRUS GENETIC MATERIAL compared to the host DNA, and to THE DIFFICULTY TO GET AN ACCURATE GENOME ASSEMBLY. "

"However, despite the relatively small size of virus genomes, THEIR SEQUENCING OFTEN REMAINS DIFFICULT. The small amount of virus genetic material compared to the host nucleic acid DECREASES VIRAL SEQUENCING OUTPUT. In addition, one have to deal with the difficulty that SEVERAL VIRAL VARIABLES COEXIST IN A SINGLE SAMPLE, PRESENTING MORE OR LESS VARIABLE SEQUENCES depending on the intrinsic mutation rate of the virus. ALL THESE POINTS BURDEN THE SEQUENCING AND THE ASSEMBLY OF VIRAL GENOME."

"Three main methods based on HTS are currently used for viral whole-genome sequencing: metagenomic sequencing, target enrichment sequencing and PCR amplicon sequencing, each showing benefits and drawbacks (Houldcroft et al., 2017). In metagenomic sequencing, total DNA (and/or RNA) from a sample INCLUDING HOST BUT ALSO BACTERIA, VIRUSES AND FUNGI is extracted and sequenced. It is a simple and cost-effective approach, and it is the only approach not requiring reference sequences. Instead, the other two HTS approaches, target enrichment and amplicon sequencing, BOTH DEPEND ON REFERENCE INFORMATION TO DESIGN BAITS OR PRIMERS. The limitation of metagenomic sequencing is that it REQUIRES A VERY HIGH SEQUENCING DEPTH TO OBTAIN ENOUGH VIRAL GENOME MATERIAL.

The target enrichment sequencing uses virus-specific capture oligonucleotides to enrich the viral genome preparation before sequencing. This method is more specific than metagenomics sequencing but implies higher costs and a more advanced technical expertise for sample preparation. Finally, the PCR amplicon sequencing is a well-established method consisting in specific viral genome amplification by PCR before sequencing. It is easily applicable on large number of samples in a routine use and so very adequate for clinical samples. The PCR amplification method, compared to the others, is particularly relevant for samples containing very low viral genetic material, IT PRESENTS SEVERAL DISADVANTAGES, though. THE SEQUENCE OF THE VIRUS OF INTEREST HAS TO BE KNOWN AND NOT TOO VARIABLE TO BE CORRECTLY AMPLIFIED BY THE SET OF DESIGNED PRIMERS. A second pitfall is due to the fact that the PCR CYCLES CAN INTRODUCE SOME AMPLIFICATION ERRORS ALONG THE SEQUENCES WHICH MAKE THE ASSEMBLY STEP MORE PRONE TO MISTAKES. Finally, this method can only be used for small genomes because of the number of PCR reactions which has to be limited."

"The bioinformatics analysis of virus sequencing data is often based on alignment, or mapping, of reads against a reference sequence followed by the consensus extraction by majority voting. However, THE ALIGNMENT STEP IS KNOWN TO INTRODUCE SOME BIASES (Archer et al., 2010, Posada-Cespedes et al., 2017). For example, IF THE STUDIED VIRUS SEQUENCE IS DIVERGENT FROM THE CHOSEN REFERENCE SEQUENCE, THE READS COVERING THE REGIONS OF DIVERGENCE COULD NOT BE ALIGNED CORRECTLY WHICH WILL BIAS THE RESULTING CONSENSUS. Moreover, the mapping step of reads in divergent, repetitive or low complexity regions is a difficult task which have to be carefully examined (Caboche et al., 2014). Finally, THE CHOICE OF THE REFERENCE SEQUENCE ITSELF IS A CRITICAL STEP FROM WHICH THE RESULTING CONSENSUS SEQUENCE WILL STRONGLY DEPEND."

https://www.sciencedirect.com/science/article/pii/S0042682219300728

From this article, we see:

-difficulty of getting accurate "viral" sequences and low load of genetic material

-several "viral variants" can coexist in a sample

-metagenomics includes host, bacterial, viral, and fungal material

-PCR requires sequence to be known and not too variable

-PCR cycles produce amplification errors which makes assembly prone to errors

-alignment is known to introduce biases

-choice of reference is crucial

Needless to say, in order for genomics to be a valuable tool for the study of "viruses," these "viruses" must be shown to exist first. There are too many errors, biases, variables, and an over reliance on references and consensus that make it a poor fit to prove and/or study something that has not been purified/isolated first.

This is what Charles Calisher WARNED about. These new tools are pretty cool and fun to look at but the information coming from them means nothing if the tried and true methods of the past are ignored.

Unfortunately for Dr. Calisher, even the old methods of Virology were prone to errors and unable to produce the proof of purified/isolated pathogenic "viruses."

https://docs.google.com/document/u/1/d/e/2PACX-1vTN6wVT5hOqXpKMZ0950u_6ogjy1GpDCZRRkt-hw3Ai_SXZI9LG3xP7QfPb6dYLHPwCnHRHcWVCiMCx/pub

Consensus Sequence

Consensus sequence models date to the earliest days of sequencing and are still in use today. Consensus sequences represent a motif as a single sequence where only one base or amino acid residue is permitted at each position. Many restriction enzyme cut sites are effectively modeled in this way (consider, e.g., the EcoRI site: G^AATC). As more sequences became available, it was common to use consensus sequences to model promoters and other DNA signals by aligning examples of the signal, and using the most common base at each position as the consensus sequence (Fig. 1). Such consensus sequences were often referred to as "boxes", for instance the Pribnow Box (Pribnow, 1975) (now known as the −10 region of the E. coli promoter), TATAAT, CAAT (Graves et al., 1986) box, TATA (Lifton et al., 1978) box, and many others.

Fig. 1

https://www.sciencedirect.com/topics/medicine-and-dentistry/consensus-sequence?fbclid=IwAR1Z9BQr1CHhq-3It_BdV82Z-r0_wbm8Ap2IwHejuAfs3tOHBjLPqAMF43o

REPRODUCIBILITY CRISIS IN GENOMICS:

It has been known since at least 2005 that much of the scientific literature being published is fundamentally flawed, non-reproducible, or outright fraudulent. Relating to the (pseudo)science of Virology, this crisis extends to the cell culturing techniques used to "isolate" the "virus" as well as the antibodies used as an indirect method to identify them. Another area closely tied to Virology is genomics, hence all the talk about genomes and variants lately with "SARS-COV-2." Just as with every other area surrounding Virology, Genomics is also embroiled in a reproducibilty crisis itself. This is highlighted from a few studies/articles with a summary at the end:

REPRODUCIBILITY CRISIS:

IS SCIENTIFIC RESEARCH 'FUNDAMENTALLY FLAWED'?

"A new report released [May 2019] by the National Academies of Sciences, Engineering, and Medicine is weighing in on a contentious debate within the science world: THE IDEA THAT SCIENTIFIC RESEARCH IS FUNDAMENTALLY FLAWED, RIFE WITH PUBLISHED FINDINGS THAT OFTEN CAN'T BE REPRODUCED OR REPLICATED BY OTHER SCIENTISTS, otherwise known as the replication and reproducibility crisis."

Common issues highlighted by these scientists have included FRAUDULENT, POORLY DONE, or OVERHYPED STUDIES, with EMBELLISHED FINDINGS based on SMALL SAMPLE SIZES."

https://geneticliteracyproject.org/2019/05/16/reproducibility-crisis-is-scientific-research-fundamentally-flawed

EASTERN GENOMICS PROMISES

"Of the many critical issues coming out of the data-rich universe that we now find ourselves in, James Taylor (Emory University) focused his talk on what feels is THE MAIN CRISIS IN GENOMICS RESEARCH REPRODUCIBILITY. With the life-sciences increasingly reliant on computational and data-driven approaches, access to the supporting data and tools and accessibility in using computational resources has not kept pace."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3491378/

THE REPRODUCIBILITY CRISIS IN SCIENCE

"However, in investigations where computation plays a large part in deriving the findings, REPRODUCIBILITY IS IMPORTANT BECAUSE IT IS ESSENTIALLY THE ONLY THING AN INVESTIGATOR CAN GUARANTEE ABOUT A STUDY. Replicability cannot be guaranteed – that question will ultimately be settled by other independent investigators who conduct their own studies and arrive at similar findings. Furthermore, many computational investigations are difficult to describe in traditional journal papers, and the only way to uncover what an investigator did is to look at the computer code and apply it to the data. In a time where data sets and computational analyses are growing in complexity, the need for reproducibility is similarly growing."

"This concern gained significant traction with a statistical argument that suggested

MOST PUBLISHED SCIENTIFIC RESULTS MAY BE FALSE POSITIVES (bit.ly/1PWAhBx). Concurrently, there have been some VERY PUBLIC FAILINGS OF REPRODUCIBILITY across a range of disciplines, from CANCER GENOMICS (bit.ly/1PWAC7a), to clinical medicine (bit.ly/1KNc4u6) and economics (bit.ly/1PWBngz) and the DATA FOR MANY PUBLICATIONS HAVE NOT BEEN MADE

PUBLICLY AVAILABLE, RAISING DOUBTS ABOUT THE QUALITY OF DATA ANALYSES. Compounding these problems is the lack of widely available and user-friendly tools for conducting reproducible research."

https://rss.onlinelibrary.wiley.com/doi/pdf/10.1111/j.1740-9713.2015.00827.x

INVESTIGATING REPRODUCIBILITY AND TRACKING PROVENANCE – A GENOMIC WORKFLOW CASE STUDY

"The scientific community has paid special attention with respect to benchmarking -omics analysis to establish transparency and reproducibility of bioinformatics studies

[11]. Nekrutenko and Taylor [12] discussed important issues of accessibility, interpretation and reproducibility for analysis of NGS data. ONLY TEN OUT OF 299 ARTICLES THAT CITED THE 1000 GENOMES PROJECT AS THEIR EXPERIMENTAL APPROACH USED THE RECOMMENDED TOOLS AND ONLY FOUR STUDIES USED THE FULL WORKFLOW. OUT OF 50 RANDOMLY SELECTED PAPERS that cited BWA [13] for alignment step, ONLY SEVEN STUDIES PROVIDED COMPLETE INFORMATION about parameter setting and version of the tool. THE UNAVAILABILITY OF PRIMARY DATA from two cancer studies [14] WAS A BARRIER TO ACHIEVE BIOLOGICAL REPRODUCIBILITY OF CLAIMED RESULTS.

IOANNIDIS et al. [15] ATTRIBUTED UNAVAILABILITY OF DATA, SOFTWARE AND ANNOTATION DETAILS AS REASONS FOR NON-REPRODUCIBILITY OF MICROARRAY GENE EXPRESSION STUDIES. Hothorn et al. [16] found that ONLY 11% of the articles conducting simulation experiments PROVIDED ACCESS TO BOTH DATA AND CODE. The authors reviewing 100 Bioinformatics journal papers [17] claimed that along with the textual descriptions, AVAILABILITY OF VALID DATA AND CODE FOR ANALYSIS IS CRUCIAL FOR REPRODUCIBILITY OF RESULTS. Moreover, the majority of papers that explained the software environment, FAILED TO MENTION VERSIONS DETAILS, WHICH MADE IT DIFFICULT TO REPRODUCE THESE STUDIES."

"Ludäscher et al. [20] reviewed common

requirements of any scientific workflow, most of which (such as data provenance, reliability and fault-tolerance, smart reruns and smart semantic links) are directly linked to provenance capture. In addition to workflow evolution [21], prospective (defined as the specification of the workflow used in an analysis) as well as retrospective (defined as the run time environment of an execution of the workflow in an analysis) PROVENANCE [22] WAS IDENTIFIED AS AN ESSENTIAL REQUIREMENT FOR EVERY COMPUTATIONAL PROCESS IN A WORKFLOW TO ACHIEVE REPRODUCIBILITY OF A PUBLISHED ANALYSIS AND ULTIMATELY ACCOUNTABILITY IN CASE OF INCONSISTENT RESULTS. Several provenance models have been proposed and implemented to support retrospective and prospective provenance [23–25] BUT THESE ARE SELDOM USED BY WMS USED IN GENOMIC STUDIES. Despite high expectations, VARIOUS EXISTING WMS [26–30] DO NOT TRULY PRESERVE ALL NECESSARY PROVENANCE INFORMATION TO SUPPORT REPRODUCIBILITY - particularly to the standards that might be expected FOR CLINICAL GENOMICS.

The inability to reproduce and use exactly the same procedures/workflows means that considerable effort and time is required on reproducing results produced by others [12, 16, 17, 31]. AT PRESENT THE CONSOLIDATION OF EXPERTISE AND BEST PRACTICE WORKFLOWS THAT SUPPORT REPRODUCIBILITY ARE NOT MATURE. Most of the time, this is due to the LACK OF UNDERSTANDING OF REPRODUCIBILITY REQUIREMENTS and INCOMPLETE PROVENANCE CAPTURE that can make it difficult for other researchers to reuse existing work. THE SUSTAINABILITY OF CLINICAL GENOMICS RESEARCH REQUIRES THAT REPRODUCIBILITY OF RESULTS GOES HAND-IN-HAND WITH DATA PRODUCTION. We, as the scientific community, need to address this gap by proposing and implementing practices that can ensure reproducibility, confirmation and ultimately extension of existing work."

THE EXPECTATION FOR SCIENCE TO BE REPRODUCIBLE IS CONSIDERED FUNDAMENTAL BUT OFTEN NOT TESTED. Every new discovery in science is built on already known knowledge, that is, published literature acts as a building block for new findings or discoveries. Using this published literature as a base, the next level of understanding is developed and hence the cycle continues. Therefore, IF WE CANNOT REPRODUCE ALREADY EXISTING KNOWLEDGE FROM THE LITERATURE, we are wasting a lot of effort, resources and time in doing potentially wrong science [53] RESULTING IN “REPRODUCIBILITY CRISIS” [54]. If a researcher claims a novel finding, SOMEONE ELSE, interested in the study, SHOULD BE ABLE TO REPRODUCE IT. REPORTS ARE ACCUMULATING THAT MOST OF THE SCIENTIFIC CLAIMS ARE NOT REPRODUCIBLE, HENCE QUESTIONING THE RELIABILITY OF SCIENCE AND

RENDERING LITERATURE QUESTIONABLE [55, 56]. THE TRUE REPRODUCIBILITY OF EXPERIMENTS IN DIFFERENT SYSTEMS HAS NOT BEEN INVESTIGATED RIGOROUSLY IN SYSTEMATIC FASHION. For computational work like the one described in this paper, reproducibility not only requires an in depth understanding of science but also data, methods, tools and computational infrastructure, making it a non-trivial task. The challenges imposed by large-scale genomics data demand complex computational workflow environments. A key challenge is how can we improve reproducibility of experiments involving complex software environments and large datasets."

"Keeping in view the critical application of the data generated, it is safe to state that ENTIRE PROCESS LEADING TO SUCH BIOLOGICAL COMPREHENSIONS MUST BE DOCUMENTED SYSTEMATICALLY TO GUARANTEE REPRODUCIBILITY OF THE RESEARCH. However a generalised set of rules and recommendations to achieve THIS IS STILL A CHALLENGE TO BE MET as workflow implementation, storage, sharing and reuse SIGNIFICANTLY VARIES depending on the choice of approach and platform used by the researcher."

"REPRODUCIBILITY OF COMPUTATIONAL GENOMICS STUDIES HAS BEEN CONSIDERED AS A MAJOR ISSUE IN RECENT TIMES. In this context, we have characterised workflows on the basis of approach used for their definition and implementation. To evaluate reproducibility and provenance requirements, we implemented a complex variant discovery workflow using three exemplar workflow definition approaches. WE IDENTIFIED NUMEROUS IMPLICIT ASSUMPTIONS INTERPRETED THROUGH THE PRACTICAL EXECUTION OF THE WORKFLOW, LEADING TO RECOMMENDATIONS FOR REPRODUCIBILITY AND PROVENANCE, as shown in Table 1."

doi: 10.1186/s12859-017-1747-0.

In Summary:

-the idea that scientific research is fundamentally flawed, rife with published findings that often can’t be reproduced or replicated by other scientists, otherwise known as the replication and reproducibility crisis

-common issues highlighted by these scientists have included fraudulent, poorly done, or overhyped studies, with embellished findings based on small sample sizes

-the main crisis in GENOMICS research is REPRODUCIBILITY

-reproducibility is important as it is the only thing an investigator can guarantee about a study

-data for many publications have not been made publicly available, RAISING DOUBTS ABOUT THE QUALITY of data analyses

-ONLY TEN OUT OF 299 ARTICLES that cited the 1000 Genomes project as their experimental approach used the recommended tools and only four studies used the full workflow

-out of 50 randomly selected papers that cited BWA for alignment step, ONLY SEVEN STUDIES PROVIDED COMPLETE INFORMATION about parameter setting and version of the tool

-the UNAVAILABILITY OF PRIMARY DATA from two cancer studies was a BARRIER TO ACHIEVE BIOLOGICAL REPRODUCIBILITY OF CLAIMED RESULTS

-Ioannidis et al. attributed unavailability of data, software and annotation details as reasons for NON-REPRODUCIBILITY of microarray gene expression studies

-Hothorn et al. found that ONLY 11% of the articles conducting simulation experiments PROVIDED ACCESS TO BOTH DATA AND CODE -the authors reviewing 100 Bioinformatics journal papers claimed that along with the textual descriptions, AVAILABILITY OF VALID DATA AND CODE FOR ANALYSIS IS CRUCIAL FOR REPRODUCIBILITY OF RESULTS

-the MAJORITY of papers that explained the software environment, FAILED TO MENTION VERSION DETAILS, which made it DIFFICULT TO REPRODUCE these studies

-PROVENANCE was identified as an ESSENTIAL REQUIREMENT for every computational process in a workflow TO ACHIEVE REPRODUCIBILITY of a published analysis and ultimately ACCOUNTABILITY IN CASE OF INCONSISTENT RESULTS

-several provenance models have been proposed and implemented to support retrospective and prospective provenance BUT THESE ARE SELDOM USED BY WMS USED IN GENOMIC STUDIES

-various existing WMS DO NOT TRULY PRESERVE ALL NECESSARY PROVENANCE INFORMATION TO SUPPORT REPRODUCIBILITY - particularly to the standards that might be expected for clinical genomics

-at present the consolidation of expertise and best practice workflows that support reproducibility ARE NOT MATURE

-most of the time, this is due to the LACK OF UNDERSTANDING OF REPRODUCIBILITY REQUIREMENTS and INCOMPLETE PROVENANCE CAPTURE

that can make it difficult for other researchers to reuse existing work

-the sustainability of clinical genomics research REQUIRES THAT REPRODUCIBILITY OF RESULTS GOES HAND-IN-HAND WITH DATA PRODUCTION

-the expectation for science to be reproducible is considered fundamental BUT OFTEN NOT TESTED

-HIGHLIGHTING PERFECT SUMMARY HERE:

"Every new discovery in science is built on already known knowledge, that is, published literature acts as a building block for new findings or discoveries. Using this published literature as a base, the next level of understanding is developed and hence the cycle continues. Therefore, if we cannot reproduce already existing knowledge from the literature, we are wasting a lot of effort, resources and time in doing potentially wrong science resulting in "reproducibility crisis"

-if a researcher claims a novel finding, SOMEONE ELSE, interested in the study, SHOULD BE ABLE TO REPRODUCE IT

-reports are accumulating that MOST OF THE SCIENTIFIC CLAIMS ARE NOT REPRODUCIBLE, hence questioning the reliability of science and RENDERING LITERATURE QUESTIONABLE

-the true reproducibility of experiments in different systems HAS NOT BEEN INVESTIGATED RIGOROUSLY in systematic fashion

-it is safe to state that ENTIRE PROCESS leading to such biological comprehensions MUST BE DOCUMENTED systematically to guarantee reproducibility of the research

-they conclude that reproducibility of computational genomic studies HAS BEEN CONSIDERED AS A MAJOR ISSUE in recent times

-they identified numerous implicit assumptions interpreted through the practical execution of the workflow which led to recommendations for reproducibility and provenance

https://www.google.com/url?q=https://docs.google.com/document/d/e/2PACX-1vSxLx4BvanrtRtxsJxnxCgkqDMGwf71K2WNkimw3Aa7xNiy3KSKIJ53eaGMTAZZBVAYG1-lJ0e2rVLb/pub&sa=D&source=editors&ust=1629750302819000

&usg=AOvVaw3Loga6K0TR3MJ0X95XNE1v

_____

CONTAMINATION IN GENOMES - A WIDESPREAD PROBLEM:

"Most clinical specimens and tissue culture samples to be used for viral genome sequencing ARE USUALLY CONTAMINATED WITH HUMAN CELLS, OTHER MICROORGANISMS AND NAKED DNA AND RNA FROM DISRUPTED CELLS."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2638583/

When talking about the proof for the existence of "viruses," it is logical to require that the particles believed to be a "virus" are purified (i.e. free of contamination, pollutants, foreign material) and isolated (separated from everything else). Only through the process of purification/isolation of particles believed to be "viruses" can one actually demonstrate that those specific particles exist in reality and are the only possible substance that could potentially be the cause of disease. Unfortunately for Virology, these two logical requirements are never met. "Viruses" are never taken directly from humans and purified free of contamination nor are they ever isolated from everything else. The cell culture process they put the sample through is the exact opposite of purification/isolation as can be seen in the above quote. Without purification/isolation, there is no proof the particles exist as the EM images could be of numerous identical substances and there can be no proof of pathogenicity as any of the toxic ADDITIVES to the culture can produce disease by themselves. This lack of purified/isolated particles leads to many issues including contamination of the supposed genome.

I have included some highlights from a few studies that showcase the widespread problem in regards to the contamination of genomes.

From a July 2020 Nature article:

CONTAMINATION IN SEQUENCE DATABASES

"Biological sequences in public databases are indispensable resources for life science research. DESPITE OUR EVERY DAY RELIANCE ON THESE DATABASES, THERE ARE GAPS, ERRORS AND CONTAMINATION IN THE DATA. “One of our research efforts for the past several years has been the detection of pathogens in humans by using metagenomic shotgun sequencing,” says Martin Steinegger, who was a member in Steven L. Salzberg’s lab at Johns Hopkins University and is now at the Seoul National University. “UNFORTUNATELY, IN MANY CASES WE HAVE FOUND THAT CONTAMINATION WITHIN THE GENOME SEQUENCES PRODUCES FALSE POSITIVES.”

This motivated Steinegger and Salzberg to start a project to assess contamination in the GenBank, RefSeq and NR databases. Using recent fast algorithms, they developed a tool called Conterminator that enables searching for contamination across kingdoms and scales linearly. “The version of GenBank we evaluated had a size of 3.3 terabytes and contained 400 million sequences. Aligning them all-against-all would require hundreds of years using classic methods,” says Steinegger. “Our algorithm only required 12 days to process all of GenBank on a single 32-core server.”

THEY EXPECTED TO SEE A FEW THOUSAND CONTAMINATED SEQUENCES BUT ENDED UP WITH MILLIONS. 2,161,746, 114,035 and 14,148 contaminated sequences were detected in GenBank, RefSeq and NR, respectively. “This single most surprising finding was the presence of a piece of a bacterium, Acidithiobacillus thiooxidans, in an alternative scaffold of the CURRENT VERSION OF THE HUMAN REFERENCE GENOME (GRCh38),” says Steinegger. “THE HUMAN GENOME HAS BEEN AROUND FOR SUCH A LONG TIME, AND SO MANY RESEARCHERS USE IT ON A DAILY BASIS, THAT WE DIDN'T EXPECT TO SEE ANY CONTAMINANTS THERE.”

Steinegger hopes Conterminator can help researchers who sequence genomes and database managers to detect contamination. As a word of caution to users of genome sequences, “MANY OF THE GENOMES CONTAIN CONTAMINATION. ONE PARTICULAR PROBLEM THAT ARISES, AGAIN AND AGAIN, IS THAT CONTAMINATION LEADS TO INCORRECT CLAIMS ABOUT HORIZONTAL GENE TRANSFER,” says Steinegger."

https://www.nature.com/articles/s41592-020-0895-8

In Summary (Part 1):

-despite everyday reliance on these databases, there are GAPS, ERRORS and CONTAMINATION in the data

-"Unfortunately, in many cases we have found that contamination within the genome sequences PRODUCES FALSE POSITIVES"

-they expected to see a few thousand contaminated sequences BUT ENDED UP WITH MILLIONS

-the single most surprising finding was THE PRESENCE OF A PIECE OF A BACTERIUM, Acidithiobacillus thiooxidans, in an alternative scaffold of the CURRENT VERSION OF THE HUMAN REFERENCE GENOME (GRCh38)

-“The human genome has been around for such a long time, and so many researchers use it on a daily basis, that WE DIDN'T EXPECT TO SEE ANY CONTAMINANTS THERE

From a March 2020 study:

CONTAMINANT DNA IN BACTERIAL SEQUENCING EXPERIMENTS IS A MAJOR SOURCE OF FALSE GENETIC VARIABILITY

"CONTAMINANT DNA IS A WELL-KNOWN CONFOUNDING FACTOR IN MOLECULAR BIOLOGY AND IN GENOMIC REPOSITORIES. Strikingly, analysis workflows for whole-genome sequencing (WGS) data COMMONLY DO NOT ACCOUNT FOR ERRORS POTENTIALLY INTRODUCED BY CONTAMINATION, which could lead to the wrong assessment of allele frequency both in basic and clinical research."

"We used a taxonomic filter to remove contaminant reads from more than 4000 bacterial samples from 20 different studies and performed a comprehensive evaluation of the extent and impact of contaminant DNA in WGS. WE FOUND THAT CONTAMINATION IS PERVASIVE AND CAN INTRODUCE LARGE BIASES IN VARIANT ANALYSIS. WE SHOWED THAT THESE BIASES CAN RESULT IN HUNDREDS OF FALSE POSITIVES AND NEGATIVE SNPs, EVEN FOR SAMPLES WITH SLIGHT CONTAMINATION."

"While many factors are taken into account when developing SNP calling pipelines, surprisingly, THE POTENTIAL ROLE OF CONTAMINATION IS SELDOMLY CONSIDERED [13]. However, MISINTERPRETATION OF CONTAMINATED DATA CAN

LEAD TO DRAW INCORRECT CONCLUSIONS ABOUT BIOLOGICAL PHENOMENA [14, 15].

GENOMIC DATABASES ARE KNOWN TO ENCOMPASS CONTAMINATED SEQUENCES, WITH ASSEMBLED GENOMES THAT CAN CONTAIN LARGE GENOMIC REGIONS FROM NON-TARGET ORGANISMS [16, 17]. Strikingly, a recent study revealed that DEPOSITED BACTERIAL AND ARCHAEAL ASSEMBLIES ARE CONTAMINATED BY HUMAN SEQUENCES THAT CREATED THOUSANDS OF SPURIOUS PROTEINS [18]. While the potential impact of contaminants has been considered in fields like metagenomics or transcriptomics, most bacterial WGS analysis pipelines lack specific steps aimed to deal with contaminant data. THIS SITUATION LIKELY ORIGINATES FROM THE ASSUMPTIONS THAT MICROBIOLOGICAL CULTURES ARE MOSTLY FREE OF NON-TARGET ORGANISMS AND THAT EVEN IF PRESENT, CONTAMINANT SEQUENCES ARE UNLIKELY TO MAP TO THE REFERENCE GENOMES OR ARE REMOVED USING STANDARD FILTER CUTOFFS. To date, THE EXTENT OF CONTAMINATION AND ITS IMPACT in bacterial re-sequencing pipelines HAS NOT BEEN COMPREHENSIVELY ASSESSED."

"WE FOUND THAT CONTAMINATION EVENTS ARE FREQUENT ACROSS BACTERIAL WGS STUDIES AND CAN INTRODUCE LARGE BIASES IN VARIANT ANALYSIS DESPITE THE USE OF STRINGENT MAPPING AND VARIANT CALLING CUTOFFS. IMPORTANTLY, THIS IS NOT ONLY TRUE FOR CULTURE-FREE SEQUENCING STRATEGIES, BUT ALSO FOR EXPERIMENTS SEQUENCING FROM PURE CULTURES. We show that THE EFFECT SIZE IS NOT DEPENDENT ON THE AMOUNT OF CONTAMINATION and that SAMPLES WITH EVEN LOW-LEVEL CONTAMINATION CAN ACCUMULATE DOZENS OF ERRORS, particularly for non-fixed SNPs."

"WHEN LOOKING AT THE MTB DATA SET, WE ALSO OBSERVED CONTAMINATION TO BE COMMON ACROSS STUDIES (Fig. 1b). As expected, DIRECT SEQUENCING FROM CLINICAL SPECIMENS and early positive mycobacterial growth indicator tubes (MGIT), which are inoculated with primary clinical samples, PRESENT HIGHER LEVELS OF CONTAMINATION IN TERMS OF BOTH THE NUMBER OF SAMPLES CONTAMINATED AND THE PROPORTION OF NON-TARGET READS WITHIN THEM. Common contaminants for these samples comprise human DNA, and bacteria usually found in oral and respiratory cavities like Pseudomonas, Rothia, Streptococcus, or Actinomyces, AND CAN CONSTITUTE VIRTUALLY ALL READS IN SOME SAMPLES. However, as observed for the bacterial dataset, CONTAMINATION WAS ALSO DETECTED IN STUDIES IN WHICH THE SEQUENCED DNA CAME FROM PURE CULTURE ISOLATES. For instance, Bacillus, Negativicoccus, and Enterococcus

represented up to 68%, 58%, and 32%, respectively, of different samples from the KwaZulu study. Strikingly, 17 out of 73 MTB samples from the Nigeria study were identified as Staphylococcus aureus (92 to 99% of reads). The high-depth dataset was mostly free of contamination, with the exception of two samples for which 3.32% of A. baumannii and 2.83% of non-tuberculous mycobacteria (NTM) were identified (representing 795,887 and 920,379 reads, respectively)."

"Remarkably, EVEN A 5% OF CONTAMINATING READS CAN INTRODUCE A LARGE NUMBER OF FALSE POSITIVE vSNPs. As expected, the erroneous calls produced by such small contamination fall mainly in conserved regions. However, in agreement with the results shown in Fig. 4a, SPURIOUS SNPs CAN BE CALLED ACROSS THE GENOME (Additional file 8: Figure S2)."

"WE SHOW THAT PRESENCE OF SEQUENCING READS FROM CONTAMINATING ORGANISMS IS FREQUENT, EVEN WHEN SEQUENCING IS PERFORMED FROM PURE CULTURE ISOLATES (Fig. 1). Beyond INAPPROPRIATE LABORATORY PRACTICES, there are several potential sources of contamination which depend on different factors such as THE TYPE OF SAMPLE PROCESSED AND ITS ORIGIN, or THE PROTOCOLS FOLLOWED FOR CULTURE, DNA EXTRACTION, AND SEQUENCING. For instance, Salter et al. demonstrated that CONTAMINATING DNA IN LABORATORY REAGENTS CAN CRITICALLY IMPACT MICROBIOME ANALYSIS FROM LOW-BIOMASS SAMPLES [19]. Culture-free sequencing approaches for unculturable or slow-growing pathogens, such as T. pallidum or MTB, ENTAIL THE PRESENCE OF HIGH AMOUNTS OF CONTAMINATING DNA FROM THE HOST ORGANISM. Other sources unrelated to sample handling are also possible. For example, the S. aureus samples supposed to be MTB from the Nigeria study are most likely an ERROR DURING DATA SUBMISSION TO THE GENOMIC REPOSITORY. Regardless of the source of contamination, the shared consequence is the PRESENCE OF NON-TARGET READS IN THE SEQUENCING FILES THAT MIGHT IMPACT THE RESULTS OF GENOMIC ANALYSIS."

"CONTAMINATION HAS BEEN RECOGNIZED AS A MAJOR SOURCE OF ERROR IN GENOME ASSEMBLIES AND OTHER FIELDS LIKE METAGENOMICS [16, 19]. However, the role of contamination in re-sequencing pipelines is USUALLY NEGLECTED. Whereas some groups are already aware of this issue, most bacterial re-sequencing pipelines are STILL LACKING CONTAMINATION-CONTROL STRATEGIES OR, IF ANY, THESE ARE RARELY DETAILED IN PUBLISHED WORKS."

https://bmcbiol.biomedcentral.com/articles/10.1186/s12915-020-0748-z

In Summary (Part 2):

-contaminant DNA is a WELL-KNOWN CONFOUNDING FACTOR in molecular biology and in genomic repositories

-analysis workflows for whole-genome sequencing (WGS) data commonly DO NOT ACCOUNT FOR ERRORS POTENTIALLY INTRODUCED BY CONTAMINATION, which could lead to the wrong assessment of allele frequency both in basic and clinical research

-they found that contamination is PERVASIVE and can INTRODUCE LARGE BIASES IN VARIANT ANALYSIS

-they also showed that these biases can result in HUNDREDS OF FALSE POSITIVE AND NEGATIVE SNPs, even for samples with SLIGHT CONTAMINATION

-they admit the potential role of contamination is seldomly considered

-misinterpretation of contaminated data can lead to drawing INCORRECT CONCLUSIONS about biological phenomena

-genomic databases ARE KNOWN TO ENCOMPASS CONTAMINATED SEQUENCES, with assembled genomes that can contain large genomic regions from non-target organisms

-a recent study revealed that deposited bacterial and archaeal assemblies are CONTAMINATED BY HUMAN SEQUENCES that created thousands of spurious proteins

-they believe that this situation likely originates from the ASSUMPTIONS that microbiological cultures are mostly free of non-target organisms and that even if present, contaminant sequences are unlikely to map to the reference genomes or are removed using standard filter cutoffs

-to date, the EXTENT OF CONTAMINATION AND ITS IMPACT in bacterial re-sequencing pipelines HAS NOT BEEN COMPREHENSIVELY ASSESSED

-they found that CONTAMINATION EVENTS ARE FREQUENT across bacterial WGS studies and CAN INTRODUCE LARGE BIASES in variant analysis despite the use of stringent mapping and variant calling cutoffs

-they state that this is not only true for culture-free sequencing strategies, but also for experiments sequencing from PURE CULTURES

-the effect size is NOT DEPENDENT on the amount of contamination and that SAMPLES WITH EVEN LOW-LEVEL CONTAMINATION can accumulate dozens of

errors, particularly for non-fixed SNPs

-when looking at the MTB dataset, they also observed CONTAMINATION TO BE COMMON ACROSS STUDIES

DIRECT SEQUENCING FROM CLINICAL SPECIMENS and early positive mycobacterial growth indicator tubes (MGIT), which are inoculated with primary clinical samples, PRESENT HIGHER LEVELS OF CONTAMINATION in terms of both the number of samples contaminated and the proportion of non-target reads within them

-Common contaminants for these samples:

1. Human DNA

2. Bacteria usually found in oral and respiratory cavities such as:

*Pseudomonas

*Rothia

*Streptococcus

*Actinomyces

-these common contaminants can constitute VIRTUALLY ALL ROADS in some samples

-as observed for the bacterial dataset, contamination was also detected in studies in which the sequenced DNA CAME FROM PURE CULTURE ISOLATES

-even a 5% of contaminating reads can introduce a large number of false positive vSNPs

-spurious SNPs can be called across the genome

-they conclude that they have shown that presence of sequencing reads from contaminating organisms is FREQUENT, EVEN WHEN SEQUENCING IS PERFORMED FROM PURE CULTURE ISOLATES

-Reasons for Contamination:

1. Inappropriate laboratory practices

2. Type of sample processed and its origin

3. The protocols followed for culture, DNA extraction, and sequencing

4. Error during data submission to the genomic repository

-CONTAMINATING DNA IN LABORATORY REAGENTS can critically impact microbiome analysis from low-biomass samples

-culture-free sequencing approaches for unculturable or slow-growing pathogens, such as T. pallidum or MTB, entail the presence of HIGH AMOUNTS OF CONTAMINATING DNA FROM THE HOST ORGANISM

-the consequences of contamination is the PRESENCE OF NON-TARGET READS in the sequencing files that might impact the results of genomic analysis

-contamination has been recognized as a MAJOR SOURCE OF ERROR in genome assemblies and other fields like metagenomics

-the role of contamination in re-sequencing pipelines is usually NEGLECTED

-they are still lacking CONTAMINATION-CONTROL STRATEGIES or, if any, these are rarely detailed in published works

From a February 2020 study:

PREVALENCE AND IMPLICATIONS OF CONTAMINATION IN PUBLIC GENOMICS RESOURCES: A CASE STUDY OF 43 REFERENCE ARTHROPOD ASSEMBLIES

"ERRORS DUE TO CONTAMINATION ARE PARTICULARLY WORRYING; THEY ARE WIDESPREAD, PROPAGATE ACROSS DATABASES, AND CAN COMPROMISE DOWNSTREAM ANALYSES, especially the detection of horizontally-transferred sequences. However WE STILL LACK CONSISTENT AND COMPREHENSIVE ASSESSMENTS OF CONTAMINATION PREVALENCE IN PUBLIC GENOMIC DATA."

"SCIENTISTS TYPICALLY RE-USE SEQUENCE DATA GENERATED BY OTHERS, AND ARE THEREFORE DEPENDENT ON THE RELIABILITY OF THE AVAILABLE GENOMIC RESOURCES. For this reason, the problem of public data quality in molecular biology has long been identified as a crucial issue (Lamperti et al. 1992; Mistry et al. 1993; Binns 1993). THE PROBLEM IS EVEN MORE ACUTE NOWADAYS WITH THE ADVENT OF HIGH-THROUGHPUT SEQUENCING TECHNOLOGIES,

WHEN MOST DATASETS GENERATED IN GENOMIC RESEARCH ARE SIMPLY NOT AMENABLE TO MANUAL CURATION BY HUMANS. This brings a new challenge to current methodologies in genomic sciences, namely, the development of automated approaches to the detection and processing of errors (e.g., Andorf et al. 2007; Schmieder and Edwards 2011; Parks et al. 2015; Delmont and Eren 2016; Drăgan et al. 2016; Tennessen et al. 2016; Laetsch and Blaxter 2017; Lee et al. 2017).

DATA QUALITY ISSUES IN GENOME SEQUENCES INCLUDE SEQUENCING ERRORS, ASSEMBLY ERRORS AND CONTAMINATION, AMONG OTHER THINGS. Errors due to contamination are particularly worrying for several reasons. First, THEY CAN LEAD TO SERIOUS MIS-INTERPRETATIONS OF THE DATA, as illustrated by recent, spectacular examples. POTENTIAL PROBLEMS INCLUDE MIS-CHARACTERIZATION OF GENE CONTENT AND RELATED METABOLIC FUNCTIONS (e.g., Koutsovoulos et al. 2016; Breitwieser et al. 2019), IMPROPER INFERENCE OF EVOLUTIONARY EVENTS (e.g., Laurin-Lemay et al. 2012; Simion et al. 2018), AND BIASES IN GENOTYPE CALLING AND POPULATION GENOMIC ANALYSES (e.g., Ballenghien et al. 2017; Wilson et al. 2018). Second, CONTAMINATION IS SUSPECTED TO BE WIDESPREAD. IT OCCURS NATURALLY IN MOST SEQUENCING PROJECTS DUE TO FOREIGN DNA INITIALLY PRESENT IN THE RAW BIOLOGICAL MATERIAL (e.g., symbionts, parasites, ingested food; Salzberg et al. 2005; Starcevic et al. 2008; Artamonova and Mushegian 2013; Driscoll et al. 2013; Martinson et al. 2014; Cornet et al. 2018), or entering the process in wet labs and sequencing centers (Longo et al. 2011; Salter et al. 2014; Wilson et al. 2018). Third, CONTAMINATION ERRORS EASILY PROPAGATE ACROSS DATABASES IN A SELF-REINFORCING VICIOUS CIRCLE. If a DNA sequence from species A is initially assigned to the wrong species B due to a contamination of B by A, it is likely to keep its incorrect status for a while, and may even be identified as a contamination of A by B when the genome of A is eventually sequenced (Merchant et al. 2014). Despite all the possible problems stemming from contamination in genomic resources, most studies addressing this issue so far have focused on one particular genome (e.g., tardigrades) and/or one particular source of contaminants (e.g., humans). Only two studies that we are aware of have consistently screened more than one genome assembly. Merchant et al. (2014) focused on the bovine genome but also applied their pipeline to eight randomly drawn draft genomes (five animals, two plants, one fungus), with contrasted results. Cornet et al. (2018) analyzed 440 genomes of Cyanobacteria and uncovered a substantial level of contamination in >5% of these. THERE IS OBVIOUSLY A NEED FOR FURTHER ASSESSMENT OF THE PROBLEM OF CONTAMINATION IN PUBLICLY AVAILABLE GENOMIC DATA."

https://academic.oup.com/g3journal/article/10/2/721/6026299

In Summary (Part 3):

-ERRORS DUE TO CONTAMINATION are particularly worrying; they are WIDESPREAD, PROPAGATE ACROSS DATABASES, and can COMPROMISE DOWNSTREAM ANALYSES, especially the detection of horizontally-transferred sequences

-still LACKING CONSISTENT AND COMPREHENSIVE ASSESSMENTS OF CONTAMINATION PREVALENCE in public genomic data

-scientists typically re-use sequence data generated by others, and are therefore DEPENDENT ON THE RELIABILITY OF THE AVAILABLE GENOMIC RESOURCES

-the problem is even more acute nowadays with the advent of high-throughput sequencing technologies, when most datasets generated in genomic research are SIMPLY NOT AMENABLE TO MANUAL CURATION BY HUMANS

-data quality issues in genome sequences include sequencing errors, assembly errors and contamination, among other things

-contamination can lead to serious mis-interpretations of the data

-Potential problems include:

1. Mis-characterization of gene content and related metabolic functions
2. Improper inference of evolutionary events
3. Biases in genotype calling and population genomic analyses

-contamination is suspected to be WIDESPREAD

-it occurs NATURALLY in most sequencing projects due to FOREIGN DNA

-contamination errors EASILY PROPAGATE ACROSS DATABASES in a self-reinforcing vicious circle

-they conclude that there is obviously A NEED FOR FURTHER ASSESSMENT of the problem of contamination in publicly available genomic data

Anyone claiming that the existence of a genome is proof of a purified/isolated "virus" is completely mistaken. The contamination of genomes is admittedly a widespread problem and one that is only getting worse. The problem of REPRODUCIBILITY is

another crisis in Genomics. Both contamination and the lack of reproducibility affect the initial step of cell culturing as well. For anyone wondering why there are over 2 million variants (and counting) for "SARS-COV-2," look no further than the highly CONTAMINATED processes used to culture the "virus" and sequence the genome.

https://docs.google.com/document/d/e/2PACX-1vQdAHg0zAxrAYN5lCvcgnvaL8MZ6kUbuWqctNWqIJwh_eWnTCb5x1aGOef6Y3hy0jhYGZWHgbfFugEY/pub

For the alignment of a virus, virologists always need a given genetic strand of a virus. For this, however, they always use **a genetically / computationally** generated genetic strand and never a real one, one found in reality. In doing so, they never attempt to check whether or not so-called genetic information could also be constructed from the existing data set, including "viral" genetic material strands of completely different viruses.

Illustration:

Bioinformatics is nebulous. Bioinformaticians do not check whether the results of software processing are found in reality. What Bioinformaticians do is check something that doesn't exist in reality with something that doesn't exist in reality. Virology + Bioinformatics = Zero Science.

Illustration: picture number 3

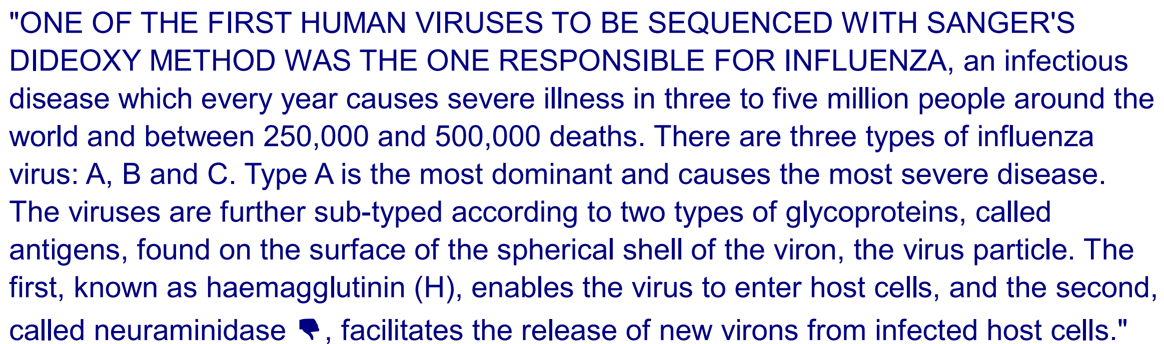
https://t.co/U431QN8Xqm?amp=1

HOW ACCURATE IS THE INFLUENZA GENOME?:

In the 1980's, the new Sanger Dideoxy method was used to sequence "viruses" in an attempt to add further INDIRECT evidence that these invisible invaders are real. Influenza was one of the first "viruses" lucky enough to get this treatment. After going through the supposed "isolations" (or lack thereof) of Influenza A, B, C and the 1976 Swine Flu, I wanted to see just how the Influenza genomes were created knowing that they would be without purified/isolated "viruses" coming directly from sick patients.

Below is what I could uncover:

SEQUENCING THE GENOME OF THE INFLUENZA VIRUS



"ONE OF THE FIRST HUMAN VIRUSES TO BE SEQUENCED WITH SANGER'S DIDEOXY METHOD WAS THE ONE RESPONSIBLE FOR INFLUENZA, an infectious disease which every year causes severe illness in three to five million people around the world and between 250,000 and 500,000 deaths. There are three types of influenza virus: A, B and C. Type A is the most dominant and causes the most severe disease. The viruses are further sub-typed according to two types of glycoproteins, called antigens, found on the surface of the spherical shell of the viron, the virus particle. The first, known as haemagglutinin (H), enables the virus to enter host cells, and the second, called neuraminidase 👎, facilitates the release of new virons from infected host cells."

"The first project to sequence the influenza virus was launched in the late 1970s by George Brownlee, Sanger's former doctoral student. It focused on the H1 subtype of the human influenza A strain. One of the aims in sequencing the virus was to understand the genetic mechanism that underlies the outbreak of new influenza pandemics. THE INFLUENZA GENOME IS UNUSUAL IN THAT IT IS MADE UP OF SEVERAL RNA SEGMENTS RATHER THAN ONE CONTINUOUS GENOME. The H1A virus was known to contain eight single-stranded RNA segments, totalling 14,000 nucleotides. It was these segments Brownlee's team set out to sequence. A key objective of the project was

to establish the degree to which the exchange of RNA segments, which naturally occurs between different strains of influenza, contribute to the initiation of new influenza pandemics. They wanted to find out whether this simple segment exchange was the main mechanism facilitating the emergence of new pandemic strains)."

"Soon after launching the influenza project, Brownlee spent a year in Australia where he began testing the Maxam-Gilbert method for sequencing the virus. Winter and Fields decided, however, to continue using the dideoxy method then being promoted by Sanger and his team within the LMB. Still rather new, THE DIDEOXY TECHNIQUE PROVED RATHER TEMPERAMENTAL INITIALLY. As Winter recalled, it worked 'beautifully on some days' AND THEN COLLAPSED SUDDENLY FOR NO OBVIOUS REASON. This could have been caused by any number of factors, such as a batch of enzymes going off OR IMPURITIES IN THE DNA. SOMETIMES THE METHOD FAILED TO WORK FOR WEEKS AT A TIME. Nor were Winter and Fields the only ones to face this problem, RESEARCHERS IN SANGER'S LABORATORY ENCOUNTERED SIMILAR DIFFICULTIES. Whenever the system collapsed, Winter remembered everyone went 'around trying each other's batch of enzymes, RUSHING FROM ONE CONCLUSION TO ANOTHER. The minute anyone got it working we would watch what it was that they were doing different[ly].' As he pointed out, in fact everyone was doing the same thing, and IT WAS MORE A QUESTION OF 'PSYCHOLOGY THAN ANYTHING [ELSE], TRYING TO OUTGUESS WHAT TYPE OF JUJU [HAD] BEEN PUT ON YOUR WORK'S. Over time, however, the method began to work more consistently, aided by improvements in the quality of enzyme batches (Winter, 2011).

INITIALLY, THE TEAM ATTEMPTED TO SEQUENCE THE RNA DIRECTLY, BUT THEY SOON SWITCHED TO CLONING THE RNA IN THE BACTERIOPHAGE M13, and then sequencing the product with the dideoxy technique used by Sanger and his team for sequencing the human mitochondrial genome. By 1981 the team had successfully SEQUENCED THE GENE in the influenza virus type A that coded for the neuraminidase protein found on its surface. The gene was 1,413 nucleotides long (Fields, Winter, Brownlee, 1981).

The genome segment sequenced by Brownlee’s group was a major achievement. It was the first complete sequence of the neuraminidase gene of the influenza virus, and the encoded protein later became the target for development of drugs such as Relenza used to treat influenza infections. HOWEVER, THE NEURAMINIDASE SEGMENT REPRESENTED ONLY ONE OF THE EIGHT SEGMENTS OF THE COMPLETE GENOME OF THE HUMAN INFLUENZA A VIRUS, which totals about 14,000 nucleotides. In due course Brownlee, Winter and Fields completed the sequence of all segments of the same strain and thereby the genome of this strain. Further work largely

focused on sequencing short fragments from the haemagglutinin or neuraminidase segments from different strains which were thought to play a role in its antigenic variation. LITTLE OF THIS WORK PROVIDED COMPLETE SEQUENCES OF THE VIRUS, HOWEVER. IN PART THIS REFLECTED THE TECHNICAL DIFFICULTY OF DEVELOPING AN EFFICIENT SEQUENCING PIPELINE FOR THE RNA BASED ORGANISM. This situation changed with the setting up of the Influenza Genome Project (IGP) in 2005 by an international consortium of scientists with funding from the U.S. National Institutes of Health. The IGP continues to this day, and sequences and analyses many different types of influenza viruses. By 2015 the IGP had sequenced over 6,000 genomes for human influenza virus A, and just under 2,000 for the human virus B. The sequencing data is being used to understand the rate of mutation underlying the evolution of the virus and to monitor the effectiveness of vaccines. (Ghedin, E, Sengamalay, Shumway et al, 2005; J Craig Venter Institute)."

https://www.whatisbiotechnology.org/index.php/exhibitions/sanger/sequencing

In Summary (Part 1):

influenza was one of the first "viruses" sequenced by Sanger's dideoxy method

-the influenza genome is unusual in that it is MADE UP of several RNA segments rather than one continuous genome

-the dideoxy technique proved rather temperamental initially

-it would collapse suddenly for no obvious reason

-problems arose from IMPURITIES in the DNA

-sometimes the method failed to work for weeks at a time

-researchers in Sanger's Laboratory encountered similar difficulties

-whenever the system collapsed, they would rush from one conclusion to another

-Winter felt it was "more a QUESTION OF 'PSYCHOLOGY than anything [else], trying to outguess WHAT TYPE OF JUJU [had] been put on your work'

-initially, the team attempted to sequence the RNA directly, but they soon SWITCHED TO CLONING THE RNA IN THE BACTERIOPHAGE M13

-by 1981 the team had sequenced THE GENE in the influenza "virus" type A that coded

for the neuraminidase protein found on "its" surface

-however, the neuraminidase segment represented ONLY ONE OF THE EIGHT SEGMENTS of the complete genome of the human influenza A "virus," which totals about 14,000 nucleotides (yet without sequencing the complete genome, how would they know this..?😲)

-little of this work provided complete sequences of the "virus,"

-in part this reflected the technical difficulty of developing an efficient sequencing pipeline for the RNA based organism

To start off with, looking at just one of their papers for the sequencing of the haemagglutinin gene (https://doi.org/10.1038/292072a0) shows the "isolate" Brownlee and Co. were using was given to them by M.A. Robertson and was the A/PR/8/34 "virus" first "discovered" by Thomas Francis Jr. in 1934. As previously discussed, this was not a purified/isolated "virus" and consisted of unpurified serially passaged ground up lung/nasal tissues injected into ferrets/mice:

https://docs.google.com/document/d/e/2PACX-1vSBvLcEQgr-kchptgHBm6rPBFg1ETMd4L4mG3-b4DiDavR3kR0NmesH7ptZhNCcu6vkdwgPuw1nHvc1/pub

Unfortunately, I could not copy/paste the relevant passages from the study but included them in the images.

Secondly, disregarding that they were able to somehow DEDUCE that the influenza "virus" is made up of 8 single RNA segments rather than one continuous genome without ever having sequenced the genome to begin with, it's apparent that the team sequencing the small RNA segments of the influenza "virus" ran into many problems with Sanger's Dideoxy method. With this being very new technology at the time, it would be safe to question the accuracy of anything stemming from it, especially as they seemed unsure about the many pitfalls they kept running into during their research and seemed to believe it amounted to "bad Juju." Fortunately, with the gift of time, we can elucidate many of these problems they most likely would have encountered:

"Sanger sequencing has a NUMBER OF LIMITATIONS that can lead to problems with results and difficulty using the method in general:

Sanger methods can only sequence short pieces of DNA--about 300 to 1000 base pairs.

THE QUALITY OF A SANGER SEQUENCE IS OFTEN NOT VERY GOOD in the first 15 to 40 bases because that is where the primer binds.

SEQUENCE QUALITY DEGRADES after 700 to 900 bases.

If the DNA fragment being sequenced has been cloned, SOME OF THE CLONING VECTOR SEQUENCE MAY FIND ITS WAY INTO THE FINAL SEQUENCE."

https://www.news-medical.net/amp/life-sciences/Challenges-with-Sanger-Sequencing.aspx

"In some cases, chromatograms resulting from a Sanger sequencing can present some problems, concerning the following: 1) the lack of sequence data due to the absence of the priming site, degraded primers, inefficient primer binding, INSUFFICIENT AMOUNT OF DNA TEMPLATE, DEGRADED DNA, and INHIBITORY CONTAMINANT IN THE SAMPLE (i.e., salts, phenol, EDTA, and ethanol); 2) low peaks throughout because of an insufficient amount of DNA template or INHIBITORY CONTAMINANT IN THE SAMPLES (i.e., salts, phenol, EDTA, and ethanol), insufficient amount of primer, and inefficient primer binding; 3) poor sequence at the start followed by weak signal attributed to self-complementarity of the primers; 4) overlapping peaks in the sequence data because of multiple priming sites, residual primers, POOR PURIFICATION DURING PRIMER SYNTHESIS, mixed plasmid prep, and insertion or a deletion in PCR product; 5) sequence starting well but signal weakening gradually because of the formation of secondary structures or too much template; 6) overlapping peaks following a stretch of mononucleotide sequence attributed to the enzyme slippage giving varying lengths of the same sequence following this region; and 7) ARTIFACTS WITH LARGE PEAKS OBSCURING THE REAL SEQUENCE due to dye blogs caused by unincorporated dye.4"

"Many sequencing problems have been already described, and answers have been proposed for each of them, including the lack of sequence data, low peaks, poor sequence, weak signal, overlapping peaks, signal weakening, and artifacts with large peaks obscuring the real sequence."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4502656/

In Summary (Part 2):

-quality of the sequence is not very good

-the sequence quality degrades

-cloned sequences (as in the original Influenza gene sequences) may find their way into final sequence

-lack of sequence data

-low peaks

-poor sequences

-weak signal

-overlapping peaks

-signal weakening

-artifacts with large peaks obscuring the real sequence

It seems safe to say that there should be questions as to the accuracy of these 8 short sequences that were glued together in a computer database to create a whole influenza "virus." This is setting aside that there is no purified/isolated "viruses" that they are sequencing from but either cell cultured supernatant from the unpurified A/PR/8/34 strain or cloned RNA from bacteriophages. In any case, it was admitted that even the "whole" genome coming from these experiments in the 80's and 90's were lacking so the Influenza Genome Project took a stab at rectifying this in 2005:

"The research was done by a large team, with Elodie Ghedin of TIGR listed as the first author. They published their report the same day other teams reported on the re-creation of the deadly 1918 pandemic flu virus and a finding that the 1918 virus closely resembled avian flu viruses.

Ghedin and colleagues say that until now, scientists had fully mapped and published the genomes OF ONLY A FEW STRAINS of human flu viruses. MOST OF THE PUBLISHED DATA PERTAIN TO SHORT FRAGMENTS OF THE GENES FOR THE

VIRUS'S TWO KEY SURFACES PROTEINS, hemagglutinin and neuraminidase.

"As a result of this project, the number of complete human H3N2 influenza virus genomes in GenBank [a public online database] HAS ALREADY GROWN FROM JUST SEVEN GENOMES to over 200," the article says.

The samples analyzed include 207 H3N2 viruses and two H1N2 isolates, which were gathered in New York state over five flu seasons, from 1998-99 through 2003-04. "The sequenced strains were not preselected because of their virulence or unusual characteristics, giving researchers an unbiased view of flu virus evolution as it moved through a varied human population," the NIAID statement said.

THE VIRUSES WERE SURPRISINGLY VARIED. "Even within a geographically constrained set of isolates, we have found SURPRISING GENETIC DIVERSITY, indicating that the reservoir of influenza A strains in the human population—and the concomitant potential for segment exchange between strains—may be greater than was previously suspected," the researchers write."

https://www.cidrap.umn.edu/news-perspective/2005/10/researchers-map-209-flu-virus-genomes

From their 2005 study:

LARGE-SCALE SEQUENCING OF HUMAN INFLUENZA REVEALS THE DYNAMIC NATURE OF VIRAL GENOME EVOLUTION

"All samples for this study were collected by the Virus Reference and Surveillance Laboratory of the Wadsworth Center in Albany, New York, which maintains a repository of human influenza samples dating back to 1992. Virus samples were received as part of outbreak investigations, through the reference function of the laboratory, and, since 2001, as part of a sentinel physician influenza programme."

"Viral RNA isolation

ISOLATES WERE AMPLIFIED IN TUBE CULTURES OF PRIMARY RHESUS MONKEY KIDNEY (pRhMK) CELLS before extracting 140 µl of culture supernatant."

"RNA ligation

RNA was circularized overnight at 4 °C with T4 RNA ligase (Epicentre). Before the ligation step, the RNA was FIRST TREATED WITH TOBACCO ACID PYROPHOSPHATASE (20 U TAP in a 15-μl reaction, incubated at 37 °C for 1 h). TAP treatment is usually used to remove molecules from the 5′ end of RNA, mostly plus-strand RNA. ALTHOUGH NO SUCH MOLECULES ARE EXPECTED TO BE PRESENT ON THE INFLUENZA GENOMIC RNA SEGMENTS, LIGATION WAS MORE EFFICIENT WITH THIS TREATMENT THAN WITHOUT. The circularized RNA was cleaned again with the RNeasy Mini kit (Qiagen)."

"For most of the segments, ALL FULL-LENGTH AND NEARLY FULL-LENGTH SEQUENCES FROM 1980 TO THE PRESENT WERE ALIGNED AND USED FOR PRIMER DESIGN. For others, more stringent criteria were used in order to reduce the number of sequences in the set to a more manageable number."

https://www.nature.com/articles/nature04239#MOESM1

From the Supplementary Material:

"When designing the high-throughput sequencing pipeline for the eight RNA molecules that comprise the influenza virus, we strove to create a method that would be robust, consistent, and flexible. We needed to minimize the number of finishing steps required to obtain full genomic sequences, TO AUTOMATE AS MANY STEPS AS POSSIBLE, and to accommodate changes in primer design and protocols that might arise later."

"First, TRIMMING THE NON-INFLUENZA SEQUENCE FROM EACH SEQUENCE "READ" WAS A CRITICAL STEP. IN ADDITION TO THE NORMAL M13 TAGS, SEQUENCE FROM THE DEGENERATE PRIMERS MUST ALSO BE TRIMMED. This is important because if an amplification primer contains a base that does not match the sequence of the isolate being amplified, AN INCORRECT BASE COULD BE INCORPORATED INTO THE PCR PRODUCT AND SUBSEQUENTLY SEQUENCED."

"ONE NOVEL ELEMENT OF THE ALGORITHM IS THAT WE WERE ABLE TO USE A REFERENCE GENOME AS A GUIDE TO ASSEMBLY, which allows the assembler to tolerate much shorter overlap between reads than normal. Thus, reads that overlapped by only 1-2 bases could be successfully assembled together. FURTHER AUTOMATION WAS ACCOMPLISHED BY THE AutoEditor program2, WHICH USES THE ALIGNED READS TO CORRECT ERRORS MADE BY THE BASECALLING SOFTWARE.This program CORRECTS APPROXIMATELY 80% OF THE MIS-CALLS that otherwise would have to be reviewed by a human editor. Following AutoEditor, all genomes went

through at least one round of manual review by human editors before being submitted to GenBank."

"Sequence editing. AFTER THE EIGHT SEGMENTS OF AN ISOLATE WERE ASSEMBLED INDIVIDUALLY, THEY WERE MANUALLY EDITED USING CloE (Closure Editor), A TIGR PROGRAM FOR EDITING ASSEMBLIES. The editors checked all apparent polymorphisms AGAINST REFERENCE DATA and REPAIRED FRAME SHIFTS AND OTHER SEQUENCING ERRORS whenever they were discovered. After editing, each isolate was submitted to a validation program, which checked segment length, ALIGNMENTS WITH REFERENCE SEQUENCES, and fidelity of reading frames."

In Summary (Part 3):

-only a few strains were said to have been mapped and had genomes published by 2005 and most of this pertained to the two short fragments of hemagglutinin and neuraminidase

-from this one study, they claim they went from just 7 genomes to over 200

-they were surprised by the variations in the genomes (in other words, not identical)

-they found surprising GENETIC DIVERSITY (again, not identical)

-isolates were amplified in tube CULTURES OF PRIMARY RHESUS MONKEY KIDNEY (pRhMK) CELLS before extracting 140 µl of culture supernatant

-RNA was first treated with TOBACCO ACID PYROPHOSPHATASE (20 U TAP in a 15-µl reaction, incubated at 37 °C for 1 h). TAP treatment is usually used to remove molecules from the 5′ end of RNA, mostly plus-strand RNA

-they state that although NO SUCH MOLECULES ARE EXPECTED TO BE PRESENT on the influenza genomic RNA segments, ligation was more efficient with this treatment than without

-in other words, they didn't need to chemically treat the RNA with Tobacco Acid Pyrophosphatase but it made their job easier so why not...

-they created a method to AUTOMATE as many steps as possible

-trimming the NON-INFLUENZA SEQUENCE from each sequence “read” was a critical step

-in other words, "non-influenza" sequences reiterates that these are not a purified nor

isolated

-in addition to the normal M13 tags (from the bacteriophages used for cloning), sequence from the degenerate primers also needed to be trimmed

-degenerate primers are a PCR primer sequence where some of its positions have several possible bases

https://pubmed.ncbi.nlm.nih.gov/15882141/

-if this was not done, an incorrect base could be incorporated into the PCR product and subsequently sequenced

-the algorithm that they used allowed a REFERENCE GENOME as a guide to ASSEMBLY

-in other words, these new genomes are only as accurate as the reference genome...of which they stated only a few existed and contained data mostly from the two short fragments of hemagglutinin and neuraminidase

-after the eight segments of an "isolate" were ASSEMBLED INDIVIDUALLY, they were MANUALLY EDITED using CloE (Closure Editor), a TIGR program for editing assemblies

-the editors checked all apparent polymorphisms AGAINST REFERENCE DATA and repaired frame shifts and other SEQUENCING ERRORS whenever they were discovered

-after editing, each "isolate" was submitted to a validation program, which checked segment length, ALIGNMENTS WITH REFERENCE SEQUENCES, and fidelity of reading frames

Well, what could possibly go wrong when using cell-cultured clones of "virus" isolates which contained non-influenza sequences which were either computationally or manually edited/corrected/fixed/etc. against reference genomes from the 80's created from the Thomas Francis Jr. "discovered" A/PR/8/34 strain of ground up lung/nasal turbinates passed between ferrets/mice/chick embryos?

I'm sure it's all highly accurate, minus all the questionable accuracy issues of the Sanger Dideoxy method...

Just to highlight the incompleteness of the 2005 influenza genomes, I present some passages from this Nature article from 2007:

THE EVOLUTION OF EPIDEMIC INFLUENZA

"MAJOR GAPS IN CURRENT GENOME SEQUENCE DATA include virus samples from tropical regions, from transmission chains, and FROM WITHIN INDIVIDUAL HOSTS."

"It is also striking that, despite the huge amount of sequence data that has been generated for influenza A virus, STUDIES OF INTRA-HOST GENETIC VARIATION ARE LARGELY ABSENT. However, the high rates of mutation and replication that are common to most RNA viruses mean that intra-host population diversity is likely to be extensive, even in viruses that cause acute infections 84. Furthermore, if the population bottleneck at inter-host transmission is not particularly severe, multiple viral lineages, including reassortants, viruses with new antigenic characteristics or even defective viruses 85, are likely to be transmitted among hosts. A crucial task for future studies in influenza virus evolution is therefore TO QUANTIFY THE EXTENT OF INTRA-HOST GENETIC VARIATION WITHIN SINGLE INDIVIDUALS to determine whether this includes isolates that are antigenically distinct, and reveal how much genetic diversity is transmitted among hosts and how this might differ among avian and mammalian influenza viruses."

https://www.nature.com/articles/nrg2053

And from the CDC in 2018:

"FOR DECADES, SCIENTISTS WHO WANTED TO RESEARCH THE GENOME OF RNA VIRUSES, SUCH AS INFLUENZA, HAD TO DO SO USING AN INDIRECT AND TIME-CONSUMING METHOD THAT INVOLVED FIRST CONVERTING THE SINGLE-STRANDED RNA INTO DOUBLE-STRANDED DNA. This method, often referred to as “reverse transcription polymerase chain reaction” (RT-PCR), works well for clinical purposes, such as identifying specific viruses from respiratory samples taken from sick patients. However, SCIENTISTS BELIEVE THAT CERTAIN SMALL FEATURES OF THE VIRUS MAY GET LOST DURING THE CONVERSION FROM RNA TO DNA.

The new method described in this study has the potential to allow researchers to decode the genome of an RNA virus with greater detail (AND LESS DISTORTION) than ever before. For example, compare an original photograph to a copy of the same photograph. THE COPY WILL GIVE YOU A PRETTY GOOD IDEA OF THE ORIGINAL (THE SAME CAN BE SAID OF RT-PCR), BUT THE COPY MAY LACK THE RESOLUTION AND GRANULARITY OF ALL THE DETAILS FOUND IN THE ORIGINAL

PHOTO."

https://www.cdc.gov/flu/spotlights/scientists-sequence-entire-rna-genomes.htm

In Summary (Part 4):

-MAJOR GAPS IN CURRENT GENOME SEQUENCE DATA include virus samples from tropical regions, from transmission chains, and FROM WITHIN INDIVIDUAL HOSTS

-for decades, scientists who wanted to research the genome of RNA "viruses," such as influenza, had to do so using an INDIRECT and time-consuming method that involved first converting the single-stranded RNA into double-stranded DNA

-scientists believe that CERTAIN SMALL FEATURES OF THE "VIRUS" MAY GET LOST during the conversion from RNA to DNA

-the copy will give you a pretty good idea of the original (the same can be said of RT-PCR), but the copy MAY LACK THE RESOLUTION AND GRANULARITY OF ALL THE DETAILS FOUND IN THE ORIGINAL PHOTO

So is there an accurate genome for the influenza "virus?" Without proper purification/isolation of the particles assumed to be the influenza "virus," it would seem impossible to know where the RNA is coming from and how it should be put together. A genome is only as good as it's reference genome. And the reference genome is dependent on a properly purified/isolated "virus." Without this, there can be no claim to an accurate genome and every variant/mutation/antigenic drift/shift is just excuses for why they are unable to sequence the same "virus" every time.

https://docs.google.com/document/d/e/2PACX-1vRzyM5U4QOtmyc314HzG8p2vcRWwLuUU8fXavymB4FwXKsExZUOEnneMmQywAQ1K4M1jDDLYyUANbpA/pub

THE CHALLENGES RELATED TO RNA EXTRACTION FOR GENOME SEQUENCING:

The critical first step to generate a genome after cell culturing is to extract the RNA from the mixture. The purpose of this is to break down and isolate RNA from any other cellular components and impurities that are within the culture supernatant. Through this process, they are not separating whole "viral" particles from everything else but breaking down the RNA in order to establish a DNA library for genome sequencing.

Many get confused when reading virology papers that when they state that they purified RNA that this means that the "virus" was properly purified/isolated. Do not get confused with the use of the word purification as they are only speaking in terms of purifying RNA from various sources, not "viral" particles. Even then, this "purified" RNA can be in a degraded form and/or full of contaminants.

"OBTAINING HIGH-QUALITY RNA IS THE FIRST, AND OFTEN THE MOST CRITICAL, STEP IN PERFORMING MANY MOLECULAR TECHNIQUES such as reverse transcription real-time PCR (RT-qPCR), transcriptome analysis using next-generation sequencing, array analysis, digital PCR, northern analysis, and cDNA library construction. To generate the most sensitive and biologically relevant results, the RNA isolation procedure must include some important steps before, during, and after the actual RNA purification."

https://www.thermofisher.com/us/en/home/references/ambion-tech-support/rna-isolation/general-articles/the-basics-rna-isolation.html

The extraction of RNA is done through various methods and it must be done quickly and carefully as RNA is not as stable as DNA and can degrade rather easily. The 3 main methods used are outlined below along with their pros and cons:

THE TOP PROS AND CONS OF DIFFERENT RNA EXTRACTION METHODS

"Isolating high-quality RNA is the most critical step for successfully performing a broad range of assays, from RT-qPCR or microarray analysis to cDNA library preparation, as well as Northern blot studies. It is even critical for high-throughput transcriptome analysis using next-generation sequencing techniques.

Therefore, getting the most from your RNA isolation procedure is a must. HIGH-QUALITY EXPERIMENTS REQUIRE HIGH-QUALITY SAMPLES, AND MAXIMIZING YIELD OF NON-DEGRADED RNA ISOLATION IS KEY. In this article, we will discuss three of the most common RNA extraction techniques and go over the pros and cons for

each strategy.

THE ORGANIC EXTRACTION METHOD

Organic extraction of nucleic acids is historically the most common, tried-and-true method for RNA isolation and removing cellular proteins. This technique requires homogenization of your sample in a phenol-containing solution (usually phenol-chloroform). The phenol-chloroform mixture is immiscible with water, therefore when centrifuged, the samples form two distinct phases.

The lower (organic) phase and phase interface contain denatured proteins, while the less-dense upper (aqueous) phase contains nucleic acids. Importantly, the phase extraction of DNA and RNA is pH-dependent, when the pH is greater than 7.0, both RNA and DNA will resolve in the aqueous phase. A pH less than 7.0, DNA more readily denatures and precipitates into the organic phase and phase interface, with RNA remaining in the aqueous phase.

The aqueous phase containing your RNA is then carefully removed by pipetting (WITH CARE NOT TO TOUCH THE INTERFACE OR ORGANIC PHASE, AS THIS CAN CONTAMINATE YOUR SAMPLE) and RNA is then precipitated with alcohol and rehydrated for further analysis.

THE PROS:

Organic extraction is the gold standard.

Protocols are well-established and routinely used, making the procedure straightforward for novice researchers.

Proteins are rapidly denatured and RNA is quickly stabilized.

The process is applicable to larger samples (such as human or animal tissues) as well as smaller samples from cell culture based experiments.

THE CONS:

Not very amenable to high-throughput processing and difficult to automate.

Manual processing of samples can be laborious.

Use of hazardous chemical and chlorinated organic waste must be managed carefully.

THE SPIN COLUMN EXTRACTION METHOD

This is a solid phase extraction technique to bind and isolate RNA within filter-based spin columns. These spin columns utilize membranes that contain silica or glass fiber to bind nucleic acids. Samples are lysed in a buffered solution containing RNase inhibitors and a high concentration of chaotropic salt. The lysates are passed through the silica membrane using centrifugal force, with the RNA binding to the silica gel at the appropriate pH.

The membrane containing residual proteins and salt is then washed to remove impurities, with flow-through discarded. RNA is subsequently eluted with RNase-free water, as RNA is stable at a slightly acidic environment.

THE PROS:

Simple, straightforward procedure to perform.

A ready to use kit format, which adds convenience.

Amenable to large-scale and high-throughput processing, including automated methods.

Flexible for use with both centrifugation or vacuum based systems.

THE CONS:

Starting with too much sample or incomplete homogenization can clog the membrane and/or RESULT IN CONTAMINATION WITH PROTEINS OR GENOMIC DNA.

Incomplete cellular lysis can lead to low yields.

Automation systems for centrifugation or vacuum can be expensive and complex to set up.

MAGNETIC PARTICLE EXTRACTION METHOD

This strategy for bioseparation utilizes beads with a paramagnetic core (in other words, they have properties of magnetism only when in proximity to an external magnetic field) coated with, most commonly, a matrix of silica for binding nucleic acids. In this method, cells are lysed in a buffer with RNase inhibitors and then incubated with the magnetic

beads, allowing the particles to bind RNA molecules.

The magnetic beads can then be quickly collected by being placed in proximity to an external magnetic field. The supernatant is removed and then subsequently washed and resuspended with removal of the magnetic field. This process can be easily repeated for multiple washes. The RNA is eluted from the magnetic beads with RNase-free water into solution, and the supernatant (containing the pure RNA) can then be transferred.

THE PROS:

RNA isolation technique is most amenable to automation and high-throughput methods.

The magnetic collection and resuspension steps are rapid and simple to perform.

Rapid and simple magnetic collection and resuspension steps.

Non-filter method reduces concern for clogging.

No organic solvent hazardous waste.

THE CONS:

Viscous samples can impede migration of magnetic beads.

While more easily amenable to automation, this technique can be laborious when performed manually with large numbers of samples.

RISK OF CONTAMINATION OF RNA SAMPLES WITH RESIDUAL MAGNETIC BEADS."

https://www.lifescience.roche.com/en_us/blog/lab-life/dna-and-rna-purifation/the-top-pros-and-cons-of-different-rna-extraction-methods.html

As can be seen, each method has its own advantages as well as limitations and drawbacks. These drawbacks are further outlined in the following article:

THE PHENOL/CHLOROFORM METHOD

“Attention should be paid not to disturb the phases formed during the process [TO PREVENT CONTAMINATION OF RNA WITH DNA OR PROTEINS], so a good handling

ability is required," says Beretta."

THE SPIN COLUMN/COLUMN CHROMATOGRAPHY METHOD

"It is important to use an appropriate amount of input material since using too much sample may reduce lysis efficiency, INTRODUCE EXCESSIVE AMOUNTS OF CELLULAR COMPONENTS OTHER THAN RNA, and compromise RNA binding to the RNA purification column," says Danielle Freedman, senior product marketing manager at NEB."

THE MAGNETIC BEADS METHOD

"The beads are incubated with cell lysate and RNase inhibitors, then anchored in place using a magnetic field while the supernatant (CONTAINING UNWANTED DEBRIS AND IMPURITIES) is removed, and the beads ARE WASHED TO REMOVE LINGERING IMPURITIES."

"COMMON PITFALLS AND CONCERNS

INHIBITING RNases

The first concern in any RNA workflow is to GUARD AGAINST DEGRADATION BY RNases. "Work in an environment which is as RNase-free as possible, so wash surfaces and pipettes, use RNase-free (DEPC-treated) water, and change gloves a lot," says Beretta. Also, add the power of RNase inhibitors as needed. "RNase control is key, so the use of inhibitors specifically, or knowing which conditions cause inactivation of RNases, such as lysis buffers or transport media, together with use of RNase-free consumables, helps maintain [RNA] integrity," says Andrew Gane, genomics and diagnostics solutions strategy and technology manager at Cytiva.

SAMPLE LYSIS

The sample type will dictate the appropriate lysis stringency, which can vary widely. This may require optimization, as INSUFFICIENT LYSIS MEANS AN INCOMPLETE YIELD, WHILE OVERLY STRINGENT LYSIS CAN DEGRADE RNA MOLECULES. "The lysis efficiency can be fine-tuned by combining chemical lysis with enzymatic lysis and physical lysis via heat and/or mechanical disruption," says Markus Sprenger-Haussels, VP, head of sample technologies product development life sciences at QIAGEN. "THESE PARAMETERS HAVE TO BE WELL BALANCED TO AVOID NEGATIVE

IMPACT ON RNA INTEGRITY."

ELUTION

Elution conditions should be optimized to find the best elution buffer for long-term RNA stability, and also TO AVOID INTERFERENCE WITH SUBSEQUENT DOWNSTREAM APPLICATIONS. For example, azide can affect quantification by spectrophotometry, EDTA can impact PCR efficiency, pH can affect enzymatic reactions, and "addition of carrier RNA might impact [spectrophotometric] quantification or oligo(dT)-primed downstream reactions," says Sprenger-Haussels.

CONTAMINATION WITH gDNA

REMOVAL OF RESIDUAL GENOMIC DNA (gDNA) FROM RNA PREPARATIONS IS ALSO AN IMPORTANT CONSIDERATION FOR SOME DOWNSTREAM APPLICATIONS, AND OPTIMIZING WORKFLOWS CAN HELP TO REDUCE gDNA CONTAMINATION. "Genomic DNA may be carried over from the interphase of organic extractions, or when solid-phase RNA purification methods are overloaded," says Freedman. "To remove traces of genomic DNA from RNA preparations, samples should be treated with DNase I."

https://www.biocompare.com/Editorial-Articles/572190-How-to-Purify-High-Quality-RNA

There are obviously a few issues which can potentially throw off the RNA extraction process. Any of these alone would be enough to undermine the remaining sequencing processes leading to a contaminated genome. In order to paint a better picture, here are a few more looks at the various ways this process can potentially go wrong.

From a study in 2014:

PITFALLS OF RNA ISOLATION FROM SPUTUM IN COPD

"The quality and the quantity of sputum RNA depends on several factors during the isolation. However, THE BIGGEST CHALLENGE IS THE ELIMINATION OF BACTERIAL DNA, which is of high importance, SINCE CONTAMINATING BACTERIAL BACKGROUND MIGHT MASK THE HUMAN RNA IN GENE EXPRESSION STUDIES."

https://erj.ersjournals.com/content/44/Suppl_58/P995

TROUBLESHOOTING RNA ISOLATION

"1. Problem: Genomic DNA in the RNA

The RNA elutes with genomic DNA as evidenced by high molecular weight smearing, or it appears clean on a gel but -RT controls amplify when PCR is performed.

Cause: NO MATTER WHAT METHOD YOU USE FOR RNA ISOLATION, TRACES OF DNA ALWAYS CARRY THROUGH. This is true with TRIzol (phenol) preps and with silica spin filters. This can be caused by insufficient shearing of the genomic DNA during homogenization. If using phenol method, the pH of the phenol is key (it should be acidic) and YOUR SKILL IN PIPETTING ONLY THE AQUEOUS PHASE WILL RESULT IN MORE OR LESS DNA CONTAMINATION."

"2. Problem: Degraded RNA/ low integrity

Cause: Degradation occurred at some point during processing. THIS CAN BE DIFFICULT TO PINPOINT. It could have happened DURING COLLECTION and STORAGE, or possibly DURING EXTRACTION. It could also have OCCURRED POST-ISOLATION."

"3. Problem: Inhibitors in the RNA

Cause: A LOW 260/230 IN AN RNA PREP IS INDICATIVE OF GUANIDINE SALT CARRY OVER INTO THE SAMPLE OR ORGANIC INHIBITORS (such as humic acids or polysaccharides if the sample is environmental). Guanidine salts are used in TRIzol and in silica preps. These salts inactivate RNases, but will also inhibit proteins such as RT enzymes if present in the final RNA. A low 260/280 measurement INDICATES PROTEIN CONTAMINATION."

"4. Problem: Low yields of RNA

The yield of RNA is lower than expected- either based on your previous results, or, based on reported yields for a certain tissue or cell type. RNA YIELDS CAN VARY GREATLY BETWEEN DIFFERENT CULTURED CELL TYPES AND IN DIFFERENT TISSUES. For blood RNA, it can vary from person to person.

Cause: If the yield of RNA is lower than you expected or know it should be, and the RNA is intact (read: not degraded) , then the homogenization may not have been complete. To isolate RNA, a strong lysis is key. Tissues stored in RNALater will tend to be a little

more difficult to homogenize. LOW YIELDS COULD BE CAUSED BY MISTAKES IN WEIGHING OF TISSUE OR IN THE CELL COUNTS FOR CULTURED CELLS. You may have less cells than you think. With blood RNA, the buffy coat can vary based on your skill in collecting the white cell layer and each individual patient."

https://bitesizebio.com/2345/troubleshooting-rna-isolation/

In Summary:

-obtaining high-quality RNA is the first, and often the most critical, step in performing many molecular techniques such as reverse transcription real-time PCR (RT-qPCR), transcriptome analysis using next-generation sequencing, array analysis, digital PCR, northern analysis, and cDNA library construction

-high-quality experiments require high-quality samples, and maximizing yield of non-degraded RNA isolation is key

-the 3 main methods are organic extraction, spin column extraction, and magnetic particle extraction

-each method has its own drawbacks and limitations

-with the organic extraction method, the aqueous phase containing RNA needs to be carefully removed by pipetting WITH CARE NOT TO TOUCH THE INTERFACE OR ORGANIC PHASE, AS THIS CAN CONTAMINATE THE SAMPLE

-with the spin column extraction method, starting with too much sample or incomplete homogenization can clog the membrane and/or RESULT IN CONTAMINATION WITH PROTEINS OR GENOMIC DNA

-with the magnetic particle extraction method, there is a RISK OF CONTAMINATION of RNA samples with residual magnetic beads

-OTHER ISSUES INCLUDE:

*inhibiting RNase leading to the degradation of RNA

*insufficient lysis leading to an incomplete yield or overly stringent lysis degrading RNA molecules

*improper elution conditions leading to long-term RNA instability and interference with subsequent downstream applications

*carryover of contaminating genomic DNA from the interphase of organic extractions, or

when solid-phase RNA purification methods are overloaded

-regarding the extraction of RNA from sputum, one study found that eliminating bacterial DNA contamination was the biggest challenge which can ultimately mask human RNA

-NO MATTER WHAT METHOD USED for RNA isolation, TRACES OF DNA ALWAYS CARRY THROUGH

-skill in pipetting only the aqueous phase will result in MORE OR LESS DNA CONTAMINATION

-degraded RNA/ low integrity is difficult to pinpoint and can occur during collection and storage, during extraction, or it can occur post-isolation

-a low 260/230 in an RNA prep is indicative of guanidine salt carry over into the sample or organic inhibitors and INDICATES PROTEIN CONTAMINATION

-RNA yields can vary greatly between different cultured cell types and in different tissues

-low yields could be caused by mistakes in weighing of tissue or in the cell counts for cultured cells

It is clear that the methods used for the extraction of RNA either from cell culture or straight from clinical samples are fraught with potential problems regarding contamination and RNA degradation. Any errors in this first crucial step will affect the following steps in the sequencing process and lead to problems with the reliability and accuracy of sequencing a genome. If the pieces of the puzzle are incorrect, what does that say about the puzzle?

https://docs.google.com/document/d/e/2PACX-1vRhekRLZlJufN-f3YdlvSFjy8gNyl1ez8K4gAhU6ZB4GWf-fjYhtxdJJNyDtVItqWp8My6n5wk41rgJ/pub

The Slippery Slope of Reference Genomics:

The genome for "SARS-COV-2" was created using multiple reference genomes. One of them was RaTG13, a bat "coronavirus:"

"Similarity plot based on the full-length genome sequence of 2019-nCoV WIV04. Full-length genome sequences of SARS-CoV BJ01, bat SARSr-CoV WIV1, BAT CORONAVIRUS RaTG13 and ZC45 were USED AS REFERENCE SEQUENCES."

"We then found that a short region of RNA-dependent RNA polymerase (RdRp) FROM A BAT CORONAVIRUS (BatCoV RaTG13)—which was previously detected in Rhinolophus affinis from Yunnan province—SHOWED HIGH SEQUENCE IDENTITY TO 2019-nCoV. We carried out full-length sequencing on this RNA sample (GISAID accession number EPI_ISL_402131). SIMPLOT ANALYSIS SHOWED THAT 2019-nCoV WAS HIGHLY SIMILAR THROUGHOUT THE GENOME TO RaTG13 (Fig. 1c), WITH AN OVERALL GENOME SEQUENCE IDENTITY OF 96.2%."

https://www.nature.com/articles/s41586-020-2012-7

What is RaTG13?

"Bat coronavirus RaTG13 is a SARS-like betacoronavirus that infects the horseshoe bat Rhinolophus affinis.[2][3] It was discovered in 2013 in bat droppings from a mining cave near the town of Tongguan in Mojiang county in Yunnan, China. AS OF 2020, IT IS THE CLOSEST KNOWN RELATIVE OF SARS-CoV-2, THE VIRUS THAT CAUSES COVID-19.[4][5]"

https://en.m.wikipedia.org/wiki/RaTG13

This bat "Coronavirus" is the closet known relative of "SARS-COV-2." But what happens if it turns out that RatG13 doesn't exist? What if it is a highly faulty sequenced genome? What does that mean for "SARS-COV-2," a "virus" which is its closest relative and which used it as a reference genome?

"Scientists claim SERIOUS DATA DISCREPANCIES IN RaTG13 sequence

"A new preprint* published in September 2020 by molecular biologists at the All India Institute of Medical Sciences, New Delhi, and the Indraprastha Institute of Information Technology Delhi discusses the current issues with the bat coronavirus (CoV) strain that is often considered to have very close homology with the above-mentioned virus, CONCLUDING THAT THERE ARE INADEQUATE GROUNDS TO CONSIDER IT TO BE THE ANCESTRAL POOL OF SARS-CoV-2."

"Many scientists mention the genome sequence of this bat CoVs, RaTG13, as being part of the ancestral descent of the current virus. A recent paper in the journal Nature also mentions its 96.2% homology with SARS-CoV-2, CONSIDERING IT TO BE A FOSSIL RECORD OF A STRAIN WHOSE CURRENT EXISTENCE IS DOUBTFUL, BUT WHICH MAY HAVE BEEN THE ORIGINAL POOL FROM WHICH THE CURRENT VIRUS DEVELOPED."

"The scientists assembled the viral genome from scratch, performed a metagenomic analysis, and looked at data quality. THEY CONCLUDED THAT THE RaTG13 GENOME HAD SERIOUS ISSUES AND ALL DATA RELATED TO IT REQUIRED A FULL REVIEW."

"De novo RaTG13 Assembly NOT POSSIBLE

The researchers found that using the available data, THEY WERE UNABLE TO DETECT ANY CONTIGUOUS SEQUENCES LARGER THAN 17 kb, using several different settings. Several matching sequences were found, BUT NONE OVER A FIFTH OF THE LENGTH OF THE REPORTED SEQUENCE. A gap spanning 111 positions was found, AND IT IS UNCLEAR ON WHAT BASIS THIS WAS FILLED IN THE PUBLISHED SEQUENCE.

CONTAMINATION LIKELY

The researchers also UNCOVERED PROOF THAT DNA CONTAMINATION IS LIKELY TO HAVE OCCURRED. For instance, the largest contig contains genetic material with 98% similarity to the full-length mitochondrial sequence of the Chinese rufous horseshoe bat (Rhinolophus sinicus), AN UNLIKELY EVENT SINCE A COMPLETE ASSEMBLY OF SUCH A SEQUENCE IS TYPICALLY INTERRUPTED BY STOP CODONS.

Secondly, NON-ADAPTER-RELATED REPETITIVE SEQUENCES WERE FOUND IN MOST READS, often at the same end of the read, comprising one G-quadruplex sequence and its reverse complement. THIS IS UNLIKELY TO HAPPEN ON THE SAME END OF AN RNA SAMPLE SINCE ONLY ONE STRAND IS DOMINANT. The researchers say MORE INFORMATION ABOUT HOW THE EXPERIMENTS WERE CARRIED OUT IS CRUCIAL TO RULE OUT THE POSSIBILITY OF GROSS RNA SAMPLES CONTAMINATION BY DNA.

POOR DATA QUALITY

The researchers also calculated that the average coverage is 9.73, INDICATING A LOW VALUE. This may be why ONLY PARTIAL SEGMENTS OF THE RaTG13 SEQUENCE ARE ASSEMBLED. The coverage is only 2 or less for about 3,000 bases, WHICH COULD MARKEDLY IMPAIR THE ACCURACY. They draw attention to multiple ambiguous bases in the first end that could PREVENT DE NOVO ASSEMBLY, AND TO MANY UNRELIABLE SECOND END READS AS WELL."

"EXPERIMENTAL PROCEDURAL CONCERNS

THE SIGNIFICANTLY LARGE DIFFERENCES IN THE BACTERIAL CONTENT OF THE TWO REFERENCED DATASETS ARE SURPRISING, say the researchers, SINCE BOTH PURPORT TO BE FROM SIMILAR SITES, fecal and oral samples. One has only 0.65% bacteria, and ~68% Eukaryota, with the rest being unidentified. The other is ~91% bacteria and ~4% Eukaryota. THIS CONCERN HAS BEEN RAISED BEFORE.

Again, 0.1% of the first dataset IS SIMILAR TO PLANT GENOMES LIKE RICE AND MAIZE, WHICH IS UNEXPECTED FROM BAT SAMPLES from creatures like the intermediate horseshoe bat Rhinolophus affinis. The researchers attribute this to CONTAMINATION BY POSSIBLE INDEX HOPPING BECAUSE OF EVIDENCE THAT THE SAME PLATFORM HAS BEEN USED TO SEQUENCE MAIZE EARLIER. Multiplex sequencing of maize and the CoV genome of interest COULD LEAD TO SUCH CONTAMINATION.

Again, THE DATASET ALSO CONTAINS MATERIAL IDENTICAL TO THAT OF THE MALAYAN PANGOLINS Manis javanica, A TOTALLY DIFFERENT ORDER. This again could be DUE TO INDEX HOPPING OF SOME FRAGMENTS for the same reason. THIS COULD HAVE MISDIRECTED THE DISCUSSION ON THE ORIGIN OF THE NOVEL CoV, as some have reported that pangolin CoV genomic sequences also have close homology with that of the former.

Thus, the inference could also be that CONTAMINATION ACCOUNTS FOR THE PRESENCE OF VARIOUS PORTIONS OF THE RaTG13 IN THE DATASET, accounting for 0.0008% of the total."

IMPLICATIONS

While most work on the origins of SARS-CoV-2 has focused on the human CoV sequence, the current study shows that EQUAL IMPORTANCE MUST BE GIVEN TO THE OTHER HALF OF THE EQUATION, NAMELY, RaTG13, in order to justify giving it a role in the narrative. Secondly, discussions may instead BE WITHHELD, WHILE THE PRECISE DETAILS OF THE METHODS USED TO GENERATE THE RaTG13 ARE AWAITED. And thirdly, THIS GENOME SHOULD NOT BE USED IN FURTHER STUDIES UNTIL ITS SCIENTIFIC

RELIABILITY IS ESTABLISHED IN ENTIRETY, BY INDEPENDENT RESEARCHERS WITH ACCESS TO THE FULL DATASETS AND METHODS USED FOR ITS GENERATION."

https://www.news-medical.net/news/20200910/Scientists-claim-serious-data-discrepancies-in-RaTG13-sequence.aspx?fbclid=IwAR1vrhAFA0JHfDW6B5X5kQD12GH3kguAcyGiBMpblIEqk-tUc7BANcZV2E0

Some very heavy IMPLICATIONS about the numerous errors in RaTG13 which was used in the creation of the "SARS-COV-2" genome.

Why is it important that the REFERENCE GENOME be accurate?

"Perhaps one of the biggest drawbacks IS THE NEED FOR A REFERENCE GENOME FOR COMPARISON WITH YOUR SEQUENCE. If you don't have one of these to compare your results to, YOU HAVE NO REAL WAY OF DETERMINING WHAT IS NORMAL AND WHAT IS UNIQUE ABOUT YOUR SAMPLE. Good luck identifying an insertion mutation without an unaltered genome to compare to! While de novo sequencing for when a reference is not available is possible, IT CAN LEAD TO MORE ERRORS SINCE YOU HAVE NOTHING TO COMPARE TO."

https://bitesizebio.com/37159/good-tbad-expensive-whole-genome-sequencing/?fbclid=IwAR0HeuOeIlOUNCIu662e3npGy88xPEKyNACzcqS_tYm_M8kan1ki4iZEXD0

"Additionally, with the current suite of PRIMARILY SEQUENCE SIMILARITY-BASED PATHOGENIC IDENTIFICATION TOOLS, the ability to detect novel pathogens is WHOLLY DEPENDENT ON HIGH-QUALITY REFERENCE DATABASES (22). There is a TREND TOWARD REQUIRING A COMPLETE GENOME SEQUENCE WHEN A DESCRIPTION OF A NOVEL VIRUS IS BEING PUBLISHED, and we agree that this is a good goal"

https://mbio.asm.org/content/5/3/e01360-14

If the reference genome is not accurate, there is no way the genome

created by using it is accurate as well.

https://docs.google.com/document/d/e/2PACX-1vQQTtEitoPXzw4X_sKVBsGe2JRRwntgcRLFW1HOuPINUTbZzJiWACW226A6oUJE8s5f2HRwhxj7bkaq/pub

Problems with Reference Genomes:

Are they "variants" or sequencing artefacts?

ARTEFACTS (Genomics):

"In genetics a RESULT THAT DOES NOT REPRESENT THE TRUE BIOLOGICAL MATERIAL or function but ARISES FROM A TECHNICAL, OFTEN ARTIFICIAL, PROCESS."

"ARTEFACTS CAN LEAD TO MISLEADING RESULTS FROM SEQUENCING. To avoid giving patients incorrect results during data analysis and validation, ANY DATA THAT COULD BE AN ARTEFACT FROM THE SEQUENCING PROCESS IS THOROUGHLY INVESTIGATED."

https://www.genomicseducation.hee.nhs.uk/glossary/artefact/

"WE INVESTIGATE ODDITIES IN THE SARS-CoV-2 GENOME SEQUENCES from GISAID. Many putative sequencing issues seem specific to genomic ends and to certain samples, and are easily filtered out. However, MANY MUTATIONS SEEM TO ARISE MANY TIMES along the phylogenetic tree (are highly homoplasic), AND SEEM MORE LIKELY THE RESULT OF CONTAMINATION, RECURRENT SEQUENCING ERRORS, OR HYPERMUTABILITY, than selection or recombination. Some homoplasic substitutions seem LABORATORY-SPECIFIC, SUGGESTING THAT THEY MIGHT ARISE FROM SPECIFIC COMBINATIONS OF SAMPLE PREPARATION, SEQUENCING TECHNOLOGY, AND CONSENSUS CALLING APPROACHES."

"Finally, as other groups have already suggested (see e.g. [12]), we recommend filtering out sequences that: HAVE TOO FEW RESOLVED CHARACTERS (our somewhat arbitrary threshold is about 29,400 reference bases), ARE TOO DIVERGED (as can be tested using TreeTime), HAVE UNUSUAL LOCALLY HIGH DIVERGENCE (as can be tested using ClonalFramML), HAVE MISSING/INCOMPLETE SAMPLING DATE INFORMATION, OR THAT ARE DISTANT FROM ANY OTHER SEQUENCE IN THE DATASETS (we use a custom script to remove all sequences that are at least three substitutions away from any other sequence). We don't provide a current list as this is quite long and varies as the number of publicly shared SARS-CoV-2 genome sequences increases."

"A few samples have extremely long terminal branches (Figure 2), suggesting either evolutionary events leading to many substitutions (e.g. recombination events or large mutation events), OR SEQUENCING/CALLING ARTEFACTS in specific samples."

"Given that these mutations are not observed in any other samples, THEY COULD

REPRESENT ARTEFACTS in the corresponding sequence."

"To test for the possibility that some of THESE HOMOPLASIES MIGHT BE THE RESULT OF PHYLOGENETIC ERRORS, we masked the most common homoplasies from these datasets one part at a time, to see if other homoplasies would disappear. This had almost no effect.

Another possibility is that these homoplasies might be CAUSED BY SOME OF THE SAMPLES BEING PARTICULARLY ENRICHED IN RECURRENT ARTEFACTS, RATHER THAN ARTEFACTS DISTRIBUTED UNIFORMLY ACROSS ALL SAMPLES."

"We will now discuss some of the most common homoplasies. As mentioned above, G11083T is the most frequent, appearing 679 times and apparently mutating 21 times forward and 7 times reverting to the original T allele. The T allele is observed in different sequencing technologies and different countries. This is a new non-synonymous (L to F) mutation (ORF1a 3606 nsp6 37) and also is considered one of the best candidates for positive selection (https://observablehq.com/@spond/natural-selection-analysis-of-sars-cov-2-covid-19? https://observablehq.com/@spond/revised-sars-cov-2-analytics-page 20), but this homoplasy has also been interpreted in literature as the result of frequent recombination [7]. It appears in all samples from the Diamond Princess cruise ship. Notably the mutation is next to the longest non-terminal homopolymer in the genome, further extending it from 8 consecutive T's to 10 (Figure 9). The mutation also appears in samples as a within-host polymorphism, as can be seen from the presence of isolated N's (17 times) and K's (9 times) in the alignment (see e.g. Figure 9). We also observe this from read files from the Sequence Read Archive (see next section), where the mutation appears as within-patient polymorphism 16 times, and even more often with less stringent filtering and variant calling. WHEN APPLYING MORE STRINGENT READ FILTERING, THE FREQUENCY OF THE T ALLELE SEEMS TO CONSISTENTLY DECREASE. Considering all of these observations, we think that G11083T might be a particularly frequent mutation OR ARTEFACT. It is unlikely to be the result of positive selective pressure at the amino acid level, as the mutation seems apparently to revert to the original allele many times, and as the same amino acid substitution L→F would also be obtained with the substitution G11083C, which however we never observed in our data."

"As a consequence of the observations above, WE SUSPECT FREQUENT HOMOPLASIES SPECIFIC TO DATASET C COULD BE ARTEFACTS (or possibly normal mutations that appear as homoplasic due to phylogenetic errors). These include T13402G, which is a nonsense mutation appearing in 51 sequences; its neighbour 13408, in which all three non-reference alleles are observed; and A4050C, a nonsynonymous mutation appearing in 18 sequences. Others are less frequent, and it is particularly unclear if their homoplasy might be caused by phylogenetic errors; these

include T8022G (nonsynonymous, appearing in 5 samples), T28785G and C3130T (appearing only in 4 and 3 samples respectively). Recently the dataset C-specific mutations at positions 24389-24390 have been suggested to be strongly affected by local recombination [9], BUT THIS MIGHT BE A PHYLOGENETIC ARTEFACT CAUSED BY OTHER HOMOPLASIES."

"To test which of these homoplasies might (also) be actual inherited viral mutations, AND WHICH ARE MORE LIKELY TO BE NON-INHERITED SEQUENCING ARTEFACTS, we measured the phylogenetic signal present in the most homoplasic and/or lab-specific variants using the methods of Borges et al 2018"

"MOST OF THE HOMOPLASIES, in particular those appearing in only a few sequences, SHOW CLOSE TO NO PHYLOGENETIC SIGNAL (Figure 11), SUPPORTING THE HYPOTHESIS THAT THEY ARE ARTIFICIAL. On the other hand, many homoplasies, including site 11083, show strong phylogenetic signal, suggesting that they originated, at least once, as a true mutational event. Of course, this analysis has substantial limitations, it ignores uncertainty in the tree and NOISE FROM POSSIBLE ARTEFACTS WITHIN THE REMAINING SITES. It also does not tell us if a variant is both a true mutational event AS WELL AS THE RESULT OF RECURRENT SEQUENCING BIASES, as possible for position 11083."

"For example, at reference position 10779, an A/T polymorphism was observed in 7 out of 8 samples from Shen et al 2020, usually with A being the minor allele, and once being the majority allele. In the 8th sample, allele A appeared fixed. We observed allele A only in read ends (see Figure 12), and after trimming read ends this polymorphism was no longer detected. THIS SUGGESTS THAT RECURRENT ARTEFACTS MIGHT BE PRESENT NOT ONLY AT THE LEVEL OF SUBSTITUTIONS BETWEEN CONSENSUS SEQUENCES, BUT ALSO AS FREQUENT, APPARENT WITHIN-HOST VARIANTS."

"Generally, most samples contain 0–5 variants (median 1, mean 8.2). However, some samples have many more, REACHING 564 AND 433 in two cases (SRR11494637 and SRR11494664 respectively). Coverage or mixed infection/contamination do not seem the issues. Instead, THESE SAMPLES WERE MOSTLY MADE OF CLIPPED READS, i.e. READS THAT ONLY PARTLY MAP ON THE REFERENCE GENOME. Removing these reads eliminated these extreme cases (Figure 13), but other samples with extreme numbers of variants can still be found, for example SRR11494662 with 359 within-host variants. As samples with an extreme number of within-host variants persist, a more rigorous filtering procedure might still be required before we can confidently

interpret within-host variant calls. WE THEREFORE OFFER A WORD OF CAUTION WHEN ATTEMPTING TO INTERPRET THE RESULTS OF SUCH VARIANT CALLING METHODS, and minimally recommend a stringent set of filters (as outlined above), as well as removing samples with more than 2% of “N”s within reads."

"The mutational spectrum of within-host variants seems extremely shifted toward G→T variants, even more than when comparing consensus sequences (Figure 6) SUGGESTING THAT MOST OF THESE VARIANTS MIGHT BE THE RESULT OF ILLUMINA SEQUENCING ERRORS AND/OR SEQUENCING BIASES, or otherwise that most of these new G→T mutations do not reach fixation due to selective forces. WE HAVE NOT YET INVESTIGATED POSSIBLE NANOPORE SEQUENCING BIASES FROM WITHIN-HOST VARIATION DATA."

"It is not clear to us if these OBSERVATIONS SUGGEST THAT SEQUENCING ERRORS MIGHT BE COMMON AT THIS POSITION due to the poly-T."

https://virological.org/t/issues-with-sars-cov-2-sequencing-data/473

Sequencing artefacts, biases, errors = not sequencing the same "virus" every time = "variants."

https://docs.google.com/document/u/1/d/e/2PACX-1vSS7fUK6sg5nuQjZypLzh5dUzQ_GyZi3EWwQ_tcf8WeDwjT7WwhXSdxTB453bAOoBcDTuURWC1YpUwt/pub?fbclid=IwAR0PCU_lftFXjdHHPAQV1F_qhPN5_whRy2M7_DYiY_4n_59z2gunSBFqgxM

Related posts on the problems with Reference Genomes:

https://m.facebook.com/story.php?story_fbid=10158048691828576&id=502548575

https://m.facebook.com/story.php?story_fbid=10158048953873576&id=502548575https://docs.google.com/document/d/e/2PACX-1vTrL7E4fyBEemz-4u8JA6Qpz2yWmvZAOQ4NSyYkPhLcBFJylrrTQ4W1H97w-TErIOJQhi8piTOx2IkJ/pub

WHO's Genomic Sequencing of SARS-COV-2 Manual:

Some interesting highlights from the WHO's Genomic Sequencing of SARS-COV-2 Manual:

6.5.1 Metagenomic analyses of uncultured clinical samples

"Despite such measures, SAMPLES MAY STILL CONTAIN HIGH QUANTITIES OF OFF-TARGET HOSTS DNA/RNA THAT MAY ALSO BE SEQUENCED."

"Metagenomic sequencing typically PRODUCES HIGH NUMBERS OF OFF-TARGET, NON-VIRUS READS."

"The number of sequencing reads per sample that must be generated to obtain the full genome will DEPEND ON SAMPLE TYPE, PRETREATMENT PROCEDURES TO REMOVE HOST MATERIAL AND LEVEL OF VIRAEMIA."

6.5.2 Metagenomic approaches following cell culture

"For samples with a low viral load, the proportion of viral genetic material CAN THEORETICALLY BE INCREASED by allowing the virus to replicate in cell culture."

"In addition, PASSAGE IN CELL CULTURE CAN RESULT IN ARTIFICIAL MUTATIONS IN THE SEQUENCES, WHICH WERE NOT PRESENT IN THE ORIGINAL CLINICAL SAMPLE. This can have major implications for subsequent analyses. Using cell culture solely for the purpose of amplifying virus genetic material for SARS-CoV-2 sequencing SHOULD THEREFORE BE AVOIDED, especially now that other bait-capture and amplicon-based approaches are available to improve sequencing sensitivity."

https://www.who.int/publications/i/item/9789240018440

https://www.google.com/url?sa=t&source=web&rct=j...

Just wanted to highlight this one more time for emphasis:

"PASSAGE IN CELL CULTURE CAN RESULT IN ARTIFICIAL MUTATIONS IN THE SEQUENCES, WHICH WERE NOT PRESENT IN THE ORIGINAL CLINICAL SAMPLE."

In image 3 below, under "Viral" Isolate from clinical sample:

"Levels high following culture, BUT CULTURE MAY INDUCE ARTIFICIAL VARIANTS"

They openly admit to the creation of "Viral" variants through cell culture and sequencing.

Also, remember the definitions of purification/isolation?

PURIFICATION:

1. to make pure; free from anything that debases, pollutes, adulterates, or CONTAMINATES:

2. to FREE FROM FOREIGN, EXTRANEOUS, or OBJECTIONABLE ELEMENTS:

https://www.dictionary.com/browse/purification

ISOLATION:

the act of SEPARATING SOMETHING FROM OTHER THINGS : the act of isolating

something

https://www.merriam-webster.com/dictionary/isolation

It is clear these are not purified/isolated "viruses" they are sequencing genomes from. Also note in the images the high "non-viral" content in each sample type which can only be REDUCED (as they do not claim it is ELIMINATED) through centrifugation/filtration.

https://docs.google.com/document/d/e/2PACX-1vRi5OJP-hIifqDSz57IWOLlmZecd9C35HGVsaBqyRo6j1f7qQqpD9ZX1rI9lrXNeqlaLIgkU0uYn1RL/pub

CHALLENGES IN GENOME LIBRARY CONSTRUCTION:

CHALLENGES IN GENOME LIBRARY CONSTRUCTION:

After RNA has been extracted and "purified," converted to cDNA, and fragmented, the task turns to building the library used for sequencing the genome. According to the Britannica, the library is essentially "a collection of DNA fragments that make up the full-length genome of an organism. A genomic library is created by isolating DNA from cells and then amplifying it using DNA cloning technology."

There are many issues with creating the library which are directly related to the initial steps that are used to prepare the DNA fragments for sequencing. These problems are highlighted with excerpts from the following three articles:

LIBRARY CONSTRUCTION FOR NEXT-GENERATION SEQUENCING: OVERVIEWS AND CHALLENGES

Considerations in NGS library preparation: Complexity, bias, and batch effects

"THE MAIN OBJECTIVE WHEN PREPARING A SEQUENCING LIBRARY IS TO CREATE AN LITTLE BIAS AS POSSIBLE. Bias can be defined as the SYSTEMATIC DISTORTION OF DATA DUE TO THE EXPERIMENTAL DESIGN. Since it is IMPOSSIBLE TO ELIMINATE ALL SOURCES OF EXPERIMENTAL BIAS, the best strategies are: (i) know where bias occurs and take all practical steps to minimize it and (ii) pay attention to experimental design so that the sources of bias that cannot be eliminated have a minimal impact on the final analysis.

THE COMPLEXITY OF AN NGS LIBRARY CAN REFLECT THE AMOUNT OF BIAS CREATED BY A GIVEN EXPERIMENTAL DESIGN. In terms of library complexity, the ideal is a highly complex library that reflects with high fidelity the original complexity of the source material. THE TECHNOLOGICAL CHALLENGE IS THAT ANY AMOUNT OF AMPLIFICATION CAN REDUCE THIS FIDELITY. Library complexity can be measured by the number or percentage of duplicate reads that are present in the sequencing data (39). Duplicate reads are generally defined as reads that are exactly identical or have the exact same start positions when aligned to a reference sequence (40). One caveat is that the frequency of duplicate reads that occur by chance (and represent truly independent sampling from the original sample source) increases with increasing depth of sequencing. Thus, it is critical to understand under what conditions duplicate read rates represent an accurate measure of library complexity."

"However, the point is the same—THE GOAL IN PREPARING A LIBRARY IS TO PREPARE IT IN SUCH A WAY AS TO MAXIMIZE COMPLEXITY AND MINIMIZE PCR OR OTHER AMPLIFICATION-BASED CLONAL BIAS. This is a significant challenge for libraries with low input, such as with many ChIP-seq experiments or RNA/DNA samples derived from a limited number of cells. It is now technologically possible to perform genomic DNA and RNA sequencing from single cells. THE KEY POINT IS THAT THE LEVEL OF EXTENSIVE AMPLIFICATION REQUIRED CREATES BIAS IN THE FORM OF PREFERENTIAL AMPLIFICATION OF DIFFERENT SEQUENCES, AND THIS BIAS REMAINS A SERIOUS ISSUE IN THE ANALYSIS OF THE RESULTING DATA. One approach to address the challenge is a method of digital sequencing that uses multiple combinations of indexed adapters to enable the differentiation of biological and PCR-derived duplicate reads in RNA-seq applications (41,42). A version of this method is now

commercially available as a kit from Bioo Scientific (Austin, TX).

When preparing libraries for NGS sequencing, IT IS ALSO CRITICAL TO GIVE CONSIDERATION TO THE MITIGATION OF BATCH EFFECTS (43–45). It is also IMPORTANT TO ACKNOWLEDGE THE IMPACT OF SYSTEMATIC BIAS RESULTING FROM THE MOLECULAR MANIPULATIONS REQUIRED TO GENERATE NGS DATA; for example, the bias introduced by sequence-dependent differences in adaptor ligation efficiencies in miRNA-seq library preparations. BATCH EFFECTS CAN RESULT FROM VARIABILITY IN DAY-TO-DAY SAMPLE PROCESSING, SUCH AS REACTION CONDITIONS, REAGENT BATCHES, PIPETTING ACCURACY, AND EVEN DIFFERENT TECHNICIANS. Additionally, batch effects may be observed between sequencing runs and between different lanes on an Illumina flow-cell. Mitigating batch affects can be fairly simple or quite complex. When in doubt, consulting a statistician during the experimental design process can save an enormous amount of wasted money and time.``

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4351865/

"LIBRARY CONSTRUCTION

After RNA isolation and extraction, the next step is library construction of transcriptome sequencing. Library construction usually begins with the depletion of ribosomal RNA (rRNA) or the enrichment of mRNA enrichment, because most of the total RNA of cellular or tissue is rRNA. For eukaryotic transcriptomes, polyadenylated mRNAs are usually extracted by oligo-dT beads, or rRNAs are selectively depleted. Unlikely, prokaryotic mRNAs are not stably polyadenylated. Hence, oligo d(T)-mediated messenger enrichment is not suitable; there is only the second option. Then, RNA is usually fragmented to a certain size range by physical or chemical method. The subsequent steps differ among experimental design and NGS platforms. However, STUDIES INDICATED THAT MOST OF THE PROTOCOLS CURRENTLY USED FOR LIBRARY CONSTRUCTION MAY INTRODUCE SERIOUS DEVIATIONS. For example, RNA FRAGMENTATION CAN INTRODUCE LENGTH BIASES OR ERRORS, SUBSEQUENTLY PROPAGATING TO LATER CYCLES. Furthermore, LIBRARY AMPLIFICATION MAY ALSO BE AFFECTED BY PRIMER BIAS, such as primer bias in multiple displacement amplification (MDA) [15], primer mismatch in PCR amplification [16, 17]. As a consequence, IT MAY INTRODUCE NONLINEAR EFFECTS AND INEVITABLY COMPROMISE THE QUALITY OF RNA-seq DATASETS, LEADING TO THE RESULT OF ERRONEOUS INTERPRETATION. Consequently, in the next section, we will describe and summarize the bias sources of library preparation, including mRNA enrichment, fragmentation, primer bias, adapter ligation, reverse transcription, and especially PCR. A sum up suggestions for improvement is presented in Table 3."

https://www.hindawi.com/journals/bmri/2021/6647597/

IDENTIFYING AND MITIGATING BATCH EFFECTS IN WHOLE GENOME SEQUENCING DATA

"Large sample sets of whole genome sequencing with deep coverage are being generated, however ASSEMBLING DATASETS FROM DIFFERENT SOURCES INEVITABLY INTRODUCES BATCH EFFECTS. These batch effects ARE NOT WELL UNDERSTOOD and can be DUE TO CHANGES IN THE SEQUENCING PROTOCOL OR BIOINFORMATICS TOOLS used to process the data. NO SYSTEMATIC ALGORITHMS OR HEURISTICS EXIST TO DETECT AND FILTER BATCH EFFECTS OR REMOVE ASSOCIATIONS IMPACTED by batch effects in whole genome sequencing data."

"RESEARCHERS CURRENTLY DO NOT HAVE EFFECTIVE TOOLS TO IDENTIFY AND MITIGATE BATCH EFFECTS in whole genome sequencing data."

"FACTORS LEADING TO BATCH EFFECTS ARE ILL-UNDERSTOOD AND CAN ARISE FROM MULTIPLE SOURCES making it difficult to develop systematic algorithms to detect and remove batch effects."

"IDENTIFYING A METHOD TO DETECT BATCH EFFECTS that have an impact on downstream association analyses IS CRUCIAL as researchers need to know upfront WHETHER WGS DATASETS CAN BE COMBINED OR IF CHANGES IN SEQUENCING CHEMISTRY WILL RESULT IN SEQUENCES THAT CAN NO LONGER BE ANALYZED TOGETHER."

"While sequencing costs are decreasing, many thousands of samples are necessary to have sufficient power to identify novel variants associated with common complex diseases [45]. In order to collect enough cases for diseases, multiple groups often work collaboratively by contributing samples to a consortium. In order to analyze these cases an even greater number of controls are desired [46]. THUS THE NEED TO COMBINE SAMPLES THAT HAVE BEEN PROCESSED INDEPENDENTLY IS CLEAR, AS IS THE UNAVOIDABLE INTRODUCTION OF BATCH EFFECTS."

"Batch effects in WGS data are not well understood and perhaps because of this, WE WERE NOT ABLE TO FIND AN EXISTING METHOD OR DEVELOP A NOVEL METHOD THAT REMOVED ALL SITES IMPACTED BY BATCH EFFECTS WITHOUT IMPACTING THE POWER TO DETECT TRUE ASSOCIATIONS."

"BATCH EFFECTS WILL ARISE AS INDEPENDENT GROUPS ATTEMPT TO COMBINE SEQUENCING DATA GENERATED AND PROCESSED FROM DIFFERENT SOURCES – this collaboration is necessary particularly to attain power to detect new

disease-associated variants. Large-scale resources are spent by research, industry, and government organizations CREATING DATABASES THAT CANNOT EASILY BE MERGED."

https://bmcbioinformatics.biomedcentral.com/articles/10.1186/s12859-017-1756-z

In Summary:

-the main objective when preparing a sequencing library is to create AS LITTLE BIAS AS POSSIBLE

-bias can be defined as the SYSTEMATIC DISTORTION OF DATA due to the experimental design

-it is IMPOSSIBLE TO ELIMINATE all sources of experimental bias

-the complexity of an NGS library can reflect the amount of bias created by a given experimental design

-the technological challenge is that ANY AMOUNT OF AMPLIFICATION can reduce fidelity

-the goal in preparing a library is to prepare it in such a way as to maximize complexity and MINIMIZE PCR OR OTHER AMPLIFICATION-BASED CLONAL BIAS

-the key point is that the level of EXTENSIVE AMPLIFICATION REQUIRED CREATES BIAS in the form of preferential amplification of different sequences, and this BIAS REMAINS A SERIOUS ISSUE in the analysis of the resulting data

-it is also critical to give consideration to the mitigation of BATCH EFFECTS

-It is also important to acknowledge the impact of SYSTEMATIC BIAS RESULTING FROM THE MOLECULAR MANIPULATIONS required to generate NGS data

BATCH EFFECTS can result from variability in day-to-day sample processing, such as reaction conditions, reagent batches, pipetting accuracy, and even different technicians

-studies indicate that MOST OF THE PROTOCOLS currently used for library construction may INTRODUCE SERIOUS DEVIATIONS

-RNA fragmentation can INTRODUCE LENGTH BIASES OR ERRORS, subsequently propagating to later cycles

-library amplification may also be AFFECTED BY PRIMER BIAS, such as primer bias in multiple displacement amplification (MDA) and primer mismatch in PCR amplification

-as a consequence, it may introduce nonlinear effects and inevitably compromise the quality of RNA-seq dataset, LEADING TO THE RESULTS OF ERRONEOUS INTERPRETATION

-datasets from different sources INEVITABLY introduces batch effects

-these batch effects are NOT WELL UNDERSTOOD and can be due to changes in the sequencing protocol or bioinformatics tools used to process the data

-NO SYSTEMATIC ALGORITHMS OR HEURISTICS EXIST TO DETECT AND FILTER BATCH EFFECTS OR REMOVE ASSOCIATIONS IMPACTED by batch effects in whole genome sequencing data

-researchers currently DO NOT HAVE effective tools to identify and mitigate batch effects in whole genome sequencing data

-factors leading to batch effects are ILL-UNDERSTOOD and can ARISE FROM MULTIPLE SOURCES making it difficult to develop systematic algorithms to detect and remove batch effects

-identifying a method to detect batch effects that have an impact on downstream association analyses IS CRUCIAL

-researchers need to know upfront WHETHER WGS DATASETS CAN BE COMBINED or if CHANGES IN SEQUENCING CHEMISTRY will result in sequences that can no

longer be analyzed together

-when trying to identify novel variants, the need to combine samples that have been processed independently is clear, AS IS THE UNAVOIDABLE INTRODUCTION OF BATCH EFFECTS

-batch effects WILL ARISE as independent groups ATTEMPT TO COMBINE SEQUENCING DATA generated and processed FROM DIFFERENT SOURCES

-large-scale resources are spent by research, industry, and government organizations CREATING DATABASES THAT CANNOT EASILY BE MERGED

It is obvious that every step in the preparation of the sequencing library is littered with bias and batch effects. They openly admit that bias can not be eliminated and can only be mitigated and that batch effects are inevitable with no way to detect/remove them. Knowing the various issues of contamination, errors, artefacts, and biases inherent in the steps leading to the creation of the library, how can the data generated by these libraries be considered accurate or reliable?

https://docs.google.com/document/d/e/2PACX-1vT-0pv03brnfYCXbmsWyrOOxKY0dmci6370SpOsULhuzp84MRA2fYJiP5Yflk5XOeDh5ia7DE8sbNU-/pub

I WOULD ADD TO THIS THAT NO "VIRUS" GENOME EXIST BECAUSE YOU NEED TO PROVE YOU HAVE A VIRUS BEFORE YOU CAN MAP ITS "GENOME" WHICH IS WHY THEY SEE PIECES OF "VIRUS'" "RNA/DNA" ALL OVER THE "HUMAN GENOME" OF WHICH HAS BEEN CONTESTED AS A MEANINGLESS ATTEMPT TO UNDERSTAND THE COMPLEXITIES OF HUMAN BIOLOGY AND THE SAME IDEA FOR THE "HUMAN VIROME" JUST IN REVERSE - SO IS THE HUMAN FULL OF THE VIROME OR THE HUMAN

GENOME FULL OF THE VIROME - OR IS MOST OF THIS ASSUMED INDESCRIPTIVE LANGUAGE THAT TELLS LITTLE OF WHICH IT CLAIMS TO.

The Human Genome Is FULL OF VIRUSES

Anyone doubting that "SARS-COV-2" could be nothing more than endogenous RNA and come from within us, you may want to research the human genome:

HEADLINE: The Human Genome Is FULL OF VIRUSES

HIGHLIGHTS:

"APPROXIMATELY 8% OF THE HUMAN GENOME IS MADE UP OF ENDOGENOUS RETROVIRUSES (ERVs), which are viral gene sequences that have become a permanent part of the human lineage after they infected our ancient ancestors."

"Viruses are powerful, ancient, and vital to our existence, but they are extremely simple constructions. They tend to be nothing more than a few pieces: a protein capsid, which is a simplistic and protective shell; a protein called a polymerase, which carries out most of the functions related to replicating the viral genome; and a sequence of nucleotides — either RNA or DNA — that encode for the previously mentioned viral proteins."

"Viruses don’t technically have a body during their dormant phase — THEY ARE NOTHING MORE THAN A STRING OF LETTERS IN THE BOOK OF THE GENOME."

"Even beyond these rhythmic cycles, certain kinds of viruses don’t need a physical form at all. These disembodied viruses are called transposable elements, or transposons. True viruses have a body made from proteins, BUT TRANSPOSONS ARE MOBILE GENETIC ELEMENTS — SEQUENCES OF DNA THAT PHYSICALLY MOVE IN AND OUT OF GENOMES. For this reason, they are often referred to as “jumping genes.” TRANSPOSONS DO VERY MUCH THE SAME THING AS TRUE VIRUSES, i.e. THEY COPY AND PASTE THEMSELVES THROUGHOUT GENOMES. They are so similar to true viruses that SOME ENDOGENOUS RETROVIRUSES (ERVs) ARE THEMSELVES

TRANSPOSONS. As stated above, ~8% of the human genome is made up of ERVs, BUT NEARLY 50% OF THE HUMAN GENOME IS MADE OF TRANSPOSONS! HUMANS ARE BASICALLY JUST BIG PILES OF VIRAL-LIKE SEQUENCES."

"A biological virus (whether it is a true virus, an endogenous retrovirus, or a transposon) CAN LITERALLY LAY DORMANT IN A WORD DOCUMENT AS A STRING OF As, Ts, Cs, AND Gs. In other words, viruses can exist independently of genetics, solely in the SYMBOLIC DIMENSION of evolution. A VIRUS IS NOTHING MORE THAN AN IDEA until it finds a host within which it can replicate itself."

https://medium.com/medical-myths-and-models/the-human-genome-is-full-of-viruses

Notice what is admitted here:

-8% of the genome is made up of ENDOGENOUS (come from within us) retroviruses

-"Viruses" are nothing more than a string of letters in a database

-Humans are a big pile of VIRAL-LIKE sequences

-"Viruses" are just a dormant word document of As, Ts, Cs, and Gs

-"Viruses" exist in the SYMBOLIC DIMENSION

-"Viruses" are nothing more than an IDEA

"Viruses" are nothing but imaginary constructions made up of random RNA sequences from our genome.

https://docs.google.com/document/d/e/2PACX-1vSPp3ZH2S_160J1HtvqStAMh19fuz2Me7AZacfn8RvpzIYxVPw_WL_4HdL_4xNYB2p9Y9BKu_opEJiU/pub

THE HUMAN VIROME:

What are "viruses?"

"Viruses are microscopic parasites, generally much smaller than bacteria. THEY LACK THE CAPABILITY TO THRIVE AND REPRODUCE OUTSIDE OF A HOST BODY."

https://www.google.com/.../amp/53272-what-is-a-virus.html

When most people think of "viruses," they think of invisible floating invaders which inhabit the body taking over host cells and multiplying out of control until disease occurs. They are under the false assumption that what are referred to as "viruses" do not belong to our own bodies (endogenous) but must come from some outside source (exogenous). However, this is clearly not the case as the human body is full of what Virologists call "viruses" and they clearly do not understand how these "viruses" work and interact within us.

When you look at the evidence given to us today, the human genome is primarily made up of "viruses:"

"The human genome contains billions of pieces of information and around 22,000 genes, but not all of it is, strictly speaking, human. EIGHT PERCENT OF OUR DNA CONSISTS OF REMNANTS OF ANCIENT VIRUSES, AND ANOTHER 40 PERCENT IS MADE UP OF REPETITIVE STRINGS OF GENETIC LETTERS THAT IS ALSO THOUGHT TO HAVE A VIRAL ORIGIN."

https://www.cshl.edu/the-non-human-living-inside-of-you/

The discovery of these vast amounts of repetitive strings of "viral" origin has given way to what is now known as the human virome:

"MANY HOST DISTRICTS OF THE HUMAN BODY AND ITS MUCOUS MEMBRANES ARE HEAVILY ‘COLONIZED’ BY VIRUSES THAT ARE NOT ASSOCIATED WITH ANY DISEASE. This has led to the CONCEPT OF THE VIROME, which can be considered as the set of all viruses, eukaryotic and prokaryotic, present in the human body. The virome includes viruses that infect host cells, viruses that infect the majority of other types of microorganisms harboured by the body, and VIRUS-RELATED GENETIC ELEMENTS IN OUR CHROMOSOMES [1]. VIRUSES, WHICH CAN NO LONGER BE INVARIABLY CONSIDERED PATHOGENS, INTERACT WITH THE HOST AND OTHER MEMBERS OF THE MICROBIAL COMMUNITIES (Archaea, bacteria and eukaryotes)

IN A VARIETY OF COMPLEX AND MEANINGFUL WAYS."

https://www.clinicalmicrobiologyandinfection.com/.../full...

These "viruses" that make up our virome are not considered pathogenic, are integrated elements in our genome, and interact with the body in complex and MEANINGFUL ways. However, the virome is vastly understudied:

"If you think you don't have viruses, THINK AGAIN.

It may be hard to fathom, but the human body is occupied by large collections of microorganisms, commonly referred to as our microbiome, that have evolved with us since the early days of man. Scientists have only recently begun to quantify the microbiome, and discovered it is inhabited by at least 38 trillion bacteria. MORE INTRIGUING, PERHAPS, IS THAT BACTERIA ARE NOT THE MOST ABUNDANT MICROBES THAT LIVE IN AND ON OUR BODIES. THAT AWARD GOES TO VIRUSES."

"IT HAS BEEN ESTIMATED THAT THERE ARE OVER 380 TRILLION VIRUSES INHABITING US, a community collectively known as the human virome. BUT THESE VIRUSES ARE NOT THE DANGEROUS ONES YOU COMMONLY HEAR ABOUT, like those that cause the flu or the common cold, or more sinister infections like Ebola or dengue. Many of these viruses infect the bacteria that live inside you and are known as bacteriophages, or phages for short. The human body is a breeding ground for phages, and despite their abundance, WE HAVE VERY LITTLE INSIGHT INTO WHAT ALL THEY OR ANY OF THE OTHER VIRUSES IN THE BODY ARE DOING."

"One might rightly assume that if viruses are the most abundant microbes in the body, they would be the target of the majority of human microbiome studies. But that assumption would be horribly wrong. THE STUDY OF THE HUMAN VIROME LAGS SO FAR BEHIND THE STUDY OF BACTERIA THAT WE ARE ONLY JUST NOW UNCOVERING SOME OF THEIR MOST BASIC FEATURES. This lag is due to it having taken scientists much longer to recognize the presence of a human virome, AND A LACK OF STANDARDIZED AND SOPHISTICATED TOOLS TO DECIPHER WHAT'S ACTUALLY IN YOUR VIROME."

"VIRUSES MAY INHABIT ALL SURFACES BOTH INSIDE AND OUTSIDE OF THE BODY. EVERYWHERE RESEARCHERS HAVE LOOKED IN THE HUMAN BODY, VIRUSES HAVE BEEN FOUND. Viruses in the blood? Check. Viruses on the skin? Check. Viruses in the lungs? Check. Viruses in the urine? Check. And so on. TO PUT IT SIMPLY, WHEN IT COMES TO WHERE VIRUSES LIVE IN THE HUMAN BODY, FIGURING OUT WHERE THEY DON'T LIVE IS A FAR BETTER QUESTION THAN

ASKING WHERE THEY DO."

"So the race is on to find those VIRUSES IN OUR BODIES THAT HAVE ALREADY FIGURED OUT HOW TO PROTECT US FROM THE BAD GUYS, while leaving the good bacteria intact."

https://earthsky.org/.../trillions-of-viruses-human-virome

Many people believe that these "viruses" must come from the outside but that is not the case. Take, for instance, ENDOGENOUS (internal origin) Retroviruses:

"ABOUT 8% OF OUR GENOME IS COMPOSED OF SEQUENCES WITH VIRAL ORIGIN, NAMELY HUMAN ENDOGENOUS RETROVIRUSES (HERVs). HERVs are relics of ancient infections that affected the primates' germ line along the last 100 million of years, and BECAME STABLE ELEMENTS AT THE INTERFACE BETWEEN SELF AND FOREIGN DNA. Intriguingly, HERV co-evolution with the host led to the domestication of activities previously devoted to the retrovirus life cycle, PROVIDING NOVEL CELLULAR FUNCTIONS."

https://www.frontiersin.org/.../10.../fimmu.2018.02039/full

"THE VIRAL COMPONENT OF THE HUMAN MICROBIOME IS REFERRED TO AS THE “HUMAN VIROME.” The human virome (also referred to as the “viral metagenome”) is the collection of all viruses that are found in or on humans, including viruses causing acute, persistent, or latent infection, AND VIRUSES INTEGRATED INTO THE HUMAN GENOME, SUCH AS ENDOGENOUS RETROVIRUSES."

"HUMAN ENDOGENOUS RETROVIRUSES COMPRISE GREATER THAN 8% OF THE HUMAN GENOME. They are transcribed ubiquitously in normal tissues. There has been preliminary evidence of their association with diseases, including amyotrophic lateral sclerosis, multiple sclerosis, and rheumatoid arthritis; HOWEVER, THE ASSOCIATION HAS NOT BEEN SHOWN TO BE CAUSAL."

https://www.sciencedirect.com/.../immunology.../human-virome

"Although there are exceptions, THE VAST MAJORITY OF ERVs (particularly the ancient ERVs) IS NOT CLOSELY RELATED TO KNOWN EXOGENOUS RETROVIRUSES, IS NO LONGER CAPABLE OF EXPRESSING VIRUS, and has no other associated biological or phenotypic properties to facilitate classification."

https://www.sciencedirect.com/.../endogenous-retrovirus

Of course, it is ASSUMED that Endogenous Retroviruses evolved from foreign DNA long ago but there is no proof of this. As with every aspect of Virology, the "viral" origin is assumed yet the fact remains, these "viral" sequences are a part of us and come from within. They are known to not be pathogenic.

With so much of the human body composed of what Virologists claim is of "viral" origin, it is clear that the idea of the exogenous pathogenic "virus" is the unproven exception to the rule and not the norm.

It's time to realize that "viruses" are not what we have been told they are and that they are, in fact, a part of us:

“We know a lot about the bacteria that inhabit humans,” says David Pride, an infectious disease doctor at the University of California, San Diego. IN COMPARISON, “WE KNOW ABSOLUTELY NOTHING ABOUT THE VIRUSES.”

"Virus hunters aren’t so lucky. THERE IS NO ANALOGOUS VIRUS-IDENTIFICATION TAG. Instead, to look for viruses, researchers must sequence hundreds of thousands of bits of DNA from a sample — skin swabs, saliva, feces or mucus, for example. Scientists have gotten really good at generating these DNA sequences; THE TRICK IS FIGURING OUT WHAT THEY ARE.

Some of these DNA bits come from human cells, some from bacteria and other microbes that occupy the body, such as archaea and fungi. SOME BITS MAY COME FROM VIRUSES, BUT IT IS HARD TO TELL FOR SURE, says Pérez-Brocal, BECAUSE SCIENTISTS HAVE A LIMITED SET OF CHARACTERIZED VIRUSES TO USE AS A GUIDE FOR SPOTTING NEW ONES."

"EVERY TIME FREDERIC BUSHMAN SAMPLES A NEW PERSON’S VIROME, he says, HE FINDS NEW VIRUSES. A microbiologist at the University of Pennsylvania Perelman School of Medicine in Philadelphia, Bushman has shown that no two people’s gut viruses are exactly alike (SN Online: 7/14/10). But once a person has picked up a community of bacteria-infecting phage, it tends to stick around. FULLY 80 PERCENT OF THE VIRUSES PRESENT WHEN THE RESEARCHERS FIRST STARTED TRACKING ONE MAN’S VIROME WERE STILL THERE MORE THAN TWO YEARS LATER."

"SOME VIRUSES MAY ACT AS BELLWETHERS FOR THE HEALTH OF THE IMMUNE SYSTEM.

Stephen Quake, a geneticist and Howard Hughes Medical Institute investigator at

Stanford University, and his colleagues were studying recipients of heart or lung transplants to learn why some people reject the organs. They collected blood from 96 transplant patients and examined bits of DNA floating in the samples. “We realized some of the DNA wasn’t human,” Quake says.

Of the nonhuman component of the patients’ blood, 73 PERCENT CAME FROM VIRUSES. The majority — 68 percent — of the viruses they found were anelloviruses, MYSTERIOUS GERMS THAT DON’T CAUSE SPECIFIC ILLNESS but have been linked to fevers in toddlers. Some of the transplant recipients had high levels of the viruses in their blood. IT MAY SOUND COUNTERINTUITIVE, BUT “THAT'S GOOD NEWS IF YOU HAVE AN ORGAN TRANSPLANT,” Quake says."

"IN ORGAN REJECTION, THE ANELLOVIRUSES ARE NOT THE CAUSE; THEY’RE SENTINELS. But other maladies MAY have viral instigators. FIGURING OUT WHICH VIRUSES ARE THE CULPRITS IS A DIFFICULT TASK, says Kristine Wylie, a virologist at Washington University School of Medicine in St. Louis.

"Wylie and her colleagues took blood samples and nasal swabs from infants and toddlers, some of whom had unexplained fevers. They wanted to see if DNA technology could quickly identify why the kids were sick.

The researchers found 25 different major categories of viruses, including many associated with illness, they reported in PLOS ONE in June 2012. Children with fevers tended to carry a heavier viral burden, both in the number and type of viruses. BUT EVEN HEALTHY KIDS HAD PLENTY OF VIRUSES IN THEIR NOSES AND IN THEIR BLOOD.

“HEALTHY SUBJECTS ARE JUST LOADED WITH VIRUSES,” Wylie says. EVEN VIRUSES KNOWN TO CAUSE DISEASES SUCH AS THE COMMON COLD WERE FOUND IN HEALTHY KIDS. THAT MAKES IT DIFFICULT TO DETERMINE WHETHER A PARTICULAR VIRUS IS REALLY MAKING SOMEONE SICK."

"TO FIGURE OUT WHICH VIRUSES ARE FRIENDS, FOES OR NEUTRAL PASSENGERS ON THE HUMAN BODY, SCIENTISTS FIRST NEED TO IDENTIFY THEM. RESEARCHERS STILL AREN’T VERY GOOD AT RECOGNIZING NEW VIRUSES, says Brian Jones, a molecular biologist at the University of Brighton in England. HENCE THE LARGE POOL OF UNKNOWN SAMPLES in Pérez-Brocal’s and other researchers’ virome studies. BUT EVEN IF SCIENTISTS IMPROVE THEIR IDENTIFICATION SKILLS, IT MAY TAKE A LONG TIME TO FIGURE OUT WHAT THE VIRUSES ARE DOING IN THE BODY.

Based on what researchers have learned so far about the virome, JONES IS CONVINCED THAT VIRUSES AND OTHER MICROBES “SHOULD BE VIEWED AS A

PART OF US RATHER THAN SOMETHING THAT LIVES IN OR ON US." They are part of the puzzle, the intricate ecosystem composed of human and microbial cells, all pushing and pulling at one another and subject to local conditions, such as diet and environment.

If he's right, then KNOWING OUR VIRUSES MIGHT HELP US KNOW OURSELVES."

https://www.google.com/.../www.../article/vast-virome/amp

No wonder they want us to stop breathing. We are nothing but walking, talking "viruses..."

When you realize that "viruses" have never been purified/isolated and proven to exist as a whole pathogenic entity, it is not surprising to find that the RNA/DNA that is assumed to come from exogenous (from outside the body) "viruses" is actually found abundantly inside of us. As is commonly stated when you read up on "viruses," they are literally nothing more than strings of letters in a genomic database. There is nothing physical backing up these letters and their role and purpose within our bodies is unknown. One thing that is clear: the RNA/DNA that make up "viruses" do not come from outside of us but are a part of our complex biological ecosystem:

"BIOLOGISTS ESTIMATE THAT 380 TRILLION VIRUSES ARE LIVING ON AND INSIDE YOUR BODY RIGHT NOW—10 times the number of bacteria. Some can cause illness, BUT MANY SIMPLY COEXIST WITH YOU. In late 2019, for example, researchers at the University of Pennsylvania discovered 19 DIFFERENT STRAINS OF REDONDOVIRUS IN THE RESPIRATORY TRACT; a handful were associated with periodontal disease or lung disease, BUT OTHERS COULD POSSIBLY FIGHT RESPIRATORY ILLNESSES. Scientists' rapidly expanding knowledge makes it clear that we are not made up primarily of "human" cells that are occasionally invaded by microbes; OUR BODY IS REALLY A SUPERORGANISM OF COHABITATING CELLS, BACTERIA, FUNGI AND MOST NUMEROUS OF ALL: VIRUSES. The latest counts indicate that as much as half of all the biological matter in your body is not human."

"A DECADE AGO RESEARCHERS WERE BARELY AWARE THAT THE HUMAN VIROME EXISTED. Today we see the vast virome as an integral part of the larger human microbiome, a crazy quilt of passive and active microscopic organisms that occupy almost every corner of our being. We have been mapping the virome for 10 years, and the deeper we investigate, the more THE VIROME LOOKS LIKE A PARTNERSHIP that can influence our daily lives positively as well as negatively."

"Viruses need to invade host cells to reproduce, and they are adept at exploiting all the

options in our body. A dozen years ago inexpensive genome sequencing led us to discover plentiful viruses in the mouth and gut. By 2013 or so SCIENTISTS LOCATED VIRUSES ON THE SKIN AND IN THE RESPIRATORY TRACT, BLOOD AND URINE. Most recently, we have found them in even more surprising places. In September 2019, for example, Chandrabali Ghose and our colleagues and I published details about VIRUSES THAT WE DISCOVERED IN THE CEREBROSPINAL FLUID OF ADULTS who were undergoing testing for various conditions. The viruses belonged to several different families and WERE NOT ASSOCIATED WITH ANY KNOWN DISEASE. We also found the same viruses in blood plasma, joint fluid and breast milk. Scientists knew that a few rare, infectious viruses, notably herpes, could sneak into cerebrospinal fluid, BUT FINDING RANDOM VIRUSES THAT SEEMED TO BE MERE BYSTANDERS WAS A SURPRISE. The central nervous system, which is supposed to be a sterile environment, is colonized by a somewhat diverse viral community."

https://www.scientificamerican.com/.../viruses-can-help.../

As you research further, you find what Virologists do is assume functions onto these strands of RNA/DNA which they have molded in a computer model of how they think the "virus" they discovered should look. They claim these sequences are "viral" in origin and that they could not possibly originate within us. However, these sequences are a part of our complex microbiome and are essential to healthy functioning. It's increasingly apparent that the more Virologists uncover about "viruses," the less they actually know:

"Although the microbiome is established as an important regulator of health and disease, THE ROLE OF VIRUSES THAT INHABIT ASYMPTOMATIC HUMANS (collectively, the virome) IS LESS DEFINED. While we are still characterizing what constitutes a healthy or diseased virome, an exciting next step is to MOVE BEYOND CORRELATIONS and toward identification of specific viruses and their precise mechanisms of BENEFICIAL OR HARMFUL immunomodulation."

"In comparison, VERY LITTLE IS KNOWN ABOUT EUKARYOTIC AND PROKARYOTIC VIRUSES THAT ALSO INHABIT ASYMPTOMATIC HUMANS. Given that the name virus was coined from the Latin word meaning slimy liquid or POISON and that VIRUSES ARE CONSIDERED OBLIGATE PATHOGENS, A POSSIBLY “BENEFICIAL VIROME” IS SURPRISING TO MANY."

"The late start for viruses in the commensal microorganism field is in large part DUE TO OUR INABILITY TO READILY CULTURE OR DETECT THEM, as was the case during the discovery of the influenza virus. We do not yet know the eukaryotic cell or bacterial host of most viruses, and there is no universal 16S ribosomal RNA equivalent, as in bacteria, allowing for rapid taxonomic characterization. Technologies such as metagenomics have only recently enabled identification of viruses in healthy human

tissues. This initially involved sequencing all DNA or RNA in a sample (human, bacterial, and viral), and COMPUTATIONALLY ALIGNING THE MASSIVE NUMBER OF SEQUENCES TO IDENTIFY THOSE THAT RESEMBLE KNOWN VIRAL GENE'S."

"However, many hurdles in our ability to catalog the human virome remain, making this data far from final. (1) THE VAST MAJORITY OF VIRUSES SHARE LITTLE TO NO HOMOLOGY WITH ANNOTATED VIRUSES IN REFERENCE DATABASES."

"A lesson from the microbiome field, at this juncture in virome research, would be to MOVE BEYOND CORRELATIONS and toward a detailed analysis of how certain viruses autonomously or cooperatively educate our physiology. Functional studies in mice have found that enteric viruses inhabiting a healthy host provide immune and gut homeostasis. Depletion of viruses or virus receptors in healthy mice exacerbates intestinal inflammation while treatment with viral ligands protects from disease [5]. However, PRECISE MECHANISMS BY WHICH INDIVIDUAL VIRUSES PROVIDE PROTECTION are limited. Furthermore, HOW HUMAN VIROME COMPOSITION IMPACTS HEALTH OR DISEASE REMAINS AMBIGUOUS as direct functional studies are currently lacking."

https://genomemedicine.biomedcentral.com/.../s13073-020...

As is stated above, it is far past time for Virologists to move beyond correlation equaling causation. It is time for them to stop assuming that the RNA/DNA sequences they find are "viral" and a cause of disease. It is time to stop looking for one cause for one disease. They have never been able to prove any of this scientifically.

It is time for us to realize that we are the keepers of our own health. What we put into our body and how we interact with our world has been proven to contribute to disease. Multiple factors play into illness such as the polluted air we breathe, the pesticide-laden food we eat, the flurodiated/chlorinated water we drink, the toxic pharmaceuticals/vaccines we take, the drugs and alcohol we consume, the lack of sleep/exercise we deprive our body's of, the emotional stressors we face daily, etc.

One thing is perfectly clear: The strings of RNA/DNA being falsely labelled as "viruses" have never been proven to exist in a physical state, let alone that these letters in a database cause disease.

The more we discover, the less we know.

"Science" tells us that there are 380 trillion "viruses" and 38 trillion bacteria that are within us. They tell us that we are more microbe than "human." We live in a state of symbiosis with these different bacteria, "viruses," fungi, yeast, etc. Attempting to kill them with antibiotics and antivirals is destroying the very core elements that make us what we are. Remember, Antibiotic literally means "tending to prevent, inhibit, or destroy life."

If you need evidence that these microbes are vital to our health, look no further than experiments involving animals. Animals which are raised as "germ-free" are known to be unhealthier than their counterparts:

"Due to lacking a microbiome, many germ-free organisms EXHIBIT HEALTH DEFICITS SUCH AS DEFECTS IN THE IMMUNE SYSTEM AND DIFFICULTIES WITH ENERGY ACQUISITION."

https://en.m.wikipedia.org/wiki/Germ-free_animal

We need to stop placing the blame for disease on the microbes within us and cease attacking them in a foolish attempt at elimination. We are only destroying ourselves in the process.

"The greatest danger of upsetting the equilibrium between man and his bacteria lies in anti-bacterial drug therapy . . . and in attempts to eradicate infections."

Hilary Koprowski, the renowned virologist and immunologist

"A germ-free world is an ecological absurdity, just as a perpetual motion machine is a mechanical absurdity . . . it is just nonsense to talk of eradication."

Biologist Julian Huxley

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3477854/

This is from one of the foundational texts of naturopathy "Principles and Practices of Naturopathy", written by Dr Ernest W. Cordingley, 1924.

https://docs.google.com/document/d/e/2PACX-1vSpo40_wld8n1ZuVm5qUs_6yV4gQIVo9tHyzG3N3965BUnvXsIiKfmyWPEWOvYjCPQwxIhNDHknFWDL/pu

b

4. Virologists **have never seen or isolated "viruses"** in humans, animals, plants or their fluids. They only did it seemingly, indirectly, and only ever by means of very special and artificial cell systems in the laboratory. They never mentioned the control attempts or documented whether they succeeded in **depicting** and isolating viruses in and from humans, animals, plants or their fluids.

Illustration:

5. Virologists have never isolated, biochemically characterized or obtained their supposed genetic material from the supposed viruses that they photograph using **electron microscope** images. They have never conducted or published control experiments as to whether, after isolating these structures, it was actually possible to detect "viral" proteins (the envelope of the virus) and, above all, the viral genome, which is supposed to be the central component and characteristic of a virus.

Illustration:

A huge problem for Virologists -

TEM

A huge problem for Virologists is that there is no way that they can claim what they present as evidence of a "virus" has any relation to natural particles that occur in vivo, or within a living organism. The numerous cell-altering chemicals/antibiotics and foreign animal RNA that is subsequently mixed together with the original unpurified sample (swab or BALF) from a sick individual immediately destroys any claim of purification. The fact that so much is added to the sample destroys the claim of isolation.

If the cell culturing process isn't enough to alter the sample beyond it's original state, the destruction of the natural cell morphology caused by preparing the sample for Transmission Electron Microscope images ensures that this is the case. Highlighted below are some of the steps (fixation and embedding) an already altered cell culture sample goes through in order to be prepared for imaging:

"Studies in virology go hand in hand with the development of microscopy techniques. Among them, electron microscopy (EM) has played a major role due to the small size of virus particles that, with very few exceptions, cannot be visualized by conventional light microscopy [1,2,3,4]. Prior to the invention of the electron microscope in 1931 by the German engineers Ernst Ruska and Max Knoll [5], VIRUSES WERE DETECTED INDIRECTLY e.g., BY MEANS OF THE CYTOPATHIC EFFECT THEY CAUSE IN INFECTED CELLS OR THROUGH CLINICAL MANIFESTATIONS. However, the availability of EM enabled us to visualize and identify many infectious agents causing diseases or living “in symbiosis” with other organisms. Thus, during the 20th century EM has been a standard technique for virus diagnosis (reviews by [6,7,8,9])."

"Preparation of cells for EM should follow one major goal, i.e., TO PRESERVE THE ULTRASTRUCTURE IN A STATE THAT IS AS CLOSE AS POSSIBLE TO A SNAPSHOT OF THE LIVING STATES. Quoting Gareth Griffiths, a pioneer in EM [14]: “the cell structure should be preserved exactly as it was in the living state and should be visualized at the resolution limit of the electron microscope”. To achieve this goal, several methods are available and standard recipes have been established. Although they are of great help for routine applications, they must be frequently modified when addressing particular biological questions."

"The standard method to prepare cells for routine EM involves the following steps: FIXATION, EMBEDDING and SECTIONING. These will be extensively described below

and are summarized in Figure 1."

2.1. FIXATION OF CELLS

The first and most critical step for the visualization of biological objects by EM is the fixation, because IT DETERMINES HOW CLOSELY THE IMAGE SEEN IN THE MICROSCOPE RESEMBLES THE IN VIVO STRUCTURE. THE AIM IS AVOIDING OR REDUCING ARTIFACTS CAUSED BY EXTRACTION, DENATURATION, STERIC HINDRANCE, CHEMICAL ALTERATION OF EPITOPES (especially important for immunocytochemistry analysis) AND CHANGES IN VOLUME AND SHAPE (critical for the 3D analysis methods described in this review) [14]. Fixation of cells can be carried out by two different methods: chemical fixation (Figure 1A) or cryo-immobilization (i.e., physical-fixation; Figure 1B).

"2.1.1. CHEMICAL FIXATION

Chemical fixation of cells is usually performed with buffered aldehydes. During this step the aldehydes create an inter- and intra-molecular network of covalent interactions (“cross-links”), mostly between amino groups that stabilize the biological sample. This results in the formation of a large 3D network of irreversible cross-links throughout the cytoplasm in tenths of seconds to minutes.

While most laboratories have their own preference, GLUTARALDEHYDE (GA) EITHER ALONE OR IN COMBINATION WITH PARAFORMALDEHYDE (PFA) ARE THE MOST COMMONLY USED PRIMARY FIXATIVES. However, GA induces a branched meshwork of cross-links that sterically hinder accessibility of antibodies to the antigen. For this reason, formaldehyde, which masks less the antigenicity, is mostly used for immunocytochemistry studies like immunofluorescence [14].

During all the processing steps it is very important that cells do not dry out. This is particularly important before fixation, prior to the initial contact with the fixative, when the cells are still alive. ALTHOUGH THE FIXATION PROCESS KILLS CELLS, CELLS SHOULD DIE “PROPERLY” TO ENSURE THAT, as far as possible, ALL CELL COMPONENTS ARE KEPT SO WELL PRESERVED AS WHEN THEY WERE ALIVE. To this aim, several other factors might be taken into consideration. Hence the purity of the aldehydes is also critical. Therefore it is recommended to use EM grade aldehydes provided by commercial suppliers. Furthermore, the cross-linking ability of these aldehydes is influenced by the time, concentration and temperature [19]. Routine fixation is performed during 15–30 min at room temperature using a buffer with a physiological pH (6.8–7.4) and a concentration of at least 0.1 M (mol/L) [14]. The nature of the buffer plays also a very important role during fixation: sodium cacodylate, sodium phosphate, HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) and PIPES

(piperazine-N,N′-bis(2-ethanesulfonic acid)) are, for instance, among the most widely used."

"UNFORTUNATELY THERE IS NOT A GENERAL RECIPE FOR CHEMICAL FIXATION. THE BEST RESULTS REQUIRE ADAPTATIONS TO INDIVIDUAL EXPERIMENTAL CONDITIONS. Therefore, we recommend consulting an EM specialist or check the literature to help you to choose the optimal conditions (including type and concentration of the aldehyde; type, concentration and pH of the buffer; time and temperature for fixing), tailored for your experiments.

Upon fixation cells are washed with the buffer of choice, in which cells can be stored at 4 °C until further processing. Note that cultured cells can be prepared for EM as monolayers or as pellets (Figure 1A). When the cells are further processed as pellets, they must be scraped off the cell culture dish after fixation if they are not growing in suspension. Alternatively, for cryo-EM cultured cells can be “trypsinized” before fixation (Figure 1B). IT SHOULD BE KEPT IN MIND THAT PELLETING OF THE CELLS ALTERS THEIR MORPHOLOGY AND CAN LEAD TO ARTIFACTS (Figure 2)."

"THE MAIN DRAWBACK OF CHEMICAL FIXATION IS THAT IT ALTERS THE STRUCTURE OF THE CELL BY FORMING A NETWORK OF CROSS-LINKED MOLECULES. SUCH A NETWORK IS PRONE TO ARTIFACTS, which is a major challenge for most EM-based studies (for a detailed discussion on this topic see [21]). Nevertheless, chemical fixation has been a mainstay of EM for decades as it PRESERVES THE CELL MORPHOLOGY REASONABLY WELL."

"As already pointed out by Small 34 years ago [24], THE MAJORITY OF ULTRASTRUCTURAL ALTERCATIONS MIGHT OCCUR DURING THE POST-FIXATION PROCESSING OF THE SAMPLES FOR EMBEDDING (described below), RATHER THAN DURING FIXATION. Thus, a tailored protocol must be designed for the highest preservation of cells/tissue of interest, including both optimal fixation and post-fixation conditions."

"2.1.2. CRYO-FIXATION

Freezing techniques represent an alternative to the ARTIFACT-PRONE CHEMICAL FIXATION (reviewed in [25]). The basic principle is to arrest cells by rapid cooling, a process that takes a few milliseconds, resulting in the simultaneous stabilization of all

cellular components without altering their environment. The simplest method consists of immersing cells growing on EM grids in liquid ethane or propane, by means of plunge [26,27] and jet freezing [28,29,30,31,32], respectively (Figure 1B). An inherent limitation of these rapid cooling approaches is that samples can only be vitrified to a depth of micrometers from their surface [33,34,35]. This lies in the poor heat conductance of water: high superficial cooling rates rapidly decay within the sample, reaching a low value that causes water crystallization [36,37,38]. ICE CRYSTAL'S ALTER THE CYTOPLASM ULTRASTRUCTURE BY INDUCING PHASE SEGREGATION BETWEEN WATER AND SOLUTES [37,39]. EVEN WORSE, GROWING ICE CRYSTAL'S MIGHT LEAD TO THE FORMATION OF HOLES IN MEMBRANES AND DESTROY ORGANELLES [40]."

"2.2.1. EMBEDDING OF CHEMICALLY FIXED CELLS

After chemical fixation, cells must be further processed in order to analyze them by EM. Due to their low electron scattering power biological samples are inherently of low contrast [56]. Therefore, heavy metals like osmium tetroxide (OsO4) and uranyl acetate (UA), with high affinity to many cellular structures, are used after fixation as contrasting agents in routine EM. Owing to its reactivity with unsaturated acyl chains of membrane lipids OsO4, for instance, facilitates the retention of lipids [57,58], in addition to its role in contrasting structures (especially membranes). Similarly, Silva et al. [59] have shown that UA plays a role in protecting lipids against solvent extraction."

"As stated before, the ultrastructural preservation of the cells is not only influenced by the method of fixation, but also by the subsequent processing steps (dehydration, infiltration and resin polymerization). AS A CONSEQUENCE OF THE DENATURING EFFECTS OF THESE PROCESSING STEPS THE STRUCTURES ARE RARELY PRESERVED TO THE EXTENT AS THEY APPEAR IN VIVO."

https://www.mdpi.com/1999-4915/7/12/2940/htm

Notice that both fixation and embedding are known to alter the cell morphology, introduce artefacts, and rarely preserve the structures as they would appear in vivo. Remember, these steps are further alteration that has been done to the original sample after the highly toxic cell culturing process.

There is no attempt at purification nor isolation. There are billions of identical particles that could be present. Virologists pick whatever heavily altered particle which fits the mold or idea of the "virus" they want to find and share it as proof of said "virus." They do not take into account that these particles more than likely were not a part of the original sample to begin with nor that they were created during the culturing, fixing, and embedding processes. They assume form, function, and pathogeniticity without ever

proving it.

This is why purification/isolation of an unaltered sample directly from a sick patient is the only way to ensure that what is being imaged has any relevance whatsoever.

https://docs.google.com/document/d/e/2PACX-1vRSC6Lu3VeC8zxHgt9ey0BD_8vncuW1YebFN25W-JDRrCpwohnmFTHNyniiZZ04jDwcoQ1Iqz8IXyNN/pub

MISINTERPRETING ELECTRON MICROSCOPE IMAGES:

(SARS COV2 AS AN EXAMPLE)

It should be clear to anyone being intellectually honest that purification and isolation of a particle believed to be a "virus" is essential and can not be skipped. Without separating the particle from everything else, it is easy to see that Virologists look at numerous similar particles and mistake them for the preconceived one that they are looking for from the start. Without purification/isolation, there is no way for a Virologist to know what exactly they are looking for, especially in regards to a "novel virus" no one has supposedly ever seen before.

Below are numerous examples of misinterpretations of Electron Microscope images just for "SARS-COV-2." It is a long read, but you will see that there are multiple issues when trying to identify particles believed to be "SARS-COV-2." Attempting to identify images of this "virus" is an area fraught with subjective analysis of a preconceived idea of what a "SARS-COV-2" particle is supposed to look like:

Caution in Identifying Coronaviruses by Electron Microscopy

"WE ARE CONCERNED ABOUT THE ERRONEOUS IDENTIFICATION OF CORONAVIRUS DIRECTLY IN TISSUES BY AUTHORS USING ELECTRON MICROSCOPY. Several recent articles have been published that purport to have identified severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) directly in tissue.1–⇓⇓4 MOST DESCRIBE PARTICLES THAT RESEMBLE, BUT DO NOT HAVE

THE APPEARANCE OF, CORONAVIRUSES."

"In the article by Farkash et al.,8 the electron microscopic images in their Figure 3, A–C DO NOT DEMONSTRATE CORONAVIRUSES. Rather, THE STRUCTURES DESCRIBED AS VIRUSES ARE CLATHRIN-COATED VESICLES (CCVs), normal subcellular organelles involved in intracellular transport."

"Additionally, Farkash et al.8 document their findings by referring to an article by Su et al.2 that purports to have identified coronavirus in kidney. Likewise, THAT ARTICLE SHOWS ONLY NORMAL CELL STRUCTURES THAT, to the non-electron microscopist virologist, MAY RESEMBLE CORONAVIRUS. Their interpretation has been refuted in Letters to the Editor of Kidney International."

"IDENTIFICATION OF VIRUSES IS NOT ALWAYS STRAIGHT FORWARD. Consideration should be given to the mechanism of virus production, including the location inside of cells, as well as the appearance (size, shape, internal pattern of the nucleocapsid, and surface spikes).14–⇓16 CARE SHOULD BE TAKEN TO PREVENT MISTAKING CELL ORGANELLES FOR VIRAL PARTICLES."

https://jasn.asnjournals.org/content/31/9/2223

Multivesicular bodies mimicking SARS-CoV-2 in patients without COVID-19

"MOST OF THE PUBLISHED IMAGES DEPICTING THE SUSPECTED VIRUS ARE VERY SIMILAR, IF NOT IDENTICAL, TO MULTIVESICULAR BODIES (MVBs). MVBs have been well-known since the 1960s and their appearance and occurrence is detailed in the classic monograph of Feroze Ghadially; however, their exact significance and function is unclear. WE SUSPECT THAT THE EM IMAGES OF SARS-CoV-2 PUBLISHED TO DATE ARE IN FACT MVBs."

"TRANSMISSION EM OF TISSUE SECTIONS IS NOT A SPECIFIC OR SENSITIVE METHOD FOR THE DETECTION OF VIRAL PARTICLES; THERE ARE NUMEROUS STRUCTURES FOUND BY EM THAT RESEMBLE VIRUSES (so-called viral-like particles), such as the well-known endothelial tubuloreticular inclusions (also called myxovirus-like particles). Therefore, caution is suggested when identifying a virus by EM in tissue sections."

https://www.kidney-international.org/article/S0085-2538(20)30529-9/fulltext?fbclid=IwAR1-u_utpFrHJj1fCx_krHH_ON-h6GFIFMooLtFSVsuEWvbWYArHX9PVQOw

Electron microscopy of SARS-CoV-2: a challenging task

"We read with interest the Correspondence by Zsuzsanna Varga and colleagues on the

possible infection of endothelial cells by SARS-CoV-2 using electron microscopic (EM) images as evidence. However, we believe the EM images in the Correspondence DO NOT SHOW CORONAVIRUS PARTICLES BUT INSTEAD SHOW CROSS-SECTIONS OF THE ROUGH ENDOPLASMIC RETICULUM (RER). These spherical structures are surrounded by dark dots, which might have been interpreted as spikes on coronavirus particles but are instead ribosomes."

"Just recently, there have been two additional reports in which structures that can normally be found in the cytoplasm of a cell HAVE BEEN MISINTERPRETED AS VIRAL PARTICLES. EM can be a powerful tool to show evidence of infection by a virus, BUT CARE MUST BE TAKEN WHEN INTERPRETING CYTOPLASMIC STRUCTURES TO CORRECTLY IDENTIFY VIRUS PARTICLES."

https://www.thelancet.com/.../PIIS0140-6736(20.../fulltext

Alternative interpretation to the findings reported in visualization of severe acute respiratory syndrome coronavirus 2 invading the human placenta using electron microscopy

"The authors examined the placenta by transmission electron microscopy to identify SARS-CoV-2 particles. They identified circular inclusions in the cytoplasm of several syncytiotrophoblasts that they concluded were SARS-CoV-2 virions."

"THE STRUCTURES IDENTIFIED AS SARS-CoV-2 VIRIONS LOOK EXACTLY LIKE CLATHRIN-COATED PITS OR VESICLES. Clathrin-coated vesicles are spherical structures employed by trophoblasts and other cell types to internalize cargos from the extracellular space."

"I propose that the structures identified by Algarroba et al in their journal preproof paper ARE CLATHRIN-COATED VESICLES AND NOT SARS-CoV-2 PARTICLES. This conclusion is based on the following evidence: (1) the circular structures in the electron micrographs in the paper, identified as virions, have the size and shape of clathrin-coated vesicles found in nearly all eukaryotic cells"

https://www.ajog.org/article/S0002-9378(20)30632-3/fulltext

Why misinterpretation of electron micrographs in SARS-CoV-2-infected tissue goes viral

"Nevertheless, ULTRASTRUCTURAL DETAILS IN AUTOPSY TISSUES HAVE BEEN MISINTERPRETED AS CORONAVIRUS PARTICLES IN RECENT PAPERS. Bradley

and colleagues described “coronavirus-like particles” in autopsy specimens of the “respiratory system, kidney, and gastrointestinal tract”, and in a case report Dolhnikoff and colleagues described “viral particles” in “different cell types of cardiac tissue” of a deceased child. HOWEVER, THE IMAGES IN THESE PUBLICATIONS SHOW PUTATIVE VIRUS PARTICLES THAT LACK SUFFICIENT ULTRASTRUCTURE FOR AN UNAMBIGUOUS IDENTIFICATION AS VIRUS. Some of these particles DEFINITELY REPRESENT OTHER CELLULAR STRUCTURES, such as rough endoplasmic reticulum (eg, Dolhnikoff and colleagues, figure 3B), multivesicular bodies (Bradley and colleagues, figure 5C) and coated vesicles (Bradley and colleagues, figure 5B, G). Moreover, it is remarkable that Dolhnikoff and colleagues referred to findings, described by Tavazzi and colleagues, of “viral particles” in interstitial cells, WHICH ARE CLEARLY NON-VIRAL STRUCTURES, SUCH AS COATED VESICLES. Furthermore, Bradley and colleagues quoted publications as a reference for their virus particle identification, which, in our opinion, both IDENTIFIED NON-CORONAVIRUS STRUCTURES AS CORONAVIRUS PARTICLES, as already discussed by Goldsmith and colleagues and by Miller and Brealey."

"As diagnostic EM requires both specialised staff and expensive equipment, and has been replaced by other methods (eg, immunohistochemistry) in several fields of application, its use has been in decline in the past decades, resulting in irreversible loss of expertise that now becomes dramatically overt during the SARS-CoV-2 pandemic. THIS DILEMMA OF DIAGNOSTIC EM SHOULD ALARM US ALL, AS MISLEADING INFORMATION ON THE PRESENCE OF SARS-CoV-2 IN TISSUE HAS ALREADY MADE ITS WAY INTO THE SCIENTIFIC LITERATURE AND SEEMS TO BE PERPETUATED."

https://www.thelancet.com/.../PIIS0140-6736%2820.../fulltext

Characterizing Viral Infection by Electron Microscopy

"Direct infection of extrapulmonary tissues has been postulated, and using sensitive techniques, viral RNA has been detected in multiple organs in the body, including the kidney. However, direct infection of tissues outside of the lung has been more challenging to demonstrate. THIS HAS BEEN IN PART DUE TO MISINTERPRETATION OF ELECTRON MICROSCOPY STUDIES."

"With the emergence of SARS-CoV-2, we are witnessing a renaissance in the use of electron microscopy (EM) to help identify virally infected cells and uncover the pathogenesis of this disease. Several articles have used EM to propose direct evidence of infection of the kidney and other tissues by SARS-CoV-2. These reports have fueled

speculation that direct infection of tissues throughout the body contributes to the morbidity and mortality of COVID-19.

UNFORTUNATELY, MANY OF THESE STUDIES ARE FRAUGHT WITH CONFUSION OVER DIFFERENTIATING VIRUS FROM NORMAL STRUCTURES WITHIN CELLS, LEADING TO AN EXPLOSION OF MISINFORMATION. Indeed, published articles claiming to provide direct evidence of SARS-CoV-2 virus infection in kidney cells and endothelial cells HAVE PROVOKED LETTERS TO THE EDITOR CHALLENGING THESE CLAIMS."

"Understanding the biology of viruses is essential to accurately identify viral particles by EM BECAUSE CERTAIN CELLULAR ORGANELLES THAT CAN MIMIC THE STRUCTURE OF VIRAL PARTICLES (Table 1).

The location inside the cell and the type of membrane-bound organelles with which viral particles are associated can be important clues to identifying the virus. ACCURATE INTERPRETATION OF ELECTRON MICROGRAPHS requires integration of morphology and biology."

"The putative virions detected in the kidney renal tubular epithelial cells, podocytes, and endothelial cells described in several recent publications appear as free particles in the cytoplasm, a location that would not be expected for coronavirus. In vitro studies and the rare examples of in vivo coronavirus infections reported before the current pandemic, as well as recent reports of in vitro studies and human infections for the current pandemic, all demonstrate coronavirus within membrane-bound organelles, or outside of cells. Similar problems lie with proposed virus detected in multiple cell types in the chorionic villi of the placenta, endothelial cells within the lung, endothelial cells within the skin, and cardiomyocytes and interstitial cells in the heart. THESE REPORTS DO NOT DISCUSS ALTERNATIVE EXPLANATIONS FOR THE IDENTIFIED STRUCTURES OR WHY SARS-CoV-2 INFECTION OF HUMAN TISSUES WOULD BREAK THE EXISTING PARADIGM FOR CORONAVIRUS INFECTION. THIS RAISES IMPORTANT QUESTIONS ABOUT THE INTERPRETATIONS OF THE MICROGRAPHS."

"Cellular Structures Mistaken for Virus

CELLS HAVE MANY ORGANELLES COMPARABLE IN SIZE TO THE CORONAVIRUS, with varying degrees of electron-dense material inside and surrounding them."

"CELLULAR VESICLES CAN BE DIFFICULT TO CLASSIFY ON THE BASIS OF MORPHOLOGY ALONE but can be deduced from their relationship with other membranes in the cell. Vesicles seen budding from the plasma membrane that are about 60 to 100 nm in diameter, surrounded by an electron-dense coat, and appear spiculated, are likely clathrin-coated (Figure 1B).

Vesicles that measure approximately 60 to 100 nm in diameter, have similar spiculated electron-dense coats, are found in the vicinity of ER and Golgi, and bud from these organelles are likely coatamer-coated (Figure 1, C and D). OTHER COATED VESICLES IDENTIFIED IN THE CELL CYTOPLASM CAN BE DIFFICULT TO CLASSIFY ON THE BASIS OF ULTRASTRUCTURAL MORPHOLOGY ALONE (Figure 1D)."

"Multivesicular bodies are also involved in the endocytic and exocytic functions of cells. Early endosomes pinch off molecules destined for removal or degradation into intraluminal vesicles, forming multivesicular bodies. The multivesicular bodies may fuse with autophagosomes or lysosomes to degrade the contents, or with the plasma membrane to expulse exosomes. THE INTRALUMINAL VESICLES FOUND WITHIN LARGER VESICLES (Figure 1E) HAVE BEEN CONFUSED WITH SARS-CoV-2 PARTICLES. MICROVILLI CAPTURED IN PLASMA MEMBRANE INVAGINATIONS CAN ALSO MIMIC MULTIVESICULAR BODIES AND BE CONFUSED FOR VIRAL PARTICLES (Figure 1F)."

"Proposed Criteria for Identification of Viral Infection of Tissues by Electron Microscopy in COVID-19 and Future Pandemics

TO ENSURE THE RIGOR AND REPRODUCIBILITY FOR THE IDENTIFICATION OF VIRUSES IN TISSUES BY ELECTRON MICROSCOPY, we propose that the following four criteria be met.

STRUCTURE: morphologic features of the viral particles SHOULD CONFORM TO PRIOR KNOWLEDGE OF THE VIRUS, including size and uniformity, formation of higher-order structures (aggregates/arrays/inclusions), the absence or presence of a clearly discernible membrane, and the qualities of internal (eg, nucleocapsid) and external (eg, peplomers/spikes) electron densities. If prior knowledge is lacking or incomplete, the structure of the viral particles should be established with an appropriate model system, such as electron microscopy of in vitro infected cells. For coronavirus, Goldsmith and Miller note that CORONAVIRUS SPIKES ARE OFTEN DIFFICULT TO VISUALIZE IN THIN SECTIONS USING TRANSMISSION EM, and are usually less obvious than clathrin coats. In addition, the nucleocapsid within the membrane of the

viral particle has characteristic dot-like electron densities that are typically absent from cellular vesicles (Figure 2). The reported diameter of the virus is approximately 80 nm. However, in our studies, the SARS-CoV-2 viral particles had an average diameter of 64 nm (range, 56 to 75 nm) (Figure 2). Tissue preservation is also critical, and poor preservation, as is common for autopsy material, COMPROMISES OBJECTIVE INTERPRETATION OF ELECTRON MICROGRAPHS AND THE ABILITY TO CONCLUSIVELY IDENTIFY VIRAL PARTICLES.

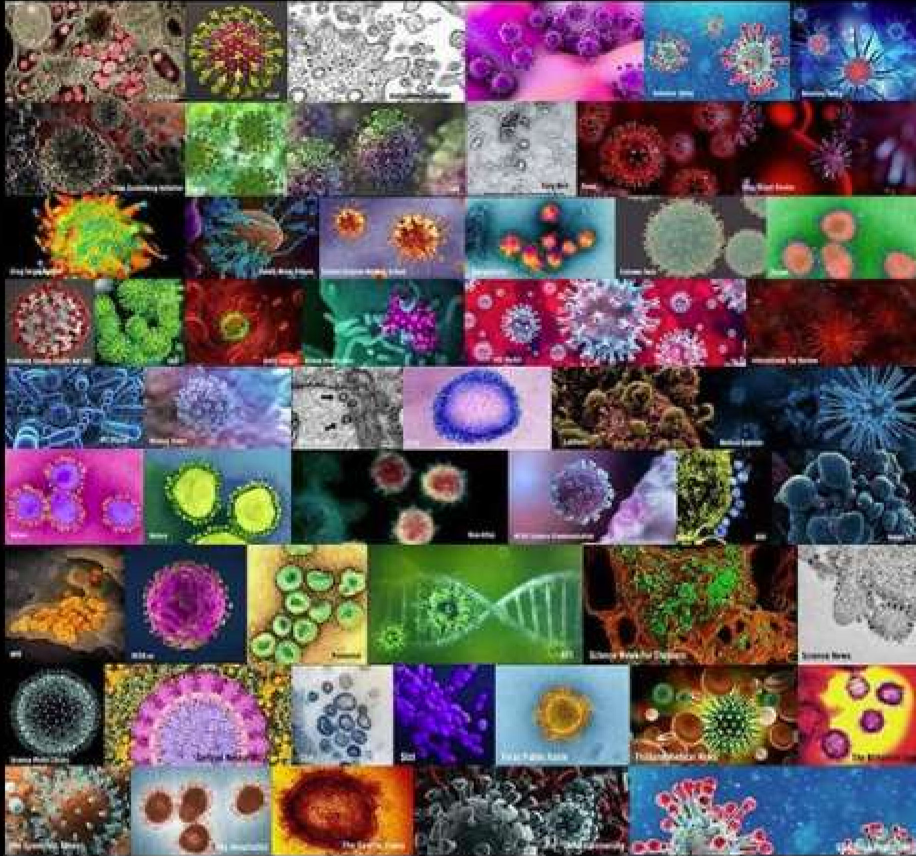
LOCATION: viral particles should be present in sites that CONFORM WITH THE KNOWN BIOLOGY OF VIRAL REPLICATION; strong supporting evidence is required when attempting to identify viral particles in tissues with suboptimal preservation, necrosis, and autolysis to differentiate these particles from normal cellular structures. Coronavirus particles are found inside the cisternae of the ER-Golgi and secretory compartment, as well as outside of cells (Figure 2).

INDEPENDENT EVIDENCE TO CORROBORATE EM FINDINGS: additional validated tests, such as PCR, immunohistochemistry, in situ hybridization, and immunoelectron microscopy, should be performed independently to confirm viral infection and further support the interpretation of the EM findings (Figure 3).

EXPERTISE: electron microscopy should be performed AND INTERPRETED by experienced individuals and aided by appropriate controls and bona fide images of the virus sought. Experience with electron microscopy for diagnosis of kidney diseases alone IS NOT SUFFICIENT TO ACCURATELY DISCERN SUBCELLULAR ORGANELLES FROM NOVEL VIRUSES, and appropriate experience should be gained or sought."

https://ajp.amjpathol.org/.../S0002-9440(20)30503-4/fulltext

As you can see, the image of "SARS-COV-2" truly is in the eye of the beholder...or at least the one interpreting the TEM images.



One of the "proofs" people try to offer in support of "SARS-COV-2" or any other "virus" is that there are pictures of them. They assume that since we can see images of these "viruses," they must exist. The images referred to are normally Transmission Electron Microscope images taken from cell cultures. However, there are numerous issues in relying on cell cultures for proof of anything as detailed here:

https://m.facebook.com/story.php?story_fbid=10158078047703576&id=502548575

Before the invention of the Electron Microscope in 1931, "viruses" were just assumed to exist and cause disease. They could not be seen visually. After the invention of EM, normally invisible particles said to be "viruses" could now be seen. However, the status

of these particles as a "virus" was still assumed as they were never properly purified/isolated from an unaltered sample from a sick patient nor proven pathogenic in a realistic way using animal models. What Virologists do is look for particles fitting the image they want of a "virus" in an unpurified cell culture and then imply pathogenicity as well as function to these particles.

This is a brief description of how the cell culture samples are prepared for imaging and the many particles that are certain to be in the sample which resemble "viruses:"

"Briefly, a 10 μl preparation is taken from the cell culture, placed on a formvar and carbon coated grid, this is followed by the addition of 10 μl of negative stain (e.g. phosphotungstic acid). The solutions are left on the grid for a few seconds to 1 minute followed by drying with filter paper. The grid is then ready to be viewed using a TEM. With this method the background is stained and particles, including intact virions are left unstained, therefore outer details of the virus are visualized against the electron-dense background. CARE MUST BE TAKEN WITH INTERPRETATION SINCE THE SAMPLE CONTAINS OTHER CELLULAR DEBRIS THAT CAN BE IN THE SIZE RANGE OF A VIRUS."

"When TEM techniques are applied to diagnostic samples it is often for the purpose of detecting a virus and thus THERE MAY BE A BIAS TO CLOSELY SCRUTINIZE THE TISSUE TO FIND A PARTICLE THAT RESEMBLES A VIRUS. Generally in cell culture the virus has been amplified to such high concentrations that detecting and identifying the virus should be relatively straightforward. IF IT IS NOT, THEN OTHER CAUSES FOR CPE IN THE CELL LINE, SUCH AS TOXICITY, SHOULD BE INVESTIGATED."

"Strict criteria must be utilized in order to be confident that a virus is responsible for the associated pathology and CAUTION MUST BE PRACTICED TO AVOID MISIDENTIFYING NORMAL CELL STRUCTURES THAT MAY RESEMBLE A VIRUS. When a viral etiology is suspected and searched for in tissue IT IS AMAZING HOW MANY CELL STRUCTURES CAN RESEMBLE A VIRUS!"

"HOST CELLULAR ORGANELLES CAN FALL INTO THE SAME SIZE RANGE AS VIRUSES AND CAN RESEMBLE VIRAL STRUCTURES, although clear differences discern cell organelles and virions. The potential for the structures in question to be related to cellular organelles in either normal or pathological states must be ruled out TO AVOID MISIDENTIFICATION OF CELLULAR STRUCTURES AS “VIRUS-LIKE”.

Several cellular components in the cytoplasm THAT MAY BE CONFUSED FOR VIRUSES include primary lysosomes, secretory granules (Figure 6a,b), transport vesicles (Figure 6c), glycogen (Figure 7), and crystalline inclusions. In the nucleus structures such as nuclear pores and perichromatin granules (Figure 8), measuring 30-35 nm, are very common and must not be confused with viruses found in the nucleus, such as herpesvirus and adenoviruses. Nuclear pores are found within the nuclear membranes and when sectioned en face the pores are clearly visible.

IN ADDITION TO NORMAL CELL STRUCTURES, PATHOLOGICAL PROCESSES CAN LEAD TO UNUSUAL CYTOPLASMIC OR NUCLEAR INCLUSIONS THAT CAN RESEMBLE VIRAL STRUCTURES."

https://www.google.com/url?sa=t&source=web&rct=j&url=https://units.fisheries.org/fhs/wp-content/uploads/sites/30/2017/08/2.1.2-Electron-Microscopy-for-Virology.pdf&ved=2ahUKEwi3poS69pnvAhVJXK0KHUC7CT44FBAWMAB6BAgEEAI&usg=AOvVaw1iLCKhWcDiQKWNMtbIxVxi

The fact that there are many micro and nanoparticles within the culture which resemble "viruses" shows why it is absolutely essential for the sample said to contain a "virus" to be PURIFIED so that a "virus" is ISOLATED from everything else which could resemble one. Exosomes, which are identical to "viruses," are some of the billions of "virus-like" particles that are within samples:

https://m.facebook.com/story.php?story_fbid=10158047459148576&id=502548575

Besides the fact that there is no realistic way for a Virologist to pick out a particle from an unpurified sample in a TEM image and claim it is the "virus" they were looking for, there are other disadvantages to TEM for "viral" identification as well:

"DISADVANTAGES OF ELECTRON MICROSCOPY

INABILITY TO ANALYZE LIVE SPECIMENS – As electrons are easily scattered by other molecules in the air, samples must be analyzed in a vacuum. This means that live specimens cannot be studied by this technique. THIS MEANS THAT BIOLOGICAL INTERACTIONS CANNOT BE PROPERLY OBSERVED, which limits the applications of electron microscopy in biological research.

BLACK AND WHITE IMAGES – Only black and white images can be produced by an

electron microscope. IMAGES MUST BE FALSELY COLORIZED.

ARTEFACTS – These may be present in the image produced. ARTEFACTS ARE LEFT OVER FROM SAMPLE PREPARATION and require specialized knowledge of sample preparation techniques to avoid."

https://www.news-medical.net/.../Advantages-and...

The process to fix and stain a sample for viewing in a TEM image also has several disadvantages:

"To be visualised by an electron microscope, biological samples need to be:

fixed (stabilised) so the electron beam doesn't destroy them dried thoroughly so the vacuum doesn't affect them.

"The first – and perhaps most important – step in the preparation process is fixation. In this step, living tissue is chemically treated to stabilise it. THIS KILLS THE TISSUE SAMPLE AT THE SAME TIME. It's important to fix a sample as quickly as possible because, AS SOON AS TISSUE IS REMOVED FROM ITS NATURAL ENVIRONMENT, IT STARTS TO CHANGE. For instance, oxygen levels start to drop as soon as tissue is removed from an organism. This causes mitochondria to start to change their appearance. Another common change in the fixation process is that lipids tend to form micelles."

"LOOKING OUT FOR ARTEFACTS OF FIXATION

Micelles and strange-shaped mitochondria are examples of artefacts – structures that are seen under the microscope but aren't found in living cells. It's very important to be aware that ARTEFACTS CAN BE INTRODUCED DURING FIXATION SO THAT YOU DON'T MISTAKEN THEM FOR REAL PARTS OF YOUR SAMPLE. Telling the difference between an artefact and a 'real' structure CAN BE DIFFICULT."

"For TEM, samples must be cut into very thin cross-sections. This is to allow electrons to pass right through the sample. AFTER BEING FIXED AND DEHYDRATED, SAMPLES ARE EMBEDDED IN HARD RESIN TO MAKE THEM EASIER TO CUT. Then, an instrument called an ultramicrotome cuts the samples into ultra-thin slices (100 nm or thinner). TEM SAMPLES ARE ALSO TREATED WITH HEAVY METALS to increase the level of contrast in the final image. The parts of the sample that interact strongly with the

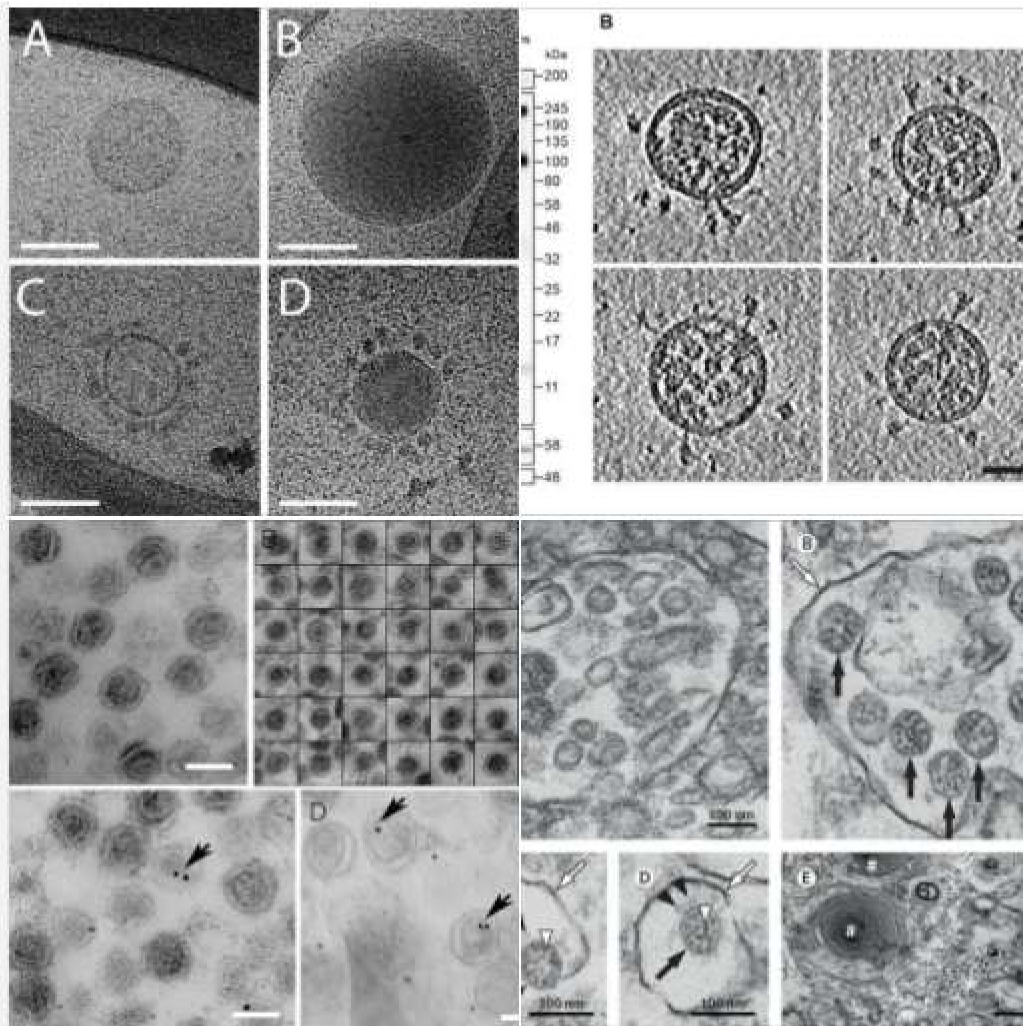
metals show up as darker areas."

https://www.sciencelearn.org.nz/.../500-preparing-samples...

There are many chemicals and procedures done to the cell culture sample before the imaging can take place. It is admitted that these processes can alter the sample which introduces artefacts. These artefacts are structures not seen in LIVING CELLS yet the sample that is seen in an TEM image is NO LONGER LIVING and has been heavily altered not only by cell culture conditions but also by the fixing and staining process. For a Virologist to claim any of these particles are "viruses" let alone that they are found in living cells is disingenuous at best and flat out lying at worst.

Look at the TEM images below. Can you tell which are "viruses" and which are exosomes or other extracellular vesicles which resemble "viruses?"

Virologists can not either.



There are many subcellular structures that can be confused with "viruses" in TEM images. To believe that they can look at sections from unpurified cell culture supernatant made up of nearly identical particles to identify a "novel virus" never before seen nor isolated is completely absurd:

"Transmission electron microscopy (TEM) seems to be a logical tool to look for SARS-CoV-2 infection, BUT SOME OF THE PUBLISHED RESULTS ARE HIGHLY CONTESTED (kidney,8, 22 endothelium,8, 9, 23-28 intestine,8 liver29-32 and placenta33-37)."

"Pathologists are good at detecting some viral infections – at least in identifying unusual inclusions on a haematoxylin and eosin (H&E)-stained slide. HOWEVER, UNLESS THERE IS A KNOWLEDGE OF VIRUS MORPHOLOGY (WHAT THEY LOOK LIKE)

AND MORPHOGENESIS (HOW AND WHERE IN THE CELL THEY ARE ASSEMBLED), IT IS DIFFICULT TO IDENTIFY THEM. Depending on the virus, we use immunohistochemistry targeting viral proteins or in-situ hybridisation to highlight their DNA or RNA. Molecular pathology techniques allow us to test for viruses in tissues when in situ techniques are not yielding results. All these techniques have been applied successfully in the context of SARS-CoV-2 (Figure 1;5, 38, 39). IT IS IMPORTANT TO NOTE THAT ANY OF THESE TESTS REQUIRE AN A PRIORI NOTION OF WHAT IS PRESENT; otherwise, it is difficult to choose the right reagent (e.g. if a herpesvirus is suspected and an anti-herpesvirus antibody is used, but the infection is caused by an adenovirus, then the test is negative and the diagnosis is no closer to being made)."

"MORPHOLOGICAL MIMICKED OF SARS-CoV-2

PHYSIOLOGICAL STRUCTURES INCLUDING COATED VESICLES, MULTIVESICULAR BODIES AND CROSS-SECTIONS OF THE ROUGH ER ARE MORPHOLOGICAL LOOKALIKES OF GENUINE CORONAVIRUSES.105

Coated vesicles (CV) are single membrane-bound vesicles of variable size (typically 50–150 nm) characterised by ‘spiny adornments on their limiting membrane’ (Ghadially106) (Figure 4E). They are involved in endocytosis and membrane trafficking (reviewed in Robinson107).

In CLATHRIN-COATED VESICLES, the best-studied example, the CV bud off from so-called coated pits on the cell surface during micropinocytosis. Clathrin and other quantitatively minor proteins provide a three-dimensional structural lattice, which is readily seen in electron micrographs. MORPHOLOGICALLY IDENTICAL STRUCTURES WITH COATS PROVIDED BY THE MAIN PROTEINS, COPI or COPII, are involved in transport processes of the trans-Golgi network.

Internalisation of SARS-CoV-2, after binding to its receptor ACE2, involves this mechanism.46, 47 WHILE CV MAY TRANSPORT VIRAL PROTEINS, as shown for vesicular stomatitis virus,108 AND MAY BE USED FOR REPLICATION OF POLIOVIRUS 109 and, further, HAVE A SIMILAR SIZE TO THAT OF CORONAVIRUS, they are not the assembled virus itself. However, ALTHOUGH THE PROJECTIONS APPEAR AS A PERFECT ‘CORONA’ IN CROSS-SECTIONS, CV lack the nucleocapsid present inside coronavirus cross-sections, and they are located within the cytoplasm and

not within vacuoles."

"Multivesicular bodies (MVB) are other structures of the endosomal pathway visible by TEM (Figure 4F) (reviewed in Huotari and Helenius110)."

"MVB ARE THE PERFECT ‘DECOY’ FOR ELECTRON MICROSCOPISTS SEARCHING FOR VIRAL PARTICLES. Some of us have been misled by them in our COVID-19 autopsy series5 BECAUSE THE ILV HAVE A SIMILAR SIZE TO SARS-CoV-2 AND ARE LOCATED WITHIN VESICLES."

"A CROSS-SECTION THROUGH ROUGH ER CAN EASILY BE MISTAKEN FOR A ‘VIRUS-LIKE PARTICLE’, but these are located within the cytoplasm and not in vesicles and lack the nucleocapsid structures inside."

"In kidneys from COVID-19 autopsies, WE ENCOUNTERED A PECULIAR SUBCELLULAR STRUCTURE CLOSELY MIMICKING SARS-CoV-2 but probably related to the ER (Figure 4C,G–I).5, 13 Larger vesicles with a smooth outside membrane contained several round to oval small vesicles with prominent electron-dense granules on the inside. These granules were bigger than SARS-CoV-2 nucleocapsid seen in our infected cell cultures and had the same size as the ribosomes visible in areas containing rough ER (ribosomes: 20–21 nm (range = 17–23 nm) versus nucleocapsid: 12 nm (range = 9–16 nm). These vesicles with ‘outside-in’ ribosomes are possibly derived from the rough ER by membrane invaginations, as suggested in some of the TEM pictures (Figure 4I). Because the particles inside are larger than nucleocapsid cross-sections, WE BELIEVE THAT THEY PROBABLY DO NOT REPRESENT ASSEMBLED VIRIONS."

"The published in-vivo data are less convincing. COATED VESICLES, MULTIVESICULAR BODIES AND SWOLLEN ROUGH ENDOPLASMIC RETICULUM ARE IMPORTANT MIMICS OF ASSEMBLED VIRIONS, all of which lack the electron-dense dots of the nucleocapsid inside the particles."

https://onlinelibrary.wiley.com/doi/10.1111/his.14264

It is apparent that what is identified in TEM images is based on guesswork and

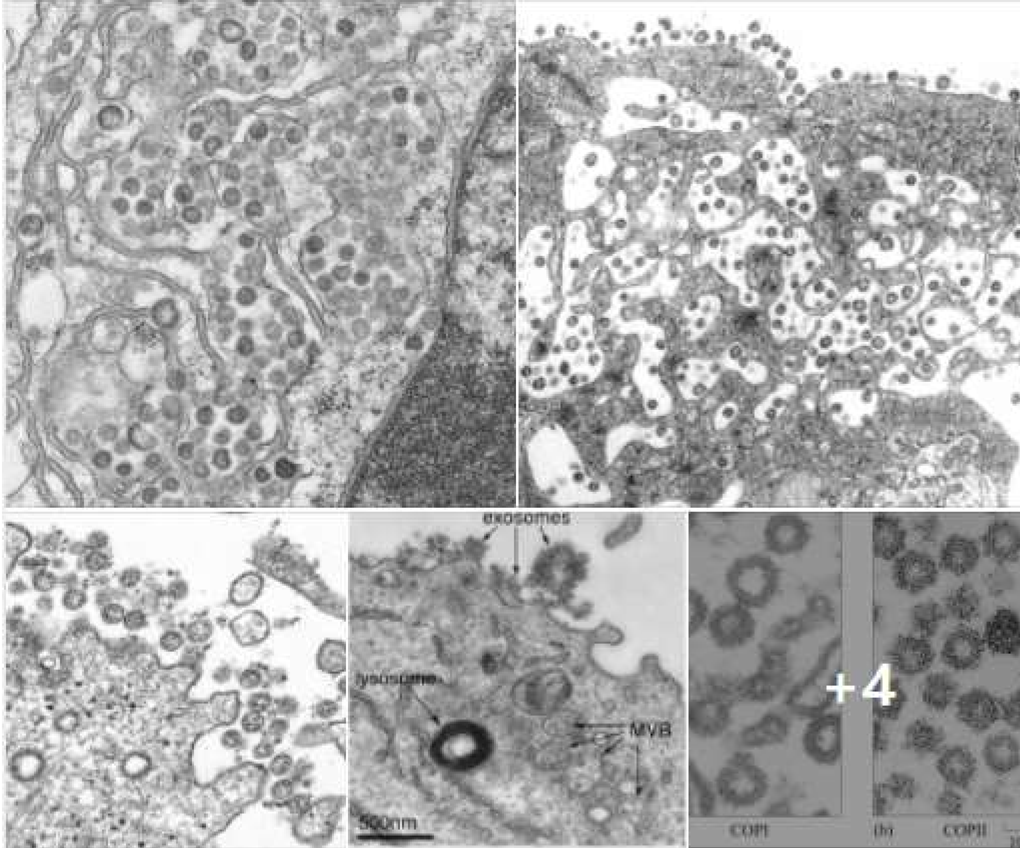
assumptions made by the subjective interpretation of the person viewing the images.

Below is a list of the various subcellular structures which mimic "viruses."

Table 1 Subcellular Structures That Can Be Confused with Viral Particles

Subcellular structure*	Virus mimic*
Perichromatin granules	Small icosahedral viruses
Improperly fixed chromatin	Nucleocapsids
Nuclear pores	Herpesvirus nucleocapsids
Melanosomes	Poxvirus
Cilia and microvilli	Enveloped viruses
Microtubules	Viruses with helical nucleocapsids
Secretory vesicles and granules	Enveloped viruses
Multivesicular bodies and exosomes	Enveloped viruses
ER/Golgi and coatomer-coated vesicles †	Enveloped viruses
Clathrin-coated vesicles †	Enveloped viruses
Granules and glycogen	Small icosahedral viruses

ER, endoplasmic reticulum.
* Personal observations and references. [1 - 22]
† Protein coats can be misinterpreted as spike proteins.



Oh look, it's SARS-COV-1...no wait, it's MERS...no, not that...maybe SARS-COV-2? I guess it could be exosomes. Possibly clathrin coated or secretory vesicles...?

Without purification/isolation, who knows...?

https://docs.google.com/document/d/e/2PACX-1vRtNF-g18mgvOL45buaMhHJp-hfTvGLYf7SZe32m69zmnauOfr7hsr_hKUuo8642z_vf-b20RvStYaJ/pub

"Viruses" or Exosomes?

First, just what is an Exosome?

"Exosomes are defined as nanometre-sized vesicles, being packages of biomolecules ranging from 40-150 nanometres in size that are RELEASED BY VIRTUALLY EVERY CELL TYPE IN THE BODY. Once thought to be a kind of refuse disposal system for cells, exosomes are now known to be far more important than that. EXOSOMES HAVE BEEN SHOWN TO BE KEY MEDIATORS OF CELL TO CELL COMMUNICATION, DELIVERING A DISTINCT CARGO OF LIPIDS, PROTEINS AND NUCLEIC ACIDS THAT REFLECTS THEIR CELLS OF ORIGIN.

The exosomes released by regenerative cells such as stem cells, for example, ARE POTENT DRIVERS OF HEALING AND REPAIR."

https://exopharm.com/what-are-exosomes-and-why-are-exosomes-important

What are the differences between "Viruses" and Exosomes? Not much.

These are two apparently different sub microscopic organisms which are impossible to tell apart:

"THE GENERIC CHARACTERIZATION OF EXTRACELLULAR VESICLES COULD ALSO BE USED AS A DESCRIPTOR OF ENVELOPED VIRUSES, highlighting the fact that extracellular vesicles and enveloped viruses ARE SIMILAR IN BOTH COMPOSITION AND FUNCTION. THEIR HIGH DEGREE OF SIMILARITY MAKES DIFFERENTIATING BETWEEN VESICLES AND ENVELOPED VIRUSES IN BIOLOGICAL SPECIMENS PARTICULARLY DIFFICULT."

https://www.microbiologyresearch.org/content/journal/jgv/10.1099/jgv.0.001193

Just how much do "Viruses" and Exosomes have in common?

"However, it has recently been found that EVs can have important biological functions and that in BOTH STRUCTURAL AND FUNCTIONAL ASPECTS THEY RESEMBLE VIRUSES. This resemblance becomes even more evident with EVs produced by cells productively infected with viruses. Such EVs CONTAIN VIRAL PROTEINS AND PARTS OF VIRAL GENETIC MATERIAL. In this article, we EMPHASIZE THE SIMILARITY BETWEEN EVs AND VIRUSES, IN PARTICULAR RETROVIRUSES. Moreover, we emphasize that in the specific case of virus-infected cells, IT IS ALMOST IMPOSSIBLE TO DISTINGUISH EVs FROM (NON INFECTIOUS) VIRUSES AND TO SEPARATE THEM."

"In contrast to EVs, the definition of viruses developed by 20th century virologists was

quite precise: both the Encyclopedia Britannica and the Oxford English Dictionary define viruses as “an infectious agent of small size that can multiply only in living cells.” EVs do not fall under this definition, because despite THEIR RESEMBLANCE TO VIRUSES IN MANY ASPECTS, they are fundamentally different, as they do not replicate. However, contemporary virology has distanced itself from this strict definition of virus by its wide use of the terms noninfectious and defective virus. Therefore, EVs generated by retrovirus-infected cells that carry viral proteins and even fragments of viral genomes ESSENTIALLY FALL UNDER THE DEFINITION OF NON INFECTIOUS VIRUSES.

Based on current knowledge, THERE ARE MANY ASPECTS IN WHICH EVs RESEMBLE VIRUSES, IN PARTICULAR RETROVIRUSES. First, although some EVs may be up to a micrometer in size, the majority of EVs are <300 nm, the size of a typical RNA virus. Like enveloped viruses, EVs are surrounded by a lipid membrane that also contains cell membrane proteins. Like many viruses, EVs are formed in the endosomal system or at the plasma membrane via defined biogenesis pathways, for example, involving the endosomal sorting complexes required for transport (ESCRT) machinery (1). Like viruses, EVs can bind to the plasma membranes of other cells, enter them either through fusion or endocytosis, and trigger specific reactions from these recipient cells (1). Finally, EVs carry genetic material, and this genetic material can change functions of the recipient cells (2, 3). Especially in the case of retroviruses, EVs generated in infected cells contain selected molecules of viral origin (4) AND CAN BE SO SIMILAR TO NON INFECTIOUS DEFECTIVE VIRUSES THAT HAVE LOST THEIR ABILITY TO REPLICATE THAT THE DIFFERENCE BETWEEN THEM BECOMES BLURRED."

"Unless more specifically defined, IT IS CURRENTLY VIRTUALLY IMPOSSIBLE TO SPECIFICALLY SEPARATE AND IDENTIFY EVs that carry viral proteins, host proteins, and viral genomic elements FROM ENVELOPED VIRAL PARTICLES that carry the same molecules."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4995926/

As you can see, the only difference between a "virus" and an Exosome (or "non-infectious virus") is that Exosomes do not replicate, which is a highly debatable difference.

So if these two sub-microscopic entities are so similar that they are impossible to tell apart in both form and function, it becomes imperative to purify any sample from a sick person in order to separate your "viruses" from your exosomes as they will both be

present in the sample. However, it appears this is a nearly impossible task:

"Nowadays, IT IS AN ALMOST IMPOSSIBLE MISSION TO SEPARATE EVs AND VIRUSES BY MEANS OF CANONICAL VESICLE ISOLATION METHODS, such as differential ultracentrifugation, BECAUSE THEY ARE FREQUENTLY CO-PELLETED DUE TO THEIR SIMILAR DIMENSION. To overcome this problem, different studies have proposed the separation of EVs from virus particles by exploiting their different migration velocity in a density gradient or using the presence of specific markers that distinguish viruses from EVs [56,58,59]. HOWEVER, TO DATE, A RELIABLE METHOD THAT CAN ACTUALLY GUARANTEE A COMPLETE SEPARATION DOES NOT EXIST."

…as it says in the study The Role of Extracellular Vesicles as Allies of HIV, HCV and SARS Viruses published in May 2020 in the journal Viruses."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7291340/pdf/viruses-12-00571.pdf

The original "SARS-COV-2" papers admitted to not purifying any particles, thus there is no way to know if what they claim is "SARS-COV-2" is really a "virus" or an exosome.

They never proved pathogenicity either.

There are no papers that have ever purified/Isolated any "virus" nor ever proven pathogenicity.

Thus, all "viruses" are nothing more than exosomes.

Can you tell the difference?

https://docs.google.com/document/d/e/2PACX-1vTjgwLt4LYP3RMDhhcmmWg_Y_moyMjepLsGZw3ulM936cSAjnlaxTTQnZUsVNd2QwCZiF59OD49GNl7/pub

"VIRUSES"/EXOSOMES: SAME PARTICLES, DIFFERENT NAMES:

Dr. James Hildreth PhD MD, proposed that "THE VIRUS IS FULLY AN EXOSOME IN

EVERY SENSE OF THE WORD.” [1]

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2248418/

It is clear to anyone looking at Virology critically and with logic that the different particles labelled as "viruses" are in fact exosomes, and vice versa. They are similar in size, function, and composition. They can not be separated by means of purification nor can they be isolated which means that any study based on one or the other is inherently contaminated and fraudulent. Two different sources highlight the similarities between these "entities" and the impossibility of purification/isolation even with the most advanced methods:

From January 2019:

VEHICLES OF INTRACELLULAR COMMUNICATION: EXOSOMES AND HIV-1

"The terms extracellular vesicles, microvesicles, oncosomes, or exosomes are OFTEN USED INTERCHANGEABLY as descriptors of particles that are released from cells and comprise a lipid membrane that encapsulates nucleic acids and proteins. Although these entities are defined based on a specific size range and/or mechanism of release, THE TERMINOLOGY IS OFTEN AMBIGUOUS. Nevertheless, these vesicles are increasingly recognized as important modulators of intercellular communication. THE GENERIC CHARACTERIZATION OF EXTRACELLULAR VESICLES COULD ALSO BE USED AS A DESCRIPTOR OF ENVELOPED VIRUSES, HIGHLIGHTING THE FACT THAT EXTRACELLULAR VESICLES AND ENVELOPED VIRUSES ARE SIMILAR IN BOTH COMPOSITION AND FUNCTION. Their high degree of similarity makes differentiating between vesicles and enveloped viruses in biological specimens particularly difficult. Because viral particles and extracellular vesicles are produced simultaneously in infected cells, it is necessary to separate these populations to understand their independent functions."

"However, the terminology and distinguishing aspects for the wide variety of vesicles released from cells are much less clear. Such vesicles are commonly referred to as exosomes, extracellular vesicles, oncosomes, microvesicles and so on. Here, we will refer to them as exosomes for simplicity.

THE WIDE RANGE OF DEFINITIONS FOR CELL-DERIVED VESICLE SUBTYPES RESULTS IN CONSIDERABLE OVERLAP WITH THE DEFINING FEATURES OF ENVELOPED VIRUSES. Functionally, viruses and cell-associated vesicles mediate

intercellular communication by circulating, binding to and entering cells, and delivering their cargo to target or recipient cells [3]. IN ADDITION TO SIMILARITIES IN FUNCTION, EXOSOMES AND VIRUSES ARE ALSO SIMILAR IN COMPOSITION, potentially due to their overlapping use of biogenesis pathways. In infected cells, VIRUSES AND EXOSOMES ARE PRODUCED SIMULTANEOUSLY, RESULTING IN THE INCORPORATION OF VIRAL MATERIAL INTO EXOSOMES. THESE FEATURES MAKE DIFFERENTIATION AND SEPARATION BETWEEN THE TYPES OF PARTICLES DIFFICULT. Distinguishing between exosomes and viruses in biological samples is important to understand their independent and dependent contributions to disease and identify potential therapeutic interventions. It is clear that exosomes may contribute to disease protection or pathogenesis, the reason for which is often unknown or cell-condition specific. PURIFICATION TECHNIQUES AIM TO ADDRESS THIS ISSUE, HOWEVER, ALL CURRENT METHODS OF SEPARATION CONTAIN CAVEATS THAT MAY INFLUENCE DOWNSTREAM APPLICATIONS [4]."

"Exosomes and enveloped viruses

By strict definition exosomes are different from viruses because of their inability to replicate their contents. Unlike viruses, exosomes are metabolically inert and cannot reproduce their contents to generate progeny from producer cells [22]. HOWEVER, AS PREVIOUSLY REVIEWED, EXOSOMES AND VIRUSES DO NOT CONFORM TO STRICT DEFINITIONS [22]. Intermediate particles exist ON THE SPECTRUM BETWEEN VIRUS AND EXOSOMES that contain both host and viral components, making it nearly impossible to classify these vesicles as either defective viruses or exosomes that contain viral components [22]. INTERMEDIATE PARTICLES ARE OFTEN CLASSIFIED AS A VIRUS OR EXOSOME DERIVATIVE, DEPENDING ON THE PREFERENCE OF THE INVESTIGATOR, but once these vesicles deviate from strict definitions they may be more accurately defined as an assortment of lipid-encased particles THAT CANNOT BE EASILY DIFFERENTIATED [22]."

"Upon release from the producer or infected cells, exosomes and viral particles share features regarding how they interact with other cells via protein binding, endocytic pathway uptake and membrane fusion [9, 85]. Following cellular entry, exosomes and HIV-1 virions act as delivery vehicles for information. Both disperse their contents into cells and influence biological processes, frequently by appropriating cellular machinery [76]. HIV-1 VIRIONS COULD BE DESCRIBED AS EXOSOMES that are unique in their ability to replicate their contents. However, this is controversial BASED ON THE DEFINITION OF REPLICATION, such as in the case of replication-incompetent viruses. These particles are still considered to be HIV-1 virions, BUT BY DEFINITION THEY ARE UNABLE TO REPRODUCE THEIR CONTENTS IN LIVING CELLS. Nevertheless, the similarities raise the question of whether or not HIV-1 virions ARE SIMPLY MODIFIED

EXOSOMES [22]. This question has led to the development of the Trojan exosome hypothesis [76], which reasons that EXOSOMES AND RETROVIRUSES CONTAIN EXTENSIVE OVERLAP IN CHARACTERISTICS because retroviruses use the exosome pathway to facilitate receptor-independent infection [86]."

"Separating virus from exosomes

THE OVERLAPPING FEATURES OF EXOSOMES AND HIV-1 PARTICLES MAKES THE PURIFICATION OF EXOSOME AND VIRUS POPULATIONS FROM THE SAME SOURCE DIFFICULT IF NOT IMPOSSIBLE, complicating determination of the composition and functions of exosomes during different stages of HIV-1 infection. Popular techniques rely on velocity gradient separation such as iodixanol, since the density of HIV-1 virions and exosomes are somewhat different, although there is considerable overlap (1.13–1.21 g l^{-1} for exosomes and 1.16–1.18 g l^{-1} for HIV-1) [4]. Thus, THESE TECHNIQUES CAN BE UNRELIABLE DUE TO THE SIMILARITY IN BIOPHYSICAL PROPERTIES AND HETEROGENEOUS NATURE OF EXOSOMES. Immuno-depletion or immuno-capture techniques have been suggested as ways to purify and concentrate exosomes from HIV-1-containing sources. Here, anti-acetylcholinesterase- and/or anti-CD45-coated beads are used to capture exosomes without binding to HIV-1 [112]. Theoretically, this technique is a means to concentrate pure exosomes from HIV-1 particles without the addition of substances influencing down-stream HIV-1 functional assays. However, as at the time of this review, THERE IS PRESENTLY NO WAY TO REMOVE EXOSOMES BOUND TO AFFINITY BEADS WITHOUT DESTROYING EXOSOME INTEGRITY, including exosome surface-associated molecules. Further, immuno-depletion or immuno-capture techniques MAY EXCLUDE SOME EXOSOMES that are surface protein-negative (or double negative) and are still capable of affecting functional studies. Exosome subpopulations contain variations in surface composition that may affect function; for example, CD63 surface protein levels from human semen-derived exosomes correlated to the inhibition of HIV-1 infection, where semen exosomes with reduced surface CD63 showed diminished ability to inhibit HIV-1 infection [64]. Similarly, depletion of the CD63-positive exosome population in herpes simplex virus-1-infected cells enhanced infection [113]. Thus, EFFICIENT METHODS OF EXOSOME AND HIV-1 SEPARATION THAT MAINTAIN VIRION AND VESICLE INTEGRITY WITHOUT THE COMPLICATION OF FUNCTIONAL ASSAYS ARE NEEDED."

https://www.microbiologyresearch.org/content/journal/jgv/10.1099/jgv.0.001193

In Summary (Part 1):

-the terms extracellular vesicles, microvesicles, oncosomes, or exosomes are OFTEN USED INTERCHANGEABLY as descriptors of particles that are released from cells and

comprise a lipid membrane that encapsulates nucleic acids and proteins

-the generic characterization of extracellular vesicles could also be used as a descriptor of enveloped "viruses," HIGHLIGHTING THE FACT THAT EXTRACELLULAR VESICLES AND ENVELOPED "VIRUSES" ARE SIMILAR IN BOTH COMPOSITION AND FUNCTION

-the wide range of definitions for cell-derived vesicle subtypes results in CONSIDERABLE OVERLAP with the defining features of enveloped "viruses"

-functionally, "viruses" and cell-associated vesicles mediate intercellular communication by circulating, binding to and entering cells, and delivering their cargo to target or recipient cells

-exosomes and "viruses" are also SIMILAR IN COMPOSITION, potentially due to their overlapping use of biogenesis pathways.

-in infected cells, "viruses" and exosomes are produced simultaneously, resulting in the incorporation of "viral" material into exosomes

-these features MAKE DIFFERENTIATION AND SEPARATION between the types of particles DIFFICULT

-exosomes and "viruses" do not conform to strict definitions

-intermediate particles exist on the spectrum BETWEEN "VIRUS" AND EXOSOME that contain both host and "viral" components, making it NEARLY IMPOSSIBLE TO CLASSIFY THESE VESICLES as either defective "viruses" or exosomes that contain "viral" components

-intermediate particles are often classified as a "virus" or exosome derivative, DEPENDING ON THE PREFERENCE OF THE INVESTIGATOR, but once these vesicles deviate from strict definitions they may be more accurately defined as an assortment of lipid-encased PARTICLES THAT CANNOT BE EASILY DIFFERENTIATED

-HIV-1 "VIRIONS" COULD BE DESCRIBED AS EXOSOMES that are unique in their ability to replicate their contents

-this is controversial BASED ON THE DEFINITION OF REPLICATION, such as in the case of replication-incompetent "viruses"

-these particles are still considered to be HIV-1 "virions," BUT BY DEFINITION THEY ARE UNABLE TO REPRODUCE THEIR CONTENTS IN LIVING CELLS

-the similarities raise the question of whether or not HIV-1 "virions" ARE SIMPLY MODIFIED EXOSOMES

-this question has led to the development of the Trojan exosome hypothesis, which reasons that EXOSOMES AND "RETROVIRUSES" CONTAIN EXTENSIVE OVERLAP IN CHARACTERISTICS because "retroviruses" use the exosome pathway to facilitate receptor-independent infection

-the overlapping features of exosomes and HIV-1 particles makes the purification of exosome and "virus" populations from the same source DIFFICULT IF NOT IMPOSSIBLE

-purification techniques can be unreliable DUE TO THE SIMILARITY in biophysical properties and heterogenous nature of exosomes

-there is presently NO WAY to remove exosomes bound to affinity beads without destroying exosome integrity, including exosome surface-associated molecules

-immuno-depletion or immuno-capture techniques MAY EXCLUDE SOME EXOSOMES that are surface protein-negative (or double negative) and are still capable of affecting functional studies

-efficient methods of exosome and HIV-1 separation that maintain 'virion' and vesicle integrity without the complication of functional assays are needed

From August 2020:

PURIFICATION METHODS AND THE PRESENCE OF RNA IN VIRUS PARTICLES AND EXTRACELLULAR VESICLES

"The fields of extracellular vesicles (EV) and virus infections are marred in a debate on whether a particular mRNA or non-coding RNA (i.e., miRNA) is packaged into a virus particle or co purifying EV and similarly, whether a particular mRNA or non-coding RNA is contained in meaningful numbers within an EV. KEY IN SETTLING THIS DEBATE, IS WHETHER THE PURIFICATION METHODS ARE ADEQUATE TO SEPARATE VIRUS PARTICLES, EV AND CONTAMINANT SOLUBLE RNA AND RNA:PROTEIN COMPLEXES. Differential centrifugation/ultracentrifugation and precipitating agents like polyethylene glycol are widely utilized for both EV and virus purifications. EV ARE KNOWN TO CO-SEDIMENT WITH VIRIONS AND OTHER PARTICULATES, such as defective interfering particles and protein aggregates.``

"For RNA viruses as well, RNAs other than full-length genomic RNA have been reported in mature virion preparations. IS IT POSSIBLE THAT EV MAY HAVE CONTAMINATED THE VIRION PREPARATIONS IN THESE STUDIES? Has there been enough experimental evidence TO RULE OUT EV CONTAMINATION?"

"Proteins, DNA, mRNA, miRNA, and other non-coding RNAs were found enclosed in these small, membrane-enclosed exosomes and microvesicles [1] (Figure 1B). THIS MAKES EV CONCEPTUALLY AND BIOCHEMICALLY SIMILAR TO VIRUSES [1,18] (Figure 1C). THE FORMATION AND EGRESS OF MICROVESICLES AND EXOSOMES SHARE SIMILARITIES TO VIRUS BIOGENESIS, such as HIV [24] and enveloped hepatitis A viruses (HAV) [25] (Figure 1), respectively. In fact, VIRUSES HAVE BEEN THOUGHT OF AS EMERGING FROM EXOSOMES OR VICE VERSA [26]. Any EV CAN PLAY AN ANALOGOUS ROLE TO A VIRUS PARTICLE in the functional transfer materials from one cell to the next, regardless of the class."

"Viruses and EV are purified by similar techniques (Table 1) [18,27]. Historically, differential centrifugation and ultracentrifugation have been the most widely used methods for concentrating viruses and EV [28]. RNAs CO-PURIFY WITH VIRUSES AND EV IN THE FORM OF (a) NON-ENCASED EXTRACELLULAR RNAs AND (b) CO-CONTAMINATION OF RNA ENCASED IN EV AND VIRIONS. For cleaner purification, RNase treatments are always recommended to remove non-EV encapsulated extracellular RNAs. Co-purified RNAs within a heterogeneous mixture can obscure definitive and functional research on EV as well as viruses. SEPARATING VIRIONS FROM EV, HOWEVER, IS MUCH MORE CHALLENGING. Thus, we will discuss how such contamination—IF ANY—can be removed by different purification methods."

"When the differences are small, i.e., between viruses, EV and protein aggregates [30], a gradient medium (i.e., sucrose, iodixanol, sorbitol, cesium chloride, etc.) is needed to increase the separation efficiency [29].

OFTEN THE VIRUSES' DENSITIES AND BUOYANCIES SO CLOSELY OVERLAP WITH EXOSOMES', THAT EVEN SEPARATION VIA DENSITY GRADIENTS IS IMPRACTICAL [18,31–33] AND LEADS TO CO-ISOLATION OF THEIR ENCASED RNAs."

"Polyethylene glycol (PEG) Precipitation - PEG has long been used to “precipitate” and purify

viruses [34,35]. It is the main reagent in several commercial kits for exosome purification [18,35]. The method consists of a precipitation step followed by low-speed centrifugation. PEG PRECIPITATION OFFERS LITTLE SEPARATION EFFICACIES, CANNOT SEPARATE VIRUSES FROM EV [36], AND OFTEN CO-PRECIPITATES OTHER MACROMOLECULAR CONTAMINANTS LIKE RNA, DNA, AND PROTEIN AGGREGATES [30,35,37,38]. Exosomes isolated by commercial kits ARE LIKELY TO BE CONTAMINATED by viruses, proteins, non-EV associated nucleic acids, and other extracellular debris [36]. This includes any molecules stuck to the outside of the EV rather than being carried inside. MANY OF THESE CONTAMINATIONS MAY CARRY RNAs."

"Chromatography- Size-exclusion chromatography (SEC), ion-exchange chromatography (IEC), and affinity chromatography (AC) are commonly used chromatography methods for virus purification [41–43]."

"Both viruses and EV can be purified by SEC and IEC [18,40] but virus-EV CROSS-CONTAMINATION IS DIFFICULT TO AVOID [30]. Thus, SEC and IEC WILL MOST LIKELY CO-ISOLATE THE ENCASED RNAs CONTAMINANTS."

"SEPARATING EV FROM VIRUS PARTICLES, PARTICULARLY EXOSOMES AND MICROVESICLES, HAS PROVEN TO BE A CONSIDERABLE HURDLE IN THE FIELD OF HOST–PATHOGEN INTERACTIONS. Chugh et al. [36] and Bess et al. [77] showed that VIRIONS AND EV CO-SEDIMENTED IN VARIOUS ISOLATION TECHNIQUES due to their similar size, density, and sedimental velocity [31]. Other studies [25,27,31,32,36,48,77] also showed that NEITHER differential centrifugation NOR commercial exosome precipitation reagents SEPARATE VIRIONS FROM EV."

"SINCE IT IS NEAR IMPOSSIBLE TO SEPARATE EV FROM VIRIONS BY BIOCHEMICAL METHODS, the absence of EV is typically demonstrated by the absence of EV protein markers. For instance, Cliffe et al. [8] checked the purity of their virion preparations by transmission electron microscopy, BUT NO IMAGE WAS INCLUDED IN THE MANUSCRIPT. Lin et al. [9] performed a Western blot and did not detect the exosome markers CD63 or CD81 in the purified virions, concluding that miRNAs were present in virions. HOWEVER, WHETHER THE WESTERN BLOT HAD THE

REQUIRED LEVEL OF SENSITIVITY IS UNKNOWN. In contrast to Lin et al., Chugh et al. [36] showed that for the same virus, the majority of miRNA are carried by EV rather than virions. Herpesvirus can switch between latent and lytic phases [78]. The RNA profiles are very different [79–81]. It is not clear whether the phase of the virus played a role in the discrepancy between Lin et al. [9] and Chugh et al. [36]. THE MAJORITY OF THE STUDIES in Table 2 [4–7,10–13,15] DID NOT INVESTIGATE POSSIBLE EV CONTAMINATIONS. THE CONCEPT OF EV TRANSFERRING FUNCTIONALITY NUCLEIC ACIDS HAS ONLY GAINED TRACTION RECENTLY [82], SO IT IS NOT SURPRISING THAT STUDIES BEFORE 2010 DID NOT CONSIDER THIS POSSIBILITY.

The problem becomes more difficult when considering that as virus-infected cells not only release virions with virus-derived RNAs, they also release EV filled with virus-encoded RNAs at the same time, as well as various species of defective interfering particles. HENCE, WE WOULD EXPECT EV TO CONTAIN VIRAL RNA UNDER MOST CIRCUMSTANCES. EV emanating from cells infected with HIV, hepatitis C virus (HCV), and various human herpesvirus viruses (HHV) can have virus-encoded RNAs present within them [83–85]. In the case of KSHV, viral miRNAs are present predominantly within exosomes, rather than mature virions [36]. Additionally, picornavirus like the EMCV and HAV can traffic the entire virion into EV [25]."

"Still, BETTER AND MORE CAREFULLY VALIDATED PURIFICATION METHODS ARE NECESSARY TO PREPARE CLEANER VIRION AND EV PREPARATIONS before many of the proposed biological functions that have been associated with EV can be accepted."

In Summary (Part 2):

-the study of extracellular vesicles (EV) and Virology are marked in a debate and the key in settling this debate is WHETHER THE PURIFICATION METHODS ARE ADEQUATE to separate "virus" particles, EV and contaminant soluble RNA and RNA:protein complexes

-EV are known to CO-SEDIMENT with "virions" and other particulates, such as defective interfering particles and protein aggregates

-for RNA "viruses," RNAs other than full-length genomic RNA have been reported in mature "virion preparations" and it is possible that EV MAY HAVE CONTAMINATED the "virion" preparations in these studies

-Proteins, DNA, mRNA, miRNA, and other non-coding RNAs were found enclosed in

these small, membrane-enclosed exosomes and microvesicles which means EV are conceptually and biochemically similar to "viruses"

-the formation and egress of microvesicles and exosomes share similarities to "virus" biogenesis

-"viruses" have been thought of as emerging from exosomes or vice versa

-any EV can play an ANALOGOUS ROLE TO A "VIRUS" PARTICLE in the functional transfer materials from one cell to the next, regardless of the class

-RNAs CO-PURIFY with "viruses" and EV in the form of (a) non-encased extracellular RNAs and (b) CO-CONTAMINATION of RNA encased in EV and "virions"

-separating "virions" from EV, however, is much more challenging and they discussed how such contamination—IF ANY—can be removed by different purification methods

-often the "viruses" densities and buoyancies SO CLOSELY OVERLAP WITH EXOSOMES', THAT EVEN SEPARATION VIA DENSITY GRADIENTS IS IMPRACTICAL AND LEADS TO CO-ISOLATION of their encased RNAs

-PEG precipitation offers little separation efficacies, CANNOT SEPARATE "VIRUSES" FROM EV AND OFTEN CO-PRECIPITATES OTHER MACROMOLECULAR CONTAMINANTS like RNA, DNA, and protein aggregates

-exosomes isolated by commercial kits are LIKELY TO BE CONTAMINATED by "viruses," proteins, non-EV associated nucleic acids, and other extracellular debris

-many of these contaminations may carry RNAs

-both "viruses" and EV can be purified by SEC and IEC but "virus"-EV CROSS-CONTAMINATION IS DIFFICULT TO AVOID so SEC and IEC WILL MOST LIKELY CO-ISOLATE THE ENCASED RNAs CONTAMINANTS

-separating EV from "virus" particles, particularly exosomes and microvesicles, has proven to be a CONSIDERABLE HURDLE in the field of host–pathogen interactions

-Chugh et al. and Bess et al. showed that "virions" and EV CO-SEDIMENTED IN VARIOUS ISOLATION TECHNIQUES due to their similar size, density, and sedimental velocity

-other studies also showed that NETHER differential centrifugation NOR commercial exosome precipitation reagents SEPARATE "VIRIONS" FROM EV

-they state it is NEAR IMPOSSIBLE TO SEPARATE EV from "virions" by biochemical methods

-the majority of the studies DID NOT INVESTIGATE POSSIBLE EV CONTAMINATIONS

-the concept of EV transferring functional nucleic acids has only gained traction recently, so it is not surprising that STUDIES BEFORE 2010 DID NOT CONSIDER THIS POSSIBILITY

-they would expect EV TO CONTAIN "VIRAL" RNA UNDER MOST CIRCUMSTANCES

-they conclude that BETTER AND MORE CAREFULLY VALIDATED PURIFICATION METHODS ARE NECESSARY to prepare cleaner "virion" and EV preparations

https://pubmed.ncbi.nlm.nih.gov/32825599/

https://www.google.com/url?sa=t&source=web&rct=j...

The bottom line: EV's are "viruses" and "viruses" are EV's. They can not be purified/isolated from each other and they share the same functions, components, size, characteristics, etc. They are all the same particles. The only differences are the names given to them and the assumptions about their purposes.

6. Virologists report **typical artifacts of dying tissue / cells and typical structures that arise when the cell's own components such as proteins, fats and the solvents** used are swirled, as viruses or viral components. Here, too, there are no control experiments with cells / tissues that were not infected but were also treated.

Illustration:

THIS WAS COVERED IN THE ABOVE CELL CULTURE CRITIQUES

7. The so-called **transmission** attempts that virologists make to prove the transmission and pathogenicity of the suspected viruses refute the entire virology. Obviously, it is the experiments themselves that trigger the symptoms, which **animal experiments** provide as evidence of the existence and effectiveness of the suspected viruses. Here, too, there are no control attempts in which exactly the same thing is done, only with non-

infected or sterilized materials.

"“Infectious experiments” with animals were carried out with the filtrated fluids from putrescent organisms or from fluids allegedly containing the proteins/enzymes which were supposed to represent the virus. The results were meant to prove that a virus was present and would cause the illness ascribed to it. However, what is never mentioned publicly is that the symptoms allegedly caused in human beings by a virus could never be replicated in animal experiments, instead there were always only “similar” symptoms, which they then claimed to be identical with the disease in humans. However, none of this has ever been proven scientifically. To date, all “infectious experiments” are missing the control experiments, i.e. the proof that the symptoms are not caused by the “treatment” of the genetic material in the so-called “infectious” experiment. In order to exclude that it was not the fluids of diseased tissue that caused the symptoms, one would have had to do an identical experiment, only with other fluids or with sterilised fluids. However, that has never happened. Extremely cruel animal experiments are carried out to date – for example in order to prove the transmissibility of measles; during these experiments, monkeys are tied up and immobilised in a vacuum chamber with a tube in their nose, and then scientists insert the allegedly infected fluids through that tube into their trachea and lungs. The exact same damage would be caused by sterile saline solution, sterilised blood, pus or saliva. The induced symptoms, which are only “similar” to those ascribed to measles, are then claimed to be measles.

Since the allegedly infected fluids are pressed through a filter which allegedly filters out bacteria and they are slightly heated, the scientists claim that the suffering and death

of the animals in those experiments cannot be caused by bacteria, but rather by smaller “pathogens”, the viruses. The concerned scientists conveniently ignored the fact already

acknowledged at that time that there are there are much more unknown bacteria than known ones, that many bacteria are heat resistant and that they form spores which cannot be filtrated. It is important to mention here that there is no evidence whatsoever that bacteria cause any disease either.

They are of course often present in the disease process, like the firemen putting out the fire. Bacteria do not cause disease, but rather they participate in biological meaningful reparation processes. As with viruses, the only so-called evidence for the apparently negative role of bacteria are the horrific animal experiments which are completely meaningless, since all control experiments are missing."

From- The Misconception of Virus Theory

TESTING PATHOGENESIS ANIMAL TRIALS

- SARS COV2 AS AN EXAMPLE

March 17th, 2021

"SCIENTISTS WORLDWIDE STRUGGLE TO IDENTIFY SUITABLE ANIMAL MODELS TO STUDY SARS-

CoV-2 INFECTIONS....

...ONE TAKE-HOME MESSAGE OF THIS REPORT IS THAT NO ANIMAL MODEL TESTED THUS FAR ENTIRELY REFLECTS HUMAN COVID-19."   -

"... to emulate human (patho)physiology, more sophisticated models are required. For example, in severe cases, COVID-19 may become a systemic disease. Whether the related extra-pulmonary organ involvement or multi-organ failure correlates to organ-specific host factor expression (for example, ACE2, TMPRSS2, Furin, CD147, Nrp1) fostering local SARS-CoV-2 propagation or

OR WHETHER IT IS CAUSED BY INDIRECT DETRIMENTAL IMMUNE ACTIVATION REMAINS ELUSIVE."

The model dilemma in biomedical research already existed pre-COVID-19 and extends into many other areas.

https://www.nature.com/articles/s41578-021-00305-z?fbclid=IwAR2o_mkJA2szbo-blf4QwNEYhi1D9Ys2ftwqtbwu6yRKz6JsEeBzPbwkBmc

From April 2nd, 2021:

. THE EXISTING SINGLE TRANSGENE MOUSE MODELS POORLY MIMIC THE CLINICAL FEATURES OF COVID-19;,,,

." THE EXISTING SINGLE TRANSGENE MOUSE MODELS POORLY MIMIC THE CLINICAL FEATURES OF COVID-19;...

"...NO ANIMALS DEVELOPED THE SEVERE SYMPTOMS SEEN IN HUMANS although a transient inflammation was OBSERVED INCONSISTENTLY in non-human primates, hamsters and mice (see below). NO CYTOKINE STORMS, COAGULOPATHY, HYPOXEMIC RESPIRATORY FAILURE, MULTIPLE ORGAN FAILURE OR DEATH WERE REPORTED."

https://www.sciencedirect.com/science/article/pii/S0006295221001398

from Mike s doc

https://docs.google.com/document/d/e/2PACX-1vRmYnE5xecj4Mbh3coSN8SObCeqPVD-vQz61HZsGIO5WIdcBh7994QPZmdXvndXGy3zaEy2AYLS-2Bn/pub

In order to prove a new pathogenic "virus" exists, Koch's Postulates must be satisfied first.

They are LOGIC-BASED rules and the bare minimum requirements needed to be fulfilled. Postulates 3 and 4 deal with the use of ANIMAL MODELS in order to prove a PURIFIED particle causes the same disease as found in a human host:

3. the pathogen from the pure culture must CAUSE THE DISEASE WHEN INOCULATED INTO A HEALTHY, SUSCEPTIBLE LABORATORY ANIMAL

4. the pathogen MUST BE REISOLATED from the new host and SHOWN TO BE THE SAME as the originally inoculated pathogen

Sadly, even the early "SARS-COV-2" researchers admitted to not fulfilling these basic postulates, specifically in regards to proving pathogenicity in an animal model:

From two of the original studies:

"ALTHOUGH OUR STUDY DOES NOT FULFILL KOCH'S POSTULATES, our analyses PROVIDE EVIDENCE IMPLICATING 2019-nCoV in the Wuhan outbreak. ADDITIONAL EVIDENCE TO CONFIRM THE ETIOLOGIC SIGNIFICANCE OF 2019-nCoV in the Wuhan outbreak include identification of a 2019-nCoV antigen in the lung tissue of patients by immunohistochemical analysis, detection of IgM and IgG antiviral antibodies in the serum samples from a patient at two time points to demonstrate seroconversion, and ANIMAL (monkey) EXPERIMENTS TO

PROVIDE EVIDENCE OF PATHOGENICITY."

https://www.nejm.org/doi/full/10.1056/NEJMoa2001017

"However, there are still many urgent questions that remain to be answered. THE ASSOCIATION BETWEEN 2019-nCoV AND THE DISEASE HAS NOT BEEN VERIFIED BY ANIMAL EXPERIMENTS TO FULFILL THE KOCH'S POSTULATES TO ESTABLISH A CAUSATIVE RELATIONSHIP BETWEEN A MICROORGANISM AND A DISEASE."

https://www.nature.com/articles/s41586-020-2012-7

In other words, they ASSUMED that the UNPURIFIED cell culture soup they created in a lab (which contains everything from monkey kidney cells, antibiotics, fetal bovine serum, etc. along with the host specimen) was a "virus" without ever proving a pathogenic "virus." They not only ignored Koch's 2nd Postulate stating the particles must be PURE, they entirely skipped over the final two crucial steps of showing that what they created in a lab caused the same disease in animals that it does in humans.

Don't just take my word for it.

From an interview with Xu Jianguo on January 10, 2020, head of an evaluation committee advising the Chinese government:

"Q: Are researchers trying to replicate the disease in lab animals TO PROVE THAT IT IS REALLY THE CAUSE OF THE OUTBREAK?

A: People have recommended that [investigators] do tests to see if the virus can cause the infection in animals, BUT THEY NEED TIME."

https://www.sciencemag.org/news/2020/01/mystery-virus-found-wuhan-resembles-bat-viruses-not-sars-chinese-scientist-says

Need time?

This is from a review published at the end of June 2020:

"THERE IS AN URGENT NEED FOR AN IDEAL ANIMAL MODEL THAT CAN REFLECT CLINICAL SYMPTOMS AND UNDERLYING ETIOPATHOGENESIS SIMILAR TO COVID-19 PATIENTS which can be further used for evaluation of underlying mechanisms, potential vaccines, and therapeutic strategies."

"THIS EMPHASIZES THE SURGE FOR A SUITABLE ANIMAL MODEL TO EXPLORE THE PATHOGENESIS and evaluation of countermeasures for the disease."

"The novel coronavirus (COVID-19) pathology is linked to viral respiratory infection, hyper-immune response, and coagulopathy (Lin et al.2020; Connors and Levy 2020), therefore, to understand the mechanism or to evaluate therapeutic countermeasures, THE ANIMAL MODELS SHOULD INVOLVE ALL THESE INTERPLAYS IN A SINGLE MODEL."

"Further, VALIDATION OF THE ANIMAL MODEL IS CRUCIAL. The error in the animal experimental study narrows the chances of the potential drugs or repurposing or repositioning drugs or vaccines to translate successfully to clinics and moreover, it is a wastage of resources. Thus, IT IS THE NEED OF THE HOUR TO VALIDATE THE ANIMAL MODEL USING DIFFERENT CRITERIA, for instance, face, construct, and predictive validity (Denayer et al.2014)."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7324485/

From September 2020:

"ANIMAL MODELS THAT FAITHFULLY REPRODUCE HUMAN COVID-19 (i.e., incorporating the most important disease mechanisms, clinical signs, and response to treatment) ARE OF UTMOST IMPORTANCE IN ORDER TO ACHIEVE THIS. As commented by others (Callaway, 2020; Cleary et al., 2020; Cohen, 2020) animal species ranging from mice to non-human primates ARE ACTIVELY BEING INVESTIGATED IN THE QUEST FOR REPRODUCIBLE AND FAITHFUL MODELS OF COVID-19."

https://www.frontiersin.org/articles/10.3389/fmicb.2020.573756/full

This is from a review published in October 2020:

"Most of the animal models of COVID-19 recapitulated the mild pattern of human COVID-19 with full recovery phenotype. NO SEVERE ILLNESS ASSOCIATED WITH MORTALITY WAS OBSERVED, SUGGESTING A WIDE GAP BETWEEN COVID-19 IN HUMANS AND ANIMAL MODELS."

https://ccforum.biomedcentral.com/articles/10.1186/s13054-020-03304-8

From a study in November 2020:

"To interpret the process of infection and understand the systematic pathology of the disease caused by SARS-CoV-2 in humans, AN EFFECTIVE SARS-CoV-2 INFECTION ANIMAL MODEL IS URGENTLY NEEDED."

https://journals.plos.org/plospathogens/article...

Clearly, we have been put under lockdown, quarantined, social distanced, masked, and now vaccinated with a rushed, experimental mRNA gene therapy for a "virus" that has never been proven to exist in a pure form nor proven pathogenic in animal models, thus failing Koch's Postulates.

Related post on Koch's Postulates/Purification:

https://docs.google.com/document/d/e/2PACX-1vSM71HNGOt63os_wsp_QRe9-E7F2uGASj--zr5zNW9HjNBxl8iC4sF1N6rmn_OAzugL_zROD4Eo26fZ/pub

Mock-infected animals were challenged with 100ul of PBS.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7184405/pdf/ciaa325.pdf?fbclid=IwAR3p5DowoVQwZZYY15lMNEzHeYCM26HQqyt4QPQKtpAp1Gi-IRt4sMdglcc

(....imediatly killed most of the animals to look for "antibodies")

"..Eight hamsters were intranasally challenged with SARS-CoV-2 (0dpi)(Supplementary Figure S2). Twenty-four hours later(1dpi), each SARS-CoV-2-challenged hamster was transferred to a new cage with each cage containing one naïve hamster as close contact. Five virus-challenged and five contact hamsters were sacrificed for viral load and histopathological studies ..."

WE WANT TO KNOW WHAT WOULD HAPPEN TO THE ANIMALS IF THE SAME PROCEDURES TOOK PLACE TO CONTROL GROUP WITHOUT A PAITIENT SAMPLE- I dont believe proper animal control experiments have ever taken place.

Leaving aside the fact that virologists never actually isolate and purify viruses—which they openly admit and which we have now explained—let's assume that the unpurified fluid they use does contain the relevant virus and, therefore, should be able to transmit infection. After "isolating" a virus, virologists have three "hosts" they can use in their attempts to prove that viruses cause illness: they can expose humans to the virus; they can expose animals to the virus; or they can use tissue cultures taken from various animal or human sources and expose the tissue cultures to the virus.

In the history of virology, most virologists have decided not to do their experiments on human subjects, as this is considered unethical. In the case of the SARS-CoV-2 virus, we know of no published study that has used humans as the test subjects. Virologists also admit that in the case of most viral infections, there are no studies available proving infection in animals. How a virus can infect and kill humans—but not animals—is left unexplained. Researchers get around this obvious biological conundrum by saying, "There are no animal models on which to test such-and-such a virus." In other words, "We know that the virus infects and kills humans even though we've never tested the virus on humans because that would be unethical. Therefore, we do our tests on animals, even though when we test animals, they don't get sick, because they are not proper 'hosts' for the virus. So, you'll just have to trust us."

In the case of SARS-CoV-2, we know of two animal model studies that used unpurified "virus," one in hamsters and one in mice. In the hamster study,10 researchers took the unpurified, lung-cancer-grown, centrifuged animal secretions and squirted them down the throats and into the lungs of a group of unfortunate hamsters. Some—but not all—of the hamsters got pneumonia, and some even died. Perplexingly, however, some of the hamsters didn't even get sick at all, which certainly doesn't square with the deadly contagious virus theory. Because there was no comparison group, we also have no idea what would have happened if the researchers had squirted plain lung cancer cells into the lungs of the hamsters; probably not anything good.

In the mouse study,11 researchers infected both transgenic mice (that is, mice genetically programmed to get sick) and wild (normal) mice with unpurified virus. None of the wild mice exposed to the "virus" got sick. Of the transgenic mice, a statistically

insignificant number either lost some fur luster or experienced weight loss. Thus, scientists have not been able to show that the Covid-19 “virus” causes harm to animals.

DR. T.C.

LACK OF "SARS-COV-2" ANIMAL MODEL:

In order to determine the pathogenicity of a "virus," animal models must be employed to see if the supposed pathogen said to be hiding in the toxic cell culture soup actually causes the same disease in healthy animals as it does in humans. However, here we are a year-and-a-half into this "pandemic" and they still have yet to find a suitable animal model that exhibits the same disease as seen in humans:

From March 17th, 2021:

COVID-19 HIGHLIGHTS THE MODEL DILEMMA IN BIOMEDICAL RESEARCH

"SCIENTISTS WORLDWIDE STRUGGLE TO IDENTIFY SUITABLE ANIMAL MODELS TO STUDY SARS-CoV-2 INFECTIONS. Interspecies-related differences, such as host specificity, divergent immune responses, or the unavailability of species-specific reagents hamper the research."

"Animal models are imperative in preclinical research; HOWEVER, MOST ANIMAL MODELS ARE POORLY PREDICTIVE OF HUMAN CONDITIONS. THE LACK OF APPROPRIATE MODELS often results in poor clinical outcomes and high failure rates of clinical trials1. In an effort to overcome this translational gap, many researchers are calling for a paradigm shift towards human-centred approaches."

"This prompted the World Health Organization to form an international panel to establish suitable animal models for vaccine and therapy testing, with the first summary report published in autumn 20202. ONE TAKE-HOME MESSAGE OF THIS REPORT IS THAT NO ANIMAL MODEL TESTED THUS FAR ENTIRELY REFLECTS HUMAN COVID-19."

https://www.nature.com/articles/s41578-021-00305-z

From April 2nd, 2021:

ANIMAL MODELS FOR SARS-Cov2/COVID19 RESEARCH-A COMMENTARY

"THERE IS AN URGENT NEED FOR NEW ANIMAL MODELS OF SARS CoV-2 INFECTION to improve research and drug development. This brief commentary EXAMINES THE DEFICITS OF CURRENT MODELS and proposes several improved alternates. THE EXISTING SINGLE TRANSGENE MOUSE MODELS POORLY MIMIC THE CLINICAL FEATURES OF COVID-19; those strains get a milder disease than human COVID-19 disease."

"However, that PROGRESS IS HAMPERED BY THE CURRENTLY AVAILABLE ANIMAL MODELS."

"A comprehensive review was recently published by Ehaideb et al., [10], [24], [25] that surveyed publications that reported data on SARS CoV-2 infections in a variety of animals. These included hamsters, non-human primates (macaques), mice, rats, ferrets, rabbits, and cats. All supported viral replication in the lung with MILD DISEASE ensuing as assessed by tissue pathology. NO ANIMALS DEVELOPED THE SEVERE SYMPTOMS SEEN IN HUMANS although a transient inflammation was OBSERVED INCONSISTENTLY in non-human primates, hamsters and mice (see below). NO CYTOKINE STORMS, COAGULOPATHY, HYPOXEMIC RESPIRATORY FAILURE, MULTIPLE ORGAN FAILURE OR DEATH WERE REPORTED."

https://www.sciencedirect.com/science/article/pii/S0006295221001398

It is clear that they have been unable to replicate the same disease symptoms associated with "Covid-19" in animals as that seen in humans. Thus, they can not claim that they have proven pathogenicity as so far they have failed all of Kochs Postulates including purification/isolation of a "virus" but especially Postulates 3 and 4 in regards to animal models:

3. The cultured microorganism should cause the SAME DISEASE when introduced into a healthy organism.

4. The microorganism must be reisolated from the inoculated, diseased experimental host and identified as being IDENTICAL to the original specific causative agent

Why is it that they are having so much trouble finding a suitable animal model to recreate the exact same disease as seen in humans? Were there not numerous reports of many animals testing positive for "SARS-COV-2?" Surely one of these animals said to have had "Covid-19" could be studied seeing as they were already determined to have caught the disease from humans.

Published April 17th, 2021:

CURRENT STATUS OF PUTATIVE ANIMAL SOURCES OF SARS-CoV-2 INFECTION IN HUMANS: WILDLIFE, DOMESTIC ANIMALS AND PETS

"Overall, current data indicate that the most

at-risk interactions between humans and animals for COVID-19 infection are those involving certain mustelids (such as minks and ferrets), rodents (such as hamsters), lagomorphs (especially rabbits), and felines (including cats). Therefore, SPECIAL ATTENTION SHOULD BE PAID TO THE RISK OF SARS-CoV-2 INFECTION ASSOCIATED WITH PETS."

"The extent of the COVID-19 pandemic in wild animals is challenging to evaluate and

REMAINS LARGELY UNCHARACTERISED. Although most domestic animals do not appear to be highly susceptible to SARS-CoV-2, THE RISK ASSOCIATED WITH PET OWNERSHIP SHOULD BE BETTER DEFINED. MANY ANIMALS (including some mustelids, rodents, and lagomorphs) ARE HIGHLY SUSCEPTIBLE TO SARS-CoV-2. Finally, since a large proportion of the human population has been or will be infected with SARS-CoV-2, there is a SIGNIFICANT CONCERN ABOUT REVERSE ZOONOSIS, i.e., THE SPREAD OF THIS VIRUS FROM INFECTED HUMANS TO NAÏVE DOMESTIC OR WILD ANIMALS."

https://www.google.com/url?sa=t&source=web&rct=j...

According to the CDC, which last updated the page on March 25th, 2021:

"SOME CORONAVIRUSES THAT INFECT ANIMALS CAN BE SPREAD TO PEOPLE AND THEN SPREAD BETWEEN PEOPLE, but this is rare. THIS IS WHAT HAPPENED WITH SARS-CoV-2, which likely originated in bats. Early reports of infections were linked to a live animal market in Wuhan, China, but the virus is now spreading from person to person."

"Based on the available information to date, the risk of animals spreading COVID-19 to people is considered to be low. MORE STUDIES ARE NEEDED TO UNDERSTAND IF AND HOW DIFFERENT ANIMALS COULD BE AFFECTED BY SARS-CoV-2."

"We are still learning about this virus, BUT WE KNOW THAT IT CAN SPREAD FROM PEOPLE TO ANIMALS IN SOME SITUATIONS, especially during close contact."

"We know that COMPANION ANIMALS LIKE CATS and DOGS, BIG CATS in zoos or sanctuaries, GORILLAS in zoos, MINK on farms, AND A FEW OTHER MAMMALS CAN BE INFECTED WITH SARS-CoV-2, BUT WE DON'T YET KNOW ALL OF THE ANIMALS THAT CAN GET INFECTED. THERE HAVE BEEN REPORTS OF ANIMALS INFECTED WITH THE VIRUS WORLDWIDE. Most of these animals became infected after contact with people with COVID-19.

A SMALL NUMBER OF PET CATS AND DOGS HAVE BEEN REPORTED TO BE INFECTED WITH SARS-CoV-2 in several countries, including the United States. One ferret was reported positive for SARS-CoV-2 in Slovenia.

SEVERAL ANIMALS IN ZOOS AND SANCTUARIES HAVE TESTED POSITIVE FOR SARS-CoV-2, INCLUDING BIG CATS (lions, tigers, pumas, cougars, snow leopards) AND NON-HUMAN PRIMATES (gorillas) AFTER SHOWING SIGNS OF ILLNESS. It is suspected that these animals became sick after being exposed to an animal caretaker

with COVID-19. In many situations, this happened despite the staff wearing personal protective equipment and following COVID-19 precautions."

"SARS-CoV-2 has been REPORTED IN MINK on farms in multiple countries' external icons, including the United States."

"Recent experimental research shows that many mammals, including CATS, DOGS, BANK VOLES, FERRETS, FRUIT BATS, HAMSTERS, MINK, PIGS, RABBITS, RACCOON DOGS, TREE SHREWS, and WHITE-TAILED DEER CAN BE INFECTED WITH THE VIRUS. Cats, ferrets, fruit bats, hamsters, racoon dogs, and white-tailed deer CAN ALSO SPREAD THE INFECTION TO OTHER ANIMALS of the same species in laboratory settings. A NUMBER OF STUDIES HAVE INVESTIGATED NON-HUMAN PRIMATES AS MODELS FOR HUMAN INFECTION. Rhesus macaques, cynomolgus macaques, baboons, grivets, and common marmosets can become infected with SARS-CoV-2 and BECOME SICK IN A LABORATORY SETTING. Laboratory MICE, chickens, and ducks DO NOT SEEM TO BECOME INFECTED or spread the infection based on results from studies.

These findings were based on a small number of animals, and do not show whether animals can spread infection to people. MORE STUDIES ARE NEEDED TO UNDERSTAND IF AND HOW DIFFERENT ANIMALS COULD BE AFFECTED BY COVID-19."

https://www.cdc.gov/coronavirus/2019-ncov/daily-life-coping/animals.html

From these two sources, it appears that many animals are said to get "infected" with "SARS-COV-2." This raises the questions:

1. Where are all of the outbreaks of "Covid-19" in the animal population?

2. If humans are transmitting "Covid-19" to animals, what stops the animals from transmitting it right back?

3. Why are none of these animals suitable as an animal model to reproduce the same disease as seen in humans seeing as to how they apparently were infected with "SARS-COV-2" from humans?

We are a year-and-a-half into this mess and we lack suitable animal midels to study this "virus" and prove its pathogenicity. The current best fit is genetically altered transgenic mice as regular mice are not susceptible to infection. It shouldn't be hard for them to find a suitable host. They have stated all of these animals can be "infected:"

1. Cats
2. Dogs
3. Bank voles
4. Ferrets
5. Fruit bats
6. Hamsters
7. Mink
8. Pigs
9. Rabbits
10. Racoon dogs
11. Tree shrews
12. White-tailed deer
13. Rhesus macaques
14. Cynomolgus macaques
15. Baboons
16. Grivets
17. Common marmosets
18. Lions
19. Tigers

20. Pumas

21. Cougars

22. Snow leopards

23. Gorillas

24. Goats

It seems that there are many animals to choose from that are apparently able to be infected with "SARS-COV-2" yet no suitable animal model exists. Why could this be?

Maybe testing animals and labelling them positive for "SARS-COV-2" with inaccurate, faulty, not-to-be-used-for-diagnosis PCR tests really does show the results are meaningless? Maybe these results help to show how we have a TESTING PANDEMIC rather than a "viral" one?

Makes sense when you think of all the other interesting items tested positive by PCR for "SARS-COV-2:" pawpaw fruit, motor oil, Coca Cola, chicken wings, ice cream, water, healthy people... 🤔

https://docs.google.com/document/d/1z7KVpYnkPyubks93GIAtjBD9NEZONvoPNg9Q1zvQxOI/edit

https://docs.google.com/document/d/e/2PACX-1vRmYnE5xecj4Mbh3coSN8SObCeqPVD-vQz61HZsGIO5WIdcBh7994QPZmdXvndXGy3zaEy2AYLS-2Bn/pub

Illustration:

In §1, the IfSG demands the scientific nature of all those involved, including those of the virologists who claim the existence of the coronavirus SARS-CoV-2.

The virologists are clearly anti-scientific and have refuted themselves, which is why the IfSG is not fulfilled, but violated and therefore the legal basis for all corona measures is withdrawn.

In all seven steps that virologists take to claim a virus, they refuse to comply with the most important scientific duty, the verification of their methods: They never document control experiments. For this reason alone, statements by virologists claiming that viruses cause illness should never be considered scientific.

THEN THERE IS THE PROBLEM OF:

BEFORE REPRODUCIBILITY MUST COME PREPRODUCIBILITY

"From time to time over the past few years, I've politely refused requests to referee an article on the grounds that it lacks enough information for me to check the work. This can be a hard thing to explain.

OUR LACK OF A PRECISE VOCABULARY — in particular the fact that we don't have a word for 'you didn't tell me what you did in sufficient detail for me to check it' — CONTRIBUTES TO THE CRISIS OF SCIENTIFIC REPRODUCIBILITY. In computational science, 'reproducible' often means that enough information is provided to allow a dedicated reader to repeat the calculations in the paper for herself. In biomedical disciplines, 'REPRODUCIBLE' OFTEN MEANS THAT A DIFFERENT LAB,

STARTING THE EXPERIMENT FROM SCRATCH, WOULD GET ROUGHLY THE SAME EXPERIMENTAL RESULT.

In 1992, philosopher Karl Popper wrote: “Science may be described as the art of systematic oversimplification — the art of discerning what we may with advantage omit.” What may be omitted depends on the discipline. Results that generalize to all universes (or perhaps do not even require a universe) are part of mathematics. Results that generalize to our Universe belong to physics. Results that generalize to all life on Earth underpin molecular biology. Results that generalize to all mice are murine biology. AND RESULTS THAT HOLD ONLY FOR A PARTICULAR MOUSE IN A PARTICULAR LAB IN A PARTICULAR EXPERIMENT ARE ARGUABLY NOT SCIENCE.

Communicating a scientific result requires enumerating, recording and reporting those things that cannot with advantage be omitted. This harks back to the idea of science as a way to build knowledge through careful experimentation. Ushering in the Enlightenment era in the late seventeenth century, chemist Robert Boyle put forth his controversial idea of a vacuum and tasked himself with providing descriptions of his work sufficient “THAT THE PERSON I ADDRESSED THEM TO MIGHT, WITHOUT MISTAKE, AND WITH AS LITTLE TROUBLE AS POSSIBLE, BE ABLE TO REPEAT SUCH UNUSUAL EXPERIMENTS”.

MUCH MODERN SCIENTIFIC COMMUNICATION FALLS SHORT OF THIS STANDARD. Most papers fail to report many aspects of the experiment and analysis that we may not with advantage omit — things that are crucial to understanding the result and its limitations, and to repeating the work. We have no common language to describe this shortcoming. I’ve been in conferences where scientists argued about whether work was reproducible, replicable, repeatable, generalizable and other ‘-bles’, and clearly meant quite different things by identical terms. CONTRADICTORY MEANINGS ACROSS DISCIPLINES ARE DEEPLY ENTRENCHED.

THE LACK OF STANDARD TERMINOLOGY MEANS THAT WE DO NOT CLEARLY DISTINGUISH BETWEEN SITUATIONS IN WHICH THERE IS NOT ENOUGH INFORMATION TO ATTEMPT REPETITION, AND THOSE IN WHICH ATTEMPTS DO NOT YIELD SUBSTANTIALLY THE SAME OUTCOME. To reduce confusion, I propose an intuitive, unambiguous neologism: ‘preproducibility’. An experiment or analysis is preproducible if it has been described in adequate detail for others to undertake it. Preproducibility is a prerequisite for reproducibility, and the idea makes sense across disciplines.

THE DISTINCTION BETWEEN A PREPRODUCIBLE SCIENTIFIC REPORT AND CURRENT COMMON PRACTICE IS LIKE THE DIFFERENCE BETWEEN A PARTIAL LIST OF INGREDIENTS AND A RECIPE. To bake a good loaf of bread, it isn't enough to know that it contains flour. It isn't even enough to know that it contains flour, water, salt and yeast. The brand of flour might be omitted from the recipe with advantage, as might the day of the week on which the loaf was baked. But the ratio of ingredients, the operations, their timing and the temperature of the oven cannot.

Given preproducibility — a 'scientific recipe' — we can attempt to make a similar loaf of scientific bread. IF WE FOLLOW THE RECIPE BUT DO NOT GET THE SAME RESULT, EITHER THE RESULT IS SENSITIVE TO SMALL DETAILS THAT CANNOT BE CONTROLLED, THE RESULT IS INCORRECT OR THE RECIPE WAS NOT PRECISE ENOUGH (things were omitted to disadvantage).

Depending on the discipline, preproducibility might require information about materials (including organisms and their care), instruments and procedures; experimental design; raw data at the instrument level; algorithms used to process the raw data; computational tools used in analyses, including any parameter settings or ad hoc choices; code, processed data and software build environments; or analyses that were tried and abandoned.

PEER REVIEW IS HAMSTRUNG BY LACK OF PREPRODUCIBILITY: REFEREES AND EDITORS CANNOT PROVIDE SERIOUS QUALITY CONTROL UNLESS THEY ARE GIVEN ENOUGH INFORMATION. Preproducibility will bring us closer to the ideals of the Enlightenment, providing crucial evidence about whether a reported result is correct and about how far the result can be generalized.

SCIENCE SHOULD BE 'SHOW ME', NOT 'TRUST ME'; IT SHOULD BE 'HELP ME IF YOU CAN', NOT 'CATCH ME IF YOU CAN'. If I publish an advertisement for my work (that is, a paper long on results but short on methods) and it's wrong, that makes me untrustworthy. If I say: "here's my work" and it's wrong, I might have erred, but at least I am honest. If you and I get different results, preproducibility can help us to identify why — and the answer might be fascinating.

Just as I have pledged not to review papers that are not preproducible, I have also

pledged not to submit papers without providing the software I used, and — to the extent permitted by law and ethics — the underlying data. I urge you to do the same. The commitment that Boyle made to the scientific community is even more crucial today."

-Philip Stark

Associate Dean, Division of Mathematical and Physical Sciences | Interim Regional Associate Dean, College of Chemistry and Division of Mathematical and Physical Sciences | Professor of Statistics | University of California

https://www.nature.com/articles/d41586-018-05256-0

Related Posts on the Reproducibility Crisis in Science:

Most of the scientific literature is non-reproducible:

Beware those who would sell you on the disease and the "cure."

We are currently being flooded with contradictory or dubious study after study in regards to this pandemic brought to you by the Pharmaceutical industry. The trustworthiness of journals and the studies they published unfortunately went out the window long ago. There were a few great articles (including one from Sanjay Gupta on CNN of all places) discussing this issue. This is from the Gupta article called "Science By Press Release:" "So this past week, I took a step back to dig deeper into the studies and look into the source of this optimism. I WAS SURPRISED AT HOW THIN THE AVAILABLE DATA ACTUALLY IS IN PEER-REVIEWED MEDICAL JOURNALS. Truth is, MOST OF WHAT WE HAVE SEEN SO FAR HAS COME IN THE FORM OF PRESS RELEASES OR PRE-PRINT REPORTS THAT HAVE NOT UNDERGONE THE SCIENTIFIC SCRUTINY OF INDEPENDENT REVIEW. In fact, despite all the enthusiasm around vaccines, there is only one published study of a vaccine trialed in humans -- from the Chinese company CanSino Biologics." "ADDING TO THE WHIPLASH AND CONFUSION IS JUST THE

SHEER NUMBER OF STUDIES COMING OUT. According to Grabowski, who based her estimate on the NIH's iSearch COVID-19 Portfolio, APPROXIMATELY 35,000 ARTICLES HAVE BEEN AMASSED TO DATE ON THE TOPIC -- AND THEY KEEP COMING." "So, what does this all mean for you? "I think that someone reading, viewing, watching, listening SHOULD NEVER MAKE ANY DECISIONS BASED ON A SINGLE REPORT THEY READ, WHETHER IT'S A STUDY OR A NEWS REPORT ON A STUDY," Oransky said. "PARTICULARLY IF THAT NEWS REPORT DOESN'T PUT INTO CONTEXT EVERYTHING THAT'S COME BEFORE AND DOESN'T EXPLAIN WHAT WE STILL DON'T KNOW." https://www.cnn.com/.../science-by-press.../index.html Then there was this article discussing the many ethical conflicts involving Big Pharma, Dr.'s, Universities, Researchers, Journals, etc. It makes the case that much of science is potentially wrong or at the very least highly biased. Here are some highlights but the whole article (and attached links within) is a great read: "The 2 most prestigious journals of medicine in the world are The Lancet and The New England Journal of Medicine. Richard Horton, editor in chief of The Lancet said this in 2015 “The case against science is straightforward: much of the scientific literature, PERHAPS HALF, MAY SIMPLY BE UNTRUE” Dr. Marcia Angell, former editor in chief of NEJM wrote in 2009 that, “It is simply NO LONGER POSSIBLE TO BELIEVE MUCH OF THE CLINICAL RESEARCH THAT IS PUBLISHED, or to rely on the judgment of trusted physicians or authoritative medical guidelines. I take no pleasure in this conclusion, which I reached slowly and reluctantly over my two decades as an editor” Well, Dr. Relman another former editor in chief of the NEJM said this in 2002 “THE MEDICAL PROFESSION IS BEING BOUGHT BY THE PHARMACEUTICAL INDUSTRY, not only in terms of the practice of medicine, but also in terms of teaching and research. The academic institutions of this country are allowing themselves to be the paid agents of the pharmaceutical industry. I think it’s disgraceful” The people in charge of the system — the editors of the most important medical journals in the world, gradually learn over a few decades that their life’s work is being slowly and steadily corrupted. Physicians and universities have allowed themselves to be bribed." "Evidence based medicine depends entirely upon having a reliable base of evidence (studies). IF THE EVIDENCE BASE IS TAMPERED WITH, AND PAID FOR, THEN EBM AS A SCIENCE IS COMPLETELY USELESS. Indeed, the very editors whose entire careers have been EBM HAVE NOW DISCOVERED IT TO BE WORTHLESS." "So here’s a damning list of all the problems of EBM Selective Publication Rigged outcomes Advertorials Reprint Revenues Bribery of Journal Editors Publication Bias Financial Conflicts of Interests"

https://drjasonfung.medium.com/the-corruption-of-evidence-based-medicine-killing-for-profit-41f2812b8704

This is a long study that looked into publication bias as well as how piecemealing evidence/studies is a problem: "Requiring multiple studies to establish a fact is no panacea, however. The same processes that allow publication of a single incorrect result CAN ALSO LEAD TO THE ACCUMULATION OF SUFFICIENTLY MANY

INCORRECT FINDINGS TO ESTABLISH A FALSE CLAIM AS FACT (McElreath and Smaldino, 2015). This risk is exacerbated by publication bias."
https://elifesciences.org/articles/21451#bib25

And this is from a great statistical analysis by John Ioannidis: "There is increasing concern that in modern research, FALSE FINDINGS MAY BE THE MAJORITY OR EVEN THE VAST MAJORITY OF PUBLISHED RESEARCH CLAIMS [6–8]. However, this should not be surprising. IT CAN BE PROVEN THAT MOST CLAIMED RESEARCH FINDINGS ARE FALSE. Here I will examine the key factors that influence this problem and some corollaries thereof." "Several methodologists have pointed out [9–11] that the HIGH RATE OF NON REPLICATION (LACK OF CONFIRMATION) OF RESEARCH DISCOVERIES IS A CONSEQUENCE OF THE CONVENIENT, YET ILL-FOUNDED STRATEGY OF CLAIMING CONCLUSIVE RESEARCH FINDINGS SOLELY ON THE BASIS OF A SINGLE STUDY" https://journals.plos.org/plosmedicine/article?id=10.1371%2Fjournal.pmed.0020124

Right now it seems pretty apparent that the Pharmaceutical industry runs the show. They either own or have massive influence over the CDC, NIH, WHO, the MSM, many Dr.'s, Researchers, Medical Journals, etc. The very companies promoting the "cures" and technology in regards to this "virus" are the ones pushing the science to sell us on the "virus" in the first place. It's all one sick circular carousel of corruption.

https://docs.google.com/document/d/e/2PACX-1vQnl8aBMGbDsNfRW1S9CH-s7bKatf9AQ_Ff9CvgqGpbiR_IcZ_zxf5tJdvDuSkvx6dUPJy5cfiJy9Ej/pub

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THE

CLAIMS OF "VIRUS" THOUGH OUT THE

YEARS

Up to the year 1949, the “virologists” cultivated their suspected “viruses” (proteins) by placing a piece of putrescent genetic material, which had been taken from a tissue allegedly infected by a virus, on a slice of “healthy” tissue of the same type. The visible intensification of the putrefaction process, which was transmitted from the “sick” tissue to the “healthy” tissue, was misinterpreted as proliferation and spreading of the virus, of the pathogenic poison. Due to control experiments with healthy tissue carried out for the first time in 1951, the virologists discovered that what they saw were quite normal processes of tissue decay and not a virus that would only be present in “sick” tissue. Enter John Franklin Enders. In 1949, he “discovered” by chance because he had no fresh “healthy” nerve tissue available – that other types of tissue started to decompose

as well if a piece of brain from a person who died of polio was placed on it. Previ□ously, the virologists had believed that every virus could only propagate in the organic material that it would also damage. For the alleged discovery that "viruses" propagate in other tissues as well, which they don't damage in live humans, Enders and the other involved academics were awarded the Nobel Prize for Medicine on the 10th of June 1954.

From then on, the alleged "polio virus" was propagated by mixing human foetal skin tissue and muscle with brain substance from people who had died of "polio", the mixture of which then collectively decayed. The filtrate from this mixture, then, was considered to contain a "virus". The famous Jonas Salk adopted this exact idea without naming the inventor. Salk used the filtrate of decayed human foetal tissue as a polio vaccine, the New York Times stated that the vaccine worked and would be safe, and Salk generated millions of dollars with the polio vaccine, without sharing anything with the real inventor of the idea of using decomposing human foetuses.18

For these reasons, Enders worked hard to develop another technique, for which he could take the credit from the very beginning. He chose the second most lucrative area of the germ theory of disease, namely that of the symptoms called measles. Enders used the same ideas and methods from bacteriology (in which he had graduated) and believed that the phages were the viruses of bacteria. Analogous to this technique of demonstrating how phages allegedly destroy bacteria on a Petri dish, he developed a tissue streak on which allegedly infected fluid was placed. Analogous to the dying off of the bacteria, the dying off of the tissue streak was claimed to be at the same time the presence of the suspected virus, the proof for its existence, its isolation and its multiplication. This precise protocol is still applied to date in the case of measles and, slightly modified, as "evidence" of all pathogenic viruses.19 The mixture of dying or dead cells/tissues is now called a "live vaccine". If single particles of dead tissue or synthethically produced molecules are used in vaccines, the experts call it "killed vaccine" or "inactivated vaccine".

Enders blamed the strikingly high numbers of deaths and injuries that the Salk polio vaccine caused in the population on the contamination of the vaccine with unknown human viruses, which is why he worked in his lab with tissues from monkey kidneys and foetal serum from horses and unborn calves. There are four striking and crucial differences between the evidence of the existing (bacterio)phages and Enders' alleged evidence of the hypothetic "viruses" in humans and animals. These differences clarify Enders' wrong assumptions, since he completely forgot his earlier clearly expressed doubts once he had received the Nobel prize, and so he led all of his colleagues and consequently the entire world (see the Corona panic) down the wrong path…. Or: exactly the same thing as is happening now, with the Corona-panic The entire world, except a pretty but stubborn schwabian village near lake Constanz (where Dr Lanka lives, note of the translator):

1. The (bacterio)phages have indeed been isolated in the meaning of the word "isolation" with standard methods (density gradient centrifugation). Immediately after the isolation they have been photographed in an electron microscope, their purity is determined and then their components, their proteins and their DNA have been biochemically described all at once, in one single paper.

2. With respect to all "viruses" of humans, animals or plants, however, no virus was ever isolated, photographed in an isolated form and its components were never biochemically characterised all at once, from the "isolate". In reality, there was a consensus process, taking place over quite a number of years, in which single particles of dead cells were theoretically ascribed to a totally virtual virus model. The phages served as a model for this entire interpretation process, as we can see clearly from the first drawings of a "virus".

3. The tissue and cells used for the "proof and propagation" of "viruses" are prepared in a very special manner before the act of the alleged "infection". 80% of their nutrients is withdrawn, so that they can become "hungry" and better absorb the "viruses". They are treated with antibiotics in order to exclude the possibility that bacteria, which are present always and everywhere in all tissues and serums, may cause the expected death of the cells. It was acknowledged only in 1972 by biochemistry experts that those antibiotics were damaging and killing the cells by themselves, a fact that the virologists had previously ignored. "Starvation" and "poisoning" is what kills the cells, but this was and still is misinterpreted as the presence, isolation, effect and propagation of the hypothetical viruses.

4. The control experiments that are crucial and required in science have to date not been carried out with respect to viruses; they could exclude the possibility that instead of a virus just typical cell particles were misinterpreted as a virus. The control experiments regarding the isolation, biochemical description and electron micrographs of the phages, however, were all carried out. Thus, Enders' speculations dated 1 June 195420 about the possible proof of an "agent" which could "possibly" play a role in measles became an apparently "scientific" fact and the exclusive basis for the entire new genetic virology after 1952, all because of his Nobel prize for the "human foetus/polio virus vaccine" in December 1954. A few months after having received his Nobel prize, Enders forgot or suppressed the discrepancies and doubts that he had mentioned himself in his 1954 paper. Still suffering due to the plagiarism committed by Jonas Salk, who had stolen his idea for the polio vaccine, Enders stated that all future developments of a measles vaccine would have to be based on his (Enders') technique.

Enders killed his tissue cultures himself unintentionally through the treatment with antibiotics (without negative control experiments – and this is a crucial aspect in the context of mandatory measles vaccination). Ever since Enders experimented with a smear taken from a young boy named David Edmonston who was supposedly ill from measles, the first model of a measles "virus" (hypothetically put together from particles of dead tissue) has been called the "Edmonston strain". The measles vaccine, as a toxic

sum of all those decayed pieces of tissue, is also claimed to contain the “Edmonston strain”. A part of that mixture containing dead monkey tissue and foetal bovine serum is being constantly frozen and then used regularly to “inoculate” other dying tissue/cells in order to create “measles viruses” and “live vaccines”.

POLIO

A BRIEF OVERVIEW ON POLIO:

When you look into the history of Polio, you will find that it is fraught with insane leaps in logic and assumptions and is replete with grotesquely inhumane experiments.

"THE HISTORY OF THE ETIOLOGY OF POLIOMYELITIS IS A HISTORY OF ERRORS. I mention only the “coccus era,” when several investigators were prejudiced by a supposed parallelism between poliomyelitis and meningitis epidemica.

However, all in all, bacteriological findings were negative; likewise, ATTEMPTS TO TRANSMIT THE DISEASE TO THE USUAL LABORATORY ANIMALS, SUCH AS RABBITS, GUINEA PIGS, OR MICE, FAILED. Landsteiner and Popper (14) INJECTED INTRAPERITONEALLY into two Old World monkeys (Cynocephalus hamadryas and Macacus rhesus) A SUSPENSION OF SPINAL CORD FROM A 9-YEAR-OLD BOY WHO HAD SUCCUMBED to severe poliomyelitis after four days of illness. The two monkeys, in good condition, HAD BEEN AVAILABLE FROM PREVIOUS EXPERIMENTS WITH SYPHILIS. The inoculated material, which was bacteriologically sterile, YIELDED NEGATIVE RESULTS WHEN INJECTED INTO RABBITS, GUINEA PIGS, AND MICE. The two monkeys, however, exhibited lesions in the spinal cord, medulla, pons, and brain stem that were indistinguishable from those observed in cases of human poliomyelitis. ONE OF THE MONKEYS, the rhesus monkey, DEVELOPED COMPLETE FLACCID PARALYSIS OF BOTH LEGS. Landsteiner and Popper WERE

UNABLE TO PASSAGE THE AGENT, but this was achieved soon afterward and independently in 1909 by Römer (22), Flexner and Lewis (8), Leiner and von Wiesner (15), and Landsteiner and Levaditi (13)."

"There were attempts as early as 1913 by Constantin Levaditi (16) to replicate poliovirus in tissue culture. But as Sabin and Olitsky (25) stated in their famous paper of 1936, “THERE IS NO UNEQUIVOCAL EVIDENCE THAT THE VIRUS OF POLIOMYELITIS HAS AS YET BEEN SUCCESSFULLY CULTIVATED OUTSIDE THE BODY.”

Sabin and Olitsky used various carefully dissected tissues of 3- to 4-month-old human embryos, e.g., brain and cord, lungs, kidney, liver, and spleen. The virus was the already mentioned MV (mixed virus) strain of the Rockefeller Institute, a virus mixture prepared by H. L. Amoss in 1914 and KEPT FOR DECADES THROUGH NUMEROUS INTRACEREBRAL PASSAGES IN MONKEYS (23). The authors found that the virus multiplied readily only in the presence of nervous tissue, as evidenced by experiments with monkeys, including neutralization tests. THE EXPERIMENT APPEARED INTERESTING AT THE TIME BUT OF NO PRACTICAL VALUE.

Despite this depressing failure and in view of the mounting evidence of the extraneural multiplication of poliovirus (see above), John Enders and his young collaborators Thomas Weller and Frederick Robbins made further attempts to cultivate poliovirus in vitro, in particular after Weller’s successful cultivation of mumps virus in vitro. Enders and coworkers (7) demonstrated the dramatic replication of Lansing virus (testable in mice) in human embryonic cultures composed chiefly of skin, muscle, and connective tissue from the arms and legs, in cultures of the human embryonic intestine, and in those of nervous tissue. It was Robbins who first recognized differences in cell morphology between inoculated and uninoculated cultures (24a). ENDERS COINED THE TERM CYTOPATHIC EFFECTS (CPE)."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC112492/

The above passage gives a general overview of the ridiculous methods used to try and prove a Polio "virus" existed. The next section from the amazing book Virus Mania gives a great summary of why these experiments fail to prove Polio as an infectious "virus:"

"Landsteiner and Popper instead CHOSE TO TAKE A DISEASED PIECE OF SPINAL MARROW FROM A LAME NINE-YEAR-OLD BOY, CHOPPED IT UP, DISSOLVED IT IN WATER AND INJECTED ONE OR TWO WHOLE CUPS OF IT INTRAPERITONEALLY (into the abdominal cavities) OF TWO TEST MONKEYS: one died and the other became permanently paralyzed.333 334 Their studies were plagued by a mind-boggling range of basic problems. First, THE “GLOP” THEY POURED INTO THE ANIMALS WAS

NOT EVEN INFECTIOUS, since the paralysis didn't appear in the monkeys and guinea pigs given the alleged “virus soup” to drink, or in those that had it injected into their extremities.335

Shortly after, researchers Simon Flexner and Paul Lewis experimented with a comparable mixture, INJECTING THIS INTO MONKEYS' BRAINS.336 Next, they BREWED A NEW SOUP FROM THE BRAINS OF THESE MONKEYS AND PUT THE MIX INTO ANOTHER MONKEY'S HEAD. This monkey did indeed become ill. In 1911, Flexner even boasted in a press release, that they had already found out how polio could be prevented, adding, of course, that they were close to developing a cure.337

But this experiment shows no proof of a viral infection. THE GLOP USED CANNOT BE TERMED AN ISOLATED VIRUS, even with all the will in the world. NOBODY COULD HAVE SEEN ANY VIRUS, AS THE ELECTRON MICROSCOPE WASN'T INVENTED UNTIL 1931. Also, Flexner and Lewis DID NOT DISCLOSE THE INGREDIENTS OF THEIR “INJECTION SOUP.” By 1948, it was still unknown “how the polio virus invades humans,” as expert John Paul of Yale University stated at an international poliomyelitis congress in New York City.338

Apart from that**, IT IS VERY PROBABLE THAT THE INJECTION OF FOREIGN TISSUES IN THE MONKEYS' CRANIUMS TRIGGERED THEIR POLIO-LIKE SYMPTOMS** (seeChapter 5: BSE). And when one considers the amount of injected material, it can hardly be surprising that the animals became ill. CONTROLLED TRIALS WEREN'T EVEN CARRIED OUT—that is, they neglected to inject a control group of monkeys with healthy spinal cord tissue. Neither were the effects of chemical toxins like heavy metals injected directly into the brain.339 340 ALL OF THESE FACTORS MAKE THE EXPERIMENTS VIRTUALLY WORTHLESS."

-Virus Mania

In summary, we have:

-Failure to transmit disease in the usual test animals

-Diseased spinal cord from a deceased 9-year-old boy emulsified and injected into the stomachs and brains of monkeys

-Admission in 1936 that no "virus" had ever been cultivated outside the body after nearly 30 years of studies/experiments

-"Virus" strains kept for decades through intracerebral passages in monkeys

-The unpurified glop used as Polio was non-infectious

-The ingredients of the spine soup were kept secret

-No control experiments were carried out

It's clear that they were not attempting to find a "virus" but rather to see if they could create paralysis from these horrific experiments.

Through injecting an emulsified spinal column from a deceased child into the brains of monkeys in order to "prove" an invisible "virus," the Polio "history of errors" sums up the sad unscientific state of Virology.

FLEXNER'S 1910 POLIO PAPER:

Here is another shining example of the incredibly horrific lengths they went in order to "prove" it was an invisible "virus" which was the cause of Polio. Notice the various ways they tried to experimentally cause disease by grounding up spinal cords and other tissues from deceased children and monkeys which were then injected into the brains and bodies of other monkeys. This is a long read (highlights from a 31 page study with a summary at the end) but it gives some insight into the madness of Virology.

EXPERIMENTAL EPIDEMIC POLIOMYELITIS IN MONKEYS

"Up to the present time there has existed no convincing knowledge of the nature of the agent causing epidemic poliomyelitis. VARIOUS BACTERIA AND ESPECIALLY CERTAIN COCCI HAVE from time to time BEEN ISOLATED IN CULTURES FROM FLUIDS OBTAINED BY LUMBAR PUNCTURE FROM PATIENTS SUFFERING FROM EPIDEMIC POLIOMYELITIS, or from specimens of the central nervous system removed at autopsy. These bacteria did not conform to one species or group of microorganisms and did not suffice to set up poliomyelitis in animals. THEY CAN BE ACCOUNTED FOR MORE SATISFACTORILY AS CONTAMINATIONS OR SECONDARILY INVADING BACTERIA THAN AS THE CAUSE OF THE DISEASE."

"THE FLUIDS, OBTAINED BY LUMBAR PUNCTURE, WERE INJECTED INTO

LABORATORY ANIMALS, INCLUDING MONKEYS, BUT WITHOUT SETTING UP ANY RECOGNIZABLE PATHOLOGICAL CONDITION. During the epidemic of I9O 7, we did not secure organs from a case of pure infantile paralysis and we failed, therefore, in our intention to inoculate monkeys from the spinal cord2"

"In September, I9o9 , we secured the spinal cord from two cases of infantile paralysis in human beings. For these valuable specimens we are indebted to Dr. Ridner, of Lake Hopatcong, N. J., and Dr. Le Grand Kerr, of Brooklyn, N.Y. Dr. Ridner's patient died on the fifth or sixth day following the appearance of the paralysis, which affected the legs. THE LUMBAR CORD WAS OBTAINED IN A STERILE CONDITION, twenty-six hours after death, AND EMPLOYED FOR INOCULATION OF ANIMALS. Dr. Kerr's patient had been widely paralyzed and died on the fourth day of the disease. The lesions in the cord were wide-spread and severe and affected gray matter and white. THE ENTIRE SPINAL CORD WAS CONTAINED TWELVE HOURS AFTER DEATH AND INOCULATED INTO ANIMALS FOUR HOURS LATER."

"IN ORDER TO FAVOR THE TRANSMISSION OF THE DISEASE TO MONKEYS, WE CHOSE THE BRAIN AS THE SITE OF INOCULATION, which was made under ether anesthesia, through a small trephine opening. After the operations, the animals were at once lively and normal. THE INJECTED MATERIAL CONSISTED, FIRST, OF EMULSIONS IN SALT SOLUTION OF THE SPIRAL CORD FROM THE CHILDREN AND, LATER, OF EMULSIONS AND FILTRATES FROM THE SPINAL CORD AND OTHER ORGANS FROM THE MONKEYS DEVELOPING PARALYSIS."

"THE MATERIAL EMPLOYED FOR INOCULATION OF TWO MONKEYS CONSISTED OF THE EMULSIFIED SPINAL CORD IN SALT SOLUTION OBTAINED FROM A CHILD NINE YEARS OLD, who died on the fourth day of attack from infantile paralysis. THE EMULSION WAS INJECTED INTO THE PERITONEAL CAVITY OF THE MONKEYS. One of the latter became severely sick on the sixth day and died on the eighth day after inoculation. The other monkey became paralyzed on the seventeenth day and was~ killed on the nineteenth day after inoculation. The spinal cord of the first monkey was not used for further inoculation, WHILE THAT FROM THE SECOND MONKEY WAS USED TO INOCULATE, PROBABLY BY INTRAPERITONEAL INJECTION, TWO OTHER MONKEYS THAT DID NOT, HOWEVER, DEVELOP THE SYMPTOMS OF THE DISEASE. APPARENTLY THEY WERE UNAFFECTED BY THE INJECTION."

"OWING TO THEIR INABILITY TO TRANSMIT THE DISEASE FROM ONE MONKEY TO ANOTHER, Landsteiner and Popper discuss the three following possible explanations of the failure, namely : (I) whether the disease in the first monkey was caused by a transferred poison or by the infectious agent; (2) whether a successful

transfer from monkey to monkey might not have been secured had the cord of the monkey, dying on the sixth day of the disease, been used; and (3) whether the infectious agent may not have become attenuated in virulence in its passage through the monkey and thus have lost its power of further transmission. THEY INCLINED TO THE LAST EXPLANATION."

"On December 22, 19o8, 14 hours post-mortem, the spinal cord was removed from a child of one year, who had died on the sixth day of the disease, AND 10 CENTIMETERS OF THE CERVICAL PORTION, AFTER EMULSIFICATION IN SALT SOLUTION, WERE INJECTED INTO THE PERITONEAL CAVITY OF A MACAQUES RHESUS. The first evidence of sickness was noted on the eighth day, and the first evidence of paralysis on the twelfth day, after the inoculation. ON THE FOURTEENTH DAY THE ANIMAL WAS KILLED WITH CHLOROFORM AND IO CENTIMETERS OF THE SPINAL CORD WERE RUBBED UP IN SALT SOLUTION AND THE EMULSION WAS INJECTED INTO A SECOND MONKEY (PRESUMABLY BY WAY OF THE PERITONEUM) WHICH WAS UNAFFECTED. The anatomical and histological findings agreed with those described by Landsteiner and Popper."

"IN SEVERAL INSTANCES THE MONKEYS FELL ILL AND DIED WITHOUT PARALYSIS HAVING OCCURRED OR BEEN NOTED. These animals were included among the series of successful inoculation only when the typical lesions were present in the nervous system. The incubation period in them was calculated from the inoculation to the onset of definite symptoms of illness."

"The description to be given is BASED UPON A STUDY OF 81 MONKEYS which became infected with the virus."

"The shortest period thus far noted as elapsing between the inoculation and the onset of paralysis HAS BEEN 4 DAYS AND THE LONGEST PERIOD, 33 DAYS. The average period has been 9.82 days. The number of animals developing paralysis after the twelfth day was 16, and the number DEVELOPING PARALYSIS BEFORE THE EIGHTH DAY WAS 18."

"Prodromal Signs.-THE INOCULATION OF THE VIRUS INTO THE BRAIN OR OTHER PARTS PRODUCES NO IMMEDIATE EFFECTS. As soon as the effects of the anesthetic disappear, the monkeys appear normal. This condition persists until a period of from six to forty-eight hours before the onset of paralysis when certain abnormal

signs may be noted. The animals become nervous and excitable; on being disturbed and made to move about the cages, they tire quickly; a tremor of the head, face or limbs develops; when the attention can be attracted the gaze is shifting, rather than fixed as in the normal monkey, and the face is ~wrinkled and mobile rather than smooth and placid; the hairs are erected somewhat, and the animals prefer to remain quiet. ALL THESE SYMPTOMS ARE ALMOST NEVER NOTED IN A GIVEN ANIMAL AND THEY OCCUR IN VARYING COMBINATIONS. The temperature does not rise constantly during the incubation period and gastro-intestinal symptoms rarely occur. A few animals have shown diarrhoea but this condition may well have been a coincidence."

"Termination.--This division of our subject cannot be discussed with the completeness of the other sections. Since the main object of our study required that we maintain an active virus, WE HAVE BEEN OBLIGED TO SACRIFICE A CONSIDERABLE NUMBER OF THE AFFECTED ANIMALS WITHIN TWENTY-FOUR TO FORTY-EIGHT HOURS OF THE ONSET OF THE PARALYSIS.

THE AFFECTED MONKEYS MAY RECOVER. When this happens, the paralysis reaches a maximum, becomes stationary, and then recedes more or less. When the affection has been severe, the animals appear sick for several days, but when recovery commences the general symptoms of sickness quickly disappear. The muscles which were weak, but not definitely paralyzed, regain strength. Hence, the actual paralyses are more sharply demarcated. ALTHOUGH THE VARIATIONS ARE CONSIDERABLE, it happens that within a week of a severe or critical state the animal has regained health and general strength, except for the actually paralyzed muscles. In other in- stances, two or three weeks have not sufficed for the restoration of strength to muscles apparently intact. In some instances in which the paralysis affecting a single limb appeared to be complete, it has entirely disappeared within a few weeks."

"THE ANIMALS MAY DIE. When the first force of the affection is exerted upon the medulla, death may occur within a short time of the first appearance of symptoms. When the limbs are first affected, the progress and extension of the paralysis may be very rapid and death be caused quickly through involvement of the medulla. Again, the progress may be slow and the prostrated animal may gradually grow weak and die after one or several days. WHEN THE PERIOD OF DISEASE IS PROLONGED, THEN OTHER FACTORS, SUCH AS SECONDARY INFECTIONS AND GENERAL NUTRITIVE AND TROPHIC DISTURBANCES, MUST BE CONSIDERED."

"From the figures given it follows that in 54.3 percent of the monkeys, in this series, which developed poliomyelitis, the issue would in all probability have been fatal. HENCE, THE EXPERIMENTAL DISEASE IS MORE HIGHLY FATAL THAN IS THE SPONTANEOUS DISEASE IN HUMAN BEINGS."

"It has been determined that the virus of epidemic poliomyelitis when introduced into the body by means of the blood, subcutis, peritoneum, spinal canal and large nerves, tends to localize in the spinal cord and brain and set up the specific lesions, in the same manner as when injected into the brain. It remains to be determined whether these several routes give uniformly as good results as the intracerebral route. Our impression is that infection is readily accomplished by way of the subcutis and less readily by way of the peritoneal cavity. WE HAVE MADE SEVERAL UNSUCCESSFUL ATTEMPTS TO PRODUCE INFECTION THROUGH INTRATRACHEAL INOCULATION AND BY FEEDING. But the number of experiments will have to be considerably greater before a final conclusion can be ventured on these points. ON THE OTHER HAND, IT HAS BEEN SHOWN THAT THE CEREBROSPINAL FLUID, at the beginning of the paralysis, IS CAPABLE WHEN INJECTED INTO THE BRAIN, OF SETTING UP PARALYSIS IN OTHER ANIMALS. Hence at this period, at least, THIS FLUID CONTAINS THE VIRUS."

"The blood contains the virus at the beginning of the infection, but how richly has not been accurately determined. AS LITTLE AS TWO CUBIC CENTIMETERS MAY FALL TO CAUSE INFECTION WHILE AS MUCH AS TWENTY HAS CAUSED TYPICAL PARALYSIS."

"The naso-pharyngeal mucosa also contains the virus. THE EXCUSED MEMBRANE, INCLUDING THE TONSILS AND TURBINATE MUCOSA, ON BEING RUBBED UP WITH QUARTZ SAND, MACERATED AND FILTERED, YIELDED A FLUID WHICH, ON BEING INJECTED INTO THE BRAIN, CAUSED PARALYSIS."

"To determine this point, THE SPINAL CORDS, REMOVED FROM MONKEYS JUST PARALYZED, WERE TITRATED WITH STERILE QUARTZ SAND, THOROUGHLY SHAKEN, AND PRESSED THROUGH A BERKEFELD FILTER WHICH HAD PREVIOUSLY BEEN TESTED AND FOUND BACTERIA-TIGHT. These clear and bacteriologically clear filtrates HAVE BEEN USED REPEATEDLY TO INOCULATE MONKEYS, BOTH BY THE INTRACEREBRAL AND SUBCUTANEOUS ROUTES, and have regularly caused paralysis. From these paralyzed animals, the virus has been transferred to other monkeys, so that it can be asserted that the effects it produces are caused NOT BY A FILTERABLE TOXIC SUBSTANCE BUT BY A TRUE VIRUS OR LIVING MICROORGANISM."

"The clear fluids obtained by filtration when examined under the dark field microscope show innumerable bright, dancing points devoid of definite size and form and not truly motile. This fluid prepared and stained by means of Loeffler's flagella stain shows minute particles of roundish or oval form which were absent from a similar filtrate prepared with the nervous system of a rabbit. THAT THE PARTICLES REPRESENT THE MICROORGANISM OF POLIOMYELITIS CANNOT BE AFFIRMED AT PRESENT."

"THE PARASITE OF EPIDEMIC POLIOMYELITIS IS, THEREFORE, VERY MINUTE AND CANNOT, FOR THE MOMENT, BE FURTHER CLASSIFIED, since the precise position among living things held by the filterable viruses has not been determined."

"THERE IS AT PRESENT NO RELIABLE WAY OF ESTIMATING THE DEGREE OF ACTIVITY OF THE VIRUS SINCE THE NUMBER OF ORGANISMS INOCULATED IS NOT SUBJECT TO CONTROL. The inoculated materials consisted of heavy suspensions in salt solution of the spinal cord, for preparing which portions from several levels were employed, or filtrates obtained from the suspensions, OF WHICH FROM TWO TO FOUR CUBIC CENTIMETERS WERE INJECTED."

"The incubation period has been worked out ON THE SUPPOSITION THAT THE SPONTANEOUS DISEASE IN MAN IS CONTAGIOUS. WE OBSERVED NO INSTANCE AMONG OUR MONKEYS OF A SPONTANEOUS TRANSFER OF THE INFECTION. However, we made no purposive experiments to test this point, AND YET THE INOCULATED AND UNINOCULATED ANIMALS WERE NOT KEPT CAREFULLY SEPARATE."

"Experimental poliomyelitis in the monkey is a severe and highly fatal disease and EXCEEDS IN THE LATTER RESPECT THE SPONTANEOUS DISEASE IN MAN."

https://doi.org/10.1084/jem.12.2.227

In summary:

-Bacteria and other organisms were found in lumbar fluid but were quickly determined not to be the cause of disease

-Fluids from lumbar puncture were injected into monkeys and other animals without any effect

-Spinal cords from deceased children were ground up and emulsified to be injected into the brains of monkeys

-The brain was chosen in order to favor transmission of disease to monkeys

-Emulsions of spinal cords and other organs from diseased monkeys were mixed together and injected into other monkeys

-The emulsified monkey goo did not make two other monkeys sick after being injected in their stomachs

-Other studies were unable to transfer disease from one monkey to another and the assumption was made that the "virus" lost virulence upon passages

-There were several instances where the monkeys died without paralysis

-Illness began anywhere from 4 days to 33 days after injection in the brain

-Symptoms were highly variable and were never noted to be in all monkeys

-It was noted with prolonged disease, factors other than a "virus" such as secondary infections or the nutritive state and trophic disturbances should be considered

-The experimental disease in monkeys is more fatal than seen in man

-There were many unsuccessful attempts to produce disease through injections into the throat or by feeding monkeys the emulsified goo

-Quartz sand was rubbed all over samples

-Particles seen in microscope could not be confirmed as Polio "virus"

-It was determined the "virus" was too small to be seen and could not be characterized

-There were NO CONTROLS at all

-They assume the disease is contagious among man but could not produce contagion among monkeys

LANSING STRAIN OF POLIO:

The Lansing strain of Polio is one of three strains used in the Polio vaccine. It was created through the emulsified brain and spinal cord of an 18-year-old boy from Lansing, MI. The emulsified goo was injected into the brains of monkeys which then had their brains and spinal cords emulsified and transferred into other monkeys 15 times. This process was repeated into cotton rats and eventually into the white mouse. This continually passaged goo was widely used for Polio research and was the one used by John Franklin Enders during his Polio tissue/cell culture experimemts which lead to the discovery of the "cytopathogenic effect" still used today to indirectly state that a "virus" is present in the cell culture soup.

Below are two studies by Charles Armstrong which detail the grotesque Lansing strain transfer from boy to monkeys to rats to mice which led to cheaper test animals being

used for Polio experimentation:

THE EXPERIMENTAL TRANSMISSION OF POLIOMYELITIS TO THE EASTERN COTTON RAT, SIGMODON HISPIDUS HISPID US

"Through the courtesy of Dr. Max Peet, of the Department of Surgery, University of Michigan, we received on August 28, 1937, A SAMPLE OF BRAIN AND CORD FROM AN 18-YEAR OLD BOY, one of several bulbar cases of poliomyelitis which occurred at Lansing, Mich., during that summer. A STRAIN OF VIRUS WAS RECOVERED FROM THE MATERIAL WHICH HAS NOW BEEN THROUGH 15 MONKEY PASSAGES and which clinically, and pathologically as reported by Surgeon R. D. Lillie, IS APPARENTLY A STRAIN OF POLIOMYELITIS. NEUTRALIZATION TESTS WITH THIS VIRUS HAVE NOT BEEN DONE.

On November 8, 1937, several species of rodents, including a cotton rat received through the courtesy of Dr. A. Packehanian, of the National Institute of Health, WERE INOCULATED WITH A FOURTH MONKEY PASSAGE OF THE VIRUS. The cotton rat remained apparently well until the twenty-fifth day, when it appeared nervous and tremulous. On the following day it was paralyzed in both hind legs and was sacrificed.

Pathologist R. D. Lillie, who has made all the pathological studies, reported "polioencephalitis." ELEVEN COTTON RATS WERE INOCULATED WITH THIS STRAIN OF POLIOMYELITIS VIRUS during the winter of 1938, OF WHICH RAT No. 9, inoculated on February 14, BECAME PARALYZED IN BOTH HIND LEGS 29 DAYS LATER. BRAIN AND CORD EMULSION WAS PASSED TO RAT No. 13 and symptoms appeared on the sixteenth day. On the following day there was paralysis in the right front leg. ATTEMPTS AT FURTHER PASSAGE WERE WITHOUT SUCCESS.

Efforts were again made, however, during the poliomyelitis season of 1939, and up to the time of this report the Lansing strain of virus has been carried in series through 7 cotton rat transfers and animals of the eighth transfer are developing symptoms. RAT BRAIN AND CORD FROM THE SECOND AND FIFTH PASSAGES CONVEYED TYPICAL POLIOMYELITIS SYMPTOMS WHEN INTRODUCED INTO MONKEYS. The details of these transfers are shown in table 1. Further transfers are under way.

THE INOCULUM UTILIZED WAS A 5 PERCENT SUSPENSION OF BRAIN AND CORD AND THE DOSAGE HAS BEEN APPROXIMATELY 0.06 cc. INTRACEREBRALLY, 0.06 cc. INTRANASALLY, AND 0.25 cc. SUBCUTANEOUSLY, for each animal. THE MINIMAL INFECTIVE DOSE HAS NOT BEEN DETERMINED, since it was necessary to conserve our limited supply of cotton rats and we preferred, more-over, to wait until the virus had become somewhat adapted to the host. The virus at the sixth serial transfer seems to be gaining in virulence. A more detailed report of the results will be made later.

SUCCESSFUL TRANSMISSION TO DATE HAS BEEN SECURED WITH THE LANSING STRAIN OF VIRUS ONLY. Limited attempts at transmission were carried out with two strains of virus from Niagara Falls and with P. M. virus during the winter of 1938, AT WHICH TIME WE ALSO HAD ONLY FAILURES WITH THE STRAIN WHICH NOW IS GIVING RESULTS.

The first symptoms noted in the cotton rats consist of a roughened appearance of the fur and a tendency to react by violent jumping when agitated. Paralysis of a flaccid type has developed in all animals WHICH WE HAVE CONSIDERED AS AFFECTED. The legs may be paralyzed in all combinations and respiratory difficulty has developed in several, with the respiratory rate falling as low as 30 per minute in some.Two rats with respiratory failure died; the others were etherized.

A number of other rodents have been inoculated with the virus utilized in the course of this study, including groups of Swiss mice with successive transfers, BUT NO POSITIVE RESULTS HAVE BEEN SECURED IN ANIMALS OTHER THAN THE COTTON RATS.'

The eastern cotton rat is not vicious and it multiplies readily in captivity. It is hoped, therefore, that when a sufficient supply becomes available and the most susceptible age is determined the cotton rat may prove to be a cheap, convenient, and useful laboratory animal for the study of poliomyelitis.

IT IS CONCEIVABLE, HOWEVER, THAT THE RESULTS SECURED MAY BE DUE TO SOME PECULIARITIES OF THIS PARTICULAR STRAIN OF VIRUS."

https://doi.org/10.2307/4583031

From this first study, notice:

-the numerous passages of emulsified brain/spinal cord from boy to monkey to rodent

-the odd "apparently a strain of poliomyelitis" admittance

-no neutralization tests were done

-out of 11 inoculated rats, only 2 became paralyzed and no further passages were successful

-the minimal infective dose of the goo was never determined

-only the Lansing strain gave results during this study but also gave no results during studies conducted in 1938

-no positive results in animals other than cotton rats

-the admittance that the results secured may be due to some peculiarities with the "strain"

This next study is where Armstrong transferred the "virus" from rat to mouse:

SUCCESSFUL TRANSFER OF THE LANSING STRAIN OF POLIOMYELITIS VIRUS FROM THE COTTON RAT TO THE WHITE MOUSE'

By CHARLES ARMSTRONG, Senior Surgeon, United States Public Health Service

In an earlier paper (1) the successful transmission of a strain of poliomyelitis to the eastern cotton rat, Sigmodon hispidus hispidus, was recorded. THIS STRAIN HAS NOW BEEN CARRIED THROUGH 26 SERIAL TRANSFERS IN THIS SPECIES to which it has become progressively better adapted. The incubation period has shown a tendency to stabilize at from 3 to 5 days when the inoculating dose is maintained at 0.06 cc. OF A 5 PERCENT SALINE SUSPENSION OF VIRUS-INFECTED FRESH CORD AND BRAIN, ADMINISTERED INTRACEREBRALLY. ATTEMPTS TO TRANSMIT THE INFECTION BY THE INTRANASAL ROUTE HAVE SO FAR BEEN WITHOUT SUCCESS. Cotton rats are apparently quite uniformly SUSCEPTIBLE TO INTRACEREBRAL INOCULATIONS. Eighty-nine cotton rats of various ages trapped in nature have been inoculated FOR THE PURPOSE OF "CARRYING" THE LANSING STRAIN OF VIRUS FROM THE SEVENTH TO TWENTY-FIFTH GENERATIONS, of which 1 animal died of unknown cause, POSSIBLY POLIOMYELITIS, on the fourth day, while of the remaining 88 only 1 failed to develop flaccid paralysis. The clinical and pathological manifestations are more pronounced than in earlier transfers and the majority of rats die within 2 to 4 days after symptoms appear, UNLESS SACRIFICED EARLIER.

INTRACEREBRAL INOCULATION INTO MONKEYS OF BRAIN AND CORD MATERIAL (1 cc. of a 5 percent suspension) FROM THE THIRD, SIXTH, AND FIFTEENTH COTTON RAT TRANSFERS was followed by severe clinical and pathological poliomyelitis in all cases.

Three neutralization tests have been attempted employing cotton rats, recent passage strains of the virus, and poliomyelitis antisera, one of which sera (P. C. M. S. XII) was received through the courtesy of Dr. E. H. Lennette, one (M-1791) from Dr. Lloyd Aycock, and one of our own (M-409) from a monkey which had recovered from an attack of poliomyelitis following inoculation with the P. M. strain of virus.

These tests, WHILE OF A PRELIMINARY EXPERIMENTAL CHARACTER, all indicate that two of the sera possess neutralizing properties for the virus, while the serum from Dr. Aycock's monkey is apparently almost or completely inert. The results of the last trial are shown in detail in table 1. In this test a 1:15 emulsion in buffered saline, pH 7.6, of cord and brain from cotton rats 452 and 453 (23 transfers) was centrifuged at 1,200 r. p. m. for 5 minutes and 1 part of the supernatant fluid was added to 2 parts of the respective sera to be tested. The mixtures were incubated in the hot room at 37.5 C. for 2 hours, then placed at 50 to 80 C. for 45 minutes. Four cotton rats were each INOCULATED INTRACEREBRALLY with 0.06 cc. of each serum virus mixture.

Attempts to adapt additional strains of poliomyelitis to the cotton rat are under way. One rat inoculated with our "Bush" strain isolated from a case of poliomyelitis at Niagara Falls, N. Y., in 1938, developed paralysis in the right front leg, first noted on the forty-first day. Sufficient time has not yet elapsed to indicate whether or not subtransfers will succeed.

TRANSFER OF THE VIRUS TO WHITE MICE

Since it was thought that a strain of virus adapted to the cotton rat might be pathogenic for other rodent species, transfers were made into white mice. Suggestive results were not obtained until 30 days after the seventh cotton rat transfer of virus was so inoculated, WHEN 1 OF 5 INTRACEREBRALLY INOCULATED MICE WAS FOUND TO BE PARALYZED in the left front paw and left hind leg. The following day, October 20, 1939, the left front and both hind legs were completely paralyzed. BRAIN AND CORD EMULSION FROM THIS MOUSE WAS TRANSFERRED TO 4 GROUPS (2 Swiss and 2 ordinary) of 6 half grown to adult white mice and to cotton rat 353. TWELVE OF THE 24 MICE DEVELOPED PARALYSIS in one or more legs in from 3 to 12 days and the cotton rat developed typical symptoms on the eighth day and was completely paralyzed on the tenth day, when it was etherized and the brain and cord submitted for pathological study. Dr. R. D. Lillie reported POLIOMYELITIS SIMILAR TO THAT OBSERVED in direct cotton rat transfers.

Successful mouse inoculations have now been carried through 12 SUCCESSIVE TRANSFERS. The virus is showing a tendency to affect a higher proportion of mice in later passages. For instance, of 36 mice inoculated on the ninth transfer, 28 developed paralyses on from the second to twentieth days. An incubation period of 3 to 7 days is most common.

The symptoms in mice consist of flaccid paralysis, most obvious when one or more legs or the respiratory muscles are involved. Except when respiration is affected, the mice

usually appear to be SLEEK AND WITHOUT SYMPTOMS OTHER THAN THE PARALYSES.

Pathological examination of a limited number of affected mice has been made by Surgeon R. D. Lillie, who reports lesions consistent with those of poliomyelitis in other species.

BRAIN AND CORD EMULSION FROM THE FOURTH MOUSE TRANSFER WAS INJECTED INTRACEREBRALLY INTO MONKEY 610 which developed a continuous fever from the fifth to eleventh days, reaching 41 C. on the sixth and seventh days. The animal was nervous and tremulous, BUT RECOVERED WITHOUT PARALYSIS.

Monkey 618, SIMILARLY INOCULATED WITH SIXTH MOUSE TRANSFER VIRUS, developed fever on the fourth day with tremors and definite weakness of the hind legs. THE ANIMAL WAS SACRIFICED ON THE EIGHTH DAY AND A SUB INOCULATION OF CORD EMULSION WAS MADE INTO MONKEY 620 which developed severe symptoms followed by complete paralysis on the tenth day. Lesions typical of moderately severe and severe poliomyelitis were reported for the respective animals by Pathologist J. H. Peers.

AN EMULSION OF CORD FROM MONKEY 620 WAS TRANSFERRED ON DECEMBER 11, 1939, TO COTTON RATS 459 AND 460 AND TO 5 WHITE MICE. The cotton rats developed typical symptoms on December 17 and 18 followed by complete paralysis and death on December 20 and 22, respectively.

Up to December 26, 1939, two of the white mice had developed symptoms. One showed flaccid paralysis in both hind legs on December 17 and died on December 22. A second became paralyzed in the left front and right hind leg on December 25 and was still living on December 26.

THAT THE VIRUS IN MICE IS THE SAME AS THE COTTON RAT STRAIN is further indicated by the successful transfer of the third, ninth, and eleventh mouse generations of virus again to cotton rats with the development of characteristic symptoms and pathology for that species and by the fact that primary mouse inoculations from the fourteenth, fifteenth, sixteenth, eighteenth, nineteenth, twenty-fourth, and twenty-fifth successive transfers in the cotton rat have uniformly produced flaccid paralysis IN A PORTION OF THE INOCULATED MICE.

THE VIRUS HAS CERTAIN MARKED SIMILARITIES TO, AS WELL AS MARKED DIFFERENCES FROM, THE SPONTANEOUS MOUSE VIRUS first described by Theiler in 1934 (2), with which it is hoped to compare it immunologically in the near future.

SUMMARY

The Lansing strain of poliomyelitis virus after adaptation to the eastern cotton rat has been successfully transmitted THROUGH TWELVE

GENERATIONS in white mice."

https://doi.org/10.2307/4583135

From this study, notice:

-26 serial transfers in the cotton rat

-the continued passage of emulsified brain and spinal cord goo from monkey to rat to mouse and back again

-the continued use of the unnatural intracerebral (brain) inoculation and the lack of success of intranasal (nose) inoculation

-of 89 cotton rats used to passage "virus" from the 7th to the 25th generation, 1 died of "possible poliomyelitis"

-monkeys were injected in the brain with passaged rat brain/spine goo

-preliminary and experimental neutralization tests were carried out on the unpurified, non-isolated "virus" goo

-only 1 of 5 mice given cotton rat goo became paralyzed and the brain/spine was then emulsified and given to 24 other mice, of which only 12 became paralyzed

-intracerebrally injected mice appear normal except for paralysis

-emulsified mice brain/spine goo was injected into the brains of monkeys with little effect which were then emulsified back into rats and mice

-the mice strain is assumed the same as the rat strain as it caused flaccid paralysis in a portion of the mice injected intracerebrally

As can be seen from these two studies, the Lansing strain is nothing but a mixture of emulsified brain and spinal cord goo transferred from a deceased boy to monkeys, rats, and mice and back again. It is not a purified/isolated "virus." They assume a "virus" is present and being transferred every time they inject animals in the brain with foreign toxic sludge and then grind up and transfer the goo from the "infected" animal to another one, repeating the horrific process over and over again. They never consider that the act of drilling a hole in the brains of these animals and injecting them with goo from other

animals is the cause of the sickness/paralysis rather than a "virus."

Keep in mind, this serially passaged toxic brain/spine goo is one of three used in the polio vaccine:

"Lansing virus: Type 2 poliovirus. Named after the city in Michigan where the first patient lived who was found to have this virus. There are two other strains of poliovirus: Type 1 (known as the Brunhilde virus) and Type 3 (known as the Leon virus). Immunity to one strain does not provide protection against the other two. ALL THREE STRAINS ARE THEREFORE INCLUDED IN THE POLIOVIRUS VACCINE."

https://www.medicinenet.com/lansing_virus/definition.htm

ENDERS 1949 POLIO PAPER:

Below are some highlights from John Franklin Enders cultivation of the Lansing Polio "virus" in 1949. It was through these experiments that Enders observed what he called cytopathogenic effects in the cultures. This is what Virologists to date claim as evidence of "viral" particles hijacking cells and causing morphological changes when in reality it is the breakdown of the cell due to starvation, poisoning, and environmental stress (among other factors). Notice the similarities to many of the cell culture techniques used today as well as the grotesque nature of the materials used:

Cultivation of the Lansing Strain of Poliomyelitis Virus in Cultures of Various Human Embryonic Tissues

"An extraneural site for the multiplication of the virus of poliomyelitis has been considered by a number of investigators (2, 6). THE EVIDENCE THAT THIS MAY OCCUR IS ALMOST ENTIRELY INDIRECT, although recent data indicate that Theiler's mouse encephalomyelitis virus as well as various mouse pathogenic poliomyelitis-like viruses of uncertain origin may multiply in non nervous tissue (1,3). DIRECT ATTEMPTS BY SABIN AND OLITSKY (4) TO DEMONSTRATE IN VITRO MULTIPLICATION OF A MONKEY-ADAPTED STRAIN OF POLIOMYELITIS VIRUS (MV strain) IN CULTURES COMPOSED OF CERTAIN NON NERVOUS TISSUES FAILED. They obtained, however, an increase in the agent in fragments of human embryonic brain."

"The technique was essentially the same as that recently described for the cultivation of mumps virus (6). The cultures consisted of tissue fragments suspended in 3 cc of a MIXTURE OF BALANCED SALT SOLUTION (3 parts) AND OX SERUM ULTRAFILTRATE (1 part). Tissues from embryos of 2.5 to 4.5 months as well as from a premature infant of 7 months' gestation were used. These were: THE TISSUES OF THE ARMS AND LEGS (without the large bones), THE INTESTINE, AND THE BRAIN. Each set of cultures included 4 or more inoculated with virus, and usually a similar number of uninoculated controls. THE PRIMARY INOCULUM CONSISTED OF 0.1 cc OF A SUSPENSION OF MOUSE BRAIN INFECTED WITH THE LANSING STRAIN OF POLIOMYELITIS VIRUS.4 The identity of the virus was verified by (a) THE CHARACTER OF THE DISEASE IT PRODUCED IN WHITE MICE FOLLOWING INTRACEREBRAL INOCULATION; and (b) its neutralization by specific antiserum. SUBCULTURES WERE INOCULATED WITH 0.1 cc OF POOLED CENTRIFUGED SUPERNATANT FLUIDS REMOVED FROM THE PREVIOUS SET OF CULTURES."

The procedure of cultivation differed from that usually followed by other workers in that the NUTRIENT FLUID WAS REMOVED AS COMPLETELY AS POSSIBLE AND REPLACED AT PERIODS RANGING FROM 4 TO 7 DAYS. Subcultures to fresh tissue were prepared at relatively infrequent intervals, ranging from 8 to 20 days.

Two experiments have been carried out employing cultures composed chiefly of skin, muscle and connective tissue from the arms and legs. The findings in each have been essentially the same. In the first, a series of

culture has now been maintained for 67 days. During this interval, in addition to the original set, THREE SUCCESSIVE SUBCULTURES HAVE BEEN MADE TO FRESH TISSUE AND THE FLUIDS HAVE BEEN REMOVED AND REPLACED 10 TIMES (Table 1). ASSUMING that at each change of fluid a dilution of approximately 1:15 was effected and that at the initiation of each set of cultures the inoculum was diluted 30 times, it has been calculated that the 10% suspension of infected mouse brain used as the primary inoculum had been diluted approximately 10^17 times in the following observations: (a) FLUIDS FROM EACH SET OF CULTURES PRODUCED PARALYSIS AND DEATH IN MICE AFTER INTRACEREBRAL INOCULATION; (b) the agent present in the fluids of the second set of subcultures was neutralized by antiserum specific for the Lansing strain; (c) FOLLOWING INTRACEREBRAL INOCULATION, THE FLUIDS FROM THE THIRD SET OF SUBCULTURES PRODUCED FLACCID PARALYSIS WITHIN 7 AND 10 days, RESPECTIVELY, IN TWO RHESUS MONKEYS. Microscopic examination of the spinal cords of these animals revealed lesions characteristic of poliomyelitis.

CULTURES OF INTESTINAL TISSUE WERE PREPARED WITH FRAGMENTS FROM

THE ENTIRE INTESTINE OF HUMAN EMBRYOS, EXCEPT IN ONE EXPERIMENT IN WHICH JEJUNUM OF A PREMATURE INFANT WAS USED. In the latter, the bacteria were eliminated in the majority of cultures by thorough washing of the tissue AND BY THE INCLUSION IN THE ORIGINAL NUTRIENT FLUID OF 100 units/cc of PENICILLIN AND OF STREPTOMYCIN.

In one experiment with embryonic intestine, which INCLUDED TWO SUBCULTURES AND 7 CHANGES OF NUTRIENT FLUID, the calculated dilution of the original inoculum was of the order of 10^13.7 times. On the basis of the mouse LD, of the original inoculum and that of the last supernatant fluid, it was calculated that the virus had increased about 10^12.7 times. THE IDENTITY OF THE AGENT THUS CULTIVATED IN INTESTINAL TISSUE HAS NOT YET BEEN CONFIRMED BY NEUTRALIZATION TESTS OR MONKEY INOCULATION, BUT IT ELICITS A RESPONSE IN THE MOUSE TYPICAL OF THE LANSING VIRUS.

THE CULTURES PREPARED WITH INTESTINE OF THE PREMATURE INFANT HAVE, so far, BEEN MAINTAINED 17 DAYS WITH 3 CHANGES OF NUTRIENT FLUID. Virus has been demonstrated, by mouse inoculation, in the fluids removed during the course of the experiment, including that of the 17th day. The calculated multiplication of the virus was approximately 10' times. This finding suggests that multiplication occurred in this tissue which, from the embryologic point of view, is more mature."

"On microscopic examination of fragments of the three types of tissue, removed after about 30 days of cultivation, differences have been observed in cell morphology between those derived from inoculated and uninoculated cultures. Many of the fragments from uninoculated cultures contained cells which appeared to be viable at the time of fixation, as indicated by the normal staining properties of the nuclei and cytoplasm. In contrast, the nuclei of the majority of the cells in fragments from inoculated cultures showed marked loss of staining properties. Since the amount of material which has been studied is as yet relatively small, ONE CANNOT CONCLUDE THAT THE CHANGES OBSERVED IN THE INOCULATED CULTURES WERE CAUSED BY THE VIRUS."

DOI: 10.1126/science.109.2822.85

In summary:

-The culture consisted of a salt solution, Ox Serum Ultrafiltrate, and tissues from the arms/legs/intestines/brains of 2-4 month old fetuses and one 7 month old premature baby

-The inoculation consisted of emulsified mouse brain assumed to have the Lansing

strain

-The confirmation that the Lansing strain was in the brain goo inoculate was due to the type of disease the mouse suffered from after intracerebral injection from a previous diseased host tissues

-Subcultures were inoculated with POOLED supernatant from all cultures

-Main difference from previous cultures was the continual removal and replacements of nutrients at different intervals

-The culture fluids consisting of human embryos, oxblood, and mouse brains caused death and paralysis in mice after intracerebral inoculation (shocking, right?)

-Penicillin and Streptomycin were used on the cultures

-The agent in the intestinal cultures was not "confirmed" by neutralization tests or monkey inoculations

-The "virus" was assumed to be in the culture due to the mice paralysis and killing effect it had after intracerebral injection

-The CPE observed could not be concluded to be caused by a "virus"

There was no "virus" ever purified/isolated from these culture experiments. In fact, "virus" was only assumed and estimated:

"Next, THE TEAM NEEDED TO FIGURE OUT HOW MUCH VIRUS HAD BEEN PRODUCED, USING A TECHNIQUE CALLED ENDPOINT DILUTION. On the 16th day of the 67-day period, some of the infected fluids were taken from the muscle, skin, and connective tissue cultures and infected into mice and monkeys. Another set of mice and monkeys were infected with the original virus solution for comparison.

THE AMOUNT OF VIRUS WAS ESTIMATED BASED ON SOMETHING CALLED AN LD50, WHICH MEANS "THE DOSE THAT KILLS 50% OF THE INFECTED ANIMALS AFTER A SPECIFIED TIME." The volume of virus solution needed to kill 50% of the test animals was 10^15 (10 trillion) times lower after 16 days compared to day 1, meaning it was more concentrated after 16 days. So, the virus had been successfully grown to very high levels. In the intestinal cells, that factor was 10^14 and in the nerve cell controls it was 10^12 ."

https://sciworthy.com/humble-laboratory-methods-behind.../

Sick culturing practices + illogical assumptions - purified/isolated "virus" = Polio

WAS POLIO EVER PROPERLY PURIFIED/ISOLATED?

When talking about Polio, the usual names come up in the conversation about the discovery process. You will most likely hear about Karl Landsteiner and Erwin Popper as the ones who originally "discovered" the "virus" in 1908 through grotesque experiments on monkeys. You will also hear about Charles Armstrong's research with the Lansing strain in 1939 which was the precursor to John Franklin Enders "successful" cultivation of the strain in non nervous tissue cultures in 1949. This apparently paved the way for Jonas Salk to create the Polio vaccine in 1953 as well as Albert Sabin's oral vaccine in 1961.

However, two people who seem to be consistently left out of the Polio discovery conversation are Hubert Loring and Carlton Schwerdt, which is odd considering these two are given credit for the purification/isolation of Polio in 1946-1947. Trying to find out much about the methods of their work or even digging up some of their publications was a challenging task yet presented below is what I could find on the supposed purification/isolation of Polio:

"Loring's laboratory was characterised by a friendly atmosphere and subdued excitement. With his students, he was involved in two major areas during this time – THE PURIFICATION OF THE POLIOMYELITIS VIRUS and the structure and metabolism of ribonucleic acids.

Along with his student Schwerdt, Loring spent three years searching for the poliovirus. THEIR EFFORTS LED TO THE "SUCCESSFUL" ISOLATION OF THE LANSING STRAIN OF THE POLIOVIRUS IN 1946. Schwerdt completed his Ph.D. in biochemistry by the time their results were announced on January 10, 1947.

Tempers excitement

LORING AND SCHWERDT WERE ABLE TO OBTAIN THE VIRUS WITH AT LEAST 80% PURITY. They were able to extract it from cotton rats, the only species then known to contract polio other than primates. Even though they had opened the door to further experimentation and the development of a vaccine against polio, Loring tempered the excitement, cautioning that the path ahead might still be long."

"Working alongside his colleagues at Berkeley, SCHWERDT DEVELOPED A METHOD

TO PURIFY THE POLIOVIRUS AND ALSO PHOTOGRAPHED IT FOR THE FIRST TIME IN PURE FORM IN 1953. He was involved in crystallising the pure virus in 1955 and ALSO PURIFIED ALL THREE KNOWN MAJOR STRAINS OF POLIOVIRUS IN 1957."

https://www.google.com/.../lorin.../article33410310.ece/amp/

This initial article gives credit to Loring and Schwerdt for successful purification/isolation of Polio in 1946, with the caveat that it was taken from cotton rats and was only 80% pure. Below are excerpts from the original paper claiming 80% purity.

From Loring's 1946 "Paper Isolation of a Macromolecular Constituent with Properties of the Lansing Strain of Poliomyelitis Virus:"

"If the normal protein is present in the infectious extracts to the same extent it is found in normal tissue, IT IS EVIDENT THAT THE MAXIMUM AMOUNT OF NORMAL MACROMOLECULAR IMPURITY PRESENT IN THE PURIFIED VIRUS IS OF THE ORDER OF 5 TO 20%."

"Conclusions. The results outlined above including (a) the presence in the purified samples of a uniform and high specific activity,(b) the demonstration that NORMAL MACROMOLECULAR CONSTITUENTS ARE LARGELY ELIMINATED by the purification procedures, and (c) the fact that the samples are relatively monodisperse in the transparent ultracentrifuge PROVIDE STRONG EVIDENCE THAT THE PREPARATIONS CONSIST OF ESSENTIALLY PURE LANSING VIRUS."

doi: 10.3181/00379727-62-15452.

Even from this study, Loring admits that normal macromolecular impurity exists within their "pure" sample of the Lansing strain and that it is strong evidence that the preparations are "essentially pure" Lansing "virus." In other words, this is not evidence of a purified/isolated Lansing Polio strain as any of the estimated macromolecular impurities could potentially be the "virus" particles they are looking for as the cause of disease.

From Lorings 1946 Paper "Electron Microscopy of Purified Lansing Virus:"

"ALTHOUGH ALL THE MICROGRAPHS HAVE NOT YIELDED UNIFORM OR

CONCLUSIVE RESULTS, they have in general shown the presence of spherical or possibly slightly asymmetrical particles RANGING IN SIZE from 12 to 34 mp."

"OF PARTICULAR INTEREST IS THE ABSENCE IN ALL OF THE PURIFIED LANSING VIRUS PREPARATION OF ASYMMETRICAL OR THREAD-LIKE PARTICLES AS FOUND BY GARD FOR BOTH MURINE (Theiler's virus) AND HUMAN POLIOMYELITIS VIRUS and as suggested by Bourdillon for the SK mouse virus. While the results of Gard appear conclusive in the case of Theiler's virus, his conclusions with respect to human poliomyelitis were admittedly based on only a few experiments and OPEN TO OTHER INTERPRETATIONS. The latter purified preparations from both infected tissues and feces CONTAINED COMPONENTS WITH SEDIMENTATION RATES OF THE ORDER OF MAGNITUDE DESCRIBED IN THE PRECEDING PAPER FOR THE LANSING VIRUS. Similarly while some filamentous particles were observed in his electron micrographs, THERE WAS ALSO AMPLE EVIDENCE FOR SMALL, APPROXIMATELY SPHERICAL, PARTICLES. We feel, therefore, that Gard's results on human poliomyelitis ARE NOT NECESSARILY INCOMPATIBLE WITH THOSE PRESENTED HERE.

Conclusions.

The results mentioned in the preceding paper as well as those given here lead to the conclusion that the Lansing virus, UNLIKE THEILER'S VIRUS AND PROBABLY THE SK MOUSE VIRUS, IS A RELATIVELY SPHERICAL OR SLIGHTLY ASYMMETRICAL PARTICLE OF ABOUT 25 MP AVERAGE PARTICLE DIAMETER. It may be recalled that a somewhat similar conclusion was reported for the MV virus.5 It appears to the present authors that such values for the size of these strains of poliomyelitis virus of human origin are also more in accord with the filtration end-point data and the conditions that have been found necessary for sedimentation in the ultracentrifuge."

doi: 10.3181/00379727-62-15453.

This paper was presented after the previous Loring paper and seems to admit that the images obtained are not uniform and consistent with sizes ranging from 12 to 34 mp. Loring goes on to talk about the differences his team observed over other teams attempting purified/isolated "virus." It is clear from his comments that there are other asymmetrical or thread-like particles that Gard observed which were not in their images. However, he admits there were small spherical particles in Gards images as well which line up with their work, thus they are deciding on these small spherical particles as the "Lansing virus" even though there are other particles in the sample.

After the experiments with Loring, Carlton Schwerdt went off to attempt further purity of

the "virus." Notice from these sources the admittance on the lack of completely purified samples from the previous efforts which only achieved purification of 1% of the "virus:"

"With another biochemist, CARLTON E. SCHWERDT, at the University of California, Berkeley, Dr. Bachrach developed a method to isolate two strains of the virus and purify them so they could be studied under an electron microscope. PAST EFFORTS HAD BEEN ABLE TO PURIFY 1 PERCENT OF THE VIRUS; the Bachrach and Schwerdt research HAD ACHIEVED A PURIFICATION LEVEL OF 10 PERCENT.

The greater purity enabled others to develop more exacting tests for the efficacy of polio vaccines. In 1955, Dr. Jonas Salk introduced a successful vaccine, known as the “killed virus” vaccine, using a form of the polio virus itself."

https://www.nytimes.com/2008/07/25/us/25bachrach.html

"In 1954, before discovering the FMD vaccine, Bachrach left a lasting mark on the fight against polio. Working with Carlton Schwerdt, THE TWO WERE ABLE TO ISOLATE AND “PURIFY” 10% OF THE POLIO VIRUS, CUTTING OUT THE PERIPHERAL JUNK THAT PREVENTED SCIENTISTS FROM STUDYING THE DISEASE. RESEARCHERS PREVIOUSLY HAD ONLY BEEN SUCCESSFUL IN PURIFYING 1% OF THE VIRUS, and the additional clarity led to several more advancements, culminating in 1952 with Jonas Salk’s vaccine."

https://www.nationalscienceandtechnologymedalsfoundation....

Notice the word "PURIFY" in quotations. Even after several years, Schwerdt was only able to achieve 10% purification/isolation of the Polio "virus." Here are some excerpts from his work:

From Schwerdt's 1953 Paper "Purification Studies on Lansing Poliomyelitis Virus:"

"Purified concentrates of the Lansing strain of poliomyelitis virus have been PREPARED FROM COTTON RAT CNS with specific infectivities increased 20,000 times, on the average, above that of the original infected tissue. The fraction of total virus infectivity recovered in the purified concentrates averaged 40 to 50%.

The virus in the concentrates of high specific infectivity has been identified by analytical electron microscopy to be a spherical particle 28 mμ in diameter. This identification was made through correlative experiments relating particle counts to infectivity and is discussed in detail. The concentration of the virus in CNS tissue of paralytic cotton rats

is calculated to be about 0.2 µg per gram of tissue. It is shown that about 21,000 virus particles or 3.0 × 10-13 g of virus are consistently present in one LD50 inoculum for cotton rats, BUT IT IS NOT KNOWN WHETHER ALL OR A CONSTANT FRACTION OF THESE PARTICLES ARE INFECTIOUS. The electron microscopic evidence shows that the virus is highly uniform in size and shape."

https://www.jimmunol.org/content/72/1/30.long

Unfortunately, I am unable to access the full article but highlights from a study done a year later gives a good idea of what was done.

From Schwerdt's 1954 paper "Morphology of Type I1 Poliomyelitis Virus (MEFI) as Determined by Electron Microscopy:"

"Previous attempts to determine by electron microscopy the size and shape of particles of human poliomyelitis virus have been equivocal BECAUSE OF UNCERTAINTY AS TO THE IDENTITY OF THE VIRUS PARTICLE AMONG VARIOUS OBJECTS OF SIMILAR APPEARANCE FOUND IN THE ELECTRON MICROGRAPHS (1-6). Improved methods of purification applicable to poliomyelitis virus PROPAGATED IN CENTRAL NERVOUS SYSTEM TISSUE IN VIVO, OR IN MONKEY KIDNEY TISSUE CULTURE, now yield virus concentrates CONTAINING AT MOST 2 CLASSES OF PARTICLES and frequently only one(7,8). Furthermore, the examination of such purified virus concentrates by analytical electron microscopy has resulted in the establishment of a constant ratio of numbers OF THE PARTICLE BELIEVED TO BE THE VIRUS to the infectivity titer (8). In a preliminary determination this particle has been found to be approximately spherical and about 28 mp in diameter, but its successful identification seems to justify at this time a more definitive examination of its shape and size."

"Materials and methods.

The virus used in this study is a TISSUE CULTURE MEFI STRAIN RECEIVED FROM DR. JONAS SALK in 1952. It has been PASSED 3 TIMES IN MONKEY KIDNEY TISSUE CULTURE in this laboratory, and the third passage has been used AS A VIRUS SEED POOL for the production of liter quantities of tissue culture virus suspension for purification purposes. The method of purification employed was similar to that described by Bachrach and Schwerdt for the purification of Lansing virus FROM INFECTED COTTON RAT BRAINS AND CORDS (8)."

"Diameter measurements of individual frozen dried particles offer further evidence of uniformity in that the variation of the measured diameters is NOT UNEQUIVOCALLY GREATER THAN THE ESTIMATED UNCERTAINTY OF MEASUREMENT. These

conclusions are IN DISTINCT CONTRAST TO THE RESULTS PUBLISHED BY SABIN, Hennessen and Warren(6) for a type I1 virus WHERE THE ELECTRON MICROGRAPH SHOWS A GREATER VARIATION OF PARTICLE SIZE."

"The absolute value of the diameter of the virus particles is SUBJECT TO SEVERAL UNCERTAINTIES, OWING TO POSSIBLE ARTIFACTS OF PREPARATION, THE FINITE RESOLVING POWER OF THE ELECTRON MICROSCOPE, THE CALIBRATION OF MAGNIFICATION, AND THE LIMITATIONS OF THE MEASUREMENT OF THE PHOTOGRAPHIC IMAGES. If we ASSUME THAT NO APPRECIABLE SHRINKAGE OF INDIVIDUAL PARTICLES HAS OCCURRED during the formation of an array like Fig. 1, the average diameter of the particles measured in a row is least susceptible to errors of image formation and measurement."

"If individual particles are measured, the most reliable diameter is probably secured from the frozen-dried preparations where no sign of surface-tension distortion is seen. OUR ESTIMATE OF THE UNCERTAINTY in the value given for the particle diameter is +-2 mp.

Summary.

The particles of the MEFI poliomyelitis virus, GROWN IN MONKEY-KIDNEY TISSUE CULTURE, and subsequently purified by chemical and physical means, are spherical in shape and highly uniform in diameter. The diameter of individual particles, prepared by normal air-drying from a 0.1 31 ammonium acetate suspension, is found to be 31 mp. The diameter of particles packed in a crystalline array, or frozen-dried from a 0.1 31 ammonium bicarbonate suspension, is found to be 27 mp. It is believed that the latter diameter is the more significant and reliable figure."

doi: 10.3181/00379727-86-21082.

From this paper, you can see that the images and "virus" come from monkey kidney tissue cultures from which a "virus" is assumed to be contained within which is then passaged several times. This is already an unpurified mixture. It is clear that they are assuming a certain size/shape of the particles they see in an EM image are the "virus" they are searching for. However, they even admit they are uncertain that these particles are the "virus." They estimate size/shape based on various measures but there are several limitations noted such as artefacts, technological limitations, and assumptions which are made. They also state that the images obtained are in direct contrast to the ones by Sabin who supplied the material that they used for this study.

It's apparent just from looking at the work of both Hubert Loring and Carlton Scwerdt that the complete purification/isolation of a Polio "virus" never took place. It is admitted that only 10% purity was ever achieved. The vaccine was created from impure particles

assumed to be "virus" taken from monkey kidney cultures. This in effect caused many health issues due to the admitted impurity of the monkey SV40 "virus" (foreign animal DNA) contained within them during production from 1955 to 1961.

Below are two images of supposed purified Polio for which we now know are not 100% purified and never confirmed as "virus" in the first place. The first is from Loring's 1946 study and the second from Schwerdt's 1954 study.

FURTHER EVIDENCE OF THE LACK OF PURIFIED/ISOLATED POLIO:

In 1959, Carlton Schwerdt wrote a review of the purification of polio. It's a long 46 pages but there are some interesting nuggets contained within. Remember that it was later admitted that Schwerdt only achieved a 10% purification yet it was claimed at that time that the "virus" was purified.

PURIFICATION AND PROPERTIES OF POLIOVIRUS

"Studies of the physical and chemical properties of the human polioviruses by direct means require, as is the case with any virus, preparations of the greatest possible degree of purity. THIS POSES THE PROBLEM OF ESTABLISHING CRITERIA OF PURITY THAT CAN BE MET EXPERIMENTALLY. Perhaps the ideal operational concept of a pure virus preparation is one in which all particles are identical with respect to measurable physical properties and each possesses the property of infectivity. THIS CONCEPT MAY NOT NECESSARILY BE ADEQUATE FOR ALL VIRUSES, since virus particles of detectable chemical and physical heterogeneity may nevertheless possess the same infectivity. An approach based on this concept may be applied, however, to the problem of purifying some of the small viruses, including the polioviruses, which seem to be relatively uniform in size and shape. Until recently the usual source of human poliovirus for purification studies was limited to portions of the central nervous system (CNS) of infected primates (Loring and Schwerdt, 1942; Stulberg et al., 1948) or the entire CNS of rodents infected with Type 2 polioviruses (Gard, 1943; Loring and Schwerdt, 1946; Bachrach and Schwerdt, 1952, 1954).

ATTEMPTS TO PURIFY VIRUS FROM SUCH SOURCES WERE SEVERELY HANDICAPPED BY THE LOW PHYSICAL CONCENTRATION OF VIRUS IN THE CNS TISSUE, AS WELL AS BY THE PRESENCE OF LARGE AMOUNTS OF SO-CALLED NORMAL, MACROMOLECULAR TISSUE PARTICLES OF PHYSICAL AND CHEMICAL CHARACTERISTICS SIMILAR TO THOSE OF THE VIRUS. With the advent of poliovirus propagation in tissue culture (Enders et al., 1949), however, purification

studies progressed more rapidly. Tissue culture not only provided an abundant source of virus for purification, RELATIVELY FREE of contaminating, nonviral components (Schwerdt and Schaffer, 1955,1956), but also a means of accurate assay (Dulbecco and Vogt, 1954), which has served as an aid in following purification steps and in IDENTIFYING THE PHYSICAL PARTICLE WITH WHICH INFECTIVITY IS ASSOCIATED (Schwerdt and Fogh, 1957)."

"Other organic solvents as well as ether, notably N-butanol (Bachrach and Schwerdt, 1952), chloroform (Polson and Selzer, 1954), and fluorocarbon (Manson et al., 1957), have been used to extract or denature nonviral

materials. The usual experience has been that emulsification of crude aqueous suspensions of poliovirus with these immiscible organic solvents results in the removal of AS MUCH AS 90% OR MORE OF THE NON VIRAL CONTAMINANTS, including lipids, without concomitant loss in total infectivity from the aqueous phase."

"The physical methods applied to poliovirus concentration and PARTIAL PURIFICATION have included ultrafiltration (Clark et al., 1933), pervaporation (Polson and Hampton, 1957), electrophoresis (Polson, 1953a) , and ultracentrifugation (Schultz and Raffel, 1937; Loring and Schwerdt, 1942; Gard, 1943). ULTRAFILTRATION SERVED ONLY TO CONCENTRATE THE VIRUS WITHOUT SIGNIFICANT PURIFICATION. Similarly, pervaporation achieved a 1000-fold reduction in volume, permitting subsequent purification by dialysis and high-speed centrifugation. Preparative electrophoresis of the zone or convection type has found only limited application to poliovirus purification as yet, although it may be a potentially powerful tool for isolating the virus from suspensions in which it is present in fairly high concentrations.

Preparative vacuum ultracentrifugation has had perhaps the greatest appeal as a physical method of purification and concentration, as evidenced by its incorporation in many multi step purification procedures, some of which are described in Section 11,B. It permits one to take advantage of the relatively large size of the virus particle for separating it from many of the cellular contaminants. Its limitations are, of course, THE INABILITY TO REMOVE SELECTIVELY THOSE SO-CALLED “NORMAL MACROMOLECULAR” CONSTITUENTS WITH SEDIMENTATION CHARACTERISTICS SIMILAR TO THE VIRUS COMPONENT (Sharp, 1953). Melnick (1946) concentrated poliovirus present in suspensions in low concentration by means of sedimentation in the Sharples centrifuge.

This method, while effective, has the disadvantage of producing aerosols, possibly contaminated with virus, if the continuous-flow bowl is used. Baron (1957) sedimented poliovirus, also from highly dilute suspensions, in the vacuum type ultracentrifuge, using calf serum or gelatin to minimize thermal convection or mechanical mixing. Both of

these methods are more useful for the isolation of small amounts of poliovirus in low concentration than for concentrating virus for purification studies."

"Among the earlier concerted efforts to this end are those of Loring and Schwerdt (1942, 1946) and Gard (1943). The former investigators ATTEMPTED TO PURIFY the MV and Lansing strains of poliovirus from monkey spinal cords and cotton rat CNS, respectively. Their procedure involved extraction of virus from CNS tissues with saline, freezing and thawing of these extracts, ether extraction, and several cycles of high- and low-speed centrifugation. Preparations obtained in this way were characterized by specific infectivity assays, sedimentation velocity analysis, and electron microscopy.

Gard (1943) reported in detail studies on both mouse encephalomyelitis virus and human poliovirus purification. His source of human poliovirus was either feces or brain and spinal cord from infected man. Precipitation with ammonium sulfate was included in his procedure, in addition to most of the steps employed by the former investigators. Infectivity was found to sediment within the range of 150 to 195 S (Svedberg units), in remarkably good agreement with those found by later workers (Melnick et al., 1951; Polson and Selzer, 1952; Schwerdt and Schaffer, 1955). Analytical ultracentrifugal analyses of these preparations revealed several components. Electron microscopy revealed filamentous particles 15 mp in diameter and of greatly varying lengths which, however, were also isolable from normal CNS and feces. These filaments are now thought to be fragments of bacterial flagella (Gard, 1955). IT IS CLEAR IN RETROSPECT THAT THESE EARLY ATTEMPTS, ALTHOUGH PROMISING, DID NOT YIELD PREPARATIONS CONSISTING SOLELY OF HOMOGENEOUS POLIOVIRUS PARTICLES."

"ROUGH ESTIMATES OF THE ABSOLUTE DEGREE OF PURITY HAVE BEEN MADE from physicochemical (Schwerdt and Schaffer, 1956) as well as from serological studies (Mayer et al., 1957) AND HAVE BEEN FOUND TO BE 50% OR GREATER. Such estimates are made on the basis of mass of characteristic physical particles without reference to the infectivity of these particles and DO NOT, THEREFORE, STRICTLY FULFILL OUR CRITERIA FOR VIRUS PURITY."

"IDENTIFICATION OF POLIOVIRUS PARTICLES

The identification of a virus particle requires the ESTABLISHMENT OF A RELATIONSHIP BETWEEN INFECTIVITY AND AN ADEQUATELY DESCRIBED PHYSICAL PARTICLE. With polioviruses, this relationship has been demonstrated for a spherical particle of 27-30 mp diameter (the major infective component). THERE IS

SOME SUGGESTION, HOWEVER, THAT POLIOVIRUS INFECTIVITY CAN ALSO BE ASSOCIATED WITH A SMALLER PARTICLE."

"FREQUENT ATTEMPTS HAVE BEEN MADE TO OBSERVE POLIOVIRUS PARTICLES BY ELECTRON MICROSCOPY, BUT, AS RHIAN et al. POINTED OUT IN 1949, NO CHARACTERISTIC PHYSICAL PARTICLE HAD BEEN UNEQUIVOCALLY IDENTIFIED AS THE INFECTIVE VIRUS PARTICLE UP TO THAT TIME. Not until Bachrach and Schwerdt (1954) correlated infectivity with physical particle count by analytical electron microscopy has any direct evidence been presented for the identity of the virus particle. In this instance, Lansing virus was PURIFIED FROM INFECTED COTTON RAT CNS TISSUE. Characteristic spherical particles, approximately 28 mp in diameter, were observed and counted in these preparations by electron microscopy, using the spray droplet technique (Backus and Williams, 1950). These particles were not found in concentrates prepared from uninfected CNS tissue suspensions. The quantitative data from virus concentrates yielded a constant ratio of particle count to 50% infective doses (ID60) in cotton rats of approximately 21,000 from preparation to preparation. Furthermore, it was not possible to dissociate infectivity from these particles by separation cell experiments using the ultracentrifuge."

"The arguments for the existence and significance of the small infectious component of poliovirus have not been accepted without question, as evidenced by the discussion following the presentation of the investigations of the South African group at the 1956 Ciba Conference (Kipps et al., 1957). It must be emphasized that all the published work was with the virus from mouse brain. Whether the phenomenon is general for all tissues or for neural tissue, or is simply limited to mice or rodents, is not known. In a careful search of the literature on the small infectious component of poliovirus and other viruses, NO MENTION COULD BE FOUND OF CRITICAL EXPERIMENTS TO ESTABLISH THE ANTIGENIC RELATIONSHIP OF THE SMALL AND LARGE INFECTIOUS PARTICLES. Problems associated with mixtures of viruses in single specimens in identification and diagnosis of human enteric viruses have been discussed by Melnick (1957). IT IS NOT INCONCEIVABLE THAT POLIOVIRUS AND SOME OTHER (SMALLER) VIRUS PATHOGENIC TO MICE COULD BE MAINTAINED IN QUASI-EQUILIBRIUM THROUGH MANY PASSAGES, and that separation could be achieved by selective passage or by physical techniques."

"THE CONCEPT OF INFECTIOUS UNITS SMALLER THAN THE USUALLY ACCEPTED VIRUS PARTICLE IS OF FUNDAMENTAL IMPORTANCE, especially in view of the current concepts of virus structure and infectivity of RNA (Williams, 1957; Fraenkel-Conrat et al., 1957). It is hoped that further experimental evidence will be offered to resolve the questions of existence and nature of the small infectious component. In this respect it would be preferable to use the less complex tissue culture

systems, if such are applicable, and to achieve purification to such a degree that physical and chemical properties may be determined directly arid the results expressed in terms of specific infectivities. Serological techniques should be used to establish the viral identity of units of different size separated by physical means."

"A. ELECTRON MICROSCOPY

The general problems of associating virus infectivity with particles observed by electron microscopy have been discussed by Williams (1954) and Rang (1955). Prior to the positive identification of infectivity of the Lansing strain with 28 mp particles (Bachrach and Schwerdt, 1954), ELECTRON MICROSCOPY OF POLIOVIRUS PREPARATIONS WAS OPEN TO QUESTION."

"Electron microscopy should play an important part in the eventual elucidation of the substructure of the poliovirus particle. HOWEVER, THE TECHNIQUES APPLIED SO FAR HAVE NOT ALLOWED PROPER CORRELATION WITH OTHER PHYSICAL OR CHEMICAL MEASUREMENTS OR WITH THE BIOLOGICAL PROPERTIES OF THE VIRUS. Experiments should be performed in such a manner that the morphological observations by electron microscopy would be directly or quantitatively related to alteration of other properties, such as sedimentation, nucleic acid content, infectivity, or antigenicity."

"8. VIRUS-CELL INTERACTION

INTRACELLULAR VISUALIZATION OF POLIOVIRUS PARTICLES BY ELECTRON MICROSCOPY HAS NOT BEEN CLEARLY DEMONSTRATED. The identification of such small particles in the complex cellular background IS ALMOST IMPOSSIBLE, unless one can find definite aggregates such as those observed in cells infected with adenovirus (Morgan et al., 1956)."

"These observations by Ruska et al., Reagan et al., and Mu contribute nothing to our knowledge of the morphology and structure of the poliovirus particle, and little if anything to knowledge of virus-cell interactions, BUT SERVE TO ILLUSTRATE THE PITFALLS ONE MAY ENCOUNTER IN UNCRITICAL USE OF THE ELECTRON MICROSCOPE IN ATTEMPTS TO IDENTIFY VIRUS PARTICLES."

"V. CHEMICAL PROPERTIES

Except for the nucleic acid component, ALMOST NOTHING IS KNOWN OF THE CHEMISTRY OF POLIOVIRUS BECAUSE THE USUAL CHEMICAL TECHNIQUES REQUIRE APPRECIABLE QUANTITIES OF MATERIAL AND ARE DESTRUCTIVE OF

THAT MATERIAL.

By analogy with other viruses, poliovirus would most certainly be primarily nucleoprotein in nature. Some information may also be drawn by inference from the action of certain agents upon the infectivity or other properties of the virus."

"ALTHOUGH COMPLETE CHEMICAL CHARACTERIZATION OF POLIOVIRUSES MAY NEVER BE MADE, there remains much that can be accomplished in the elucidation of chemical components, subunits, or groups related to the immunological properties of the virus and to the fundamental virus-cell interactions."

"In connection with rodent-adapted Type 2 poliovirus, it should be noted that in purified preparations of Lansing virus from infected cotton rat CNS, BACHRACH AND SCHWERDT (1954) OBSERVED PARTICLES OF ABOUT 12 mμ IN DIAMETER, IN ADDITION TO THE CHARACTERISTIC VIRUS PARTICLES. The CF test was not applied to the material examined by electron microscopy and NO SIGNIFICANCE WAS ATTACHED TO THE SMALL PARTICLES, SINCE SIMILAR PARTICLES WERE OBSERVED IN NORMAL CNS CONTROLS."

https://doi.org/10.1016/S0065-3527(08)60491-1

In summary:

-there was no standardized criteria for purity

-attempts to purify directly from CNS tissues of primates and rodents was hampered by low "virus" and high amounts of normal macromolecular particles similar to "viruses"

-tissue culture was used to get "virus" that was "relatively free" of contaminants

-use of organic solvents only resulted in as much as 90% of non-viral contamination removal

-tools used for PARTIAL PURIFICATION included ultrafiltration, pervaporation, electrophoresis, and ultracentrifugation

-ultra-filtration did not lead to significant purification

-ultra-centrifugation was limited by the inability to remove normal macromolecular particles similar in size/shape to "viruses"

-many attempts were made to purify polio which did not meet the requirements with estimated purity ranging from 50% or greater and non-homogeneous particles

-there is evidence a smaller particle than what is assumed to be the polio "virus" could

be causing infectivity

-up to 1949 (a few years after Loring and Schwerdt announced 80% purity for polio with EM images), no particle had been unequivocally identified as polio in EM images

-admittance that there are smaller particles in the samples from mice brains that could be infectious yet no studies were carried out to determine if they were or not

-prior to Schwerdt's own 1954 paper, he claims earlier EM images of supposed Polio "virus" were open to question

-EM images could not be correlated with other physical and chemical measurements or with other biological properties of the "virus"

-intracellular visualization of the particles by EM had not been achieved and is considered almost impossible

-almost nothing is known about the chemical properties of the "virus" as appreciable quantities of "virus" are needed and the methods are destructive to these particles

-admittance that complete chemical composition of polio may never be made

-Schwerdt also noticed particles smaller than the assumed purified Polio "virus" in 1954 but considered them inconsequential as they were present in the controls

Excerpts from a letter by Virologist Thomas Rivers regarding the Polio vaccine by Jonas Salk:

Amazing how in 1953, they were more concerned about vaccine "safety and effectiveness" than they are now.

"I have been kept informed of the progress of these investigations, and it seems to me that the recent paper by Dr. Salk provides substantial evidence that a practical vaccine against human paralytic poliomyelitis can be achieved BY THE USE OF VIRUS PROPAGATED IN TISSUE CULTURE, RENDERED NONINFECTIOUS BY TREATMENT WITH A SOLUTION OF FORMALDEHYDE, and administered in the form of a mineral oil emulsion. It is evident that any investigator who possesses a promising preparation for the prevention of a human disease is faced with a decision either to conduct innumerable small-scale studies with relatively fewsubjects, in an effort to develop more effective preparations before widespread application, or to employ the experimental preparation in large numbers of human subjects EVEN THOUGH THE PREPARATION MAY NOT YET POSSESS ALL OF THE REFINEMENTS ULTIMATELY

DESIRED. THE TEMPTATION WILL BE GREAT TO URGE THAT THE EXPERIMENTAL VACCINE STUDIED BY Dr. SALK BE PREPARED FOR IMMEDIATE WIDESPREAD USE. SUCH ENTHUSIASM, HOWEVER, SHOULD BE TEMPERED NOT ONLY BY THE REALIZATION OF WHAT WE DO KNOW BUT, PERHAPS EVEN MORE, BY WHAT WE DO NOT KNOW."

"At this meeting, it was our privilege to hear from Dr. Salk a full and detailed account of the studies he has carried out to date. As a result of the critical evaluation that followed, this group recommended that (1) BEFORE LARGE-SCALE FIELD TRIALS ARE INITIATED, ADDITIONAL STUDIES INVOLVING INCREASING NUMBERS OF PERSONS BE UNDERTAKEN TO EXTEND THE EXPERIENCE ALREADY ACCUMULATED; (2) such studies be limited to Allegheny County, Pennsylvania; (3) SUCH STUDIES BE STOPPED FOR THAT PART OF THE SUMMER OF 1953 DURING WHICH POLIOMYELITIS MIGHT BE PREVALENT IN ORDER TO AVOID INSTANCES OF POLIOMYELITIS OCCURRING SHORTLY AFTER VACCINATION BEING ERRONEOUSLY ATTRIBUTED TO THE IMMUNIZATION PROCEDURE; and (4) these investigations be resumed on an ever-increasing scale after the poliomyelitis season is passed and that they be conducted in a sufficient number of communities to permit a controlled evaluation of the effectiveness of this preparation during the summer of 1954.

There is every indication that the preparation in question is as safe as any other vaccine now widely used against diseases other than poliomyelitis. HOWEVER, ONLY BY GRADUAL EXTENSION IN EVER-INCREÂSING GROUPS OF PERSONS AND IN A SYSTEMATIC FASHION AS HEREIN INDICATED CAN THIS BE ESTABLISHED WITH THE CERTAINTY REQUIRED BEFORE TESTING THE VALIDITY OF THE EXPERIMENTAL VACCINE AGAINST POLIOMYELITIS UNDER EPIDEMIC CIRCUMSTANCES.``

"This letter is written with the knowledge and approval of the persons who attended the special meeting to consider this problem. The opinions herein expressed are conveyed to you in the hope that every assistance will be given to Dr. Salk so that it may be determined whether the preparation that he has developed is the long-sought, practical means for the control of human paralytic poliomyelitis. In conclusion I would like to emphasize Dr. Salk's own statement that, while the results obtained in his studies "can be regarded as encouraging, THEY SHOULD NOT BE INTERPRETED TO INDICATE THAT A PRACTICAL VACCINE IS NOW AT HAND."

Thomas M. Rivers, M.D.

Director, Hospital of the Rockefeller

Institute for Medical Research

New York 21.

doi:10.1001/jama.1953.02940140068025

Sadly, even with this increased vaccine precaution, things went horribly wrong with the Salk (and later Sabin) vaccine:

"In April 1955 more than 200 000 children in five Western and mid-Western USA states RECEIVED A POLIO VACCINE IN WHICH THE PROCESS OF INACTIVATING THE LIVE VIRUS PROVED TO BE DEFECTIVE. WITHIN DAYS THERE WERE REPORTS OF PARALYSIS AND WITHIN A MONTH THE FIRST MASS VACCINATION PROGRAMME AGAINST POLIO HAD TO BE ABANDONED. Subsequent investigations revealed that the vaccine, manufactured by the California-based family firm of Cutter Laboratories, HAD CAUSED 40 000 CASES OF POLIO, LEAVING 200 CHILDREN WITH VARYING DEGREES OF PARALYSIS AND KILLING 10."

The Cutter incident led to the replacement of Salk's formaldehyde-treated vaccine with Sabin's attenuated strain. Though Sabin's vaccine had the advantages of being administered orally and of fostering wider `contact immunity', it could also be re-activated by passage through the gut, resulting in occasional cases of polio (STILL CAUSING PARALYSIS IN SIX TO EIGHT CHILDREN EVERY YEAR in the 1980s and 1990s, when a modified Salk vaccine was re-introduced). As Offit observes, `IRONICALLY, THE CUTTER INCIDENT—BY CREATING THE PERCEPTION AMONG SCIENTISTS AND THE PUBLIC THAT SALK'S VACCINE WAS DANGEROUS —LED IN PART TO THE DEVELOPMENT OF A POLIO VACCINE THAT WAS MORE DANGEROUS'.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1383764/

And then there was the issue of the SV40 contamination:

"SOME OF THE POLIO VACCINE ADMINISTERED FROM 1955–1963 WAS CONTAMINATED WITH A VIRUS, CALLED SIMIAN VIRUS 40 (SV40). THE VIRUS CAME FROM THE MONKEY KIDNEY CELL CULTURES USED TO PRODUCE THE VACCINE. Most, but not all, of the contamination was in the inactivated polio vaccine (IPV). Once the contamination was recognized, steps were taken to eliminate it from future vaccines. RESEARCHERS HAVE LONG WONDERED ABOUT THE EFFECTS OF THE CONTAMINATED VACCINE ON PEOPLE WHO RECEIVED IT. Although SV40 HAS BIOLOGICAL PROPERTIES CONSISTENT WITH A CANCER-CAUSING VIRUS, it has not been conclusively established whether it might have caused cancer in humans. Studies of groups of people who received polio vaccine during 1955–1963 provide evidence of no increased cancer risk. However, because THESE EPIDEMIOLOGIC STUDIES ARE SUFFICIENTLY FLAWED, the Institute of Medicine's

Immunization Safety Review Committee CONCLUDED THAT THE EVIDENCE WAS INADEQUATE TO CONCLUDE WHETHER OR NOT THE CONTAMINATED POLIO VACCINE CAUSED CANCER. IN LIGHT OF THE BIOLOGICAL EVIDENCE SUPPORTING THE THEORY THAT SV40-CONTAMINATION OF POLIO VACCINES COULD CONTRIBUTE TO HUMAN CANCERS, the committee recommends continued public health attention in the form of policy analysis, communication, and targeted biological research."

https://pubmed.ncbi.nlm.nih.gov/25057632/

Today, we have a rushed, experimental mass vaccination campaign for a never approved mRNA gene therapy that began while in "season" on a large-scale population with limited safety/efficacy data. Reports from VAERS are proving that the "vaccines" themselves are dangerous.

Seems we are incapable of learning from the past.

SALK'S 1953 POLIO VACCINE RESEARCH PAPER:

Presented below are some of the highlights from an 18-page report by Jonas Salk that was provided as evidence for the success of vaccination against Polio. Remember when reading this that the Salk vaccine was pulled off the market after being considered too dangerous as well as for being unpurified and contaminated with SV40, a monkey "virus." This, in turn, led to the use of Sabin's oral vaccine which was even more dangerous. The vaccines were (and still are) causing the disease they were supposed to prevent.

STUDIES IN HUMAN SUBJECTS ON ACTIVE IMMUNIZATION AGAINST POLIOMYELITIS

"The discovery by Enders, Weller, and Robbins u and of Evans and his associates15 that the virus of poliomyelitis could be propagated in CULTURES OF NON-NEURAL

TISSUES not only MADE AVAILABLE NEW TECHNIQUES FOR DEVELOPMENT OF A SOURCE OF VIRUS for immunologie studies but influenced profoundly and brought evidence to bear on the question of whether the poliomyelitis virus is strictly neurotropic. This discovery supported the hypothesis that the virus may multiply in non-neural tissue and only thereafter be disseminated from the portal of entry or site of multiplication to the central nervous system. This hypothesis suggests further that the paralytic sequel of infection might be avoided if an antibody barrier could be created in the blood stream to intercept the virus before invasion of the central nervous system. Perhaps the first decisive experimental demonstration that this might be possible was the observation by Morgan 16 that antibody induced by subcutaneous vaccination of monkeys with live virus reduced the frequency of paralysis in animals subsequently inoculated intramuscularly with fully potent virus. Similar observations were made with a variety of strains in the course of the program on immunologie classification.17"

"The reasonable immunologic explanation for the once mysterious epidemiological behavior of poliomyelitis is now well established. Only recently, however, has it been possible to begin a detailed and quantitative elucidation of the activities of the poliomyelitis virus under natural circumstances. SUCH SYSTEMATIC INVESTIGATIONS CAN NOW BE CARRIED OUT THROUGH THE APPLICATION OF ENDERS' TISSUE CULTURE TECHNIQUES NOT ONLY FOR THE ISOLATION OF VIRUS BUT ALSO FOR THE QUANTITATIVE ESTIMATION OF ANTIBODY FOR ALL THREE IMMUNOLOGIC TYPES."

"Among the earliest reports on immunization of monkeys and then of man were those of Brodie 5 and of Kolmer 6 in the early 1930's. THEIR STUDIES WERE CONDUCTED BEFORE PRECISE KNOWLEDGE REGARDING PATHOGENESIS AND IMMUNOLOGIC COMPLEXITY WERE AVAILABLE, AND BEFORE THERE HAD ACCUMULATED THE VAST EXPERIENCE WITH THE MANY STRAINS THAT POSSESS DIFFERENT PATHOGENIC CHARACTERISTICS. Moreover, METHODS HAD NOT BEEN DEVELOPED FOR ADEQUATE PURIFICATION OF VIRUS from suspensions of central nervous system tissue, nor were the dynamics of virus inactivation or modification well enough understood."

"Another approach that has been suggested for inducing immunity is that of the feeding of attenuated living virus. Koprowski and his associates 27 have recently reported the RESULTS OF STUDIES IN 20 HUMAN SUBJECTS FED A PREPARATION OF TYPE 2 VIRUS, WHICH HAD BEEN PROPAGATED IN THE CENTRAL NERVOUS SYSTEM OF RODENTS. In their studies, an immunologic response was induced and evidence found of virus multiplication in the gastrointestinal tract. The agent used for feeding was reported to be less pathogenic for the monkey than for rodents, BUT STILL CAUSED

PARALYSIS WHEN INOCULATED INTRACEREBRALLY into the monkey."

"The basic prerequisites are simple enough. These are first, a rich source of virus REASONABLY FREE OF EXTRANEOUS ANTIGENIC MATERIAL and second, a method for DESTROYING PATHOGENICITY WITHOUT COMPLETELY DESTROYING ANTIGENIC CAPACITY. To these might be added a third, a means for enhancing antigenic activity if the richest source of virus available after treatment for destroying pathogenicity proves to be inadequate or borderline in its effectiveness. It is the purpose of this report to show how these objectives are being approached, and to present data on the degree of antibody response observed in man in the first experiments cautiously undertaken."

"VIRUS OF THE THREE IMMUNOLOGIC TYPES USED IN THESE STUDIES WAS DERIVED FROM CULTURES OF MONKEY TESTICULAR AND MONKEY KIDNEY TISSUE. The destruction of infectivity was accomplished by TREATMENT WITH FORMALDEHYDE, and in some experiments EMULSIFICATION WITH MINERAL OIL employing the technique of Freund 2B was used for enhancing antigenic activity."

"Source of Virus for Vaccine. —The vaccines employed in these studies were prepared from viruses propagated in roller tube cultures of two different tissues derived from monkeys. MONKEY RATHER THAN HUMAN TISSUE WAS DECIDED ON FOR PRODUCING THE VIRUS FOR THESE EXPERIMENTS IN HUMAN SUBJECTS BECAUSE OF THE REMOTE POSSIBILITY THAT HUMAN TISSUE MIGHT OCCASIONALLY BE CONTAMINATED WITH SUCH AGENTS AS THE VIRUS OF INFECTIOUS HEPATITIS THAT MIGHT CONCEIVABLY PROPAGATE IN CULTURES OF HUMAN TISSUE. Even though the plan was to treat the culture fluids with formaldehyde to destroy the disease-producing property of the poliomyelitis virus, THERE COULD BE NO ASSURANCE THAT CONDITIONS FOR DESTRUCTION OF THE LATTER, for which specific tests are available, WOULD SIMULTANEOUSLY DESTROY OTHER AGENTS OF HUMAN DISEASE FOR WHICH NO SIMPLE LABORATORY TESTS ARE AVAILABLE. Another consideration was the fact that methods had been worked out for producing the virus in reasonably high concentration employing relatively small amounts of monkey tissue.

Although testicular tissue was the first monkey tissue used for culturing the virus, 30 it appeared desirable to investigate other monkey tissues for comparison. ENDERS AND ASSOCIATED 81 HAD EMPLOYED SUCH HUMAN SOURCES AS THE FORESKIN, EMBRYONIC SKIN AND MUSCLE, THE KIDNEY, AND THE UTERUS. It seemed of interest, therefore, TO EXPLORE CULTURES OF MONKEY KIDNEY AND MUSCLE, which are available in much larger supply than testicular tissue. The data in table 1 indicate the relative concentrations of virus derived from cultures of the kidney, testis, muscle, and liver of a single monkey. The advantage of the testicular cultures in yielding

virus for long periods is offset by the greater abundance of virus in kidney cultures in the early days after virus inoculation. This example is presented to illustrate why the decision was made to use kidney rather than any of the other tissues in further studies on vaccines. The striking difference between cultures of kidney tissue and testicular tissue might well be due to the nature of the principal cell type, which is epithelial-like in kidney cultures and fibroblastic in testicular and muscle cultures."

"In the search for a fluid medium free of protein and still satisfactory for maintaining the viability of the tissues and for supporting virus multiplication, it was soon found that the chemically defined mixture no. 199 described by Morgan, Morton, and Parker *- and first used for similar studies by Rhodes and his associates 8S provided such a nutrient fluid. THIS MIXTURE CONSISTS OF A LARGE NUMBER OF AMINO ACIDS, CERTAIN NUCLEIC ACID CONSTITUENTS, VITAMINS, AND MINERALS, AS WELL AS OTHER ESSENTIAL GROWTH-PROMOTING OR GROWTH-SUSTAINING SUBSTANCES. Details of the use of this medium for cultivation of the three types of poliomyelitis virus in roller tube cultures will be elaborated on elsewhere. The roller cultures in which virus was propagated for the experiments to be described contained APPROXIMATELY 100 mg. OF MINCED TISSUE WITH 4 ml. OF NUTRIENT FLUID."

"The strains selected to represent types 1, 2, and 3, respectively, are known as the Mahoney, MEF-1, and Saukett strains. The Mahoney strain, which was isolated originally by Francis, was adapted to tissue culture FROM MONKEY CENTRAL NERVOUS SYSTEM TISSUE. The MEF-1 strain, which was isolated by Schlessinger and Olitsky, was transferred to tissue culture FROM THE SPINAL CORD OF PARALYZED MICE. The Saukett strain was isolated in this laboratory by direct inoculation of TISSUE CULTURE WITH A FECAL SPECIMEN FROM A PATIENT PARALYZED IN 1950.

When inoculated into cynomolgus monkeys, these strains were found to have the following characteristics: The Mahoney strain is highly pathogenic by any route, whereas the MEF-1 and Saukett strains ARE ESSENTIALLY NONPATHOGENIC EXCEPT WHEN GIVEN INTRACEREBRALLY. When undiluted tissue culture fluids containing high concentrations of either of the latter two are INJECTED BY NONNEURAL ROUTES, PARALYSIS WILL OCCUR INFREQUENTLY. Of the two, the Saukett strain produces the milder disease, even when given intracerebrally."

"Safety Tests.—The first problem that had to be resolved before human subjects could be inoculated was the question of safety. IT HAS BEEN THE CONSENSUS THAT TESTS FOR SAFETY SHOULD INCLUDE THE INTRACEREBRAL INOCULATION OF MONKEYS. In all of the material employed for human beings, the absence of infectivity for the cynomolgus monkey was established in prior tests by the intracerebral inoculation of 0.5 ml. of fluid in 6 to 10 animals. As an additional safeguard, the only preparations CONSIDERED SATISFACTORY FOR USE WERE THOSE THAT HAD

BEEN EXPOSED TO THE ACTION OF FORMALDEHYDE FOR AT LEAST 24 TO 48 HOURS after the test just described indicated that infectivity had been destroyed. THE FACT THAT AMOUNTS OF VIRUS THAT PRODUCE PARALYSIS WHEN GIVEN INTRACEREBRALLY ARE NOT PARALYTOGENIC WHEN GIVEN INTRAMUSCULARLY OR INTRAVENOUSLY SUGGESTS THAT THE INTRACEREBRAL SAFETY TEST MAY PROVIDE A MORE THAN ADEQUATE MARGIN IN CERTIFYING A GIVEN PREPARATION AS SAFE FOR USE IN HUMAN SUBJECTS."

HUMAN SUBJECTS

"In extending to man studies on vaccination performed in laboratory animals, tests on more than a few individuals had to be anticipated. THE FIRST PERSONS TO PARTICIPATE IN THESE STUDIES WERE PATIENTS PARALYZED IN RECENT YEARS BY A POLIOMYELITIS INFECTION and who were in residence at the D. T. Watson Home for Crippled Children, Leetsdale, Pa. In addition to patients who had recovered from paralytic poliomyelitis, there were others who were in residence at the Watson Home FOR SUCH DISEASES AS ARTHRITIS, SPASTIC PARALYSIS, AND A VARIETY OF CONGENITAL DEFORMITIES. After the initial investigations were underway at the Watson Home, additional studies were undertaken at the Polk State School, Polk, Pa.

The numbers of subjects involved in the several experiments under way are indicated in table 3. Of the group of 98 patients at the Watson Home, 51 are participating in studies involving aqueous vaccines INOCULATED INTRADERMAL, and 27 are involved in studies on emulsified vaccines given intramuscularly. All of the 63 individuals at the Polk State School have been GIVEN EMULSIFIED VACCINES INTRAMUSCULARLY."

"POLIOMYELITIS IS NOT A SINGLE DISEASE, SINCE IT CAN BE CAUSED BY ANY ONE OF THREE IMMUNOLOGICALLY DIFFERENT VIRUSES. Therefore, PERSONS WHO HAVE HAD PARALYTIC POLIOMYELITIS ARE CONSIDERED SATISFACTORY FOR STUDIES ON ACTIVE IMMUNIZATION. From the immunologic viewpoint, these individuals are similar to others without previous history of paralytic poliomyelitis, since there are many persons who may have at one time or another been infected with one or two of the three types of virus without becoming paralyzed. INDIVIDUALS WHOSE BLOOD IS DEVOID OF ANTIBODY FOR ANY ONE VIRUS TYPE CAN NOT BE REGARDED AS IMMUNE TO THE DISEASE BECAUSE THEY HAVE NOT EXPERIENCED INFECTION WITH ALL THREE TYPES."

"Results of Infectivity Tests in Monkeys: In order that the significance of the foregoing findings may be evaluated in relation to the material inoculated, data are presented in figure 5 showing the infectious activity, for the cynomolgus monkey, of fluids from which the type 2 vaccine was derived. In this figure is indicated the fate of individual animals inoculated intracerebrally with samples of MEF-1 tissue culture pool no.15, treated with formaldehyde for different intervals.

IT WILL BE RECALLED THAT, on the days indicated, THE EXCESS FORMALDEHYDE WAS NEUTRALIZED WITH SODIUM BISULFITE. WHEN SUCH FLUIDS WERE INOCULATED INTRACEREBRALLY, NOT ALL OF THE ANIMALS COULD TOLERATE THE CONCENTRATIONS OF THE CHEMICALS CONTAINED IN THESE MIXTURES, AND SOME SUCCUMBED SHORTLY AFTER INOCULATION. In some instances the symbol for death in the chart also refers to deaths not due to poliomyelitis that occurred before termination of the 30 day observation period. The solid black symbols indicate that paralytic poliomyelitis occurred; the one symbol with the shaded area indicates that no clinical signs of infection were observed in life, but histological evidence of nonparalytic infection was observed when the spinal cords were examined at the end of the 30 day observation period."

"It is of interest to note in figure 12 that, in recently paralyzed persons, most of whom were children, a high proportion possess antibody for the type 1 virus; and it appears that when antibody is present it is usually found in high concentration. In the group of PERSONS WITHOUT A PRIOR HISTORY OF POLIOMYELITIS, some had no demonstrable antibody WHILE OTHERS HAD ANTIBODY THAT WAS READILY MEASURABLE; a point of particular interest is that, in general, the level of antibody is lower than in those who were recently paralyzed. The most reasonable explanation for this difference is that a shorter interval of time had elapsed between infection and testing in the group of recently paralyzed patients, most of whom were children, as compared with the group who had not been paralyzed, and therefore, in whom the time of infection could not be dated.

IT IS INTERESTING TO OBSERVE THAT THE 15 SUBJECT'S IN EXPERIMENT P-I HAVE ESSENTIALLY THE SAME DISTRIBUTION OF ANTIBODY AS THE GROUP OF PERSONS WITHOUT PRIOR HISTORY OF POLIOMYELITIS, from which they were drawn, except, of course, for the fact that THERE ARE NOT, in the vaccinated group, ANY WHO HAD NO DEMONSTRABLE ANTIBODY BEFORE VACCINATION. Following vaccination it appears that the distribution of antibody titers compares well with those of recently paralyzed patient."

"As before, PERSONS WITHOUT PRIOR HISTORY OF POLIOMYELITIS TEND TO HAVE LOWER LEVELS OF ANTIBODY, and some have no detectable antibody at all."

"The first of these is the question as to whether or not treatment with sodium bisulfite to arrest the action of the excess formaldehyde may in any way affect reactivation of the virus. The data presented in figure 16 suggest that reactivation does not occur. In this experiment, Mahoney pool 7, 11(T) was treated with formaldehyde for different periods of time at the temperature of melting ice; two different concentrations of formaldehyde were employed, and two samples were prepared. On the days indicated one sample was inoculated intracerebrally into monkeys and the second sample treated with sodium bisulfite.

The neutralized mixture was then returned to a melting ice bath and 18 days later was inoculated intracerebrally into groups of five monkeys. IT IS PERHAPS UNFORTUNATE THAT THE QUANTITY OF SODIUM BISULFITE NECESSARY TO NEUTRALIZE THE MORE CONCENTRATED SOLUTION OF FORMALDEHYDE COULD NOT BE TOLERATED INTRACEREBRALLY, AND MANY OF THE ANIMALS SUCCUMBED SHORTLY AFTER INOCULATION. This can now be obviated by overnight dialysis.

"THERE ARE SOME WHO MAY OBJECT TO THE USE OF KIDNEY TISSUE AS THE SOURCE OF VIRUS BECAUSE OF THE UNANSWERED QUESTIONS REGARDING ORGAN DAMAGE BY IMMUNIZATION WITH MATERIAL EMULSIFIED WITH MINERAL OIL. Although many theoretical arguments as well as examples by analogy may be brought forth to indicate that such effects are improbable with the material employed, IT STILL REMAINS FOR STUDIES THAT ARE UNDERWAY TO INDICATE WHETHER OR NOT IT WILL BE NECESSARY TO PURIFY THE VIRUS BY CHEMICAL OR PHYSICAL MEANS IN ORDER TO INSURE THAT SUCH IMMUNOLOGICAL REACTIONS WILL NOT OCCUR IF SUCH VACCINES ARE USED ON A LARGE SCALE OR IF MORE THAN ONE INJECTION HAS TO BE GIVEN."

"SUMMARY AND CONCLUSIONS

Preliminary results of studies in human subjects inoculated with different experimental poliomyelitis vaccines are here reported. For preparation of these vaccines virus of each of the three immunologie types WAS PRODUCED IN CULTURES OF MONKEY TESTICULAR TISSUE OR MONKEY KIDNEY TISSUE. Before human subjects were inoculated, THE VIRUS WAS RENDERED NONINFECTIOUS FOR THE MONKEY BY

TREATMENT WITH FORMALDEHYDE."

doi:10.1001/jama.1953.13.1081

In summary:

-Enders tissue-culture of monkey kidney cells paved the way for vaccines as enough "virus" could now be created

-previous vaccination studies were done BEFORE pathogenesis and immunological activity was understood and BEFORE there was a means of PURIFYING A "VIRUS"

-in some trials, humans were fed emulsified rat brain/spine said to contain the Lansing strain which caused paralysis when injected into the brains of monkeys

-Salk's prerequisites for a successful vaccine was a solution REASONABLY FREE of extraneous antigenic material

-vaccines were created from cultures containing either monkey testicles or monkey kidneys mixed with formaldehyde and sometimes mineral oil

-monkey tissue was decided over human tissue due to fear that other pathogens may be present in human tissues (i.e. NOT PURIFIED).

-since Enders used human foreskin, embryonic tissues, kidneys, and uterus, Salk concluded he could use various monkey organs

-vaccines were created with a mixture containing unknown amino acids, nucleic acids, vitamins, minerals, and other growth-promoting/sustaining substances

-strains in the vaccines come from tissue cultures created from monkey CNS, paralyzed mice spine, and feces from a paralyzed human

-2 of the 3 strains were essentially nonpathogenic except for causing paralysis when injected INTRACEREBRALLY

-safety tests for humans consisted of injecting the vaccine into the brains of monkeys and concluding that if the same amount which causes paralysis in the brain does not cause paralysis when injected in the muscles or veins of monkeys, it is considered safe for humans

-the first human test subjects were PARALYZED KIDS from Polio as they decided there are 3 strains and the paralyzed kids would not be immune to all 3 thus they were suitable test subjects

-Polio is not considered a single disease as there are strains that differ immunologically

-excess formaldehyde was "removed" by sodium bisulfite which was toxic and killed the test monkeys until it was sufficiently diluted

-patients with no history of Polio had measurable antibodies against it (meaning these measurements are NOT SPECIFIC to Polio)

-it is admitted that there will be objections to the use of monkey kidney tissue due to unanswered questions of organ damage

-studies were ongoing to see if a "virus" from monkey kidney tissue culture needed purification for vaccination to insure immunological reactions do not occur

At the end of the study was this prescient warning about the future of medicine:

"Laboratory Medicine.—In most branches of medicine the individual physician is still the indispensable man and his functions cannot as yet be mechanized. In the field of laboratory medicine, however, there is an increasing tendency to relegate to a secondary position the role of the individual and TO EXALT THE APPARATUS, THE TEST, AND THE REPORT. THIS ACCENTUATION OF THE INANIMATE IN MEDICINE IS A TREND TO BE DEPLORED AND STRONGLY RESISTED. Like all habits it REDUCES THE RESOURCEFULNESS OF THE INDIVIDUAL, and it is especially pernicious in that it further weakens those who are already irresolute.

There are physicians in all branches of medicine who at times are unable to reach a prompt diagnosis on clinical grounds alone. Before the era of the ascendancy of laboratory medicine such men would often call on a more experienced colleague for consultation. In most cases such tutorial help would elucidate the clinical features present and would leave the physician much richer in knowledge not only for the benefit of the patient at hand but also for the future. WITH THE RISE OF LABORATORY MEDICINE THE ROLE OF THE CLINICAL CONSULTANT HAS BEEN TO A LARGE EXTENT ERASED, AND PHYSICIANS HAVE TENDED MORE AND MORE TO RELY ON THE LABORATORY TO MAKE THE DIAGNOSIS FOR THEMSELVES. Unfortunately, those physicians whose clinical judgment is the poorest are also the ones who are most inclined to accept laboratory reports without question as being final and impeccable. SUCH A BLIND DEPENDENCE ON LABORATORY DIAGNOSIS DOES VERY LITTLE TO DEVELOP CLINICAL JUDGMENT AND SKILL."

—Marcus Rayner Caro, M.D., Diagnostic Pitfalls of Dermal Pathology, A. M. A. Archives of Dermatology and Syphilology, January, 1953.

As a final nail in the coffin to the claims of purity for the Salk Polio vaccine as well as the methods of Carlton Schwerdt (who it was later admitted only achieved purity of 10% of the "virus"), I present this study from 1961 which comes 8 years after Salk's vaccine research and 7 years after Schwerdt's initial "purification:"

DEVELOPMENT OF A PURIFIED POLIOMYELITIS VIRUS VACCINE

"IMPROVEMENTS TO BE DESIRED in commercial Salk poliomyelitis vaccine ARE THE ELIMINATION OF LOT-TO-LOT VARIATIONS IN POTENCY, increase in mean vaccine potency, AND ELIMINATION OF FOREIGN ANTIGENS, PARTICULARLY HOST (MONKEY KIDNEY) ANTIGEN."

"SALK poliomyelitis vaccine, as ordinarily prepared, EXHIBITS MARKED VARIATION IN ANTIGENIC POTENCY from lot to lot. In spite of high over-all efficacy, THE VACCINE DOES NOT PROTECT ALL INDIVIDUALS AGAINST THE DISEASE, EVEN AFTER THE FOURTH DOSE.1' 2"

"The PRINCIPAL DIFFERENCE from the ordinary Salk vaccine lies in the fact that the viral antigen in the vaccine is ESSENTIALLY PURE; the amount of viral antigen put into the vaccine is precisely measured; and THE HIGHLY NEUROVIRULENT TYPE I MAHONEY VIRUS OF THE ORDINARY VACCINE IS REPLACED BY THE RELATIVELY AVIRULENT PARKER STRAINS."

"Virus Purification.—THE VIRUS PURIFICATION PROCEDURE STEMS FROM THE EARLY PIONEERING EXPERIMENTS OF SCHWERDT AND SCHAFFER 10 who purified poliovirus by a combined methanol fractionation and differential centrifugation method. This was followed by the work of Charney et al." who devised an efficient method for poliovirus purification using initial viral concentration by precipitation with nucleic acid and combined physical and chemical separation."

"The POLIOVIRUS CONCENTRATES used to prepare the vaccine usually WERE ESSENTIALLY PURE. "C" COMPONENT WAS COMMONLY PRESENT IN THE TYPE I VIRUS PREPARATIONS. THIS VIRUS-ASSOCIATED ENTITY sedimented more slowly than did the poliovirus and IS THOUGHT to comprise the protein coat of the poliovirus particle devoid of nucleic acid."'13 SINCE THIS ENTITY WAS VIRUS-RELATED AND SINCE ITS CAPACITY TO STIMULATE NEUTRALIZING ANTIBODY WAS

UNCERTAIN, IT WAS COUNTED NEITHER AS IMPURITY NOR AS VIRAL ANTIGEN.

In certain preparations, NONDIALYZABLE, NONVIRAL MATERIAL WAS PRESENT IN TRACE AMOUNT. THIS PRESUMABLY WAS OF HOST CELL ORIGIN AND WAS CONSIDERED UNDESIRABLE INSOFAR AS THE VACCINE WAS CONCERNED. In no case, however, did the concentrates contain serologically detectable monkey kidney complement fixation (CF) antigen, even when tested in high concentration. A SMALL AMOUNT OF NUCLEIC ACID WAS SOMETIMES PRESENT IN VIRAL CONCENTRATES WHICH HAD NOT BEEN SUBJECTED TO A DOUBLE CYCLE OF HIGH SPEED CENTRIFUGATION. THIS WAS INERT MATERIAL ANTIGENICALLY AND WAS NOT COUNTED AS A SIGNIFICANT IMPURITY OF THE FINAL PRODUCT."

"Data relating to the degree of purification achieved in the process of purification FROM THE CRUDE INFECTED TISSUE CULTURE FLUIDS of the individual components of lot 11 trivalent vaccine (see Table 1) are presented in Table 2. The data are summarized in terms of reduction of extraneous protein impurity and in terms of reduction in content of monkey kidney CF antigen. The amount of nonviral protein impurity in the crude infected tissue culture fluids ranged from 98.3% to 99.2% of the total protein content of the fluids. By contrast, ONLY 13.5% OF THE TOTAL PROTEIN OF THE PARKER TYPE I PURIFIED CONCENTRATE WAS NONVIRAL and the type II and III concentrates were free of such extraneous protein."

"The degree of purity achieved may be appreciated in the light of the fact that the measurable properties of the virus concentrates routinely used for purified polio vaccine production DO NOT DIFFER SIGNIFICANTLY FROM THE CLASSICAL PREPARATIONS OF PURIFIED POLIOVIRUS DESCRIBED BY SCHWERDT AND SCHAFFER."

"During the past several years, our laboratories have sought to develop a purified polio vaccine which would exhibit uniform high potency and which would overcome the deficit in protective capacity of the ordinary preparation. This goal has been achieved. In the process, the vaccine has been FREED OF ALL SEROLOGICALLY DETECTABLE MONKEY KIDNEY SUBSTANCE and of ESSENTIALLY ALL OTHER NONVIRAL CONTAMINATING SUBSTANCES PRESENT IN THE ORDINARY CRUDE COMMERCIAL SALK VACCINE."

"The principal difference from the ordinary Salk vaccine lies in the fact that the viral antigen in the vaccine is ESSENTIALLY pure; "

"The data are summarized IN TERMS OF REDUCTION OF EXTRANEOUS PROTEIN IMPURITY and in terms of REDUCTION IN CONTENT OF MONKEY KIDNEY CF ANTIGEN."

"Substitution of the relatively avirulent Parker type I poliovirus for the highly neurovirulent type I Mahoney strain adds a "MARGIN OF SAFETY" to the purified vaccine. REMOVAL OF THE MONKEY KIDNEY ANTIGEN MINIMIZES THE POTENTIAL FOR INDUCTION OF ALLERGIC OR AUTOIMMUNE REACTIONS WHICH MIGHT CONCEIVABLY DEVELOP FOLLOWING REPEATED INJECTIONS OF VACCINES OF MONKEY KIDNEY ORIGIN."

doi:10.1001/jama.1961.03040350001001

From this study, it is clear:

-the Salk vaccine varied from lot-to-lot and was contaminated with monkey kidney antigens

-the Salk vaccine did not protect everyone, EVEN AFTER 4 INJECTIONS

-main difference from the Salk vaccine is that this on replaced the type 1 strain and was "essentially pure"

-the purification procedure used follows closely to that pioneered by Schwerdt

-they claim the poliovirus concentrates were "essentially pure" as there was another entity in the preparation they assumed was related to the "virus" and thus did not count it as an impurity

-nonviral hist cell was present in trace amounts in some preparations

-small amounts of nucleic acids were in the samples but were once again ignored as impurities

-the "purified virus" comes from the same unpurified monkey kidney tissue culture fluids thus making them impure from the start

-they claim the new vaccine is free of all serologically detectable monkey kidney cells (what about those potentially undetectable?) and "essentially" all other non viral contaminants normally found in the Salk vaccine

-the data is summarized as a REDUCTION of extraneous protein impurities and monkey kidney antigens, not elimination of them

-they wanted to remove monkey kidney antigens due to the potential of allergic or autoimmune reactions which could stem from repeated injections of foreign animal DNA

It's safe to say that the Salk vaccine was anything but pure which can clearly be seen by the amount of harm it caused. Even later studies attempting to achieve greater purity than that which could be accomplished by Schwerdt (later revealed to only be 10% purity) failed to eliminate impurities.

There is no evidence of a Polio"virus" taken directly from a sick human patient which was then purified/isolated and proven pathogenic. What we do have is a history of horrors from the grotesque brain drilling experiments to the sickening emulsified brain/spinal goo that was injected directly into them. We have disgusting tissue culture experiments which combined this goo with various infant and later monkey organs. We have a mixture of these disturbing practices put together from the brain/spine of a mouse, the emulsified CNS of a monkey, and the feces of a paralyzed patient combined into a monkey kidney tissue culture subjected to formaldehyde/sodium bisulfite as well as other unknown ingredients which was then injected directly into healthy people.

We have evidence of inhumane and criminal fraud which has taken place for well over a hundred years now.

And it needs to stop.

A few last thoughts on Polio:

Contrary to popular belief, what is known as Polio was never eradicated and still exists today. In India, Aftica, Pakistan, and Afghanistan, it is still said to be running around although the majority of the cases are "vaccine-induced" Polio:

POLIO VACCINATION CAUSES MORE INFECTIONS THAN WILD VIRUSES

"Nigeria, Democratic Republic of Congo, Central African Republic, and Angola have experienced nine new cases of polio caused by the live virus in oral polio vaccines that has mutated into an infectious form, according to statistics released last week (November 20) by the World Health Organization. That brings the global total of these types of infections to 157 for the year, AND IT MEANS THAT MORE CHILDREN ARE PARALYZED AS A RESULT OF SUCH VACCINE-DERIVED INFECTIONS THAN ILLNESSES CAUSED BY THE WILD TYPE VIRUS, which has affected 107 people this year.

OTHER COUNTRIES IN AFRICA AND ASIA HAVE ALSO REPORTED SUCH VACCINE-DERIVED INFECTIONS, which have the potential to spark new outbreaks."

“IT'S ACTUALLY CRAZY BECAUSE WE'RE VACCINATING NOW AGAINST THE VACCINE IN MOST PARTS OF THE WORLD,” Vincent Racaniello, a virologist at Columbia University, tells NPR, “not against wild polio, which is confined to Pakistan and Afghanistan.”

https://www.the-scientist.com/news-opinion/polio-vaccination-causes-more-infections-than-wild-virus-66778

In the US, Polio was said to be completely eradicated by the vaccines but looking at a "new" but not so new condition with the exact same symptoms shows that this is not the case:

ACUTE FLACCID MYELITIS:

"Acute flaccid myelitis (AFM) is a rare but serious condition that affects the spinal cord. It can cause sudden weakness in the arms or legs, loss of muscle tone, and loss of reflexes. The condition mainly affects young children.

Most children have a mild respiratory illness or fever caused by a viral infection about one to four weeks before developing symptoms of acute flaccid myelitis."

https://www.mayoclinic.org/.../symptoms-causes/syc-20493046

Acute Flaccid Myelitis: What Parents Should Know About a New, Polio-Like Illness

"A rare but FRIGHTENING POLIO-LIKE ILLNESS is striking more and more kids in the United States. It has also affected children in Australia and Asia."

https://health.clevelandclinic.org/acute-flaccid-myelitis-what-parents-should-know-about-a-new-polio-like-illness/

"Since 2014, acute flaccid myelitis (AFM), a long-recognized condition ASSOCIATED WITH POLIOVIRUSES, non polio enteroviruses, and various other viral and nonviral causes, has been reemerging globally in epidemic form. This UNANTICIPATED

REEMERGENCE is ironic, given that polioviruses, once the major causes of AFM, are now at the very threshold of global eradication and cannot therefore explain any aspect of AFM reemergence."

"AFM was first recognized around 2010 AS A SEEMINGLY NOVEL CONDITION (3, 4) and quickly grew into an alarming and important disease threat, with the first large outbreak occurring in 2014 (5). Since then, SEASONAL WAVES HAVE OCCURRED EVERY OTHER YEAR IN THE UNITED STATES, the largest occurring in 2018 (Fig. 1) (6–8)). Because of its UNCERTAIN CAUSE AND PATHOGENESIS, ENIGMATIC EPIDEMIOLOGY, and limited treatment options, the disease captured national attention and triggered considerable concern among parents of young children."

https://mbio.asm.org/content/10/2/e00521-19

Of course, they try to make the caveat that this condition (i.e. PARALYSIS) commonly associated with Polio could not possibly have anything to do with Polio today due to it being on the "threshold of global eradication." However, is Polio really on the verge of being eliminated or just reclassified from "wild type" Polio to "vaccine-derived?"

On top of that, how many Polio cases are really being diagnosed today anyways? It has been estimated that 99% of Polio cases are ASYMPTOMATIC, meaning people wouldn't even know they have "it" in order to be tested and diagnosed:

"It's estimated THAT 95 TO 99 PERCENT OF PEOPLE WHO CONTRACT POLIOVIRUS ARE ASYMPTOMATIC. This is known as subclinical polio. Even without symptoms, people infected with poliovirus can still spread the virus and cause infection in others."

https://www.healthline.com/health/poliomyelitis#symptoms

"One of the most startling statistics associated with non-paralytic polio is that up to 95 percent of polio cases HAD NO SYMPTOMS AT ALL! The vast majority of people who contracted the disease didn't even know they had it! That means that PEOPLE LIKE MANY OF US WHO GREW UP DURING THE POLIO EPIDEMIC MAY HAVE HAD POLIO WITHOUT KNOWING IT."

"LESS THAN 1 PERCENT OF ALL POLIO CASES ARE PARALYTIC, of which there are three types – spinal cord polio; polio in the brain stem (bulbar polio); or both, which is called bulbospinal polio."

https://www.argusleader.com/story/news/dell-rapids/2017/08/22/remembering-polio-epidemic-part/104718400/

So if we are to take them at their word that nearly all cases of Polio are asymptomatic, without a mass testing campaign such as we are seeing today with "SARS-COV-2," there would be no way to know how many "real" Polio cases are out there in the world today.

Minus paralysis, which occurs in LESS THAN ONE PERCENT of the cases, Polio symptoms line up exactly with the flu. How many cases which are diagnosed as the flu are actually Polio cases:

"Signs and symptoms, which can last up to 10 days, include:

Fever

Sore throat

Headache

Vomiting

Fatigue

Back pain or stiffness

Neck pain or stiffness

Pain or stiffness in the arms or legs

Muscle weakness or tenderness"

https://www.mayoclinic.org/.../symptoms-causes/syc-20376512

However, even paralysis can be associated with the flu:

"Guillain-Barré syndrome (GBS) is an autoimmune disorder—which can be a complication of a serious case of the cold OR FLU—in which the body's immune system attacks and destroys its nerves. AS A RESULT, MUSCLE WEAKNESS AND EVEN PARALYSIS CAN OCCUR."

https://www.mdlinx.com/article/5-surprising-serious-cold-and-flu-complications/2RIynpqIR8Jo4EZEIIIRmh

Neither the flu nor Polio are regularly screened for. The flu is normally diagnosed by

symptoms rather than testing, especially during the "flu season:"

"Your doctor will conduct a physical exam, look for signs and symptoms of influenza, and POSSIBLY ORDER A TEST that detects influenza viruses.

During times when influenza is widespread, YOU MAY NOT NEED TO BE TESTED for influenza. Your doctor may DIAGNOSE YOU BASED ON YOUR SIGNS AND SYMPTOMS."

https://www.mayoclinic.org/.../diagnosis.../drc-20351725

"There are several reasons for this including that ILINet does not include every health care provider and MONITORS FLU-LIKE ILLNESS, NOT LABORATORY-CONFIRMED INFLUENZA CASES."

"CDC USES MATHEMATICAL MODELING in combination with data from traditional flu surveillance systems TO ESTIMATE THE NUMBERS OF FLU ILLNESSES in the United States."

https://www.cdc.gov/flu/about/burden/faq.htm

If Polio was running around in the US, with the majority of asymptomatic cases, the familiar flu-like symptoms confounding diagnosis, and the lack of testing, would we even know?

If Polio is tested for, it is the usual INDIRECT methods:

Here is how the CDC says it is diagnosed:

"Poliovirus can be detected in specimens from the throat, feces (stool), and occasionally cerebrospinal fluid (CSF) by ISOLATING THE VIRUS IN CELL CULTURE or by detecting the virus by polymerase chain reaction (PCR).

CDC laboratories conduct testing for poliovirus, including:

Culture

Intratypic differentiation

Genome sequencing

Serology

Virus ISOLATION IN CULTURE is the most sensitive method to diagnose poliovirus infection. Poliovirus is most likely to be isolated from stool specimens. It may also be isolated from pharyngeal swabs. Isolation is less likely from blood or CSF."

"Real-time reverse transcription PCR is used to differentiate possible wild strains from vaccine-like strains (“intratypic differentiation”), USING VIRUS ISOLATED IN CULTURE as the starting material."

"PARTIAL GENOME SEQUENCING IS USED to confirm the poliovirus genotype and determine its likely geographic origin."

"DETECTION OF POLIOVIRUS IN CSF IS UNCOMMON. CSF usually contains an increased number of leukocytes [from 10 to 200 cells/mm3 (primarily lymphocytes)] and a mildly elevated protein (from 40 to 50 mg/dL). THESE FINDINGS ARE NONSPECIFIC AND MAY RESULT FROM A VARIETY OF INFECTIOUS AND NONINFECTIOUS CONDITIONS."

https://www.cdc.gov/.../what.../lab-testing/diagnostic.html

As can be seen, it is the usual cell culture tricks where they claim "isolation" by adding numerous chemicals/foreign DNA together with a sample (faces or snot) or they do a PARTIAL genome sequencing of a "virus" which, in over 100 years, has never been properly purified/isolated in order to get an accurate genome nor proven pathogenic.

Finally, Polio cases were already well on a decline before the toxic vaccines were introduced and there are alternative explanations as to the cause of Polio other than an unproven "virus," such as DDT. I won't go into too much detail but it is clear to see the overlap between the two:

"The National Foundation for Infantile Paralysis (NFIP)—the nation’s large and powerful polio-fighting philanthropy—was “RUSHING THREE SPECIALLY EQUIPPED TRUCKS AND THREE PUMPS TO THE ROCKFORD AREA” TO COAT THE CITY WITH DDT, the new war-developed pesticide. It would be “the first time,” said Gunderson, “that the powerful insecticide WOULD BE USED TO COMBAT POLIOMYELITIS.”2

Rockford’s anti-polio DDT deployment, carried out by the US Army and a team of Yale polio researchers with support from the NFIP, WAS PART OF A ROUGHLY HALF DECADE OF EXPERIMENTATION WITH THE USE OF DDT TO FIGHT POLIO."

"DDT became available to the American public as a disease preventive at the tail end of

an era dominated by germ theory, at the advent of the golden era of US biomedicine, and at the beginning of a new era of ecological understanding of health. All at once, DDT was considered a modern tool, dubbed a wonder drug and a miracle akin to the atom bomb, and also LABELED A DISINFECTANT AND EMPLOYED IN AN APPROACH TO DISEASE, namely sanitation, that belonged more to the late nineteenth century than the middle of the twentieth. ITS USE TO FIGHT POLIO thus illustrates the tensions among competing ideas about disease explanation and prevention in the years immediately following World War II.

ALTHOUGH POLIO'S AND DDT's HISTORIES OVERLAP AND INTERSECT, in the historiographies on each, the other warrants only passing mentions at best."

"In David Oshinky's work on polio, DDT makes two brief appearances: first, as one of many tools employed in desperation against a 1949 outbreak in San Angelo, Texas, and again a few chapters (and three years) later, AS A SUSPECT IN POLIO'S SPREAD, GIVEN ITS ENVIRONMENTAL UBIQUITY.7 This contrast—between DDT AS A POSSIBLE SOLUTION TO AND POSSIBLE CAUSE OF POLIO—paired with polio's reputation as the last disease of a bygone era, captures how the two's shared history points to an important moment of transition in both public health and etiologies of disease."

https://academic.oup.com/envhis/article/22/4/696/4057684

WHEN TEXAS SPRAYED DDT ON CITIZENS TO PREVENT POLIO

"YES, THIS HAPPENED in San Antonio, Texas. The director of the city's Department of Health, H.L. Crittenden, ordered the spraying of DDT ALONG EVERY ONE OF OVER A THOUSAND STREETS in May, 1946 in an ill-fated attempt to wipe out polio.

A HANDFUL OF OTHER CITIES JOINED IN, like Rockford, Illinois, and Paterson, New Jersey. Such an event came from the misguided notion that polio was spread by mosquitoes or other insects."

https://allthatsinteresting.com/when-texas-sprayed-ddt

Where is DDT still in use today?

"DDT IS STILL USED TODAY in SOUTH AMERICA, AFRICA, AND ASIA for this purpose. Farmers used DDT on a variety of food crops in the United States and

worldwide. DDT was also used in buildings for pest control."

http://npic.orst.edu/factsheets/ddtgen.pdf

"Neurotoxic effects of DDT include tremors, convulsion, PARALYSIS, decreased central nervous system lipid, phospholipid and cholesterol content. DDT exposure early in life causes decreased growth, MORTALITY, decreased levels of brain muscarinic acetylcholine receptors, increased spontaneous motor activity in adulthood and decreased learning."

https://www.ewg.org/.../humanto.../chemicals/chemical.php...

In summary:

-the symptoms of disease known as Polio still exist today in "wild" and "vaccine-induced" varieties

-Acute Flaccid Myelitis, which is the exact same symptoms of disease as Polio, exists in the US

-99% of Polio cases are ASYMPTOMATIC

-Polio has the same symptoms as the flu

-Polio is tested for in indirect ways such as cell culture and partial genome sequencing

-Polio cases were declining well before the introduction of the vaccines

-DDT is a possible alternative explanation for cases of paralysis other than an unproven "virus"

https://docs.google.com/document/d/e/2PACX-1vQyfgROsQRAug4hL_5FfjIx6HzgGcGUZa2_HFF8atnYr0jmEfFBKoBPbVgVHN8jk4kVDyq1h4-d4OxH/pub

CHICKENPOX AND SMALLPOX

All Chickenpox studies - includes the following

https://docs.google.com/document/d/e/2PACX-1vQ4-hxzXo4tbiOwxEPxz_G-U3x1JZLlplkNSexAB9S8R5Db79bBdT6vuLJiOJmI00I5O2_08e81stzj/pub

HEBERDEN 1767 CHICKENPOX PAPER:

HEBERDEN 1767 CHICKENPOX PAPER: While the symptoms of disease now known as Chickenpox had been known for centuries, it wasn't until William Heberden provided a description of them in 1767 that these symptoms started to be seen as a distinct disease. Up to that time, and even for some time after Heberden's paper, people believed that Chickenpox was nothing more than a mild form of Smallpox. A few excerpts provide some interesting background on this subject: "While herpes zoster infection has been recognized since antiquity, CHICKENPOX (varicella) WAS CONFUSED WITH SMALLPOX UNTIL THE 1800s, when both illnesses became better understood."

"The name “chickenpox” was used by Richard Morton (1637-1698) WHO CHARACTERIZED IT AS A MILD FORM OF SMALLPOX. The exact origin of the name “chickenpox” remains unclear. Samuel Johnson suggested that SINCE THE DISEASE WAS “OF NO GREAT DANGER,” IT WAS A “CHICKEN” FORM OF SMALLPOX. Other reasons given are that the scars remaining after chickenpox looked as though chickens had pecked at the skin." "WILLIAM HEBERDEN IS CREDITED WITH DISTINGUISHING THE DIFFERENCES BETWEEN SMALLPOX AND CHICKENPOX. In his address to the College of Physicians in London in 1768, Heberden described the clinical distinction between the two disorders [4], underscoring the importance of recognizing that a history of chickenpox did not confer immunity to smallpox. While today it is understood that chickenpox, as compared to smallpox, has no or mild prodrome, and lesions at different stages of development and generally a more centripetal distribution, HEBERDEN'S INSIGHTS REGARDING THE DISTINCTION BETWEEN SMALLPOX AND CHICKENPOX WERE NOT ACCEPTED IMMEDIATELY."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4628852/

"Interest in the eruptive fevers during this period also produced, in 1767, THE FIRST CLINICAL DESCRIPTION OF CHICKEN-POX AS A SEPARATE ENTITY DISTINCT FROM SMALLPOX. Written by the elder Heberden 88 it is a lucid and definitive account. Its appearance was prompted, HEBERDEN POINTS OUT, BY THE DANGER OF THIS RELATIVELY MILD COMPLAINT BEING CONFUSED WITH SMALLPOX, AND THUS LULLING ITS VICTIMS INTO A FALSE SENSE OF SECURITY IN THE BELIEF THAT THEY WOULD HENCEFORTH BE IMMUNE TO SMALLPOX.89 In fact, there can be

little doubt that varicella had been known for centuries, AND IT MAY HAVE BEEN DESCRIBED AS A MILD FORM OF SMALLPOX not only by Rhazes and Avicenna, but also by Indian writers."

https://core.ac.uk/download/pdf/192051336.pdf

"English physician William Heberden was the first to give a DETAILED DESCRIPTION THAT DISTINGUISHED CHICKENPOX FROM SMALLPOX. He wrote: 'These pocks break out on many without any illness or previous sign: in others they are preceded by a little degree of chillness, lassitude, cough, broken sleep, wandering pains, loss of appetite and feverishness for three days… MOST OF THEM ARE OF THE COMMON SIZE OF THE SMALLPOX but some are less. I never saw them confluent nor very numerous. The greatest number which I ever observed was about twelve in the face, and two hundred over the rest of the body.' Heberden also noted that those who had previously had chickenpox "were not capable of having it again."

https://www.historyofvaccines.org/content/chickenpox-disease-distinguished-smallpox

"YOU SAY VARICELLA, I SAY VARIOLA DESPITE THE FACT THAT HEBERDEN HAD SHOWN THAT THE VARICELLA AND VARIOLA VIRUSES WERE UNRELATED, PEOPLE CONTINUED TO BELIEVE OTHERWISE. This led not only to fear of chickenpox, but also prevented some from being inoculated for smallpox believing that they had immunity conferred from chickenpox. It was our very own William Osler (1849-1919) who, DECADES AFTER HEBERDEN'S PROOF, helped change this impression once and for all stating that "There can be no question that varicella is an affection quite distinct from and without at present any relation whatsoever to it."

http://www.doctorsreview.com/history/thinking-outside-pox/

It seems very clear from these few sources that Chickenpox was very much considered a mild form of Smallpox. They were seen as the same disease. It wasn't until Heberden provided his "proof" that they became separate illnesses caused by separate "viruses." Keep in mind that at this point in time, "viruses" could not be seen and both their presence as well as the role of infectiousness were based on assumptions. So did Heberden really prove Chickenpox and Smallpox are two separate diseases?

Unfortunately, I could only find the attached images from his 1767 paper "ON THE CHICKEN-POX" so I can not post highlights from it but I will provide a brief summary and you can judge for yourselves whether or not he succeeded: In Summary: -most of the pustules are the same size as those seen in Smallpox -at their maturity, there is a yellowish liquor that very much resembles Smallpox -they eventually erupt, normally by accidental rubbing and/or itching, and scab over just as is seen in Smallpox -the patients rarely suffered anything throughout the illness other than loss of strength, spirit, and appetite which he attributed to being locked in their rooms -Heberden states the infection lays in the body much as it does as in the case of Smallpox -the ways he

"distinguished"

Chickenpox from Smallpox are:

1. The appearance on the second or third day from the eruption of the vesicle full of serum on the top of the pock

2. The crust, which covers the pocks on the fifth day, at which time those of Smallpox are not at the height of their suppuration 3. Just kidding, there was no #

3...that was it...1 and 2 were apparently enough to distinguish "Chickenpox" from "Smallpox" in order to claim definitive proof they are two different illnesses caused by two separate "viruses" -unlike Heberden, others who spoke of Chickenpox considered it a mild form of Smallpox -he states Chickenpox is "surely totally different" from Smallpox based on his above two descriptive differences as well as those who have had Smallpox may get Chickenpox while those who have had Chickenpox never get it again -he states he proved this difference by taking Chickenpox pustule and putting it in the incision of a boy who had previously had Chickenpox and the incision healed up without further problems -he states that even though Chickenpox and Smallpox are very similar, he believes Chickenpox is mislabeled as Smallpox due to untrained observers -Heberden ends by assuming that cases of reinfection with Chickenpox in England were actually Smallpox in disguise but could have been distinguished by the eruptions of the pocks

https://archive.org/details/b24976957_0001/page/426/mode/2up

Did Heberden meet the burden of proof required to claim Chickenpox was a distinct disease separate from Smallpox? Did this mild form of Smallpox really require vaccination to "defeat" it? Did Heberden have an annoying lisp or just a horrible editor? You be the judge.

https://docs.google.com/document/d/e/2PACX-1vQwkzIaN5BfCg_W0O6WZle2vZA9NqhQyOEN6bxkkEWIbANoXsF83VGux2vcc6Uv_IIDCTT6_budncjf/pub

EDWARD JENNER 1798 SMALLPOX PAPER:

EDWARD JENNER 1798 SMALLPOX PAPER:

Edward Jenner is considered the "Father of Immunology" for his work with both Cowpox and Smallpox. While inoculations were known and used before him, Jenner made the case that he could create immunity to Smallpox by inoculating people with Cowpox due to the similarities present in both diseases. These inoculations entailed taking the pus

from the infected scabs of people suffering Cowpox, cutting open the skin of a healthy subject with a lancet, and spreading this impure material into the wound. If they were to develop disease and recover, they would then be considered immune to Smallpox. This would be tested by the same method of inoculation but made up of the pus from those considered to be Smallpox cases.

Jenner presented a mini booklet outlining numerous cases detailing this process. I highlighted a few below. A little background before some excerpts from his report:

"The 1798 publication had three parts. In the first part Jenner presented his view regarding the origin of cowpox as a disease of horses transmitted to cows. THE THEORY WAS DISCREDITED DURING JENNER'S LIFETIME. He then presented the hypothesis that infection with cowpox protects against subsequent infection with smallpox. The second part contained the critical observations relevant to testing the hypothesis. The third part was a lengthy discussion, in part polemical, of the findings and a variety of issues related to smallpox. THE PUBLICATION OF THE INQUIRY WAS MET WITH A MIXED REACTION IN THE MEDICAL COMMUNITY."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1200696/

As can be seen, part of Jenner's theory about Cowpox coming from horses was discredited during his lifetime. There are many other reasons to discredit his observations when looking at his report. This is a lengthy one so bear with me. There is much I had to cut out due to space constraints so I highly recommend reading the whole report. Highlights below:

VACCINATION AGAINST SMALLPOX

AN INQUIRY INTO THE CAUSES AND EFFECTS OF THE VARIOLE VACCINE, OR COW-POX. 1798

"There is a disease to which the horse, from his state of domestication, is frequently subject. The farriers have called it the grease. IT IS AN INFLAMMATION AND SWELLING IN THE HEEL, FROM WHICH ISSUES MATTER POSSESSING PROPERTIES OF A VERY PECULIAR KIND, WHICH SEEMS CAPABLE OF GENERATING A DISEASE IN THE HUMAN BODY (AFTER IT HAS UNDERGONE THE MODIFICATION WHICH I SHALL PRESENTLY SPEAK OF), WHICH BEARS SO STRONG A RESEMBLANCE TO THE SMALLPOX THAT I THINK IT HIGHLY

PROBABLE IT MAY BE THE SOURCE OF THE DISEASE."

"In this dairy country a great number of cows are kept, and the office of milking is performed indiscriminately by men and maid servants. ONE OF THE FORMER HAVING BEEN APPOINTED TO APPLY DRESSINGS TO THE HEELS OF A HORSE AFFECTED WITH THE GREASE, AND NOT PAYING DUE ATTENTION TO CLEANLINESS, INCAUTIOUSLY BEARS HIS PART IN MILKING THE COWS, WITH SOME PARTICLES OF THE INFECTIOUS MATTER ADHERING TO HIS FINGERS. When this is the case, it commonly happens that a disease is communicated to the cows, and from the cows to the dairymaids, which spreads through the farm until the most of the cattle and domestics feel its unpleasant consequences. This disease has obtained the name of the cow-pox. It appears on the nipples of the cows in the form of irregular pustules. AT THEIR FIRST APPEARANCE THEY ARE COMMONLY OF A PALISH BLUE, or rather of a colour somewhat approaching to livid, AND ARE SURROUNDED BY AN ERYSIPELATOUS INFLAMMATION. These pustules, unless a timely remedy be applied, frequently degenerate into phagedenic ulcers, which prove extremely troublesome. [Footnote: They who attend sick cattle in this country find a speedy remedy for stopping the progress of this complaint in those applications which act chemically upon the morbid matter, such as the solutions of the vitriolum zinci and the vitriolum cupri, etc.] The animals become indisposed, and the secretion of milk is much lessened. INFLAMED SPOTS NOW BEGIN TO APPEAR ON DIFFERENT PARTS OF THE HANDS OF THE DOMESTICS EMPLOYED IN MILKING, and sometimes on the wrists, which quickly run on to suppuration, FIRST ASSUMING THE APPEARANCE OF THE SMALL VESICATIONS PRODUCED BY A BURN."

"THUS THE DISEASE MAKES ITS PROGRESS FROM THE HORSE [Footnote: Jenner's conclusion that "grease" and cow-pox were the same disease has since been proved erroneous; but this error has not invalidated his main conclusion as to the relation of cow-pox and smallpox.--EDITOR.] TO THE NIPPLE OF THE COW, AND FROM THE COW TO THE HUMAN SUBJECT."

"MORBID MATTER OF VARIOUS KINDS, WHEN ABSORBED INTO THE SYSTEM, MAY PRODUCE EFFECTS IN SOME DEGREE SIMILAR; but what renders the cow-pox virus so extremely singular is that the person who has been thus affected is forever after secure from the infection of the small-pox; neither exposure to the variolous effluvia, nor the insertion of the matter into the skin, producing this distemper."

"[Footnote: IT IS A REMARKABLE FACT, AND WELL KNOWN TO MANY, THAT WE ARE FREQUENTLY FOILED IN OUR ENDEAVOURS TO COMMUNICATE THE SMALLPOX BY INOCULATION TO BLACKSMITHS, who in the country are farriers.

THEY OFTEN, AS IN THE ABOVE INSTANCE, EITHER RESIST THE CONTAGION ENTIRELY, OR HAVE THE DISEASE ANOMALOUSLY. Shall we not be able to account for this on a rational principle?]"

"CASE VII.--Although the preceding history pretty clearly evinced that the constitution is far less susceptible of the contagion of the cow-pox after it has felt that of the smallpox, and although in general, as I have observed, they who have had the smallpox, and are employed in milking cows which are infected with the cow- pox, either escape the disorder, OR HAVE SORES ON THE HANDS WITHOUT FEELING ANY GENERAL INDISPOSITION, yet the animal economy is subject to some variation in this respect, which the following relation will point out:"

"CASE IX.--Although the cow-pox shields the constitution from the smallpox, and the smallpox proves a protection against its own future poison, YET IT APPEARS THAT THE HUMAN BODY IS AGAIN AND AGAIN SUSCEPTIBLE OF THE INFECTIOUS MATTER OF THE COW-POX, as the following history will demonstrate."

"Mr. Abraham Riddiford, a farmer at Stone in this parish, IN CONSEQUENCE OF DRESSING A MARE THAT HAD SORE HEELS, WAS AFFECTED WITH VERY PAINFUL SORES IN BOTH HIS HANDS, tumours in each axilla, and severe and general indisposition. A surgeon in the neighbourhood attended him, who KNOWING THE SIMILARITY BETWEEN THE APPEARANCE OF THE SORES UPON HIS HANDS AND THOSE PRODUCED BY THE COW-POX, and being acquainted also with the effects of that disease on the human constitution, assured him that he never need to fear the infection of the smallpox; BUT THIS ASSERTION PROVED FALLACIOUS, for, ON BEING EXPOSED TO THE INFECTION UPWARDS OF TWENTY YEARS AFTERWARDS, HE CAUGHT THE DISEASE, which took its regular course in a very mild way. THERE CERTAINLY WAS A DIFFERENCE PERCEPTIBLE, although it is not easy to describe it, IN THE GENERAL APPEARANCE OF THE PUSTULES FROM THAT WHICH WE COMMONLY SEE. Other practitioners who visited the patient at my request agreed with me at this point, though there was no room left for suspicion as to the reality of the disease, as I inoculated some of his family from the pustules, who had the smallpox, with its usual appearances, in consequence."

"CASE XVII.--The more accurately to observe the progress of the infection I selected a healthy boy, about eight years old, for the purpose of inoculation for the cow-pox. THE MATTER WAS TAKEN FROM A SORE ON THE HAND OF A DAIRYMAID [Footnote: From the sore on the hand of Sarah Nelmes. See the preceding case.], who was

infected by her master's cows, AND IT WAS INSERTED, on the 14th of May, 1796, INTO THE ARM OF THE BOY BY MEANS OF TWO SUPERFICIAL INCISIONS, barely penetrating the cutis, each about half an inch long.

On the seventh day he complained of uneasiness in the axilla, and on the ninth he became a little chilly, lost his appetite, and had a slight headache. During the whole of this day he was perceptibly indisposed, and spent the night with some degree of restlessness, but on the day following he was perfectly well.

THE APPEARANCE OF THE INCISIONS IN THEIR PROGRESS TO A STATE OF MATURATION WERE MUCH THE SAME AS WHEN PRODUCED IN A SIMILAR MANNER BY VARIOLOUS MATTER. The only difference which I perceived was in the state of the limpid fluid arising from the action of the virus, which assumed rather a darker hue, and in that of the efflorescence spreading round the incisions, which had more of an erysipelatous look than we commonly perceive when variolous matter has been made use of in the same manner; but the whole died away (leaving on the inoculated parts scabs and subsequent eschars) without giving me or my patient the least trouble.

In order to ascertain whether the boy, after feeling so slight an affection of the system from the cow--pox virus, was secure from the contagion of the smallpox, HE WAS INOCULATED THE 1st OF JULY FOLLOWING WITH VARIOLOUS MATTER, IMMEDIATELY TAKEN FROM A PUSTULE. Several slight punctures and incisions were made on both his arms, and the matter was carefully inserted, but no disease followed. The same appearances were observable on the arms as we commonly see when a patient has had variolus matter applied, after having either the cow--pox or smallpox. Several months afterwards he was again inoculated with variolus matter, but no sensible effect was produced on the constitution."

"A MARE, the property of a person who keeps a dairy in a neighbouring parish, BEGAN TO HAVE SORE HEELS the latter end of the month of February, 1798, which were occasionally WASHED BY THE SERVANT MEN OF THE FARM, Thomas Virgoe, William Wherret, and William Haynes, WHO IN CONSEQUENCE BECAME AFFECTED WITH SORES IN THEIR HANDS, followed by inflamed lymphatic glands in the arms and axillae, shiverings succeeded by heat, lassitude, and general pains in the limbs. A single paroxysm terminated the disease; for within twenty--four hours they were free from general indisposition, nothing remaining but the sores on their hands. Haynes and Virgoe, WHO HAD GONE THROUGH THE SMALLPOX FROM INOCULATION,

DESCRIBED THEIR FEELINGS AS VERY SIMILAR TO THOSE WHICH AFFECTED THEM ON SICKENING WITH THAT MALADY. Wherrett never had had the smallpox. Haynes was daily employed as one of the milkers at the farm, and the disease began to shew itself among the cows about ten days after he first assisted in washing the mare's heels. Their nipples became sore in the usual way, with bluish pustules; but as remedies were early applied, they did not ulcerate to any extent."

"CASE XVIII.--John Baker, a child of five years old, was inoculated March 16, 1798, WITH MATTER TAKEN FROM A PUSTULE ON THE HAND OF THOMAS VIRGOE, one of the servants who had been infected from the mare's heels. He became ill on the sixth day with symptoms similar to those excited by cow--pox matter. On the eighth day he was free from indisposition. THERE WAS SOME VARIATION IN THE APPEARANCE OF THE PUSTULE ON THE ARM. ALTHOUGH IT SOMEWHAT RESEMBLED A SMALLPOX PUSTULE, yet its similitude was not so conspicuous as when excited by matter from the nipple of the cow, or when the matter has passed from thence through the medium of the human subject.

This experiment was made to ascertain the progress and subsequent effects of the disease when thus propagated. We have seen that the virus from the horse, when it proves infectious to the human subject, is not to be relied upon as rendering the system secure from variolous infection, but that the matter produced by it upon the nipple of the cow is perfectly so. WHETHER ITS PASSING FROM THE HORSE THROUGH THE HUMAN CONSTITUTION, AS IN THE PRESENT INSTANCE, WILL PRODUCE A SIMILAR EFFECT, REMAINS TO BE DECIDED. This would now have been effected, but the boy was rendered unfit for inoculation from having felt the effects of a contagious fever in a workhouse soon after this experiment was made."

"CASE XX.-From William Summers the disease was transferred to William Pead, a boy of eight years old, who was inoculated March 28th. On the sixth day he complained of pain in the axilla, and on the seventh WAS AFFECTED WITH THE COMMON SYMPTOMS OF A PATIENT SICKENING WITH THE SMALLPOX FROM INOCULATION, which did not terminate till the third day after the seizure. SO PERFECT WAS THE SIMILARITY TO THE VARIOLOUS FEVER THAT I WAS INDUCED TO EXAMINE THE SKIN, conceiving there might have been some eruptions, but none appeared. THE EFFLORESCENT BLUSH AROUND THE PART PUNCTURED IN THE BOY'S ARMS WAS SO TRULY CHARACTERISTIC OF THAT WHICH APPEARS ON VARIOLOUS INOCULATION that I have given a representation of it. The drawing was made when the pustule was beginning to die away and the areola retiring from the centre."

"CASE XXI.-April 5th: Several children and adults were inoculated from the arm of William Pead. The greater part of them sickened on the sixth day, and were well on the seventh, BUT IN THREE OF THE NUMBER A SECONDARY INDISPOSITION AROSE IN CONSEQUENCE OF AN EXTENSIVE ERYSIPELATOUS INFLAMMATION WHICH APPEARED ON THE INOCULATED ARMS. It seemed to arise from the state of the pustule, which spread out, accompanied with some degree of pain, to about half the diameter of a sixpence. One of these patients was an infant of half a year old. By the application of mercurial ointment to the inflamed parts (a treatment recommended under similar circumstances in the inoculated smallpox) the complaint subsided without giving much trouble.

Hannah Excell, an healthy girl of seven years old, and one of the patients above mentioned, RECEIVED THE INFECTION FROM THE INSERTION OF THE VIRUS UNDER THE CUTICLE OF THE ARM IN THREE DISTINCT POINTS. The pustules which arose in consequence so much RESEMBLED, on the twelfth day, THOSE APPEARING FROM THE INFECTION OF VARIOLOUS MATTER, THAT AN EXPERIENCED INOCULATOR WOULD SCARCELY HAVE DISCOVERED A SHADE OF DIFFERENCE AT THAT PERIOD. Experience now tells me that almost the only variation which follows consists in the pustulous fluids remaining limpid nearly to the time of its total disappearance; and not, as in the direct smallpox, becoming purulent."

"THESE EXPERIMENTS AFFORDED ME MUCH SATISFACTION; THEY PROVED THAT THE MATTER, IN PASSING FROM ONE HUMAN SUBJECT TO ANOTHER, THROUGH FIVE GRADATIONS, LOST NONE OF ITS ORIGINAL PROPERTIES, J. Barge being the fifth who received the infection successively from William Summers, the boy to whom it was communicated from the cow."

"With respect to the opinion adduced "THAT THE SOURCE OF THE INFECTION IS A PECULIAR MORBID MATTER ARISING IN THE HORSE," ALTHOUGH I HAVE NOT BEEN ABLE TO PROVE IT FROM ACTUAL EXPERIMENTS CONDUCTED IMMEDIATELY UNDER MY OWN EYES, yet the evidence I have adduced appears sufficient to establish it.

They who are not in the habit of conducting experiments may not be aware of the coincidence of circumstances necessary for their being managed so as to prove perfectly decisive; nor how often men engaged in professional pursuits are liable to interruptions which disappoint them almost at the instant of their being accomplished:

however, I feel no room for hesitation respecting the common origin of the disease, BEING WELL CONVINCED THAT IT NEVER APPEARS AMONG THE COWS (except it can be traced to a cow introduced among the general herd which has been previously infected, or to an infected servant) UNLESS THEY HAVE BEEN MILKED BY SOME ONE WHO, AT THE SAME TIME, HAS THE CARE OF A HORSE AFFECTED WITH DISEASED HEELS.

The spring of the year 1797, which I intended particularly to have devoted to the completion of this investigation, proved, from its dryness, remarkably adverse to my wishes;-for it frequently happens, while the farmers' horses are exposed to the cold rains which fall at that season, THAT THEIR HEELS BECOME DISEASED, AND NO COW-POX THEN APPEARED IN THE NEIGHBORHOOD."

"The active quality of the virus from the horses' heels is greatly increased after it has acted on the nipples of the cow, as it rarely happens that the horse affects his dresser with sores, and as rarely that a milkmaid escapes the infection when she milks infected cows. It is most active at the commencement of the disease, even before it has acquired a pus-like appearance; indeed, I am not confident whether this property in the matter does not entirely cease as soon as it is secreted in the form of pus. I am induced to think it does cease [Footnote: It is very easy to procure pus from old sores on the heels of horses. This I have often inserted into scratches made with a lancet, on the sound nipples of cows, and have seen no other effects from it than simple inflammation.], and that it is the thin, darkish- looking fluid only, oozing from the newly-formed cracks in the heels, similar to what sometimes appears from erysipelatous blisters, which gives the disease. Nor am I certain that the nipples of the cows are at all times in a state to receive the infection. The appearance of the disease in the spring and the early part of the summer, when they are disposed to be affected with spontaneous eruptions so much more frequently than at other seasons, INDUCES ME TO THINK THAT THE VIRUS FROM THE HORSE MUST BE RECEIVED UPON THEM WHEN THEY ARE IN THIS STATE, IN ORDER TO PRODUCE EFFECTS: EXPERIMENTS, HOWEVER, MUST DETERMINE THESE POINTS. But it is clear that when the cow-pox virus is once generated, that the cows cannot resist the contagion, in whatever state their nipples may chance to be, IF THEY ARE MILKED WITH AN INFECTED HAND.

WHETHER THE MATTER, EITHER FROM THE COW OR THE HORSE, WILL AFFECT THE SOUND SKIN OF THE HUMAN BODY, I CANNOT POSITIVELY DETERMINE; PROBABLY IT WILL NOT, unless on those parts where the cuticle is extremely thin, as on the lips, for example. I have known an instance of a poor girl who produced an ulceration on her lip by frequently holding her finger to her mouth to cool the raging of a cow-pox sore by blowing upon it. THE HANDS OF THE FARMERS' SERVANTS HERE,

FROM THE NATURE OF THEIR EMPLOYMENTS, ARE CONSTANTLY EXPOSED TO THOSE INJURIES WHICH OCCASION ABRASIONS OF THE CUTICLE, TO PUNCTURES FROM THORNS, AND SUCH LIKE ACCIDENTS; SO THAT THEY ARE ALWAYS IN A STATE TO FEEL THE CONSEQUENCE OF EXPOSURE TO INFECTIOUS MATTER."

"Elizabeth Wynne, WHO HAD THE COW-POX IN THE YEAR 1759, WAS INOCULATED WITH VARIOLOUS MATTER, WITHOUT EFFECT, IN THE YEAR 1797, AND AGAIN CAUGHT THE COW-POX IN THE YEAR 1798. When I saw her, which was on the eighth day after she received the infection, I found her affected with general lassitude, shiverings, alternating with heat, coldness of the extremities, and a quick and irregular pulse. These symptoms were preceded by a pain in the axilla. On her hand was one large pustulous sore, which resembled that delineated in Plate No. I. (Plate appears in original.)"

"THERE ARE CERTAINLY MORE FORMS THAN ONE, WITHOUT CONSIDERING THE COMMON VARIATION BETWEEN THE CONFLUENT AND DISTINCT, IN WHICH THE SMALLPOX APPEARS IN WHAT IS CALLED THE NATURAL WAY. About seven years ago a species of smallpox spread through many of the towns and villages of this part of Gloucestershire: IT WAS OF SO MILD A NATURE THAT A FATAL INSTANCE WAS SCARCELY EVER HEARD OF, and consequently SO LITTLE DREADED by the lower orders of the community that they scrupled not to hold the same intercourse with each other AS IF NO INFECTIOUS DISEASE HAD BEEN PRESENT AMONG THEM. I never saw nor heard of an instance of its being confluent. The most accurate manner, perhaps, in which I can convey an idea of it is by saying that had fifty individuals been taken promiscuously and infected by exposure to this contagion, THEY WOULD HAVE HAD AS MILD AND LIGHT A DISEASE AS IF THEY HAD BEEN INOCULATED WITH VARIOLOUS MATTER IN THE USUAL WAY. The harmless manner in which it shewed itself could not arise from any peculiarity either in the season or the weather, for I watched its progress upwards of a year without perceiving any variation in its general appearance. I CONSIDER IT THEN AS A VARIETY OF THE SMALLPOX."

"A medical gentleman (now no more), who for many years inoculated in this neighbourhood, FREQUENTLY PRESERVED THE VARIOLOUS MATTER INTENDED FOR HIS USE ON A PIECE OF LINT OR COTTON, WHICH, IN ITS FLUID STATE, WAS PUT INTO A VIAL, CORKED, AND CONVERTED INTO A WARM POCKET; A SITUATION CERTAINLY FAVOURABLE FOR SPEEDILY PRODUCING PUTREFACTION IN IT. IN THIS STATE (not unfrequently after it had been taken several days from the pustules) IT WAS INSERTED INTO THE ARMS OF HIS PATIENTS, and brought on inflammation of the incised parts, swellings of the axillary glands, fever, and

sometimes eruptions. BUT WHAT WAS THIS DISEASE? CERTAINLY NOT THE SMALLPOX; for the matter having from putrefaction lost or suffered a derangement in its specific properties, was no longer capable of producing that malady, THOSE WHO HAD BEEN INOCULATED IN THIS MANNER BEING AS MUCH SUBJECT TO THE CONTAGION OF THE SMALLPOX AS IF THEY HAD NEVER BEEN UNDER THE INFLUENCE OF THIS ARTIFICIAL DISEASE; AND MANY, UNFORTUNATELY, FELL VICTIMS TO IT, WHO THOUGHT THEMSELVES IN PERFECT SECURITY. THE SAME UNFORTUNATE CIRCUMSTANCE OF GIVING A DISEASE, SUPPOSED TO BE THE SMALLPOX, WITH INEFFICACIOUS VARIOLOUS MATTER, having occurred under the direction of some other practitioners within my knowledge, and probably from the same incautious method of securing the variolous matter, I avail myself of this opportunity of mentioning what I conceive to be of great importance; and, as a further cautionary hint, I shall again digress so far as to add another observation on the subject of inoculation.

WHETHER IT BE YET ASCERTAINED BY EXPERIMENT THAT THE QUANTITY OF VARIOLOUS MATTER INSERTED INTO THE SKIN MAKES ANY DIFFERENCE WITH RESPECT TO THE SUBSEQUENT MILDNESS OR VIOLENCE OF THE DISEASE, I KNOW NOT; but I have the strongest reason for supposing that if either the punctures or incisions be made so deep as to go through it and wound the adipose membrane, THAT THE RISK OF BRINGING ON A VIOLENT DISEASE IS GREATLY INCREASED. I have known an inoculator whose practice was "TO CUT DEEP ENOUGH (to use his own expression) TO SEE A BIT OF FAT." and there to lodge the matter. THE GREAT NUMBER OF BAD CASES, INDEPENDENT OF INFLAMMATIONS AND ABSCESSES ON THE ARM'S, AND THE FATALITY WHICH ATTENDED THIS PRACTICE, WAS ALMOST INCONCEIVABLE; and I cannot account for it on any other principle than that of the matter being placed in this situation instead of the skin."

"I do not mean to insinuate that exposure to cool air, and suffering the patient to drink cold water when hot and thirsty, may not moderate the eruptive symptoms and lessen the number of pustules; yet, to repeat my former observation, I cannot account for the uninterrupted success, or nearly so, of one practitioner, and the wretched state of the patients under the care of another, where, in both instances, THE GENERAL TREATMENT DID NOT DIFFER ESSENTIALLY, WITHOUT CONCEIVING IT TO ARISE FROM THE DIFFERENT MODES OF INSERTING THE MATTER FOR THE PURPOSE OF PRODUCING THE DISEASE. As it is not the identical matter inserted which is absorbed into the constitution, but that which is, by some peculiar process in the animal economy, generated by it, is it not probable that different parts of the human body may prepare or modify the virus differently? Although the skin, for example, adipose membrane, or mucous membranes are all capable of producing the variolous virus by the stimulus given by the particles originally deposited upon them, yet I am induced to conceive that each of these parts is capable of producing some variation in the qualities

of the matter previous to its affecting the constitution. WHAT ELSE CAN CONSTITUTE THE DIFFERENCE BETWEEN THE SMALLPOX WHEN COMMUNICATED CASUALLY OR IN WHAT HAS BEEN TERMED THE NATURAL WAY, OR WHEN BROUGHT ON ARTIFICIALLY THROUGH THE MEDIUM OF THE SKIN?

After all, are the variolous particles, possessing their true specific and contagious principles, ever taken up and conveyed by the lymphatics unchanged into the blood vessels? I imagine not. WERE THIS THE CASE, SHOULD WE NOT FIND THE BLOOD SUFFICIENTLY LOADED WITH THEM IN SOME STAGES OF THE SMALLPOX TO COMMUNICATE THE DISEASE BY INSERTING IT UNDER THE CUTICLE, OR BY SPREADING IT ON THE SURFACE OF AN ULCER? YET EXPERIMENTS HAVE DETERMINED THE IMPRACTICALITY OF ITS BEING GIVEN IN THIS WAY; although it has been proved that variolous matter, when much diluted with water and applied to the skin in the usual manner, will produce the disease. But it would be digressing beyond a proper boundary to go minutely into this subject here."

"Its rise in this country may not have been of very remote date, as the practice of milking cows might formerly have been in the hands of women only; which I believe is the case now in some other dairy countries, and, consequently, that the cows might not in former times have been exposed to the contagious matter brought by the men servants from the heels of horses. [Footnote: I have been informed from respectable authority that IN IRELAND, although dairies abound in many parts of the island, THE DISEASE IS ENTIRELY UNKNOWN. The reason seems obvious. THE BUSINESS OF THE DAIRY IS CONDUCTED BY WOMEN ONLY. WERE THE MEANEST VASSAL AMONG THE MEN EMPLOYED THERE AS A MILKER AT A DAIRY, HE WOULD FEEL HIS SITUATION UNPLEASANT BEYOND ALL ENDURANCE.] Indeed, a knowledge of the source of the infection is new in the minds of most of the farmers in this neighbourhood, but it has at length produced good consequences; and it seems probable, from the precautions they are now disposed to adopt, that the appearance of the cow-pox here may either be entirely extinguished or become extremely rare.

Should it be asked whether this investigation is a matter of mere curiosity, or whether it tends to any beneficial purpose, I should answer that, notwithstanding THE HAPPY EFFECTS OF INOCULATION, with all the improvements which the practice has received since its first introduction into this country, IT NOT VERY INFREQUENTLY PRODUCES DEFORMITY OF THE SKIN, AND SOMETIMES, UNDER THE BEST MANAGEMENT, PROVES FATAL."

"Several instances have come under my observation which justify the assertion that the DISEASE CANNOT BE PROPAGATED BY EFFLUVIA. The first boy whom I inoculated

with the matter of cow-pox slept in a bed, while the experiment was going forward, with two children who never had gone through either that disease or the smallpox, WITHOUT INFECTING EITHER OF THEM.

A young woman who had the cow-pox to a great extent, several sores which maturated having appeared on the hands and wrists, slept in the same bed with a fellow-dairymaid who never had been infected with either the cow-pox or the smallpox, BUT NO INDISPOSITION FOLLOWED.

Another instance has occurred of a young woman on whose hands were several large suppurations from the cow-pox, who was at the same time a daily nurse to an infant, BUT THE COMPLAINT WAS NOT COMMUNICATED TO THE CHILD."

"THERE ARE MANY WHO, from some peculiarity in the habit, RESIST THE COMMON EFFECTS OF VARIOLOUS MATTER INSERTED INTO THE SKIN, and who are in consequence haunted through life with the distressing idea of being insecure from subsequent infection."

https://biotech.law.lsu.edu/cphl/history/articles/jenner.htm

In Summary:

-Jenner believed that the morbid pustule from the sore heels of horses infected cows after the men who worked on the horses milked the cows without washing their hands

-the cows had palish blue markings (bruises perhaps?) along the nipples with inflammation and decreased milk production

-the workers had small burn markings on their hands (friction burns perhaps?)

-Jenner's (discredited) theory was the virus went from horse to the nipples of cows to humans

-he states that morbid matter of many kinds absorbed into the system can produce similar symptoms of disease

-he admits that it is a well-known fact that Blacksmiths can not be given Smallpox through inoculation although he theorizes some may have it "anomalously"

-Jenner presents the case of a man who worked with the diseased heels of horses and ended up with Cowpox-like lesions on his hands

-the man was told he would be immune to Smallpox since he had had Cowpox

-however, 20 years later, the man developed Smallpox thus Jenner concluded the man

did not really have Cowpox originally

-Jenner presents the case of an 8-year-old boy inoculated in 2 spots with the pus from a dairy maid said to have Cowpox and the boy developed similar symptoms 7 days later

-the boy was later inoculated with pus from Smallpox but this did not produce disease thus Jenner concluded the boy was now immune to it

-3 servent men on a farm washed the sore heels of a mare and developed lesions on their hands considered to be Cowpox

-2 of the men who had SMALLPOX FROM INOCULATION said it felt similar

-a 5-year-old boy was inoculated with pustule from the hand of a man and developed Cowpox-like symptoms 6 days later

-another 8-year-old boy was inoculated with Cowpox and had fever/symptoms identical to that caused by Smallpox inoculation

-a number of adults and children were inoculated and 3 of them developed secondary infections from the inoculation

-a 7-year-old girl was inoculated in 3 places and developed symptoms identical to those acquired from Smallpox inoculation

-the many people sickened by "passing" pustule from one human to another through 5 gradations and not losing potency satisfied Jenner

-Jenner was convinced that the morbid matter from horses was the cause of both Cowpox/Smallpox even though he admitted he could not prove it experimentally

-Jenner stated Cowpox never affected cows unless the person milking them had worked on the diseased heels of horses

-in 1797, even though many horses had diseased heels, no cows came down with Cowpox and Jenner could not conclude his investigation that year

-Jenner was convinced cows could not escape Cowpox if they were milked by hands who had worked on diseased horse heels

-Jenner believed that the pustule from diseased horses/cows could not cause disease on healthy human skin but only if the hands were already sore/cut from hard labor

-Jenner shares the case of a woman who had Cowpox in 1759, was inoculated with Smallpox in 1797, and again had Cowpox in 1798

-Jenner believed there were various versions of Smallpox as 7 years before his investigation, there was an outbreak so mild no one was concerned nor took

precautions and had symptoms as light as those caused by inoculation

-Jenner discusses cases where Smallpox pustule by a Dr. was stored in such a way that those inoculated with it succumbed to severe disease and even death

-Jenner concluded it must have been a different disease other than Smallpox due to the purification process "killing" the "virus"

-he knew of "artificial" cases of Smallpox caused by inoculation but he stated the material used was non-infectious thus it was not real Smallpox

-Jenner did not know whether or not if the amount of Smallpox pustule inoculated into a subject caused worsening disease

-Jenner believed that deeper cuts down to the fat layers for inoculations caused inconceivable deaths in many cases

-he pondered what caused differences between the natural Smallpox and that created through artificial means of inoculation

-Jenner stated that Smallpox could not be induced through blood experimentally

-Jenner knew in Ireland, Cowpox was unheard of even though they had dairy farms

-he presumed it was because women were the ones milking the cows vs angry men

-Jenner admits that his inoculations causes skin deformities and death

-Jenner presents many cases were Cowpox could not be spread person-to-person

-Jenner admits there are many who are not successfully inoculated with Smallpox

Edward Jenner, through his unethical and crude experiments, proved that taking diseased pustule and spreading them into the wounds in healthy subjects could cause disease in some but not in others. He based his work on the assumption that a "virus" was present in the pustules. While he made observations about the unsanitary conditions and the need for unwashed hands with open sores in order to become "infected," he clung to an invisible "virus" as the culprit even while stating morbid material of many kinds, once introduced into the human system, can produce the same symptoms of disease. This unproven idea of creating immunity by vaccinating against an unseen foe with dead/diseased material still persists today.

https://docs.google.com/document/d/e/2PACX-1vQbZKBvvXtzUVKpwPZ2KNVQ0y1TawhdpvInyeiLBSpvCk8XhaltZRNB2Ggt4FK9e6gfw6EjXplPVTX1/pub

RUDOLPH STEINER DEMONSTRATES CHICKENPOX INFECTIOUSNESS IN 1875?:

RUDOLPH STEINER DEMONSTRATES CHICKENPOX INFECTIOUSNESS IN 1875?:

Rudolph Steiner, an Austrian philosopher, esotericist, spiritualist, claimed clairvoyant, and the founder of anthroposophy, is given credit for proving that Chickenpox was indeed caused by an infectious agent in 1875. The CDC claims this as fact:

"In 1875, RUDOLF STEINER DEMONSTRATED THAT CHICKENPOX WAS CAUSED BY AN INFECTIOUS AGENT by inoculating volunteers with the vesicular fluid from a patient with acute varicella."

https://www.cdc.gov/vaccines/pubs/pinkbook/downloads/varicella.pdf

Rudoplh Steiner taking pustules from a Chickenpox patient and subjecting healthy volunteers to it who then developed itchy bumpy rashes definitely makes for an interesting story. But did this really happen?

One thing I took away from reading interviews with Kary Mullis (inventor of PCR) is that if you make a claim about something as a fact, it should be backed up with a citation to the paper which proves that as fact. Here is his quote regarding the lack of scientific proof for HIV:

"Its not even probable, let alone scientifically proven, that HIV causes AIDS. If there is evidence that HIV causes AIDS, THERE SHOULD BE SCIENTIFIC DOCUMENTS WHICH EITHER SINGLY OR COLLECTIVELY DEMONSTRATE THAT FACT, at least with a high probability. There are no such documents."-- Kary Mullis

https://www.azquotes.com/author/10544-Kary_Mullis

If a large part of the proof that Chickenpox is an infectious disease is based upon the experiments of Rudolph Steiner in 1875, there should be papers/documents backing this claim up. We should be able to read this work for ourselves and see if the results produced are valid. So I tried to find Steiner's original paper by combing through a few sources:

"The late 1800s also saw great strides toward understanding varicella. In 1875, STEINER INOCULATED HEALTHY VOLUNTEERS WITH VEHICULAR FLUIDS FROM INDIVIDUALS WITH ACTIVE VARICELLA INFECTIONS [7], thus demonstrating that chickenpox is caused by an infectious agent."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4628852

The [7] citation in this paper led me here:

"Although chickenpox was first proved to be an infectious disease in 1875, WHEN STEINER [5] TRANSMITTED THE VIRUS FROM VESICLES TO SUSCEPTIBLE VOLUNTEERS,"

doi: 10.1093/clinids/24.5.753.

This [5] reference led me to this citation:

"Steiner P. Zur inokulation der varicellen. We in Med Wochenschr 1875; 25:306."

I also found this supposed paper by Rudolph Steiner listed in other papers as:

"Steiner G: Zur Inokulation der Varicellen. Wien Med Wochenschr 1875;25:306."

https://books.google.com/books?id=3Eh51Np2w7IC&pg=PR9&lpg=PR9&dq=steiner%20g%3A%20zur%20inokulation%20der%20varicellen.%20wien%20med%20wochenschr%201875%3B25%3A306.&source=bl&ots=rSV5BV8Zys&sig=ACfU3U2axFJFVBQr_hZ2vZVUjUDF_PahoA&hl=en&sa=X&ved=2ahUKEwix05fR_tbwAhUVITQIHWJvCsMQ6AEwBnoECAsQAg

"STEINER: Zur inokulation der Varicella. Wien Med Wochenschr 25:306, 1875"

https://doi.org/10.7326/0003-4819-89-3-375

As can be seen, the paper cited is listed as being by P. Steiner, G. Steiner, or just plain ol' Steiner, but not R. Steiner. Unfortunately, while I could find a few other papers with similar citations such as this, try as I might, I could not find the original 1875 paper neither in German nor in an English translation. I couldn't even find any actual quotes taken from Steiner's work. All I could find were the same repeated claims over and over again:

"In 1875, RUDOLF STEINER DISCOVERED THAT CHICKENPOX WAS INFECTIOUS to others after he took liquid from the chickenpox blisters of an infected individual and spread it to healthy volunteers."

https://www.nvic.org/vaccines-and-diseases/Chickenpox/history.aspx

"It took over a hundred years for another scientist, RUDOLF STEINER IN 1875, TO IDENTIFY THAT CHICKEN POX WAS CAUSED BY AN INFECTIOUS AGENT. He did so by extracting fluid from the blisters of an infected person and rubbing it on the skin of healthy volunteers; they too developed an itchy, blistering rash."

https://nouse.co.uk/2017/02/21/the-spotted-history-of-chicken-pox

"In the year 1875 it was discovered by a scientist that chickenpox was caused by an infectious agent. A man known as RUDOLF STEINER, TOOK SOME FLUID FROM THE CHICKENPOX BLISTERS OF AN INFECTED INDIVIDUAL AND DECIDED TO RUB THE FLUID UPON THE SKIN OF HEALTHY VOLUNTEERS.Those healthy volunteers also developed an itchy bumpy rash."

https://chickenpoxinformationforyou.weebly.com/origin.html

So while this paper by either P. or G. Steiner may exist, it is not readily accessible. However, there are a few other problems with the claim that Rudolph Steiner performed experiments in 1875 proving the infectiousness of Chickenpox. Steiner's first published credits are in 1883:

"Rudolf Steiner went to Weimar to edit the scientific writings of Goethe for the Kürschner edition of the “German National Literature.” Along with sorting and arranging Goethe's works, Steiner wrote introductions and commentaries that have been collected and published in English translation under the titles, Goethe the Scientist, Goethean Science or Nature's Open Secret. ORIGINALLY PUBLISHED IN GERMAN as, Einleitung Zu Goethes Naturwissenschaftliche Schriften, 1883."

https://www.rsarchive.org/Books/

On top of that, Rudolph Steiner was born on February 27th, 1861 which means he would have been 14 years old at the time these experiments were performed and this paper was supposedly written in 1875. Now while unrealistic, it is plausible a 14 year old Steiner could have done these experiments and wrote a seminal Chickenpox paper proving infectiousness, but is it probable?

Another strike against this "evidence" is that Steiner seemed very much inclined to believe that disease occurred from spiritual conflict from within and that childhood diseases were caused by the child adapting to the physical world. He believed that the mind played a significant role in "catching" and preventing disease. Granted, maybe he came to these views later in life, however they definitely contradict the belief that

"viruses" were a cause of disease. A few quotes here highlight his views:

"Rudolf Steiner generally portrayed the traditional childhood diseases as signs, aids, trials, and accompaniments of the NATURAL PROCESS OF CHILD DEVELOPMENT AND MATURING IMMUNE SYSTEMS. He helps us to understand their deeper meaning and management within human development."

QUOTES FROM STEINER:

“. . . When I see people suffering from influenza, I must always turn my attention to something other than the symptoms that the doctors pay heed to, because THE FLU IS ACTUALLY A KIND OF BRAIN ILLNESS. The flu is really an illness of the brain! I shall say more about this later."

“Treatment with a modified virus vaccine is effective in the case of diphtheria, because the body is thus given a strong impulse to become active, BUT IT HAS UNFAVORABLE AFTER-EFFECTS. Particularly if a child is treated with a vaccine, IT WILL LATER SUFFER A HARDENING OF ITS ORGANIZATION. ONE MUST THEREFORE STRIVE TO REPLACE VACCINES WITH BATHING TREATMENT, especially in the case of diphtheria, which is caused primarily by the effective activity of the skin. . . ."

"...IF THE BODY IS LEFT TOTALLY TO ITS OWN DEVICES IN CONDITIONS OF STEADY WARMTH AND LIGHT, IT CAN ITSELF ENDURE EVEN THE WORST ATTACKS OF PNEUMONIA, PLEURISY, AND PERITONITIS. The human being is capable of that. Even with the worst illnesses that display the symptoms mentioned, IT IS MORE A MATTER OF PROPER NURSING CARE THAN OF REMEDIES. . . ."

“The risk of infection is actually great in diphtheria-related disorders, but why? It is because THEY DEVELOP IN DIRECT CONNECTION WITH LEARNING TO SPEAK, and occur therefore most widely in children aged between two and four. AFTER THIS AGE CHILDREN ARE LESS SUSCEPTIBLE. But every process in the human organism that arises in the normal course of things at any particular period can also arise abnormally. THIS PROCESS, therefore, THAT IS REALLY SIMPLY A NATURAL PROCESS OF CHILDHOOD DEVELOPMENT CAN ALSO OCCUR AT A LATER AGE, ALBEIT IN A SOMEWHAT MODIFIED FORM, A METAMORPHOSIS. When diphtheria

occurs at a later age this is in a sense an infantile condition that works on in a person."

“. . . If one has a heavenly Imagination such as that of which I spoke, one knows what smallpox is, BECAUSE IT IS ONLY THE PHYSICAL PROJECTION OF WHAT IS EXPERIENCED SPIRITUALLY. AND SO IT IS, REALLY, WITH ALL KNOWLEDGE OF ILLNESS.”

"THE MENTAL FACTOR can thus play a considerable role when it comes to catching things."

“I HAVE ACTUALLY NEVER SHIED AWAY FROM EXPOSING MYSELF TO ANY DANGER OF INFECTIONS AND HAVE REALLY NEVER CAUGHT ANYTHING, HAVE NEVER GOT MYSELF INFECTED. This did show that mere awareness, powerful awareness of the existence of a disease, can bring about the disease through the astral body. POWERFUL AWARENESS OF A DISEASE CAN BE THE CAUSE OF THAT DISEASE ARISING FROM THE ASTRAL BODY."

https://www.waldorflibrary.org/articles/614-the-meaning-of-illness

Rudolph Steiner was also against vaccinations and believed that they would cut one off from the spiritual world:

”IN THE FUTURE, WE WILL ELIMINATE THE SOUL WITH MEDICINE. Under the pretext of a ‘healthy point of view’, THERE WILL BE A VACCINE by which the human body will be treated as soon as possible directly at birth, SO THAT THE HUMAN BEING CANNOT DEVELOP THE THOUGHT OF THE EXISTENCE OF SOUL AND SPIRIT.

To materialistic doctors, they will be entrusted with the task of removing the soul of humanity. AS TODAY, PEOPLE ARE VACCINE AGAINST THIS DISEASE OR DISEASE, SO IN THE FUTURE, CHILDREN WILL BE VACCINATED WITH A SUBSTANCE that can be produced precisely in such a way that people, thanks to this vaccination, will be immune to being subjected to the “madness” of spiritual life. He would be extremely smart, BUT HE WOULD NOT DEVELOP A CONSCIENCE, and that

is the true goal of some materialistic circles.

WITH SUCH A VACCINE, YOU CAN EASILY MAKE THE ETHERIC BODY LOOSE IN THE PHYSICAL BODY. Once the etheric body is detached, the relationship between the universe and the etheric body would become extremely unstable, and man would become an automaton, for the physical body of man must be polished on this Earth by spiritual will. So, the vaccine becomes a kind of arymanique force; man can no longer get rid of a given materialistic feeling. He becomes materialistic of the constitution and can no longer rise to the spiritual.” (1861-1925)"

https://thegreatwork208716197.wordpress.com/2020/11/16/more-than-a-hundred-years-ago-rudolf-steiner-wrote-the-following

“I have told you that the spirits of darkness are going to inspire their human hosts, in whom they will be dwelling, TO FIND A VACCINE THAT WILL DRIVE ALL INCLINATION TOWARD SPIRITUALITY OUT OF PEOPLE'S SOULS WHEN THEY ARE STILL VERY YOUNG, and this will happen in a roundabout way through the living body. Today, bodies are vaccinated against one thing and another; in future, children will be vaccinated with a substance which it will certainly be possible to produce, and this will make them immune, so that they do not develop foolish inclinations connected with spiritual life – ‘foolish’ here, or course, in the eyes of materialists. . . ."

“. . . a way will finally be found to VACCINATE BODIES SO THAT THESE BODIES WILL NOT ALLOW THE INCLINATION TOWARD SPIRITUAL IDEAS to develop and all their lives people will believe only in the physical world they perceive with the senses. Out of impulses which the medical profession gained from presumption – oh, I beg your pardon, from the consumption [tuberculosis] they themselves suffered – PEOPLE ARE NOW VACCINATED AGAINST CONSUMPTION, AND IN THE SAME WAY THEY WILL BE VACCINATED AGAINST ANY INCLINATION TOWARD SPIRITUALITY. This is merely to give you a particularly striking example of many things which will come in the near and more distant future in this field – the aim being to bring confusion into the impulses which want to stream down to earth after the victory of the [Michaelic] spirits of light [in 1879].” ~Rudolf Steiner

http://www.renegadetribune.com/in-1917-rudolf-steiner-foresaw-a-vaccine-that-would-drive-all-inclination-toward-spirituality-out-of-peoples-souls

So here we are, left with a bit of a conundrum. Are we to believe that a 14-year-old Rudolph Steiner performed experiments and wrote a paper proving Chickenpox infectiousness in 1875? Does this paper even exist? If so, why were there either different first name initials or a lack of one based on the citation listed? If it does exist, why does the Rudolph Steiner archive list his first published work as being done as an editor of Goethe's work in 1883? Should the writings of a 14-year-old Steiner, where he supposedly took pustules from a Chickenpox patient and transferred infection to a healthy patient by rubbing it on their skin, be taken seriously? Were these experiments validated or repeated by anyone else? Would these experiments even constitute proof of infection in the first place? If this work is such a seminal moment in the history of Chickenpox, why is this paper seemingly impossible to find?

For now, it remains a mystery that hardly constitutes proof.

https://docs.google.com/document/d/e/2PACX-1vS8HKU9nLj53oYCRay0chM9_1REdh-WL_TeTet4k9nfmyO73t1UZAThXT2kV-60Qlvyfk_FV3Lb5AtS/pub

SHINGLES = CHICKENPOX:

"Did you know? The same virus that causes chickenpox also causes shingles. ALTHOUGH SHINGLES AND CHICKENPOX ARE CAUSED BY THE SAME VIRUS, THEY ARE NOT THE SAME ILLNESS."

https://apic.org/monthly_alerts/chickenpox-versus-shingles-whats-the-difference

When looking into the history of Virology, it becomes readily apparent that the same symptoms of disease are regularly given different names which are claimed to be caused by different "viruses," bacteria, or conditions. Syphilis, HIV, and Lupus. Rubella and Measles. Influenza and "Covid-19." Pneumonia and Tuberculosis. Smallpox and Monkeypox.

And so it is with Chickenpox and Shingles (of which both can also be included alongside Smallpox/Monkeypox). The history between these two diseases seems to be a long and

complicated one. Many, if not all of the symptoms, overlap with each other. They can both be acquired at any age even though one is associated with childhood while the other with adulthood. However, unlike other diseases which are distinguished by different "viruses," bacteria, or conditions, Chickenpox and Shingles share the exact same "virus" as the root cause. However, for the longest time, they weren't even considered separate diseases. Two sources highlight this claim:

"Shingles has a long recorded history, ALTHOUGH HISTORICAL ACCOUNTS FAIL TO DISTINGUISH THE BLISTERING CAUSED BY VZV AND THOSE CAUSED BY SMALLPOX,[32] ERGOTISM, AND ERYSIPELAS. In the late 18th century WILLIAM HEBERDEN ESTABLISHED A WAY TO DIFFERENTIATE BETWEEN SHINGLES AND SMALLPOX,[96] and in the late 19th century shingles was differentiated from erysipelas."

https://en.m.wikipedia.org/wiki/Shingles#History

"Shingles has a long recorded history, but physicians and scientists were UNABLE TO DISTINGUISH THIS PARTICULAR DISEASE FROM BLISTERING CAUSED BY SMALLPOX, ERGOTISM, AND ERYSIPELAS. William Heberden finally accomplished the distinction between herpes zoster and smallpox in the late 18th century."

https://carrington.edu/blog/shingles-and-shingles-zoster-vaccine

Interestingly, both sources credit William Heberden as distinguishing Shingles from Smallpox. However, his paper was not about Shingles but was instead about Chickenpox. Granted, they later "discovered" both Shingles and Chickenpox stem from the same Herpes "virus" yet even today they are still somehow considered different diseases so I guess the confusion is warranted.

Trying to determine the history of Shingles is rather difficult. Even though it was apparently discussed throughout history, it went by different names (Herpes Zoster, Shingles, Varicella) and was confused with Smallpox. The first mention of the name Shingles as a distinct disease seems to be in 1831:

"In the mid-1700s, a scientist named WILLIAM HERBERDEN DISCOVERED HOW TO DIFFERENTIATE BETWEEN SHINGLES AND SMALLPOX. But the cause of the disease was not determined until a century later.

Shingles has existed since the Middle Ages. However, THE DISEASE WAS OFTEN CONFUSED WITH SMALLPOX, as that was one of the prevalent diseases at that time.

THE ORIGIN OF SHINGLES WAS FIRST DISCUSSED IN 1831, when a scientist named Richard Bright stated that he believed the disease was carried by the dorsal root

ganglion, or the spinal ganglion."

https://healthyliving.azcentral.com/history-of-shingles-disease-12222755.html

Meanwhile, Chickenpox was a term used long before Shingles:

"The name “chickenpox” was used by Richard Morton (1637-1698) WHO CHARACTERIZED IT AS A MILD FORM OF SMALLPOX."

"The exact origin of the term “varicella” is also uncertain. VOGEL REFERRED TO CHICKENPOX AS VARICELLA IN 1765."

"BOTH VARICELLA AND HERPES ZOSTER WERE INVESTIGATED INDEPENDENTLY UNTIL THEIR STORIES FINALLY MERGED. Their histories are complicated by several factors, including the DIFFICULTY IN INTERPRETING MEDICAL LITERATURE DESCRIBING SKIN CONDITIONS BEFORE THE 16th CENTURY, INCONSISTENT TERMINOLOGY, AND USE OF DATA FROM EARLIER MEDICAL WRITERS WITHOUT PROPER CITATION. During the 18th and 19th centuries, there was an explosion of medical writings about both diseases, but with little overlap."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4628852/

Both Shingles as Chickenpox were often confused with Smallpox. The terminology used throughout history varied and the actual conditions they were discussing along with the names used was difficult to understand due to varied terminology and the lack of proper citations. Could it be that these researchers in different eras in different parts of the world were using different names while discussing the exact same symptoms of disease?

After William Heberden distinguished Chick...ahem, Shingles from Smallpox, it fell to Viennese physician Janos Von Bokay to provide some solid observational evidence connecting these two identical diseases to the same "virus." A few sources highlight his impeccable observational skills:

"CLINICAL OBSERVATIONS OF THE RELATIONSHIP BETWEEN VARICELLA AND HERPES ZOSTER WERE MADE IN 1888 BY VON BOKAY, when susceptible children acquired varicella after contact with herpes zoster."

https://wonder.cdc.gov/wonder/prevguid/p0000108/p0000108.asp

"THE ASSOCIATION BETWEEN CHICKENPOX AND SHINGLES WAS FIRST

NOTICED BY VON BOKAY IN 1888. He noted that chickenpox SOMETIMES DEVELOPED in susceptible children after exposure to persons with acute shingles.5"

https://phdres.caregate.net/curriculum/pdf-files/Derm_refs/VZVirus.pdf

"In 1892, the Viennese physician Janos von

Bókay 5 SUGGESTED A POSSIBLE RELATIONSHIP BETWEEN ZOSTER AND VARICELLA AFTER OBSERVING THAT HOUSEHOLD EXPOSURE TO ZOSTER CASES COULD GIVE RISE TO VARICELLA IN SUSCEPTIBLE CHILDREN. Kundratitz 6 confirmed this connection in 1925 by showing that vesicle fluid from patients with either varicella or zoster could produce VARICELLA-LIKE RASH IN UNEXPOSED CHILDREN and that these children could in turn transmit chickenpox to non inoculated contacts."

DOI:10.1001/archneur.61.12.1974

Sadly, while I could find the citations for Von Bokay's papers in 1888 and 1909, as in the case with Rudolph Steiner's mysterious Chickenpox paper, I was unable to find the actual papers themselves in order to read or highlight any pertinent information from them. We must take his observations on blind faith alone. It must also be noted that due to the fact that no animal model existed for these diseases and no "viruses" could be seen, the only evidence for this connection came from both clinical and observational evidence:

"The earliest reports of vesicular rashes of the type we now recognize to be caused by herpes simplex and zoster date to the ancient civilizations. It was not until 1888, however, that a relationship between herpes zoster and chickenpox was suggested. Establishing this link represented one of the major hurdles in the history of varicella zoster virus. THERE WAS NO ANIMAL HOST AND THIS MEANT THAT MUCH OF THE EVIDENCE NEEDED TO BE OBTAINED BY CLINICAL AND EPIDEMIOLOGICAL OBSERVATION."

https://pubmed.ncbi.nlm.nih.gov/11867004/

Digging deeper beyond Von Bokay's observational hypothesis that the two identical looking diseases were caused by the same "virus," we find that there were human experiments performed by Lipschutz and Kundratitz in 1925. They found that some (not all) of the inoculated humans with vesicular fluid from patients with Herpes Zoster (Shingles) developed identical lesions to Varicella (Chickenpox). These experiments obviously led to further hypotheses about the sameness of these two diseases while

doing everything possible to attempt to keep them as separate:

"VON BÓKAY, IN 1892, FIRST SUGGESTED A RELATIONSHIP BETWEEN VARICELLA AND HERPES ZOSTER WHEN HE NOTED THE OCCURRENCE OF BOTH DISORDERS AT THE SAME TIME IN HOMES OF HIS PATIENTS. He recognized the SIMILAR APPEARANCE of the skin findings and HYPOTHESIZED THAT THE SAME VIRUS PRODUCED THE TWO, thus setting the stage for experimental analysis."

"Shortly thereafter, scientists and physicians BEGAN TO MORE FORMALLY TEST THE HYPOTHESIS THAT THE TWO DISEASES WERE RELATED. Lipschutz and Kundratitz (1925) and Bruusgaard (1932) inoculated humans with vesicular fluid from patients with herpes zoster and FOUND THAT SOME SUBJECTS DEVELOPED DIFFUSE VASCULAR SKIN LESIONS CLINICALLY AND MICROSCOPICALLY IDENTICAL TO THOSE IN VARICELLA [13]."

"Garland HYPOTHESIZED that zoster (shingles) was due to reactivation of a latent varicella zoster virus [18]."

"YET THE ASSOCIATION BETWEEN ZOSTER AND VARICELLA STILL REQUIRED CLARIFICATION. In his landmark presentation in 1965, Hope-Simpson [19] POSTULATED that zoster was due to reactivation of latent virus and that viral latency was maintained by repeated exposure to exogenous virus, based on all cases of herpes zoster he encountered during 16 years in general practice and on evidence that zoster was unrelated to epidemics. HE HYPOTHESIZED THAT VIRUS REACTIVATED WHEN ANTIBODY LEVELS FELL BELOW A CRITICAL LEVEL [20], ALTHOUGH IT HAS SINCE BEEN SHOWN THAT THE PRESENCE OF ANTI-VZV ANTIBODIES IN SERA FROM INDIVIDUALS AGED 60-94 YEARS IS VARIABLE [21], and that a declining cell-mediated immune response to VZV is more important in allowing VZV to reactivate. Overall, while primary VZV infection in childhood usually produces varicella, seronegative adults can develop Varicella (often severe) when exposed to children with varicella or adults with zoster."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4628852/

Interestingly enough, a 1925 review which discussed the 1925 Kundratitz paper found that his experiments relating Shingles and Chickenpox, while providing a connection between the two, were somewhat lacking as evidence of transmission:

Varicella and Herpes Zoster.

"SEVERAL OBSERVERS (Lipschiitz, Meineri, and others) HAVE MADE ISOLATED ATTEMPTS TO INOCULATE HUMAN VOLUNTEERS WITH HERPES ZOSTER, BUT ALWAYS WITH NEGATIVE RESULTS. Recent studies of KUNDRATITZ (32) SEEM TO SHOW that herpes zoster can be successfully transmitted to very young children. This author wished to test out VON BOKAY'S (33) HYPOTHESIS, BASED ON CLINICAL OBSERVATION, that the virus of varicella, under certain unknown conditions, may produce a typical picture of herpes zoster and that the virus from this lesion may in turn cause varicella. He therefore attempted to immunize children against varicella by the inoculation of material from herpes zoster cases. HIS FIRST RESULTS WERE NEGATIVE, BUT HIS LATER ATTEMPTS PROVED SUCCESSFUL. He now reports that he has inoculated material from TEN TYPICAL CASES of thoracic herpes zoster and HAS HAD POSITIVE RESULTS WITH THE MATERIAL FROM FIVE OF THESE CASES. POSITIVE REACTIONS WERE OBTAINED ONLY IN CHILDREN UNDER 5 YEARS OF AGE. Children who reacted positively were subsequently shown to be immune to varicella.

KUNDRATITZ'S WORK SEEMS TO INDICATE THAT THE VIRUS OF VARICELLA AND THAT OF HERPES ZOSTER ARE IDENTICAL OR, AT LEAST, CLOSELY RELATED. IT IS UNFORTUNATE THAT KUNDRATITZ DOES NOT GIVE A DESCRIPTION OF THE CASES OF HERPES ZOSTER USED BY HIM FOR INOCULATION. It would be interesting to know whether there were any clinical differences between the five cases of herpes zoster with which he was able to make successful transfers and the five cases in which transfers resulted negatively for, as Von Bokay and others have shown, the vesicles of varicella may be quite localized, RESULTING IN LESIONS RESEMBLING HERPES ZOSTER. The relation between herpes zoster and varicella will, in all probability, not be entirely cleared up until we are able to transmit either one or both of these diseases to animals."

"THE QUESTION OF THE IDENTITY OR NON-IDENTITY OF HERPES ZOSTER AND VARICELLA IS EVEN MORE DIFFICULT TO ANSWER, BECAUSE AT PRESENT NEITHER OF THESE INFECTIONS IS READILY TRANSMISSIBLE TO ANIMALS. The work of Kundratitz is extremely interesting. His observations, aside from indicating a close immunological relationship between herpes zoster and varicella, are important in that THEY SEEM TO SHOW THE PRESENCE OF A TRANSMISSIBLE VIRUS IN THE VESICLES OF HERPES ZOSTER. The only QUESTION THAT ARISES IS WHETHER THE CASES OF HERPES ZOSTER from which Kundratitz was able to make successful transfers WERE TRUE CASES OF IDIOPATHIC HERPES ZOSTER."

doi: 10.1084/jem.42.6.799.

It's interesting to note that the transfer of Herpes Zoster to humans was largely unsuccessful thus ruling out the infectiousness of the supposed "virus." That they were also unable to transfer disease to animals is just icing on the cake. The "successful" evidence for human transmission only included success in 5 out of 10 children with questions pertaining to whether they were true Herpes Zoster cases. If 50% equals a passing grade in your book, I guess you will be happy with this evidence.

So here we have observations throughout history describing the same symptoms of disease under various names yet rather than bring them all under the same umbrella, somehow it was decided that they should all be distinct diseases caused by various "viruses." It is clear that these observations, experiments, hypotheses have gone a long way towards attempting to make sure that the same symptoms of disease known as Shingles and Chickenpox are separate diseases caused by the same "virus." However, what causes them to be considered different diseases?

Is it the symptoms?

CHICKENPOX SYMPTOMS:

"Skin: blister, scab, ulcers, or red spots

Whole body: fatigue, fever, or loss of appetite

Also common: headache, itching, sore throat, or swollen lymph nodes"

https://www.mayoclinic.org/diseases-conditions/chickenpox/symptoms-causes/syc-20351282?utm_source=Google&utm_medium=abstract&utm_content=Chickenpox&utm_campaign=Knowledge-panel

SHINGLES SYMPTOMS:

'Rash characteristics include:

red patches, fluid-filled blisters that break easily, wraps around from the spine to the torso, on the face and ears itching

Some people experience symptoms beyond pain and rash with shingles. These may include:

fever, chills, headache, fatigue, muscle weakness"

https://www.healthline.com/health/shingles

It seems that the symptoms matchup fairly well. Is it because they are easily distinguishable by appearance?

"Chickenpox and shingles are two infectious diseases that ARE OFTEN CONFUSED WITH ONE ANOTHER."

https://www.medicinenet.com/difference_between_chickenpox_and_shingles/article.htm

It appears they are often confused with each other. Both diseases also seem to be confused with other skin conditions as well:

"In some cases, a person who appears to be developing chickenpox for the second time is actually having their first case of chickenpox. SOME RASHES CAN MIMIC CHICKENPOX. IT MAY BE THAT THAT PERSON ACTUALLY NEVER HAD CHICKENPOX BEFORE, BUT INSTEAD RECEIVED A MISDIAGNOSIS."

https://www.healthline.com/health/can-you-get-chickenpox-twice

"Especially if you haven't seen shingles pictures, YOU COULD EASILY MISTAKE A SHINGLES RASH FOR ANOTHER HEALTH CONDITION THAT AFFECTS THE SKIN. The shingles virus causes an outbreak of a red rash and blisters across the face and body, LIKE MANY OTHER SKIN CONDITIONS — PSORIASIS, ALLERGIES, ECZEMA, AND HIVES AMONG THEM."

https://www.everydayhealth.com/pictures/shingles-other-skin-conditions

So what could possibly separate these two conditions?

"Shingles is a condition you can develop if you've already had chickenpox. After you recover from chickenpox, THE VIRUS STAYS DORMANT INSIDE YOU. THE VIRUS CAN THEN REAPPEAR YEARS OR DECADES LATER AS SHINGLES."

"Shingles cannot be spread from person to person. SOMEONE WITH SHINGLES CAN SPREAD THE VARICELLA VIRUS TO ANOTHER PERSON, BUT THAT PERSON WOULD GET CHICKENPOX."

https://www.medicinenet.com/difference_between_chickenpox_and_shingles/article.htm

"Varicella-Zoster Virus (VZV)

VZV is a DNA virus and is a member of the herpesvirus group. Like other herpesviruses, VZV PERSISTS IN THE BODY AS A LATENT INFECTION AFTER THE PRIMARY (first) INFECTION; VZV persists in sensory nerve ganglia. PRIMARY INFECTION WITH VZV RESULTS IN VARICELLA. LATENT INFECTION CAN REACTIVATE RESULTING IN HERPES ZOSTER (shingles). The virus has a short survival time in the environment."

https://www.cdc.gov/vaccines/pubs/pinkbook/varicella.html

Ah...so it's dormant "virus" magic that separates the two.

In other words, if you get Chickenpox, you can spread Chickenpox to others but will not get it again afterwards. However, if you do somehow get it again, it is not the Chickenpox that you have, it is Shingles. If you get Shingles, you can not give Shingles to another person, but you can give them Chickenpox. Fortunately, you are immune from Chickenpox but you can become reinfected with Shingles again. This convoluted merry-go-round is all caused by passing around the same "virus."

Confused?

It's really quite simple actually.

Shingles and the Chickenpox are the EXACT SAME DISEASE.

https://docs.google.com/document/d/e/2PACX-1vQX7oznFODVWyJI-1rZpXpy5anoGH6Xza49y-RRQwOMndsHVDZ699F3B71Kq5rziioybyBsb_bfifSZ/pub

THOMAS RIVERS 1923 CHICKENPOX STUDY:

In 1923, Virologist Thomas Rivers (of the revised Koch's Postulates fame) wrote a paper on his attempts to transmit Chickenpox to animals, specifically rabbits. In it, he makes some pretty startling revelations on how Smallpox, Chickenpox, Shingles, and other similar diseases may be all caused by the same "virus" or may even be the same disease. He also outlines the numerous negative attempts by various researchers to transmit Chickenpox to animals and humans. I've tried to highlight the most important points from his 21-page report while again showcasing the horrific torture the animals were put through as well as the numerous outlandish assumptions that were made while trying to prove an invisible "virus" exists:

STUDIES ON VARICELLA.

THE SUSCEPTIBILITY OF RABBITS TO THE VIRUS OF VARICELLA.

"IN SPITE OF THE IGNORANCE REGARDING THE ETIOLOGICAL AGENTS CONCERNED IN THE CONTAGIOUS DISEASES OF CHILDREN, it has been possible, through the careful clinical observations of such men as Sydenham, to differentiate one from the other. Notwithstanding this wide clinical knowledge, it is still impossible to control the spread of these diseases, or to arrest their course once they have established themselves in susceptible individuals. It is true that some progress has been made in the prevention of measles, scarlet fever, and chicken-pox by the introduction of the method of injecting serum from convalescent patients into exposed individuals. ATTEMPTS HAVE ALSO BEEN MADE TO PREVENT CHICKEN-POX BY VACCINATION WITH FRESH VESICLE LYMPH, and while a few workers have reported positive results with this method, THE MAJORITY OF INVESTIGATORS HAVE NOT SUCCEEDED."

VARICELLA IS USUALLY CONSIDERED A MILD DISORDER, but, nevertheless, for many reasons, it has appeared to be a suitable one on which to begin study.

In the first place, chicken-pox, IN SPITE OF ITS MILDNESS, ranks high among the infectious diseases as a cause of loss of time from schools, and is very troublesome in institutions, such as orphan asylums and hospitals for children. Also, KNOWLEDGE CONCERNING CHICKEN-POX WOULD PROBABLY BE DIRECTLY APPLICABLE TO THE MORE SEVERE DISEASE, SMALLPOX. Although this disease no longer prevails to the wide extent that it did in former centuries, nevertheless, it is still common in

certain sections of the United States. According to Low (1), for the last 20 years there has been an average of 20,000 to 30,000 cases of smallpox every year. Recently, in 1 year, over 94,000 cases (2) were reported. Usually it is not difficult to differentiate smallpox from chicken-pox, BUT THERE ARE INSTANCES WHEN IT IS NOT EASY, AND MISTAKES UNDOUBTEDLY OCCUR. Although Paul (3), Tiche (4), Force and Beckwith (5), Salmon (6), and Paschen (7) have introduced certain laboratory methods of aid in diagnosis, THESE ARE NOT ENTIRELY SATISFACTORY. Furthermore, in chicken-pox there is a characteristic skin lesion which may serve as a mark of identification in case the disease were produced in animals. Although the recent work on herpes and lethargic encephalitis has aroused great interest in all diseases in which vesicular lesions on the skin are present, relatively little experimental work has been done on varicella.

THERE ARE OTHER DISEASES, such as sheep-pox, vaccina, smallpox, alastrim, herpes zoster and symptomatic herpes, WITH WHICH IT IS POSSIBLE TO COMPARE CHICKEN-POX. Certain of these diseases have been grouped under such general terms as" ectodermal ses neurotropas" by certain investigators, for instance, Levaditi and Nicolau (😎 and Lipschfitz (9). From the numerous studies on these CLOSELY RELATED DISEASES many facts are available which may be of aid in work undertaken with varicella. Upon first thought there seems to be little connection between sheep-pox and lethargic encephalitis in man. On considering all these diseases in a series, however, (Table I), studying the clinical relations existing between them and taking into consideration the results of the experimental studies which have been made, THE TWO JUST MENTIONED DO NOT SEEM SO FAR REMOVED FROM EACH OTHER.

IT IS NOW GENERALLY BELIEVED THAT CHICKEN-POX AND SMALLPOX ARE DISTINCT AND DIFFERENT DISEASES. THIS HAS NOT ALWAYS BEEN THE CASE, however, and as late as the middle of the 19th century HEBRA (10) TAUGHT THAT THEY WERE IDENTICAL. Even at the present time THERE IS A DIFFERENCE OF OPINION IN REGARD TO THE RELATION OF SMALLPOX AND VARIOLOID TO ALASTRIM on the one hand, and of chicken-pox to alastrim on the other. Von B6kay's (11) paper on the relation of chicken-pox to herpes zoster appeared in 1909, and since then A NUMBER OF OTHER PAPERS HAVE APPEARED IN WHICH THE IDEA THAT THE TWO DISEASES ARE IDENTICAL HAS BEEN SUPPORTED OR OPPOSED. For many years there has been much discussion concerning the interrelationship existing between the various kinds of herpes, and this interest has been stimulated recently by the work of Doerr (12) and others on herpes and lethargic encephalitis. IF SYMPTOMATIC HERPES AND LETHARGIC ENCEPHALITIS ARE CAUSED BY THE SAME ETIOLOGICAL AGENT, THEN THE VIRUSES OF ALL THE DISEASES LISTED IN TABLE I, with the exception of chicken-pox and herpes zoster, HAVE BEEN

RECOVERED IN SOME FORM AMENABLE TO EXPERIMENTATION.

Some attempts to transmit varicella to animals and to normal children have already been made. Salmon (6), Tyzzer (13), and Teissier, Gastinel, and Reilly (14) WERE UNABLE TO OBTAIN A SPECIFIC REACTION ON RABBITS' CORNEAS INOCULATED WITH FRESH FLUIDS FROM VARICELLA VESICLES. Swellengrebel (15) found cellular changes in rabbits' corneas inoculated with vesicle lymph, BUT THESE CHANGES WERE NOT CONSIDERED CHARACTERISTIC OF CHICKEN-POX. Bertarelli (16) and Gins (17), however, reported that, in rabbits' corneas inoculated with fresh vesicle lymph, cellular changes occurred which were specific for varicella and were not present in the controls. Levadifi (18) found Guanieri bodies in rabbits' corneas inoculated either with vaccine virus or with varicella lymph. Park (19), Martin (20), and Tyzzer (13) WERE UNABLE TO TRANSMIT CHICKEN-POX TO MONKEYS. Hess and Unger (21) FAILED TO PRODUCE VARICELLA IN NORMAL CHILDREN by inoculating them upon the mucous membranes of the nose and throat with vesicle lymph and material collected from the nose and throat of patients with chicken-pox, OR BY INOCULATING THEM INTRACUTANEOUSLY, SUBCUTANEOUSLY, OR INTRAVENOUSLY WITH FRESH VESICLE LYMPH.

Many workers have attempted to vaccinate normal children against varicella, using fresh vesicle lymph for this purpose. ALTHOUGH MOST OBSERVERS HAVE REPORTED ONLY NEGATIVE RESULTS, Steiner (22), Kling (23), Lapidus (24), Meyer (25), Gyr (26), Hotzen (27), and others have reported that the inoculations were followed by positive results IN CERTAIN CASES. These investigators state that after such inoculations SOME of the children had MILD CHICKEN-POX WITH NO LOCAL REACTION; in other children a local reaction and a mild chicken-pox occurred; IN OTHERS ONLY A LOCAL REACTION AT THE SITE OF INOCULATION APPEARED; and in still others, OFTEN THE LARGEST GROUP (26, 27), NO REACTION OF ANY KIND WAS NOTICED. All of the writers agree that the vesicle fluid should be collected during the first few days of the eruption and used shortly afterwards. Kling (23) stated that to obtain positive results AT LEAST SIX INOCULATIONS SHOULD BE MADE, AND THAT EVEN THEN ONLY ONE MAY BE SUCCESSFUL. These reports seem to indicate that the virus in the vesicles either is very dilute or has lost its virulence.

In view of the CONFLICTING REPORTS BY VARIOUS INVESTIGATORS who used vesicle fluid in their experimental work ON THE TRANSMISSION OF VARICELLA TO MAN AND TO ANIMALS, it seemed advisable to search elsewhere in the body for the virus of chicken-pox in the hope that thence IT MIGHT BE OBTAINED IN A CONDITION MORE SUITABLE FOR EXPERIMENTAL PURPOSES. Certain observations soon afforded INDIRECT EVIDENCE that the virus occurs in the blood and that the amount

there does not necessarily correspond to the number of skin lesions. It was noticed that IRRITATION SEEMED TO HAVE A DIRECT INFLUENCE on the localization of the virus in the skin as evidenced by the appearance of an unusual number of vesicles at the site of irritation. The picture was particularly striking when the irritation involved the skin of the face and extremities, parts of the body usually least affected by the eruption of chicken-pox.

The effect of irritation on the LOCALIZATION OF THE VIRUS IN THE SKIN HAS BEEN OBSERVED IN 5 OF 51 PATIENTS. A brief summary of these observations follows.

B. K. had adhesive PLASTER APPLIED TO THE ANKLES to alleviate the pain of chronic arthritis. After the patient developed varicella the adhesive was removed. Beneath it were found more vesicles than on all the rest of the body (Fig 1).

B. S and J. M. WORE NAPKINS WHICH IRRITATED THE SKIN and the eruption localized mostly in the irritated areas (Figs. 2 and 3).

J. G. WORE SOFT COLLARS WITH HIS NECKTIE DRAWN VERY TIGHT. Just beneath where the tie rested was a band of vesicles extending around the neck (Fig. 4).

V. S., a young adult, had acne over the face and upper part of the back. THE ERUPTION WAS MOSTLY MARKED OVER THESE AREAS AND ACTUALLY INVOLVED THE ACNE LESIONS.

From reports in the literature it was found that the eruption of many of the exanthemata CAN BE MADE TO LOCALIZE BY IRRITATION, provided the irritant is applied before or shortly after the appearance of the eruption. Von Pirquet (28) and Schick (29) HAVE DEMONSTRATED THIS PHENOMENON IN MEASLES, Heim and John (30) in SCARLET FEVER, Swoboda (31) in CHICKEN-POX, and Hebra (10), TiSche (32), and many others in SMALLPOX."

"THE OBSERVATIONS ON PATIENTS MENTIONED ABOVE INDICATE THAT THE VIRUS OF CHICKEN-POX PROBABLY IS PRESENT IN THE BLOOD, and it was thought that it might be possible to demonstrate the disease in animals by INJECTING

THEM WITH PATIENTS' BLOOD, THEN SHAVING AND IRRITATING THE SKIN. Before proceeding, however, with the experimental work on varicella, it seemed advisable to determine whether there is a practical method of demonstrating vaccine virus in the blood of animals previously inoculated on the skin. The following experiment, therefore, was performed.

Rabbit A was shaved on both sides of the body. THE SKIN WAS SCARIFIED GENTLY AND THEN INOCULATED WITH VACCINE VIRUS. 2 A confluent eruption occurred at the site of inoculation. 4 days after the inoculation the skin over the thorax was dissected back, 3 AND 10 cc. OF BLOOD WERE REMOVED FROM THE HEART. THIS BLOOD WAS INJECTED IMMEDIATELY INTO THE EAR VEIN OF RABBIT B. A SMALL PORTION OF SKIN WAS SHAVED AND SCARIFIED SHORTLY AFTER THE INJECTION. 5 days later several nodules (Fig. 5) appeared in the shaved skin. These developed into typical vaccine pustules. 2 weeks after the lesions had healed the rabbit was vaccinated on the skin and was found to be immune.

Although the above experiment showed that vaccine virus can be recovered from the blood of animals, IT HAS BEEN IMPOSSIBLE TO DEMONSTRATE IN THE SAME WAY THE PRESENCE OF THE VIRUS OF VARICELLA IN THE BLOOD OF PATIENTS. Other methods, then, were sought. In view of the fact that Pasteur (36) was able to grow the virus of rabies in the brain of animals, and that a similar method has recently been employed

successfully in the growth of herpes virus, AN ATTEMPT WAS MADE TO DEMONSTRATE THE VIRUS OF CHICKEN-POX BY INJECTING SMALL QUANTITIES OF FRESH BLOOD AND VESICLE FLUID FROM PATIENTS DIRECTLY INTO THE BRAIN OF YOUNG RABBITS. ONLY NEGATIVE RESULTS WERE OBTAINED WITH THIS METHOD. Then, since the TESTICLES of rabbits have already been shown to be a suitable place for the growth of spirochetes (37, 38), vaccine virus (39), and tumors (40), they were thought of as POSSIBLY BEING A SUITABLE PLACE IN WHICH TO GROW AND CONCENTRATE THE VIRUS OF VARICELLA.

Further evidence of the advantage of using the testicles as a place to cultivate a virus was found in the work of Ohtawara (41). He recently reported that he was able to demonstrate vaccine virus in the blood of rabbits vaccinated on the skin by INJECTING THE BLOOD INTO THE TESTICLES OF NORMAL RABBITS. We have confirmed his work.

RABBITS WERE VACCINATED OVER EXTENSIVE AREAS ON BOTH SIDES OF THE BODY. 4 days later the SKIN OVER THE THORAX WAS DISSECTED BACK, BLOOD WAS REMOVED FROM THE HEART, AND 1 cc. WAS INJECTED INTO EACH TESTICLE OF SEVERAL NORMAL RABBITS. After 4 days the TESTICLES WERE REMOVED, GROUND UP WITH SAND, and tested for the presence of the virus by APPLICATION OF THE EMULSION TO THE SHAVED SKIN OF NORMAL RABBITS. Confluent eruptions were obtained with the testicular material, whereas 1 cc. of blood, similar to that injected into the testicles, CAUSED NO VISIBLE REACTION WHEN GROUND UP AND SMEARED DIRECTLY ON THE SCARIFIED SKIN."

The details of the method employed in recovering a virus from the blood of varicella patients are as follows: Blood was drawn from patients with chicken-pox usually during the first 24 hours after the appearance of the eruption. THE BLOOD WAS NOT CITRATED AND BEFORE CLOTTING OCCURRED WAS INJECTED IN 2 cc. AMOUNTS INTO EACH TESTICLE OF NORMAL RABBITS (1,800 gm.). These large quantities of blood were used intentionally. AT THE TIME OF INOCULATION THE NEEDLE WAS MOVED ABOUT IN THE TISSUES TO PRODUCE A CERTAIN AMOUNT OF TRAUMA. 4 days later the TESTICLES WERE REMOVED, GROUND UP THOROUGHLY WITH STERILE, CHEMICALLY CLEAN SAND, AND MIXED WITH 10 cc. OF PHYSIOLOGICAL SALT SOLUTION. The mixture was allowed to stand until the sand settled to the bottom. Strict asepsis was observed throughout the work. Portions of the testicular emulsion were tested for the presence of ordinary bacteria by means of cultures on blood agar, in broth, and in Smith-Noguchi tubes. Other portions for future use were stored on ice either in the original state, or after the addition of equal quantities

of glycerol. Then 1 cc. of the emulsion was INJECTED INTO EACH TESTICLE OF NORMAL RABBITS. Two areas on the rabbits' skin were shaved and scarified. ONE OF THE AREAS WAS SMEARED WITH THE EMULSION, the other was used as a control. AN EYE OF EACH RABBIT WAS ALSO INOCULATED.

BOTH CORNEAS WERE SCARIFIED WITH A CATARACT KNIFE (cocaine anesthesia was always used); ONE WAS INOCULATED WITH THE TESTICULAR EMULSION, the other was used as a control. THE FIRST FEW ANIMALS IN EACH SERIES SHOWED LITTLE REACTION OTHER THAN THAT WHICH MIGHT BE EXPECTED TO FOLLOW THE TRAUMA OF THE INOCULATIONS. The skin and cornea healed rapidly. The scrotum was edematous at times for 24 to 48 hours. The testicles, when removed, were

slightly swollen. Necrotic areas

and often remains of the material injected studded the tissue in various places. THIS REACTION WAS NO MORE STRIKING THAN THAT CAUSED BY THE INJECTION OF AN EMULSION OF NORMAL TESTICLES. The first few rabbits in each series and all the rabbits in the series from which no virus was recovered served as excellent controls for the work. AFTER FOUR TO EIGHT TRANSFERS OF THE VIRUS FROM RABBIT TO RABBIT, however, IN CERTAIN SERIES, the testicles became tense and finn on the 3rd or 4th day after the inoculation, and the scrotum often remained edematous. In the inoculated eye a roughness of the cornea and a circumcorneal redness appeared on the 3rd or 4th day following the inoculation. An erythema and swelling not present in the control occurred along the lines of scarification in the skin 4 to 6 days after the inoculation (Fig. 6). The testicles, when removed from the animals, were usually swollen and hemorrhagic. Later, when the virus became more concentrated, the inoculated animals looked sick, refused to eat, lost weight, and occasionally developed diarrhea. At first the temperatures of the rabbits were not elevated. Later, however, it was not unusual for the rabbits to have temperatures of 106-107°F. on the 3rd or 4th day after the inoculation. Temperatures of 104 ° and 105 ° occurred frequently (Text-figs. 1 to 5). Occasionally, in addition to the reaction in the skin inoculated with the testicular emulsion, discrete, papular lesions appeared 5 to 11 days after the inoculation in the control areas (Fig. 7). This phenomenon SEEMED TO INDICATE that the virus invaded the blood stream of the rabbits and became localized in the irritated skin. INVASION OF THE BLOOD STREAM WAS THEN DEMONSTRATED BY REMOVING BLOOD FROM THESE RABBITS AND INJECTING IT INTO THE TESTICLES OF OTHER RABBITS (Text-figs. 1, 2, b, and 5, b).

BLOOD FROM ELEVEN PATIENTS with varicella was injected into the testicles of rabbits and transplants from each were made through at least eight rabbits. IT WAS POSSIBLE in this way TO RECOVER A VIRUS FROM FIVE OF THE PATIENTS."

"It was in the fourth rabbit of the series that a reaction was first noticed, in the eye' skin, and testicles, more severe than that USUALLY CAUSED BY THE TRAUMA OF INOCULATION."

"A WHOLE TESTICLE from Rabbit 4 was stored 29 days on ice in 50 per cent glycerol. THEN IT WAS GROUND UP AND PASSED THROUGH RABBITS IN THE USUAL WAY, and while the virus was still active, its virulence had decreased to such an extent that THREE TESTICULAR PASSAGES WERE NECESSARY BEFORE IT CAUSED A

VISIBLE REACTION IN THE SKIN."

"POSITIVE RESULTS WERE NOT ALWAYS OBTAINED IN THE SKIN, EVEN WHEN THERE WERE REASONS TO JUSTIFY A BELIEF THAT THE VIRUS WAS PRESENT IN THE MATERIAL USED FOR THE INOCULATION. Success seemed dependent in part at least upon the condition of the rabbits, the texture of the skin, and the depth of the scarifications. The best results were obtained when the rabbits were healthy and fat, when the skin was rather thick and firm, when the scarifications were deep, but not quite deep enough to cause bleeding, and when the virus was rubbed in thoroughly."

"THE PATHOLOGICAL CHANGES IN THE TESTICLES WERE DISREGARDED AT FIRST BECAUSE OF THE AMOUNT OF MATERIAL INJECTED EACH TIME. Large quantities were necessary to adapt the virus to the animals."

"In contrast to the non-specific lesions, certain other lesions occurred in the uninoculated shaved skin of rabbits into the testicles of which Virus III was injected. These lesions, red macules and papules, appeared 5 to 11 days after the inoculation, disappeared rapidly, and WERE INTERPRETED BY US as the result of a general infection with the virus."

"IT PROVED IMPOSSIBLE TO PREDICT WITH REGULARITY WHETHER A VISIBLE REACTION WOULD OCCUR IN ANY INDIVIDUAL RABBIT EVEN WHEN A CONCENTRATED VIRUS WAS SMEARED ON THE SCARIFIED SKIN. This, to a certain extent, interfered with the demonstration of an immunity in recovered

animals and with the determination of the effect of various temperatures on the virus."

"**DISCUSSION**.

A great deal has been written about the identity of chicken-pox and herpes zoster. Parounagian and Goodman (42) believe that SOME OF THE CASES OF VARICELLA AND HERPES ZOSTER REPORTEDLY AS OCCURRING SIMULTANEOUSLY IN THE SAME PATIENT are only instances of generalized herpes zoster. From observations given in the present paper IT SEEMS AT LEAST POSSIBLE that certain of the cases may be instances of the localization of the virus of varicella in areas of irritated skin in such a way as to produce zoster-like lesions. THOUGH HERPES ZOSTER AND

CHICKEN-POX MAY POSSIBLY BE IDENTICAL, convincing proof is lacking, in spite of de Lange's (43) work on complement fixation in zoster and varicella. Tiche (32) reported that irritation causes herpetiform lesions in smallpox and modified smallpox, and that such an occurrence is rare in chicken-pox. He emphasized this point in the differential diagnosis of the diseases. ONE SHOULD BE CAREFUL, HOWEVER, NOT TO PLACE TOO MUCH DEPENDENCE IN SUCH A STATEMENT, AS IN 10 PERCENT OF OUR PATIENTS WITH CHICKEN-POX IRRITATION OF THE SKIN CAUSED A LOCALIZATION OF THE VIRUS IN THE AFFECTED AREAS.

In working with MATERIAL SUPPOSED TO CONTAIN A VIRUS WHICH IS INVISIBLE, or not recognizable, and about which practically nothing is known experimentally, ONE CAN EASILY BE MISTAKEN ABOUT THE IDENTITY OF THE VIRUS OR EVEN IN REGARD TO ITS PRESENCE. In spite of these difficulties we feel warranted in saying that a virus has been recovered from patients with varicella under the conditions outlined, and that the REACTIONS OBSERVED IN RABBITS ARE MORE THAN THE RESULTS OF A NONSPECIFIC IRRITATION. FURTHER EVIDENCE MUST BE OBTAINED, however, before one can think and speak definitely of this virus AS THE ETIOLOGICAL AGENT OF VARICELLA. The ultimate proof depends upon the type of reaction this virus will cause in man and upon the possibility of protecting against varicella by inoculations of the virus.

CONCLUSIONS.

1. The localization of the virus of varicella in the human skin is INFLUENCED BY IRRITATION. This is INDIRECT EVIDENCE that the virus is in the blood.

2. Rabbits are susceptible to a virus recovered from the blood of varicella patients.

3. Testicular emulsions containing the virus are free from ordinary aerobic and anaerobic bacteria, and produce lesions in the cornea, skin, and testicles of rabbits.

4. The virus can be transmitted indefinitely from rabbit to rabbit by means of testicular inoculation, and can be preserved 29 days in 50 per cent glycerol at a low temperature."

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In Summary:

-Rivers admits to a current IGNORANCE regarding the etiological agents concerned in the contagious diseases of children

-he states attempts have also been made to prevent chicken-pox by vaccination with fresh vesicle lymph and that the majority of investigators have NOT SUCCEEDED

-he also admits that varicella is usually considered a mild disorder

-somehow, even though he claims they are separate diseases, Rivers believes knowledge concerning chicken-pox would probably be DIRECTLY APPLICABLE to the more severe disease Smallpox

-it's probably because Rivers admits that there are instances when it is not easy to differentiate Smallpox and Chickenpox and that mistakes undoubtedly occur

-certain laboratory methods of aid in diagnosis have been utilized but these are not entirely satisfactory

-he states that there are other closely related diseases, such as sheep-pox, vaccina, smallpox, alastrim, herpes zoster and symptomatic herpes, with which it is possible to compare chicken-pox

-Rivers states that it is now GENERALLY BELIEVED that chicken-pox and smallpox are distinct and different diseases but that this has not always been the case

-in the late 19th century, Hebra taught that they were identical

-a number of other papers regarding Shingles/Chickenpox have appeared in which the

idea that the two diseases are identical has been supported or opposed

-if symptomatic herpes and lethargic encephalitis are caused by the SAME ETIOLOGICAL AGENT, then the viruses of all the diseases listed in Table I, with the exception of chicken-pox and herpes zoster, have been recovered in some form amenable to experimentation (table 1 includes: Sheep-pox, Cow-pox, Horse-pox, Smallpox, Varioloid, Alastrhn, Symptomatic herpes, Lethargic encephalitis)

-Salmon, Tyzzer, and Teissier, Gastinel, and Reilly were UNABLE to obtain a specific reaction on rabbits' corneas inoculated with fresh fluid from varicella vesicles

-Swellengrebel found cellular changes in rabbits' corneas inoculated with vesicle lymph, but these changes were NOT CONSIDERED CHARACTERISTIC OF CHICKEN-POX

-Park, Martin, and Tyzzer were UNABLE to transmit chicken-pox to monkeys

-Hess and Unger FAILED TO PRODUCE VARICELLA IN NORMAL CHILDREN by inoculating them upon the mucous membranes of the nose and throat with vesicle lymph and material collected from the nose and throat of patients with chicken-pox, OR BY INOCULATING THEM INTRACUTANEOUSLY, SUBCUTANEOUSLY, or INTRAVENOUSLY WITH FRESH VESICLE LYMPH

-many workers attempted to vaccinate normal children against varicella using fresh vesicle lymph and most reported ONLY NEGATIVE RESULTS

-after inoculations, some of the children had mild chicken-pox with no local reaction; in other children a local reaction and a mild chicken-pox occurred; in others only a local reaction at the site of inoculation appeared; and in still others, OFTEN THE LARGEST GROUP, NO REACTION OF ANY KIND was noticed

-Kling stated that to obtain positive results AT LEAST SIX INOCULATIONS should be made, and that even then ONLY ONE MAY BE SUCCESSFUL

-due to the conflicting reports by various investigators who used vesicle fluid in their experimental work on the transmission of varicella to man and to animals, they decided to search elsewhere in the body to find the "virus" in the hope that it MIGHT be obtained in a condition more suitable for experimental purposes

-some observations showed INDIRECT evidence that the "virus" occurs in the blood and that the amount there does not necessarily correspond to the number of skin lesions

-they decided that at sites of skin irritation, more "virus" was likely present

-this effect was only seen in 5 of 51 patients

-in one young adult, the "Chickenpox" lesions appeared mostly with acne

-they found literature stating that irritation to the skin causes localization of eruptions

-Von Pirquet and Schick demonstrated this phenomenon in measles, Heim and John in scarlet fever, Swoboda in chicken-pox, and Hebra, TiSche, and many others in smallpox

-the observations on patients mentioned above indicated to them that the "virus" was PROBABLY present in the blood, and it was thought that it might be possible to demonstrate the disease in animals by injecting them with patients' blood and then shaving and irritating the skin

-rabbit A was shaved, skin scarified, inoculated with "virus," and had blood drained from its heart which was then injected into the ear of rabbit B which was also shaved and scarified

-while they claim this method was successful for showing vaccine "virus" in the blood of animals, it was IMPOSSIBLE to demonstrate in the same way the presence of the

"virus" of varicella in the blood of patients

-attempts were made to demonstrate the "virus" of chicken-pox by injecting small quantities of fresh blood and vesicle fluid from patients DIRECTLY INTO THE BRAIN of young rabbits

-only negative results were obtained with this method, thus they decided to try rabbit TESTICLES as a suitable place to grow "virus"

-rabbits were VACCINATED OVER EXTENSIVE AREAS ON BOTH SIDES of the body and 4 days later the skin over the thorax was dissected back, blood was removed from the heart, and 1 cc. was injected into each testicle of several normal rabbits

-after 4 days the testicles were removed, GROUND UP WITH SAND, and tested for the presence of the "virus" by application of the emulsion to the shaved skin of normal rabbits

-confluent eruptions were obtained with the testicular material, whereas 1 cc. of blood, similar to that injected into the testicles, CAUSED NO VISIBLE REACTION when ground up and smeared directly on the scarified skin

-the procedure they used is as follows:

*blood was drawn from patients with chicken-pox usually during the first 24 hours after the appearance of the eruption

*blood was not citrated and before clotting occurred was injected in 2 cc. amounts into each testicle of normal rabbits (1,800 gm.) *these large quantities of blood were used intentionally

*at the time of inoculation the NEEDLE WAS MOVED about in the tissues TO

PRODUCE A CERTAIN AMOUNT OF TRAUMA

*4 days later the testicles were removed, ground up thoroughly with sterile, chemically clean sand, and mixed with 10 cc. of physiological salt solution

*the emulsion was injected into the testicles of normal rabbits and smeared on their scarified skin

*an eye of each rabbit was also injected with the testicle emulsion

-BOTH corneas were scarified with a cataract knife (but don't worry: cocaine anesthesia was always used .....)

-the first few animals in each series showed LITTLE REACTION other than that which might be expected to follow the TRAUMA OF THE INOCULATIONS

-reactions were no more striking than that caused by the injection of an emulsion of normal testicles

-after FOUR TO EIGHT TRANSFERS of the "virus" from rabbit to rabbit, however, IN CERTAIN SERIES, the testicles became tense and finn on the 3rd or 4th day after the inoculation, and the scrotum often remained edematous

-OCCASIONALLY, in addition to the reaction in the skin inoculated with the testicular emulsion, discrete, papular lesions appeared 5 to 11 days after the inoculation in the control areas

-this phenomenon SEEMED TO INDICATE that the "virus" invaded the blood stream of the rabbits and became localized in the irritated skin

-invasion of the blood stream was said to be demonstrated by removing blood from these rabbits and injecting it into the testicles of other rabbits

-blood from 11 patients was injected into 8 rabbits and they claimed "virus" was recovered from 5 of the 11 patients

-a whole testicle from a rabbit was ground up and passed around in the "usual way" yet it took at least 3 passages between rabbits to create a visible reaction

-positive results WERE NOT ALWAYS OBTAINED in the skin, even when there were REASONS TO JUSTIFY A BELIEF THAT THE "VIRUS" WAS PRESENT in the material used for the inoculation

-the pathological changes in the testicles were DISREGARDED AT FIRST because of the LARGE amount of material injected each time

-at times lesions, red macules and papules, appeared 5 to 11 days after the inoculation, disappeared rapidly, and WERE INTERPRETED BY THEM as the result of a general infection with the "virus"

-It proved IMPOSSIBLE TO PREDICT WITH REGULARITY WHETHER A VISIBLE REACTION WOULD OCCUR in any individual rabbit even when a concentrated "virus" was smeared on the scarified skin

-Parounagian and Goodman believe that some of the cases of varicella and herpes zoster REPORTED AS OCCURRING SIMULTANEOUSLY in the same patient are only instances of generalized herpes zoster

-River states that even though he felt the evidence lacking, herpes zoster and chicken-pox may possibly be IDENTICAL

-he admits that in working with MATERIAL SUPPOSED TO CONTAIN A "VIRUS" WHICH IS INVISIBLE, or not recognizable, and about which practically nothing is known experimentally, ONE CAN EASILY BE MISTAKEN ABOUT THE IDENTITY OF THE "VIRUS" OR EVEN IN REGARD TO ITS PRESENCE

-he felt the reactions observed in rabbits are more than the results of a NONSPECIFIC IRRITATION, however further evidence must be obtained before one can think and speak definitely of this "virus" as the etiological agent of varicella

Once again, it is clear to see that the assumption is made that a "virus" is in the lab-created material. Results were consistently negative which led to ridiculous and grotesquely inhumane attempts to create the condition they wanted to see experimentally in rabbits. Rivers' work is proof that these "scientists" did everything possible to create the desired effect whilst throwing logic out the window in order to do so. Had they thought logically and critically, they would have seen that it was not an unseen invisible "virus" creating these occasional lesions and sickness in the rabbits, it was the severe trauma they were repeatedly exposed to throughout these horrific experiments.

https://docs.google.com/document/d/e/2PACX-1vTkVpSO9m5AD92bTotttwOcFIJGooQGt7psIcL3W703pYxbl0xyx_STwWQYpsMh6f-X-1dS9URcoqLa/pub

CHICKENPOX/SHINGLES: NO TRANSMISSION

In 1925, Rufus Cole, M.D., and Ann G. Kuttner, PH.D wrote a pretty scathing review on the evidence (or lack thereof) for the successful transmission of herpes zoster to both animal and humans. They felt compelled to not only share their own negative experiments but to also analyze and critique the work of others due to the growing body of work coming out claiming successful transmission yet lacking solid proof. The report is 22 pages long so obviously I could not copy/paste the whole thing here, but I tried to highlight most of the pertinent sections. I also highlighted many of the inhumane and

grotesque experiments the unfortunate animals were subjected to all in the name of "science." There is much I had to leave out so I highly recommend reading the whole report when you have the time. I provided a summary at the end:

THE PROBLEM OF THE ETIOLOGY OF HERPES ZOSTER.

"THE NATURE AND ETIOLOGY of that group of infectious diseases of which ONE OF THE FEATURES IS A VESICULAR ERUPTION ON THE SKIN ARE at the present time MUCH CONFUSED. RIVERS (1) has constructed a table indicating a POSSIBLE RELATIONSHIP BETWEEN A SERIES OF THESE DISEASES beginning with SHEEP-POX and HORSE-POX and extending through COW-POX, SMALLPOX, VARIOLOID, ALASTRIM, CHICKEN-POX, and HERPES ZOSTER to SYMPTOMATIC HERPES and LETHARGIC ENCEPHALITIS. CERTAIN OF THESE CONDITIONS RESEMBLE EACH OTHER IN THEIR CLINICAL MANIFESTATIONS, others have little in common. Certainly, the symptoms of herpes simplex have little resemblance to those of smallpox. The only FEATURE PRESENT IN ALL OF THEM, except lethargic encephalitis, is a VESICULAR ERUPTION OF THE SKIN. In most of the conditions the skin lesions SHOW SIMILAR HISTOLOGICAL CHARACTERISTICS.

At one time or another SOME RELATIONSHIP IN ETIOLOGY between various members of the group, OR EVEN AN IDENTICAL ETIOLOGY IN ALL OF THEM HAS BEEN SUGGESTED. IN NONE OF THE CONDITIONS HAS THE ECOLOGICAL AGENT BEEN CULTIVATED, but there is considerable evidence that the responsible agent in most of them is ultramicroscopic or filterable. IT IS EVIDENT THAT IN THE ABSENCE OF CULTIVATION, in order to establish the etiological relation of an ultramicroscopic virus with one of these diseases, IT IS NECESSARY TO REPRODUCE, IN ANIMALS OR MAN, LESIONS RESEMBLING THE NATURAL INFECTION. With certain of these diseases, notably smallpox and vaccinia, the EXPERIMENTAL REPRODUCTION of the disease is comparatively easy. WITH OTHERS, SUCH AS VARICELLA AND HERPES ZOSTER, ALL ATTEMPTS TO TRANSMIT THEM TO ANIMALS HAVE LED ONLY TO EQUIVOCAL AND UNCERTAIN RESULTS. In the case of HERPES SIMPLEX, although a virus has been isolated which is highly infectious for rabbits, THE CLINICAL PICTURE PRODUCED IS NOT, as will be discussed below, IDENTICAL WITH HERPES SIMPLEX IN MAN.

"In the study of diseases of this group, the finding of these characteristic nuclear changes in experimental lesions in animals or man is therefore of importance in determining whether the reaction obtained is specific. Following the observation by

Gfiiter (6) in 1920 and those of Lowenstein (7, 😎 investigators in all parts of

the world have demonstrated that THE VIRUS OF HERPES SIMPLEX, WHEN INOCULATED INTO THE SCARIFIED CORNEA OF RABBITS PRODUCES WITH GREAT REGULARITY A VESICULAR ERUPTION FOLLOWED BY AN INTENSE KERATOCONJUNCTIVITIS. INOCULATIONS INTO THE SKIN LESS FREQUENTLY GIVE RISE TO LESIONS. As Doerr and Vochting (9) first observed, corneal inoculations are frequently followed by marked nervous symptoms and death and SIMILAR SYMPTOMS CAN BE PRODUCED BY DIRECT INOCULATION INTO THE BRAIN. The inoculations of the virus into rabbits, therefore, gives rise to lesions which may resemble those seen in man, BUT IN MOST CASES THE LESIONS AND SYMPTOMS DIFFER BOTH IN CHARACTER AND SEVERITY FROM THOSE PRESENT IN THE MILD AND COMMON CONDITION IN HUMAN BEINGS KNOWN AS HERPES SIMPLEX. It is of importance, however, that in all the lesions produced in animals, including those in the cornea, the skin, and the brain, the most characteristic feature of the lesion of herpes simplex in man is reproduced; namely, the occurrence of ceils containing intranuclear inclusion bodies. THE INTRACUTANEOUS INOCULATION OF THE VEHICULAR FLUID OF HERPES SIMPLEX EITHER INTO AN INDIVIDUAL ALREADY INFECTED WITH HERPES SIMPLEX OR INTO A NORMAL PERSON HAS NOT GIVEN AS CONSTANT RESULTS as has the inoculation of this fluid into the rabbit's cornea. MAN'S SUSCEPTIBILITY TO HERPES SIMPLEX SEEMS TO DEPEND ON CERTAIN SECONDARY FACTORS WHICH ARE, at the present time, UNKNOWN.``

The successful inoculation of rabbits with herpes simplex material was followed by ATTEMPTS TO TRANSMIT VARICELLA TO ANIMALS, BUT SO FAR THESE ATTEMPTS HAVE NOT BEEN SUCCESSFUL. (For a review of the literature, see Rivers and Tillett (1).) Many attempts have been made to reproduce varicella in man by inoculating material from active cases into normal individuals. Kling (10) reported the successful vaccination of children against chicken-pox by inoculation with vesicular fluid. Certain later observers employing the method of Kling have noted the development of a local vesicle or papule at the site of inoculation, others have described the occurrence of a generalized eruption (TRUE CHICKEN-POX?), OTHERS HAVE STATED THAT NO OBVIOUS LESIONS RESULT FROM THE INOCULATION. So far as we are aware no histological study has been made of any of the lesions described. THE DIFFICULTY IN SUCCESSFULLY INOCULATING ANIMALS OR MAN WITH VARICELLA VIRUS IS OF INTEREST in view of the claims which have been made regarding the identity of the viruses of chicken-pox and herpes zoster."

Inoculation of Virus of Herpes Zoster into Animals.

Prior to 1921, ATTEMPTS TO INOCULATE ANIMALS WITH HERPES ZOSTER HAD PROVED NEGATIVE. In this year Lipschtitz (3) reported successful results. Seven cases which were apparently typical as regards clinical manifestations were studied. THE VESICULAR FLUID was obtained early in the disease and was RUBBED INTO THE SCARIFIED CORNEA OF RABBITS. In certain instances the fluid was combined with the "roofs" of vesicles. Lipschiitz considers that positive results were obtained with the material from four cases. In view of the importance of his conclusions a brief review of his cases will be given.

Case 1.--It is stated that the INOCULATION OF THE MATERIAL INTO THE RABBIT'S EYE was followed in 4 days by a slight opacity of the cornea along the lines of scarification. The eye was removed and sections were made through the cornea. Occasional giant epithelial cells were present along the lines of scarification. Under Bowman's membrane hypertrophied and swollen connective tissue cells were seen but no leukocytes. In the nuclei of the epithelial cells and also of the swollen connective tissue cells there were to be seen occasional, typical, round, sharply circumscribed and clearly demonstrable intranuclear inclusion bodies.

Cases 2 and 3.--These were also early cases and material from them was SIMILARLY INOCULATED INTO THE EYES OF FOUR RABBITS. In one of the rabbits after 4 days the cornea showed an intense circumscribed keratitis, with the appearance of a slightly elevated vesicle "eitrig getrieben," and "daherweiszlich" appearing vesicle. The microscopic examination of this eye showed marked infiltration with pus cells. NO INCLUSION BODIES WERE FOUND. In another animal a keratitis developed, BUT THE OCCURRENCE OF VESICLES OR THE PRESENCE OF INCLUSION BODIES IS NOT NOTED. THE RESULTS IN THE OTHER TWO ANIMALS WERE NEGATIVE. In these two cases, therefore, THE EVIDENCE PRESENTED IN THE PROTOCOLS WHICH INDICATES POSITIVE RESULTS IS VERY SLIGHT.

Case 4.--This was also a typical early case of herpes zoster. VESICULAR MATERIAL from this case WAS INOCULATED INTO THE EYES OF TWO RABBITS AND TWO GUINEA PIGS. THE RESULTS in the two guinea pigs and in one of the rabbits WERE NEGATIVE. In the second rabbit the inoculated eye showed on the 2nd day conjunctivitis and circumscribed corneal infiltration. The eye was enucleated on the 4th day and in microscopical sections very numerous intranuclear inclusion bodies were found in the epithelial cells.

THE REMAINING THREE CASES WERE STUDIED AT LATER PERIODS OF THE DISEASE AND THE RESULTS WERE NEGATIVE.

The experiments of Lipschtitz with material from these seven cases can, therefore, as judged from his brief protocols, BE CONSIDERED TO HAVE YIELDED POSITIVE RESULTS IN ONLY TWO ANIMALS, and in these instances the results are of importance chiefly on account of the presence of intranuclear inclusion bodies in the epithelial cells.

Lipschiitz (11) considers that his positive findings have been confirmed by Marinesco and Draganesco, Truffi, Mariani, and Blanc and Caminopetros, and that the successful transmission of herpes zoster to animals has thus been accomplished. It is therefore important to review in some detail the reports of these investigators.

Marinesco (12), and Marinesco and Draganesco (13), injected material from three cases of herpes zoster.

Case 1.--Herpes zoster localized on the thigh. VESICULAR FLUID WAS INOCULATED INTO THE SCARIFIED CORNEAS OF FOUR SMALL RABBITS, AND INTO THE SECOND CERVICAL GANGLION OF TWO SMALL CATS. THE RABBITS ALL REMAINED UNAFFECTED. The ganglia of the cats were examined after 7 days and in one of them there was evident lymphatic infiltration and atrophy of the neurons.

Case 2.--Herpes zoster of the first and second branches of the trigeminus nerve. SINCE THE VESICLES CONTAINED BUT LITTLE FLUID, SPINAL FLUID OBTAINED ON THE 6th DAY of THE DISEASE WAS USED FOR INOCULATION. THE INJECTIONS WERE MADE INTO THE ANTERIOR CHAMBER OF ONE EYE, AND INTO THE SCARIFIED CORNEA OF THE OTHER EYE IN EACH OF NINE RABBITS. Moreover, in five of these nine rabbits, in addition to the eye inoculations, 0.2 cc. OF SPINAL FLUID WAS INOCULATED INTRACEREBRALLY. The eyes of the first three rabbits SHOWED ONLY INJECTION DUE TO INJURY. On the 4th day, in Rabbit 4, in the cornea of the eye in which the injection was made into the anterior chamber, there was noted a zone of infiltration. Rabbit 5 showed two points of infiltration on the scarified cornea. RABBIT'S 6, 8, AND 9 WERE NEGATIVE. Rabbit 7, besides a febrile reaction on the 4th day, developed an area of infiltration reaching the center of the pupil. THE EMULSIFIED BRAIN AND CEREBELLUM OF THIS RABBIT WERE INOCULATED INTO THE CORNEA OF FOUR MORE RABBITS, two of which showed on the 3rd and 4th days

grayish infiltration along the lines of scarification. No statement is made concerning microscopic examination.

So far as can be judged from the protocols, therefore, INOCULATIONS MADE WITH THE MATERIAL FROM THESE TWO CASES PRODUCED NO CHARACTERISTIC LESIONS. A macroscopic infiltration of the cornea can hardly be regarded as specific.

Case 3.--Herpes zoster lesions on the thigh. VESICULAR FLUID WAS INOCULATED INTO THE SCARIFIED CORNEA OF THREE RABBITS, two of which on the 4th day showed a linear infiltration. The writer states (12) that the sections of the cornea of one of these rabbits showed swollen, edematous cells, and that here and there could be seen the " specific nuclear lesion, consisting in atrophy of the chromatin which is pushed toward the membrane, while the acidophilic mass, which has developed, offers a striking resemblance to the inclusions described by Lipschiitz in animals injected with herpes." IT IS UNDOUBTEDLY ON THIS LAST STATEMENT THAT LIPSCHTITZ BASES THE VIEW THAT HIS OWN OBSERVATIONS HAVE BEEN CONFIRMED BY MARINESCO AND DRAGANESCO. CERTAINLY NOTHING ELSE IN THE PROTOCOLS INDICATES THE OCCURRENCE OF A SPECIFIC LESION.

Truffi (14) studied three cases of herpes zoster. The results in the first two were negative. Vesicular fluid from the lesion of Case 3, cervicobrachial in distribution, was obtained on the 3rd day of the disease and inoculated into the scarified cornea of one rabbit. After 48 hours a slight opacity along some of the lines of scarification, and an intense conjunctivitis were noted. The corneal opacity disappeared rapidly and the eye regained its normal appearance by the 7th day. 22 days after inoculation the rabbit showed symptoms of encephalitis and was killed 10 days later. The microscopical examination of the brain was negative. The presence of intranuclear inclusion bodies in the brain cells is not noted. THE INOCULATION OF THE BRAIN EMULSION INTO THE SCARIFIED CORNEAS OF TWO RABBITS AND TWO GUINEA PIGS FAILED TO PRODUCE LESIONS.

MOST OF THE ATTEMPTS MADE BY MARIANI (15) TO INOCULATE THE CORNEA OF RABBITS WITH HERPES ZOSTER RESULTED NEGATIVELY. In one instance he obtained a very acute keratoconjunctivitis with hypopyon and purulent ophthalmia. In only one case did there result a keratitis which he was able to transmit in series. The lesion produced was clinically and symptomatically very similar to the keratitis produced by herpes simplex virus. No description of the case of herpes zoster from which the material for inoculation was obtained, is given. No statement concerning microscopic

examination of the corneas is made. MARIANI HIMSELF CONSIDERS THIS SINGLE EXPERIMENT INCONCLUSIVE.

MATERIAL FROM NINE CASES OF HERPES ZOSTER WAS INOCULATED by Blanc and Caminopetros (16) INTO THE EYES, CORNEA, CONJUNCTIVA, SKIN, BRAIN, AND SPINAL CORD OF A SERIES OF ANIMALS, including rabbits, mice, sheep, pigeons, monkeys, and a dog. Three monkeys (Macacus rhesus) were inoculated as follows: one into the eye, one into the spinal canal, and the third into the skin of the thoracic region which had

previously been shaved and excoriated. THE INOCULATIONS IN THE FIRST TWO MONKEYS RESULTED NEGATIVELY. The third monkey showed a slight inflammatory reaction at the site of inoculation but recovered WITHOUT THE APPEARANCE OF VESICLES. ALL THE OTHER ANIMALS EXPERIMENTS GAVE NEGATIVE RESULTS with the following exceptions; two rabbits developed a late paralysis which, however, the authors considered was probably not specific, and one rabbit and one sheep, both inoculated with material from the same case, developed a definite keratitis, which spread from the point of inoculation. THE WRITERS THINK THAT THIS LESION MIGHT EASILY BE INTERPRETED AS A REACTION RESULTING FROM THE INJECTION. THEY CONCLUDED THAT THE PROBLEM OF THE TRANSMISSION OF HERPES ZOSTER TO ANIMALS REMAINS OPEN AND THEY APPARENTLY CONSIDER THEIR OWN EXPERIMENTS NEGATIVE OR INCONCLUSIVE.

Meineri (17) claims to have produced encephalitis in a guinea pig by the intracerebral inoculation of vesicle fluid from a case of herpes zoster. A CAREFUL ANALYSIS OF HIS EXPERIMENTS, HOWEVER, IN OUR OPINION, INDICATES THAT HIS FINDINGS CAN BEST BE INTERPRETED AS THE RESULT OF TRAUMA. The writer also injected vesicle fluid obtained on the 3rd day of the disease from one of his cases of herpes zoster into the skin of the arm of the patient and into the skin of a normal man. THESE INJECTIONS IN BOTH INSTANCES WERE WITHOUT VISIBLE RESULT.

THE REVIEW OF THE PUBLICATIONS OF THOSE WRITERS WHOM LIPSCHTITZ QUOTES AS HAVING CONFIRMED HIS WORK SHOWS THAT TWO OF THE WRITERS REGARD THEIR OWN RESULTS AS INCONCLUSIVE. Only Marinesco and Draganesco found microscopic lesions which might be interpreted as specific.

On the other hand, MANY OTHER AUTHORS REPORT ENTIRELY NEGATIVE

RESULTS FOLLOWING THE INOCULATION OF HERPES ZOSTER MATERIAL INTO THE SACRIFICED CORNEAS OF RABBITS: Kraupa (18); Baum (19); LSwenstein (😎, Teissier, Gastinel, and Reilly (20) ; Kooy (21) ; Netter and Urbain

(22); Bloch and Terris (23); Simon and Scott (24); and Doerr (25).

It is evident, therefore, that the results of ATTEMPTS TO INOCULATE ANIMALS WITH MATERIAL FROM CASES OF HERPES ZOSTER MUST BE CONSIDERED AT PRESENT TO BE INCONCLUSIVE.

Herpes Simplex and Herpes Zoster.

ALTHOUGH IT HAS NOT BEEN POSSIBLE TO DEMONSTRATE CONCLUSIVELY ANY SPECIFIC VIRUS ASSOCIATED WITH HERPES ZOSTER, certain writers have presented evidence which suggests that, in certain cases at least, the symptoms and lesions of herpes zoster may result from the presence of the virus of herpes simplex.

Luger and Lauda (26) have published several papers on the PROBLEM OF THE ETIOLOGY OF HERPES ZOSTER. In their first paper they give the results obtained by inoculation with material from seven cases of typical herpes zoster, employing the technique used by Lipschfitz. IN NONE OF THE EYES INOCULATED DID ANY MACROSCOPIC REACTION OCCUR. On microscopical examination there was found fairly regularly

edematous swelling of the epithelial cells, giant cell formation, and "baUonierende" degeneration, but IN NO INSTANCES WERE CELL INCLUSIONS OR CHARACTERISTIC CHANGES OF THE NUCLEI SEEN. THEY THEMSELVES CONSIDERED THE RESULTS IN THIS SERIES OF EXPERIMENTS NEGATIVE."

"Griiter (27) inoculated material from three cases of herpes zoster into the scarified corneas of rabbits. A mild keratitis resulted. No detailed description of the lesion or results of microscopic examination are given. Griiter, however, believes the lesion obtained was specific and attributes it to herpes simplex virus of a low grade of virulence. HE STATES THAT THERE IS NO EVIDENCE FOR ASSUMING A SPECIFIC

VIRUS FOR HERPES ZOSTER. THE DATA PRESENTED, however, ARE NOT SUFFICIENT TO ESTABLISH THE ISOLATION OF A TRUE HERPES SIMPLEX VIRUS FROM THESE CASES."

"Bastai and Busacca (28), in a general article on herpes, state that they inoculated material from three cases of herpes zoster into the cornea of rabbits and into the cornea of one monkey (Macacus). Rabbits were also inoculated intracerebrally. NONE OF THE ANIMALS SHOWED ANY REACTION, with the exception of one rabbit which developed a slight keratitis. No attempts were made to transmit this lesion, and no microscopic examinations are reported. These authors also are of the opinion that herpes zoster is probably a manifestation of infection with herpes simplex virus. THE EXPERIMENTAL DATA PRESENTED, HOWEVER, ARE HARDLY SUFFICIENT TO JUSTIFY THIS POINT OF VIEW."

"Teague and Goodpasture (30) were ABLE TO PRODUCE ZOSTER-LIKE LESIONS in the skin of rabbits and guinea pigs BY THE INOCULATION OF HERPES SIMPLEX VIRUS into areas of the skin previously treated with coal tar. The study of the corresponding posterior root ganglia showed lesions comparable to those found in man in the ganglia innervating the area of zonal eruption. THE WRITERS DO NOT MAINTAIN THAT THEY HAVE REPRODUCED THE HUMAN DISEASE HERPES ZOSTER IN ANIMALS, but they believe there is a close analogy between the EXPERIMENTAL CONDITIONS PRODUCED BY THEM and true herpes zoster."

"The interesting hypothesis presented by Teague and Goodpasture (30) and by Luger and Lauda (26) concerning the relation of herpes zoster to herpes simplex does not find acceptance, however, by Lipschiitz (31). HE EMPHASIZES THE POINT OF VIEW THAT IN THE PRODUCTION OF AN EXPERIMENTAL HERPES ZOSTER IT IS OF PRIME IMPORTANCE THAT THE STARTING POINT BE A TYPICAL CLINICAL CASE AND NOT A BORDER LINE CASE.

AT THE PRESENT TIME THE EVIDENCE THAT HERPES ZOSTER MAY RESULT FROM INFECTION WITH HERPES SIMPLEX VIRUS RESTS UPON THE ISOLATION OF A

VIRUS APPARENTLY IDENTICAL WITH THAT OF HERPES SIMPLEX FROM A SMALL NUMBER OF CASES. No description of the type of case from which the material employed for inoculation was obtained is given by Griiter or by Bastai and Busacca. The

case described by Teague and Goodpasture and the first case described by Luger and Lauda belong to the intermediate type of cases. The second case of Luger and Lauda, and the so called symptomatic cases of Cipolla, seem to have been clinically typical cases of herpes zoster. It is possible, therefore, that in certain instances the virus of herpes simplex may be isolated from cases clinically characteristic of herpes zoster, BUT THE EVIDENCE FOR THIS IS NOT COMPLETE AND THE CONCLUSION THAT HERPES ZOSTER MAY BE THE RESULT OF INFECTION WITH HERPES SIMPLEX VIRUS NEEDS FURTHER VERIFICATION.``

Varicella and Herpes Zoster.

"SEVERAL OBSERVERS (Lipschiitz, Meineri, and others) HAVE MADE ISOLATED ATTEMPTS TO INOCULATE HUMAN VOLUNTEERS WITH HERPES ZOSTER, BUT ALWAYS WITH NEGATIVE RESULTS. Recent studies of KUNDRATITZ (32) SEEM TO SHOW that herpes zoster can be successfully transmitted to very young children. This author wished to test out VON BOKAY'S (33) HYPOTHESIS, BASED ON CLINICAL OBSERVATION, that the virus of varicella, under certain unknown conditions, may produce a typical picture of herpes zoster and that the virus from this lesion may in turn cause varicella. He therefore attempted to immunize children against varicella by the inoculation of material from herpes zoster cases. HIS FIRST RESULTS WERE NEGATIVE, BUT HIS LATER ATTEMPTS PROVED SUCCESSFUL. He now reports that he has inoculated material from TEN TYPICAL CASES of thoracic herpes zoster and HAS HAD POSITIVE RESULTS WITH THE MATERIAL FROM FIVE OF THESE CASES. POSITIVE REACTIONS WERE OBTAINED ONLY IN CHILDREN UNDER 5 YEARS OF AGE. Children who reacted positively were subsequently shown to be immune to varicella.

KUNDRATITZ'S WORK SEEMS TO INDICATE THAT THE VIRUS OF VARICELLA AND THAT OF HERPES ZOSTER ARE IDENTICAL OR, AT LEAST, CLOSELY RELATED. IT IS UNFORTUNATE THAT KUNDRATITZ DOES NOT GIVE A DESCRIPTION OF THE CASES OF HERPES ZOSTER USED BY HIM FOR INOCULATION. It would be interesting

to know whether there were any clinical differences between the five cases of herpes zoster with which he was able to make successful transfers and the five cases in which transfers resulted negatively for, as Von Bokay and others have shown, the vesicles of varicella may be quite localized, RESULTING IN LESIONS RESEMBLING HERPES ZOSTER. The relation between herpes zoster and varicella will, in all probability, not be

entirely cleared up until we are able to transmit either one or both of these diseases to animals."

"Case 1.--A. M. Age 13. Patient admitted to the hospital Oct. 4, 1924, suffering from subacute rheumatic fever and chronic cardiac disease. SHE GAVE NO HISTORY OF A PREVIOUS ATTACK OF HERPES ZOSTER OR CHICKEN-POX. The arthritis had almost entirely disappeared and the cardiac lesion was well compensated, when on Dec. 6, 1924, the patient complained of pain and itching over the upper scapular area, in the axilla, and posterior part of the upper arm. On examination of this area there was discovered a rash consisting of small, discrete papules and vesicles distributed in patches over a zone on the upper chest from the midline behind to the midsternal line in front, and over the inner and posterior surface of the arm. The area of distribution corresponded to Head's second and third dorsal areas. During the following days the vesicles became larger. The temperature was not higher than 99.8 ° until Dec. 11, when some of the vesicles had become pustular, and now the temperature rose to 101.4 °. THE PAIN WAS SEVERE AND CHARACTERISTIC OF HERPES ZOSTER, and the appearance and distribution of the lesions were typical. A small piece of skin was removed and microscopical sections showed characteristic vesicles with numerous intranuclear inclusion bodies in the epithelial cells.

On Dec. 9, the 3rd day of the disease, fluid was pipetted from a number of vesicles and a small piece of the involved skin was obtained. The skin was ground between two glass slides, the ground material was washed off in a small amount of normal saline solution and was added to the vesicular fluid. SMALL AMOUNTS OF THIS EMULSION WERE RUBBED INTO THE SCARIFIED 2 CORNEAS OF TWO RABBITS, Nos. 1 and 2, AND ALSO INJECTED INTRACUTANEOUSLY INTO THE SHAVED SKIN OF RABBITS 2 AND INTO THE SKIN OF GUINEA PIG 1. The area of skin in the guinea pig where the injection was made had been painted several days previously with coal tar solution. The emulsion was also rubbed into the scarified skin of Rabbit 3, which had received one painting of tar 5 days before, and into the scarified skin of a similarly tarred guinea pig, No. 2. (The rabbit and guinea pigs were painted with a refined coal tar solution obtained through the courtesy of Dr.Jas. B. Murphy. This refined coal tar was much less toxic than ordinary tar and could be applied in a fairly thick coat, so that one painting resulted in a marked reaction.) On the following day, Dec.

10, vesicular fluid was again obtained from fresh vesicles and also another piece of

skin. This material was treated in the same way as that obtained on the preceding day,

and inoculated in the following ways. The cornea of Rabbit 1 and the scarified tarred skin of Rabbit 3 and Guinea Pig 2 were reinoculated. SOME OF THE MATERIAL WAS ALSO INOCULATED INTRACEREBRALLY INTO RABBIT 4 AND INTO THE SCARIFIED CORNEA OF RABBIT 5. Thus, with fresh material obtained on the 3rd and 4th days of the disease, FIVE RABBITS AND TWO GUINEA PIGS WERE INOCULATED IN VARIOUS WAYS.

The animals were carefully observed each day following the inoculations, but in none of the animals were any macroscopic changes seen which could be ascribed to the inoculations. One of the eyes of Rabbit 2 was removed on the 3rd day and the other on the 7th day following the inoculations and sections were made through the corneas. The sections show in places what are apparently the results

of mechanical injuries and in the section of the eye removed on the 7th day, loci of slight infiltration of the substantia propria with small round cells. Some swelling of certain epithelial cells is also seen. BUT NOWHERE ARE THERE ANY SIGNS OF VESICLE FORMATION OR MARKED INFLAMMATORY REACTIONS AND NOT INCLUSION BODIES WERE FOUND.

ALTHOUGH NO DEFINITE REACTIONS WERE OBTAINED in this first series of animals it was thought that by inoculating from one cornea to another and from one brain to another, the virus might possibly become adapted to the rabbit and produce definite lesions in subsequent transfers. Therefore, starting with Rabbit 1 INOCULATIONS WERE MADE FROM ONE RABBIT TO ANOTHER BY SCRAPING THE CORNEA AND WASHING OUT THE CONJUNCTIVAL SAC WITH A SMALL AMOUNT OF SALINE AND INOCULATING THE MATERIAL THUS OBTAINED INTO THE SCARIFIED CORNEA OF ANOTHER RABBIT. FOURTEEN CORNEAL PASSAGES WERE THUS MADE, at 2 and 3 day intervals. In many of the rabbits, the scarified eye on the day following the inoculation showed a slight degree of opacity along the lines of scarification and a slight exudation. However, it was found during the course of the study that SLIGHT CHANGES OF THIS CHARACTER FREQUENTLY OCCUR FOLLOWING THE INOCULATION OF AN EMULSION OF NORMAL RABBIT CORNEA, AND EVEN AFTER SCARIFICATION ALONE WITHOUT THE INJECTION OF ANY FOREIGN MATTER WHATEVER. Except for these slight non-specific reactions no changes were observed in any of the eyes of the series. In certain instances, although no gross changes were present, the cornea was sectioned but NO LESIONS WHICH COULD BE CONSIDERED SPECIFIC AND NOT INTRANUCLEAR INCLUSION BODIES WERE FOUND.

STARTING WITH RABBIT 4 INOCULATED INTRACEREBRALLY WITH THE MATERIAL FROM THIS CASE, TEN BRAIN TO BRAIN TRANSFERS WERE MADE at 5 day intervals. Each animal was killed with ether, and the brain removed with sterile precautions. AN EMULSION OF THE BRAIN WAS MADE WITH LOCKE'S SOLUTION in a sterile mortar, the suspension centrifuged at low speed, and 0.2 cc. of the supernatant fluid INJECTED INTRACEREBRALLY INTO A NORMAL ANIMAL. At the same time, SOME OF THE BRAIN EMULSION WAS INOCULATED INTRACORNEAL AND INTRADERMAL INTO EACH OF TWO OTHER RABBITS. It was found that the inoculation of brain emulsion into the scarified cornea usually was followed by conjunctivitis of considerable severity which, however, PROVED TO BE WHOLLY NON-SPECIFIC. The temperature of the intracerebrally inoculated rabbits was taken daily, and sections of the brain of each of the inoculated animals made. NONE OF THE RABBITS SHOWED A SIGNIFICANT RISE IN TEMPERATURE AND CAREFUL STUDY OF THE BRAIN FAILED TO REVEAL ANY CHARACTERISTIC LESIONS. NO INTRANUCLEAR INCLUSION BODIES WERE FOUND.``

(The eyes of the rabbits were anesthetized locally with cocaine before inoculation. For other operations the animals were given ether.)

"In making the animal experiments we employed various methods which were suggested largely by the technique used by previous observers, especially by those who have reported results which were considered positive. In making inoculations into the corneas the technique recommended by Lipschtitz was employed as far as possible.

Young rabbits were used and the material was obtained from fresh vesicles early in the disease and inoculated with as little delay as

possible. The material injected into rabbits' eyes was obtained from seven cases and twenty-four rabbits were used. In judging the results obtained in this kind of experimentation great caution must be observed. OUR EXPERIENCE CONVINCES US THAT SLIGHT OPACITIES OCCURRING ALONG THE LINES OF SCARIFICATION AND MILD CONJUNCTIVITIS CANNOT BE HELD TO INDICATE THE EFFECT OF A SPECIFIC VIRUS. As regards the interpretation of the microscopic changes found, we were quite familiar with the appearance of intranuclear inclusion bodies as seen in the lesions of experimental herpes simplex and the filterable virus (Virus III) indigenous to rabbits described by Rivers and Tillett (5). We also had no difficulty in finding intranuclear inclusions in the sections of skin removed from patients. It is not likely,

therefore, that these structures were overlooked in our study of the sections. Briefly stated, although the material studied was satisfactory and in spite of the fact that a considerable number of animals were used for each case, WE HAVE BEEN UNABLE TO CONFIRM THE OBSERVATIONS OF LIPSCHTITZ regarding the experimental production of specific lesions in the corneas of rabbits. WE REALIZE THAT THIS IS ONLY NEGATIVE EVIDENCE and therefore not of conclusive importance in view of Lipschiitz's observations. It indicates, however, THAT THE PRODUCTION OF SPECIFIC LESIONS IN RABBITS' EYES WITH MATERIAL FROM HERPES ZOSTER VESICLES IS EXTREMELY DIFFICULT AND THAT SUCCESSFUL RESULTS MAY BE A MATTER OF CHANCE, depending, possibly, on peculiar susceptibility on the part of the rabbits. In view of the fact, however, that a careful analysis of the positive results reported by other observers shows that the conclusions were based on insufficient evidence, we believe that FURTHER WORK IS NECESSARY BEFORE THE SUCCESSFUL INOCULATION OF THE RABBITS' CORNEAS WITH HERPES ZOSTER VIRUS CAN BE ACCEPTED AS FULLY DEMONSTRATED. To make the evidence convincing SPECIFIC LESIONS SHOULD BE OBTAINED WITH A FAIR DEGREE OF REGULARITY AND THE VIRUS SHOULD BE SUCCESSFULLY TRANSMITTED THROUGH AT LEAST TWO GENERATIONS. Apparently the latter was not attempted by Lipschtitz.

Intracerebral inoculations into three rabbits with material from two cases (Nos. I and IV) were made. Two rabbits were also inoculated

intraspinal with material from one case (No. IV). NONE OF THESE ANIMALS SHOWED ANY REACTION. In the case of one of the animals inoculated into the brain (Case I) although this rabbit showed no symptoms, we thought it conceivable that the susceptibility of the species for the virus might be so slight that no obvious lesion had been produced. Nevertheless it was thought that the virus might possibly remain alive at the seat of inoculation and by repeated transfers become adapted to the rabbit. This phenomenon has been observed by Noguchi with vaccine virus, and by Rivers and Tillett with the rabbit virus

isolated by these workers. This possibility was tested by us by making serial corneal and brain inoculations. CORNEAL TRANSFERS WERE CARRIED THROUGH FOURTEEN ANIMALS IN SERIES, AND BRAIN TRANSFERS THROUGH TEN. NO SPECIFIC LESIONS DEVELOPED IN ANY OF THE ANIMALS.

The work of Teague and Goodpasture suggested that the skin might be rendered more susceptible to infection by previous treatment with tar. Material from two cases (Nos. I and VIII) was inoculated into the tarred skin of guinea pigs and rabbits. The material was

injected intracutaneously and also rubbed into the scarified skin. NO REACTION WAS OBTAINED IN ANY OF THE ANIMALS.

Finally, the TRANSMISSION OF HERPES ZOSTER TO MONKEYS WAS ATTEMPTED. Blanc and Caminopetros, and Bastai and Busacca, as discussed in the review of the literature, inoculated monkeys (Macacus) in various ways, WITHOUT SUCCESS.

It was thought possible that although monkeys of the genus Macacus might be refractory, monkeys of another genus might prove susceptible. Consequently, besides the inoculation of two Macacus monkeys, attempts were made to infect five vervets. Moreover, in view of the fact that the virus of vaccinia and the rabbit virus of Rivers and Tillett could be successfully cultivated in the testicle, intratesticular inoculations were employed. The testicles were removed at varying periods following inoculation. Numerous sections of these testicles were made and examined, BUT IN NO INSTANCES WERE ANY LESIONS FOUND WHICH COULD BE INTERPRETED AS SPECIFIC. No cells containing intranuclear inclusion bodies were found. THESE EXPERIMENTS, THEREFORE, HAVE ALSO LED TO PURELY NEGATIVE RESULTS.

THIS REPORT OF OUR WORK IS MADE AT THE PRESENT TIME BECAUSE A CONSIDERABLE AMOUNT OF LITERATURE HAS BEEN PUBLISHED WHICH GIVES THE IMPRESSION THAT HERPES ZOSTER HAS BEEN SUCCESSFULLY TRANSMITTED TO ANIMALS. Although the observations of Lipschtitz are suggestive, it is important that they be confirmed by further investigations.

UNTIL HERPES ZOSTER CAN BE REGULARLY TRANSMITTED TO ANIMALS AND CROSS-IMMUNITY TESTS BE CARRIED OUT, THE RELATION OF THE VIRUS OF HERPES ZOSTER TO THAT OF HERPES SIMPLEX REMAINS A MATTER OF SPECULATION. In view of the fact that herpes simplex can be easily and regularly transmitted to rabbits, WHEREAS IN THE HANDS OF A LARGE NUMBER OF INVESTIGATORS SIMILAR EXPERIMENTS WITH HERPES ZOSTER ARE COMPLETELY NEGATIVE, it does not seem likely that the etiological agent concerned in these two diseases can be absolutely identical.

"THE QUESTION OF THE IDENTITY OR NON-IDENTITY OF HERPES ZOSTER AND VARICELLA IS EVEN MORE DIFFICULT TO ANSWER, BECAUSE AT PRESENT NEITHER

OF THESE INFECTIONS IS READILY TRANSMISSIBLE TO ANIMALS. The work of Kundratitz is extremely interesting. His observations, aside from indicating a close immunological relationship between herpes zoster and varicella, are important in that THEY SEEM TO SHOW THE PRESENCE OF A TRANSMISSIBLE VIRUS IN THE

VESICLES OF HERPES ZOSTER. The only QUESTION THAT ARISES IS WHETHER THE CASES OF HERPES ZOSTER from which Kundratitz was able to make successful transfers WERE TRUE CASES OF IDIOPATHIC HERPES ZOSTER.

CONCLUSION.

Attempts to inoculate rabbits, guinea pigs, and monkeys with material obtained from nine cases of herpes zoster HAVE PROVED UNSUCCESSFUL."

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In Summary:

-the nature and etiology of the infectious diseases where one of the features is a vesicular eruption on the skin are confused

-Rivers stated there was a possible relationship between a series of these diseases beginning with sheep-pox and horse-pox and extending through cow-pox, smallpox, varioloid, alastrim, chicken-pox, and herpes zoster to symptomatic herpes and lethargic encephalitis

-certain of these conditions RESEMBLE EACH OTHER in their clinical manifestations while others have little in common but they all share eruptions of the skin

-in most of the conditions the skin lesions show SIMILAR HISTOLOGICAL CHARACTERISTICS

-at one time or another SOME RELATIONSHIP IN ETIOLOGY between various members of the group, OR EVEN AN IDENTICAL ETIOLOGY in all of them has been suggested

-the etiological agent had NOT BEEN CULTIVATED in any of the above conditions

-since no "viruses" had been cultivated, the only "proof" was reproducing the same lesions in animals or man

-attempts to transmit varicella and herpes zoster to animals was largely UNSUCCESSFUL

-in the case of herpes simplex, they could not reproduce an identical disease in animals to that experienced by humans

-injecting "herpes simplex" into the sacrificed eyes of rabbits consistently produced

vesicular eruptions and keratoconjunctivitis while injecting it into the skin did not regularly produce lesions

-intracutaneous inoculation of the vesicular fluid of herpes simplex either into an individual already infected with herpes simplex or into a normal person HAS NOT GIVEN AS CONSTANT RESULTS

-man's susceptibility to herpes simplex seems to depend on certain secondary factors which are UNKNOWN

-attempts to transmit varicella to animals have not been successful

-there has been much difficulty in successfully inoculating animals or man with varicella virus

-attempts using similar inoculation methods produced a papule at the site of injection or a generalized eruption (which they were unsure if it were "true" Chicken-pox) while others produced no lesions whatsoever

-attempts to inoculate animals with herpes zoster were unsuccessful

-various experiments on rabbits and Guinea pigs were performed by inoculation of vesicular fluid into their eyes

-THE EMULSIFIED BRAIN AND CEREBELLUM of one rabbit were inoculated into the cornea of four more rabbits

-in another experiment, the inoculation of the brain emulsion into the scarified corneas of two rabbits and two guinea pigs FAILED to produce lesions

-most of the attempts made by Mariani to inoculate the cornea of rabbits with herpes zoster RESULTED NEGATIVELY

-material from nine cases of herpes zoster was inoculated by Blanc and Caminopetros into the eyes, cornea, conjunctiva, skin, brain, and spinal cord of a series of animals, including rabbits, mice, sheep, pigeons, monkeys, and a dog

-three monkeys (Macacus rhesus) were inoculated as follows: one into the eye, one into the spinal canal, and the third into the skin of the thoracic region which had previously been shaved and excoriated

-inoculations in the first two monkeys resulted negatively

-the third monkey showed a slight inflammatory reaction at the site of inoculation but

recovered without the appearance of vesicles

-all the other animal experiments gave NEGATIVE RESULTS

-the writers of one study thought that the lesion brought about by the experiment might easily be interpreted as a reaction resulting from the injection

-they concluded that the problem of the transmission of herpes zoster to animals remains open and they apparently consider their own experiments negative or inconclusive

-analysis of the experiments by Meineri indicated that his findings can best be interpreted as the result of trauma

-his attempts to inoculate two humans with herpes zoster both FAILED

-2 out of the 3 researchers Lipschtitz cited as backing up his results stated their results were INCONCLUSIVE

-many other authors report entirely NEGATIVE RESULTS following the inoculation of herpes zoster material into the sacrificed corneas of rabbits: Kraupa; Baum; LSwenstein, Teissier, Gastinel, and Reilly; Kooy; Netter and Urbain; Bloch and Terris; Simon and Scott; and Doerr

-they state it has NOT BEEN POSSIBLE to demonstrate conclusively any specific virus associated with herpes zoster

-Luger and Lauda published several papers on the problem of the etiology of herpes zoster

-in their first paper they give the results obtained by inoculation with material from seven cases of typical herpes zoster, employing the technique used by Lipschfitz and in none of the eyes inoculated did any macroscopic reaction occur

-they considered the results in this series of experiments NEGATIVE

-Griiter states that there is NO EVIDENCE for assuming a specific virus for herpes zoster

-the data he presented was not sufficient to establish the isolation of a true herpes simplex virus from these cases

-Bastai and Busacca were of the opinion that herpes zoster is probably a manifestation of infection with herpes simplex virus but experimental data presented was not sufficient to justify this point of view

-none of the animals they inoculated presented any lesions

-Teague and Goodpasture were able to produce zoster-like lesions in the skin of rabbits and guinea pigs by the inoculation of herpes simplex virus into areas of the skin previously treated with coal tar

-however, they did not maintain that they reproduced the human disease herpes zoster in animals, only that there is a close analogy between the EXPERIMENTAL CONDITION PRODUCED BY THEM and true herpes zoster

-Lipschiitz did not accept the hypothesis by several researchers about the relation of herpes zoster to herpes simplex

-he emphasized the point of view that in THE PRODUCTION OF AN EXPERIMENTAL HERPES ZOSTER it is of prime importance that the starting point be a typical clinical case and not a border line case

-the evidence that herpes zoster may result from infection with herpes simplex virus rests upon the isolation of a virus apparently identical with that of herpes simplex from a small number of cases

-however, while they feel it may be possible these "viruses" are related, it is inconclusive and in need of verification

-several researchers attempted to infect humans with herpes zoster with NEGATIVE results

-Kundratitz tried to infect children yet his first attempts were negative

-his second attempts were "successful" in infecting 5 of 10 children all under the age of five

-Kundratitz did not provide any details on the herpes zoster cases used for infection

-the vesicular fluid from a girl with herpes zoster was inoculated in various ways (eyes, skin, brain) into 5 rabbits and 2 Guinea pigs

-there were no signs of vesicle formation or marked inflammatory reaction and no inclusion bodies were found

-inoculations were made from one rabbit to another by scraping the cornea and washing out the conjunctival sac with a small amount of saline and inoculating the material into the scarified cornea of another rabbit - FOURTEEN corneal passages were made in this way

-they noticed slight changes in the cornea but found that these changes frequently occur

following the inoculation of an emulsion of normal rabbit cornea, and even after scarification alone WITHOUT THE INJECTION OF ANY FOREIGN MATTER whatsoever

-no lesions which could be considered specific and no intranuclear inclusion bodies were found

-TEN brain to brain transfers were made at 5 day intervals in some rabbits

-an emulsion of the brain was made with Locke's solution in a sterile mortar, the suspension centrifuged at low speed, and 0.2 cc. of the supernatant fluid injected intracerebrally into a normal animal

-some of the brain emulsion was inoculated intracorneal and intradermally into each of two other rabbits

-NONE of the rabbits showed a significant rise in temperature and careful study of the brain failed to reveal any characteristic lesions

-no intranuclear inclusion bodies were found

-the writer states their experience convinces them that slight opacities occurring along the lines of scarification and mild conjunctivitis CANNOT BE HELD TO INDICATE THE EFFECT OF A SPECIFIC VIRUS

-their experiments indicate that the production of specific lesions in rabbits' eyes with material from herpes zoster vesicles is extremely difficult and that successful results MAY BE A MATTER OF CHANCE

-they state further work is necessary before the successful inoculation of the rabbits' corneas with herpes zoster virus can be accepted as fully demonstrated

-they determined that to make the evidence convincing, SPECIFIC LESIONS SHOULD BE OBTAINED WITH A FAIR DEGREE OF REGULARITY and the virus should be successfully transmitted through at least two generations

-material from two cases was inoculated into the tarred skin of guinea pigs and rabbits intracutaneously and also rubbed into the scarified skin yet NO REACTION WAS OBTAINED in any of the animals

-attempts to transfer herpes zoster to monkeys by various researchers were all UNSUCCESSFUL

-the writers detailed their negative experiments due to the growing amount of literature suggestive of successful transmission of herpes zoster to animals when this is not the case

-they state until herpes zoster can be regularly transmitted to animals and cross-immunity tests are carried out, the relation of the virus of herpes zoster to that of herpes simplex remains A MATTER OF SPECULATION

-neither varicella or herpes zoster had been shown to be transmissible to animals

-they question whether the cases outlined by Kundratitz were "true" herpes zoster cases

-they conclude that attempts to inoculate rabbits, guinea pigs, and monkeys with material obtained from nine cases of herpes zoster HAVE PROVED UNSUCCESSFUL

NO TRANSMISSION. PLEASE STAND BY.

https://docs.google.com/document/d/e/2PACX-1vQod3Vgy15-hXqDeJCaqULaM7EG6wIfMKfKVECkyN3-6TRSTd6FJDHJujVgetm3g0ZZbjGIPqiOhaKV/pub

THOMAS WELLER 1953 CHICKENPOX PAPER:

According to the CDC:

"In 1954, Thomas Weller USED CELL CULTURE to isolate VZV from vesicular fluid of patients with varicella or zoster."

If we are being picky, the paper was actually published in 1953 and Weller was awarded the Nobel Prize for his disgusting tissue culture practices in 1954 yet seeing how the CDC also believes a 14-year-old Rudolph Steiner performed experiments and wrote a paper in 1875 proving Chickenpox infectiousness, I should probably let this error slide. In any case, a brief history on Weller's work:

"In 1954, the Nobel Prize for Medicine was awarded to Drs John Enders, Thomas Weller, and Frederick Robbins for their watershed discovery that growth of poliomyelitis

virus OCCURRED IN CULTURES OF CELLS OF EXTRANEURAL ORIGIN, first reported in 1949. Their demonstration in 1949 that the Lansing type II strain of poliomyelitis COULD BE GROWN IN CULTURES OF HUMAN EMBRYONIC TISSUE set into motion a race to develop a vaccine for the disease that had crippled countless thousands of individuals. The discovery and subsequent recognition were only the beginning of a prolific career for Thomas Huckle Weller, who made numerous contributions to the field of virology, INCLUDING ISOLATING THE VARICELLA-ZOSTER VIRUS (VZV) FROM CASES OF CHICKENPOX AND ZOSTER, PROVIDING SUGGESTIVE EVIDENCE THAT THE SAME VIRUS IS RESPONSIBLE FOR BOTH DISEASES; isolating the human cytomegalovirus (CMV) for the first time in tissue culture and suggesting the descriptive name now used for it; establishing Coxsackie viruses as the cause of epidemic pleurodynia: and first isolating rubella virus, the cause of German measles."

https://pubmed.ncbi.nlm.nih.gov/12118846/

"Suggestive evidence." That is a very firm conclusion drawn there. Keep in mind that this "suggestive evidence" stems from the same sick tissue culture practices of aborted human embryos and foreskin tissues mixed with numerous chemicals and sources of animal DNA that was also used for the "isolation" of Polio. So did Weller really "isolate" VSV and provide "suggestive evidence" as stated here? Below is the full paper with highlights and summary:

SERIAL PROPAGATION IN VITRO OF AGENTS PRODUCING INCLUSION BODIES DERIVED FROM VARICELLA AND HERPES ZOSTER.

"IT IS GENERALLY ACCEPTED THAT SERIAL PROPAGATION IN THE LABORATORY OF THE AGENTS OF VARICELLA AND OF HERPES ZOSTER HAS NOT HERETOFORE BEEN ACCOMPLISHED. Certain of the earlier

published reports regarding their serial propagation have more recently BEEN ATTRIBUTED TO POSSIBLE CONFUSION WITH VIRUSES PATHOGENIC FOR LOWER ANIMALS. However, morphological evidence has suggested that a single passage of the agents of varicella and herpes zoster has been achieved. Thus Rivers (1,2) observed focal lesions with intranuclear inclusions in the monkey testicle following the local inoculation of varicella vesicle fluid. Goodpasture and

Anderson(3) grafted human skin on the chorioallantoic membrane of the chick embryo. ALTHOUGH NEGATIVE RESULTS WERE OBTAINED FOLLOWING INOCULATION OF VARICELLA VESICLE FLUID, in one experiment histological examination of grafts following inoculation with fluid from herpes zoster lesions revealed intranuclear inclusions. Using essentially the same technic, Blank, Coriell and Scott (4) likewise IN

ONE INSTANCE demonstrated inclusion bodies after inoculation of zoster material.

In 1948 we began an investigation of the potentialities of SUSPENDED CELL CULTURES OF HUMAN TISSUES as a medium for the isolation of the causative agent of varicella. Eosinophilic intranuclear inclusions were demonstrated on examination of tissue fragments removed from the cultures at intervals after the introduction of varicella vesicle fluid(5). EFFORTS TO MAINTAIN THE RESPONSIBLE AGENT ON SERIAL PASSAGE IN THIS TYPE OF CULTURE WERE UNSUCCESSFUL. More recently ROLLER TUBE CULTURES OF HUMAN TISSUES have been applied to the same objective. The results so far obtained are here reported in a PRELIMINARY manner. As will be shown it has been possible to isolate and maintain in serial passage cytopathogenic agents APPARENTLY DERIVED from specimens of varicella vesicle fluid as well as others APPARENTLY OBTAINED from herpes zoster vesicle fluid.

Materials and methods. Vesicle fluid specimens were collected and stored as previously described (5). Roller tube cultures were prepared as in our studies on poliomyelitis (6) UTILIZING EITHER HUMAN EMBRYONIC SKIN-MUSCLE TISSUE OR FORESKIN TISSUE OBTAINED FROM BOYS BETWEEN THE AGES OF 3 MONTHS AND 3 YEARS. In the present experiments the NUTRIENT FLUID FOR THE CULTURES CONSISTED OF BOVINE AMNIOTIC FLUID (90%), BEEF EMBRYO EXTRACT (5%), HORSE SERUM (SP), ANTIBIOTICS, SOYBEAN TRYPSIN INHIBITORS and PHENOL RED as recently described (6,7). Changes of the nutrient fluid were made at 3- or 4-day intervals. In certain experiments for histologic examination tissues were grown on coverslips COATED WITH CHICKEN PLASMA that were placed in roller tubes; these preparations were FIXED WITH ZENKERS-ACETIC ACID AND STAINED WITH HEMATOXYLIN AND EOSIN.

Experimental. 1) Studies with varicella vesicle fluid. Vesicle fluids derived from 11 cases of varicella were inoculated individually, usually IN THE FORM OF 0.1 ml ALIQUOTS OF A SUSPENSION IN MILK, into groups of roller cultures of human embryonic skin-muscle tissue. THE EXACT CONCENTRATION OF THE FLUID IN MILK CANNOT BE STATED. In every instance tissue growth was well established at the time of inoculation. As summarized in Table I, in cultures inoculated with fluids from six of these cases, focal cytopathogenic lesions of a characteristic appearance developed which were readily seen on microscopical examination of the living cultures. These lesions usually were first observed from the 6th to 8th day after inoculation. They consisted of small collections of swollen, rounded refractile cells which contrasted sharply with the surrounding fibroblastic or epithelial outgrowth. Those foci of affected cells developing in the sheets of cells of normal appearance increased slowly in size. The cells in the center of such focal areas gradually degenerated over the course of several days, while slow peripheral

extension of the lesion continued for days or weeks as contiguous cells became infected. Study of stained coverslip preparations from roller tube cultures inoculated with vesicle fluid material has shown that the changes in cellular morphology are CHARACTERISTICALLY ASSOCIATED WITH THE PRESENCE OF INCLUSION BODIES. At the margin of a focus occasional cells may be observed that show no gross morphological changes, yet contain small granular intranuclear inclusions. Rounded and swollen cells almost invariabLy possess intranuclear eosinophilic inclusions. Occasionally the larger swollen cells are multinucleated, with each nucleus containing an inclusion; such cells resemble the multinucleated giant cells described by various workers in the lesions of varicella, herpes zoster and herpes simplex (😎. Focal lesions developing in a pre-existing loose network of cells progress irregularly, but manifest similar changes. The morphological changes observed in the cultures will be reported in more detail in a future communication.

All six of the agents isolated following the

Inoculation of varicella fluids have been PROPAGATED SERIALLY IN TISSUE CULTURE as indicated by the successive development in SUBCULTURES of focal areas of cellular enlargement and degeneration. Two of the strains (McE. and Wel.) HAVE NOW BEEN MAINTAINED FOR 10 PASSAGES as summarized in Table 11. The inoculum employed to initiate the third and succeeding passages in these experiments consisted of 0.1 ml of a suspension of COARSELY GROUND TISSUE removed from the preceding set of cultures. On one occasion, the infected tissue suspension was frozen in the COz box prior to use; otherwise, the inoculum was prepared on the day it was employed. Fig. 1 to 3 depict representative cytopathic changes observed during the serial propagation of the McE. and Wel. agents. In contrast to the results obtained with tissue suspensions, ATTEMPTS TO ESTABLISH PASSAGES WITH INOCULA CONSISTING OF CENTRIFUGED FLUIDS REMOVED FROM THE INFECTED CULTURES HAVE SO FAR BEEN UNSUCCESSFUL.

STUDIES DESIGNED TO ELUCIDATE THE NATURE AND ETIOLOGICAL RELATIONSHIP OF THESE CYTOPATHOGENIC AGENTS THUS ISOLATED TO VARICELLA ARE IN PROGRESS. In one experiment, vesicle fluid material of known infectivity for cultures DID NOT PRODUCE CYTOPATHIC CHANGES when inoculated following heating at 60°C for 30 minutes. In none of more than 70 control cultures maintained during the passage experiments have focal lesions been observed; these tubes routinely received inocula consisting of a suspension of TISSUE DERIVED FROM CONTROL CULTURES OF THE PRECEDING PASSAGE. Tissue suspensions infective for cultures HAVE BEEN WITHOUT OBVIOUS EFFECT when inoculated into suckling mice or into the developing hens egg by various routes. ATTEMPTS AT IN VITRO NEUTRALIZATION OF THE CYTOPATHOGENIC EFFECT WITH CONVALESCENT

SERUM FROM CASES OF VARICELLA HAVE SO FAR BEEN UNSUCCESSFUL. It is possible that the close association of the infective agents with cell constituents may be involved in this apparent

lack of neutralizing effect. In certain preliminary experiments in which culture fluids harvested from tubes showing cytopathic changes have been employed as antigen in complement fixation tests on paired serum specimens obtained from cases of varicella, a rise in antibody titer has been observed. THE SPECIFICITY OF THIS REACTION IS UNDER INVESTIGATION.

2) Studies with herpes zoster vesicle fluid.

SIMILAR FOCAL CYTOPATHIC CHANGES have been observed in cultures inoculated with material obtained on the day of appearance of vesicles from an 80-year-old (Sto.) with thoracic herpes zoster and with fluid collected from a 30-year-old woman (Pie.) with involvement of the ophthalmic branch of the trigeminal nerve. The Sto. inoculum had been stored in the frozen state for 21 months prior to use, while the Pie. fluids were inoculated on the day of collection. SERIAL PROPAGATION IN TISSUE CULTURE OF THESE TWO AGENTS HAS BEEN ACCOMPLISHED, as summarized in Table 111, AGAIN WITH INOCULA CONSISTING OF GROUND TISSUE SUSPENSIONS. (THESE MANIPULATIONS have been performed in a separate room from that in which the varicella agents have been maintained.) THE CYTOPATHOGENIC EFFECT OBSERVED IN THE CULTURES CLOSELY RESEMBLES that obtained following the introduction of varicella vesicle fluid. Examination of stained preparations from the second tissue culture passage of the Sto. strain has also revealed that the rounded swollen cells composing the focal lesions contain intranuclear inclusions (Fig. 4). Tissue suspensions prepared from cultures of the Sto. and Pie. agents, that were successfully employed to initiate culture passages, HAVE PRODUCED NO OVERT SYMPTOMS IN NEWBORN MICE WHEN INOCULATED BY VARIOUS ROUTES.

Discussion. Inoculation of roller tube cultures of human tissues with materials derived from the eruptive lesions of varicella and of herpes zoster has revealed the presence of cytopathogenic agents capable of producing intranuclear inclusions. These, subsequently, have been maintained in serial passage.

A PECULIARITY OF THEIR BEHAVIOR IN THE CULTURE SYSTEM EMPLOYED HAS BEEN THE APPARENT FAILURE OF INFECTIOUS MATERIAL TO APPEAR IN THE FLUID PHASE. The focal lesions appear to increase in size by infection of immediately adjacent cells. On prolonged cultivation, however, an increase in the number of focal

lesions occurs. In one experiment in which infected cultures were maintained for 8 weeks approximately 90% of the proliferating tissue became involved. Even so, extension of the lesions was continuing at the end of this period. THE SIGNIFICANCE OF THESE OBSERVATIONS IS BEING INVESTIGATED. It is at present clear, however, that in the utilization of TISSUE CULTURE TECHNIQUES FOR THE ISOLATION OF UNKNOWN AGENTS, CONSIDERATION MUST BE GIVEN TO PASSAGE OF TISSUE SUSPENSIONS, AS WELL AS TO THE PASSAGE OF FLUID INOCULA.

NO EVIDENCE HAS BEEN OBTAINED THAT THE AGENTS ISOLATED ARE NOT THOSE RESPONSIBLE FOR VARICELLA AND HERPES ZOSTER. YET NO DEFINITIVE STATEMENT CAN NOW BE MADE REGARDING THEIR NATURE, THEIR ETIOLOGICAL RELATIONSHIPS, OR THE INTERESTING QUESTION OF THE POSSIBLE IDENTITY OF THE AGENTS OF HERPES ZOSTER AND VARICELLA. It is to be noted that the virus of herpes simplex when propagated in tissue cultures of the type here employed manifests an early focal tendency to induce lesions but then rapidly brings about a widespread infection of the cell population resulting in an appearance quite dissimilar to that described (9). THE FAILURE OF THESE AGENTS TO PRODUCE OBVIOUS INFECTION OF SUCKLING MICE, IN VIEW OF THE HIGH DEGREE OF SUSCEPTIBILITY OF THIS ANIMAL TO THE VIRUS OF HERPES SIMPLEX (10). ALSO INDICATES THAT THEY ARE NOT TO BE IDENTIFIED WITH THE LATTER. We have no indication that a Ricket-tsia-like agent of the type described by Sprunt and Hirst (11) was present in our cultures.

Summary

The inoculation of roller tube tissue cultures of human tissues with vesicle fluid derived from patients with varicella has resulted in the isolation of six cytopathogenic agents. Two of the strains have been maintained for 10 tissue culture passages by employing tissue suspensions as the passage material. Histologically the lesions produced consist of focal accumulations of cells which become swollen, and then degenerate: characteristically, such cells contain intranuclear inclusion bodies. From the eruptive lesions of two cases of herpes zoster, cytopathogenic agents of a similar nature have been isolated and have been propagated serially in cultures of human tissue."

https://doi.org/10.3181/00379727-83-20354

In Summary:

-Weller admits it is generally accepted that serial propagation in the laboratory of the

agents of varicella and of herpes zoster HAS NOT BEEN ACCOMPLISHED

-certain of the earlier published reports regarding their serial propagation have more recently been attributed to POSSIBLE CONFUSION with viruses pathogenic for lower animals

-NEGATIVE RESULTS were obtained following inoculation of varicella vesicle fluid yet in one experiment they found intranuclear inclusions using herpes zoster fluid (apparently the same "virus") on grafts

-Weller states that n 1948 they began an investigation of the potentialities of SUSPENDED CELL CULTURES OF HUMAN TISSUES as a medium for the isolation of the causative agent of varicella

-he admits efforts to maintain the responsible agent on serial passage in this type of culture were UNSUCCESSFUL

-he then tried ROLLER TUBE CULTURES OF HUMAN TISSUES for the same objective

-he states it has been possible to isolate and maintain in serial passage cytopathogenic agents APPARENTLY DERIVED from specimens of varicella vesicle fluid as well as others APPARENTLY OBTAINED from herpes zoster vesicle fluid

-roller tube cultures were prepared as in the studies on poliomyelitis (6) utilizing either HUMAN EMBRYONIC SKIN-MUSCLE TISSUE or FORESKIN TISSUE obtained from boys between the ages of 3 months and 3 years

-the NUTRIENT FLUID for the cultures consisted of bovine amniotic fluid (90%), beef embryo extract (5%), horse serum (SP), antibiotics, soybean trypsin inhibitor and phenol red

-in certain experiments for histologic examination tissues were grown on coverslips coated with CHICKEN PLASMA that were placed in roller tubes; these preparations were fixed with Zenkers-acetic acid and stained with hematoxylin and eosin

-vesicle fluids derived from 11 cases of varicella were inoculated individually, usually in the form of 0.1 ml aliquots of a suspension IN MILK, into groups of roller cultures of human embryonic skin-muscle tissue

-the exact concentration of the fluid in milk CANNOT BE STATED

-all six of the agents isolated following the inoculation of varicella fluids have been PROPAGATED SERIALLY IN TISSUE CULTURE as indicated by the successive development in SUBCULTURES of focal areas of cellular enlargement and degeneration

-two of the strains (McE. and Wel.) have now been MAINTAINED FOR 10 PASSAGES

-studies designed TO ELUCIDATE THE NATURE AND ETIOLOGICAL RELATIONSHIP of these cytopathogenic agents thus isolated to varicella are in progress (in other words, they had no idea if their "isolate" was the "virus" or not)

-in one experiment, vesicle fluid material of known infectivity for cultures DID NOT PRODUCE CYTOPATHIC CHANGES when inoculated following heating at 60°C for 30 minutes

-"controls" consisted of a suspension of tissue derived from control cultures of the preceding passage

-tissue suspensions infective for cultures were WITHOUT OBVIOUS EFFECT when inoculated into suckling mice or into the developing hens egg BY VARIOUS ROUTES

-attempts at in vitro neutralization of the cytopathogenic effect with convalescent serum from cases of varicella were UNSUCCESSFUL

-the SPECIFICITY of a possible antigenic response in complement fixation tests reaction (which are useless without a purified/isolated "virus" to begin with) was under investigation

-Weller states SIMILAR focal cytopathic changes had been observed in cultures inoculated with material from 2 herpes zoster cases

-serial propagation in tissue culture of these two agents was accomplished again with inocula CONSISTING OF GROUND TISSUE SUSPENSIONS

-the cytopathogenic effect observed in the cultures CLOSELY RESEMBLES (but not identical) that obtained following the introduction of varicella vesicle fluid

-Weller admits that issue suspensions prepared from cultures of the 2 herpes zoster agents, that were successfully employed to initiate culture passages, PRODUCED NO OVERT SYMPTOMS in newborn mice WHEN INOCULATED BY VARIOUS ROUTES

-Weller states that a peculiarity of their behavior in the culture system employed was the apparent FAILURE OF INFECTIOUS MATERIAL TO APPEAR in the fluid phase

-he explains changes in the tissue observed but states the SIGNIFICANCE of these observations were BEING INVESTIGATED

-Weller admits that in the utilization of tissue culture techniques for the isolation of UNKNOWN AGENTS, consideration must be given to passage of tissue suspensions (significance unknown), as well as to the passage of fluid inocula (material was not infectious)

-Weller relies on his conclusion that no evidence has been obtained that the agents

isolated ARE NOT those responsible for varicella and herpes zoster (besides the lack of infectiousness...but I guess that wasn't a deal breaker for him... 🤷)

-however, he admits NO DEFINITIVE STATEMENT can be made regarding their NATURE, their ETIOLOGICAL RELATIONSHIPS, or the INTERESTING QUESTION OF THE POSSIBLE IDENTITY of the agents of herpes zoster and varicella (in other words, they had no proof of anything)

-Weller admits to the FAILURE of these agents TO PRODUCE OBVIOUS INFECTION of suckling mice, in view of the high degree of susceptibility of this animal to the virus of herpes simplex, which indicated to him THAT THEY ARE NOT TO BE IDENTIFIED WITH THE LATTER (so this "virus" did not produce varicella, herpes zoster, or herpes simplex...good to know!)

"Suggestive evidence?" Seems more like NO EVIDENCE.

https://docs.google.com/document/d/e/2PACX-1vSPVXnpiqSdXniYmCGZW2lrAUmFt-BIbBD5ZVZ1zlNR1tjk-5PD7X3jrV_eiwkhBz88qT9hjSvoikGt/pub

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INFLUENZA

THE CREATION OF THE 1918 SPANISH FLU "VIRUS:"

In 2005, it was announced that the full genome of the 1918 Spanish flu had been recreated in a lab. There was much controversy over this as people feared a highly "virulent" strain could potentially escape from the lab and infect the population. However, if one were to dig deeper into the creation of this "virus," one would discover the hysteria was much ado about nothing. A little background first:

From the CDC's recounting of the reconstruction:

"USING REVERSE GENETICS, DR.TUMPEY TOOK THE PLASMIDS CREATED BY Dr. PALESE FOR EACH OF THE 1918 VIRUS' EIGHT GENE SEGMENTS AND INSERTED THEM INTO HUMAN KIDNEY CELLS. The plasmids then instructed the cells to reconstruct the RNA of the complete 1918 virus. For multiple weeks in July 2005, colleagues and collaborators ASKED DR. TUMPEY IF HE HAD THE 1918 VIRUS AND IF IT HAD APPEARED IN CELL-CULTURE YET.

ON THE DAY THE 1918 VIRUS APPEARED IN HIS CELL-CULTURE, Dr. Tumpey knew

history had been made, and in fact, a historic virus had been brought back from extinction. He sent a playful, Neil Armstrong-inspired email later that day to colleagues and collaborators, which simply said “That’s one small step for man, one giant leap for mankind.” Everyone then knew what had been accomplished. Dr. Tumpey had become the first man to RECONSTRUCT the complete 1918 virus. The next step was to study it and unlock its deadly secrets."

https://www.cdc.gov/flu/pandemic-resources/reconstruction-1918-virus.html

Keeping in mind that once again we have the typical cell culture soup where a "virus" is assumed to be hiding within, the below image taken from the CDC shows what Tumpey claimed was his "virus." Keep in mind that the images are nothing but cell deterioration called cytopathogenic effect stemming from the culture conditions. No "virus" is able to be seen in the images. No attempts were made to purify/isolate any "virus" in the paper. Only one EM image of particles claimed to be the "virus" was ever taken. Neither the cell culture nor the EM image were in the original Tumpey Science paper in 2005 but both appeared on the CDC website with no description for how they were taken/obtained.

Below are highlights from Tumpey's 2005 paper along with some other relevant highlights from papers he referenced to give an idea about what went into this Frankenstein-like reconstruction of an assumed "virus."

CHARACTERIZATION OF THE RECONSTRUCTED 1918 SPANISH INFLUENZA PANDEMIC VIRUS

"Genomic RNA of the 1918 virus was RECOVERED FROM ARCHIVED FORMALIN-FIXED LUNG AUTOPSY MATERIALS AND FROM FROZEN, UNFIXED LUNG TISSUES from an Alaskan influenza victim who was buried in permafrost in November of 1918 (5, 6)."

"PLASMID-BASED REVERSE GENETICS HAS ALLOWED FOR THE GENERATION OF RECOMBINANT VIRUSES containing 1918 hemagglutinin (HA) with or without the 1918 neuraminidase (NA) RESCUED IN THE GENETIC BACKGROUND OF CONTEMPORARY HUMAN H1N1 OR H3N2 INFLUENZA VIRUSES. The resulting strains were demonstrated to cause mortality in mice only at high infection doses (12, 13); however, THE VIRULENCE OF THE COMPLETE 1918 VIRUS HAS NOT BEEN EVALUATED."

"In the present study, WE GENERATED A VIRUS CONTAINING THE COMPLETE

CODING SEQUENCES OF THE EIGHT VIRAL GENE SEGMENTS from the 1918 virus in an effort to understand the molecular basis of virulence of this pandemic virus. GENES ENCODING THE 1918 INFLUENZA VIRUS WERE RECONSTRUCTED FROM DEOXYOLIGONUCLEOTIDES and corresponded to the reported coding sequences of the 1918 virus as previously described (5–11). Because the 1918 5¶ and 3¶ noncoding regions HAVE NOT BEEN SEQUENCED, THE GENES WERE CONSTRUCTED SUCH THAT THEY HAD THE NON CODING REGIONS CORRESPONDING TO THE CLOSELY RELATED INFLUENZA A/WSN/33 (H1N1) VIRUS. The 1918 virus and recombinant H1N1 influenza viruses were generated USING THE PREVIOUSLY DESCRIBED REVERSE GENETICS SYSTEM (8, 14). All viruses containing one or more gene segments from the 1918 influenza virus were generated and handled under high-containment Ebiosafety level 3 enhanced (BSL3)^ laboratory conditions in accordance with guidelines of the National Institutes of Health and the Centers for Disease Control and Prevention (15). VIRUSES WERE GROWN IN MADIN-DARBY CANINE KIDNEY CELLS (MDCK) CELLS AND/OR THE ALLANTOIC CAVITY OF 10-DAY-OLD EMBRYONATED HENS_ EGGS (table S1). The control viruses included an avian A/duck/Alberta/35/76 H1N1 virus, two contemporary human H1N1 influenza viruses, the wild-typeA/New Caledonia/20/99 (N. Cal/99, H1N1) virus and A/Texas/36/91 (Tx/91, H1N1) virus GENERATED BY REVERSE GENETICS. THE OTHER RECOMBINANT VIRUSES USED were a virus having only the HA from the Tx/91 virus with the remaining seven genes from the 1918 virus (Tx/91HA:1918); a virus having the NA from 1918 with the remaining seven genes from the Tx/91 virus (1918 NA:Tx/91); and recombinant viruses having two 1918 (1918 HA/NA:Tx/91) or five 1918 genes (1918 HA/NA/M/NP/NS:Tx/91) with the remaining genes derived from the Tx/91 virus. The HA of the 1918 viruses used throughout these studies WAS DERIVED FROM A/SouthCarolina/1/18 STRAIN that was shown to preferentially bind the a2,6 sialic acid (human) cellular receptor (16). The identity of the 1918 and Tx/91 influenza virus genes in the rescued viruses was confirmed by reverse transcription polymerase chain reaction and sequence analysis."

DOI: 10.1126/science.1119392

In Summary (Part 1):

-genomic RNA of the 1918 "virus" was RECOVERED FROM ARCHIVED FORMALIN-FIXED LUNG AUTOPSY MATERIALS AND FROM FROZEN, UNFIXED LUNG TISSUES from an Alaskan influenza victim who was buried in permafrost in November of 1918

-in other words, no "virus" was actually taken but they took RNA from the tissues and assumed it belonged to one in order to genetically engineer a genome

-PLASMID-BASED REVERSE GENETICS HAS

ALLOWED FOR THE GENERATION OF RECOMBINANT "VIRUSES" containing 1918 hemagglutinin (HA) with or without the 1918 neuraminidase (NA) RESCUED IN THE GENETIC BACKGROUND OF CONTEMPORARY HUMAN H1N1 OR H3N2 INFLUENZA "VIRUSES"

(Quick reminder on Recombinant "Viruses:"

RECOMBINANT DEFINITION:

"of or resulting from NEW COMBINATIONS OF GENETIC MATERIAL:

the genetic material produced when segments of DNA FROM DIFFERENT SOURCES ARE JOINED to produce recombinant DNA."

https://www.dictionary.com/browse/recombinant

"2a: relating to or CONTAINING GENETICALLY ENGINEERED DNA

b: PRODUCED BY GENETIC ENGINEERING"

https://www.merriam-webster.com/dictionary/recombinant

In other words, recombinant "viruses" are genetically engineered from DNA from various sources.)

-the virulence of the complete 1918 "virus" had not been evaluated

-they claim that they GENERATED a "virus" containing the complete coding sequences of the eight "viral" gene segments

-all of these 8 sequences were based on the sequencing work of other researchers over the previous 7 years using human, pig, and bird sequences (more on this later)

-genes encoding the 1918 influenza "virus" were RECONSTRUCTED from deoxyoligonucleotides and corresponded to the reported coding sequences of the 1918 "virus" as previously described (see Reference 5 and 6 later)

-because the 1918 5¶ and 3¶ noncoding regions HAVE NOT BEEN SEQUENCED, THE

GENES WERE CONSTRUCTED such that they had the noncoding regions corresponding to the closely related influenza A/WSN/33 (H1N1) "virus"

-in other words, since they did not have those regions, they constructed them off a "virus" they assumed was closely related

-the 1918 "virus" and recombinant H1N1 influenza "viruses" were generated USING THE PREVIOUSLY DESCRIBED REVERSE GENETICS SYSTEM (see Reference 😎

-"VIRUSES" WERE GROWN IN MADIN-DARBY CANINE KIDNEY CELLS (MDCK) CELLS AND/OR THE ALLANTOIC CAVITY OF 10-DAY-OLD EMBRYONATED HENS_ EGGS

-the control "viruses" included an avian A/duck/Alberta/35/76 H1N1 "virus," two contemporary human H1N1 influenza "viruses," the wild-typeA/New Caledonia/20/99 (N. Cal/99, H1N1) "virus" and A/Texas/36/91 (Tx/91, H1N1) "virus" GENERATED BY REVERSE GENETICS

-the other RECOMBINANT "VIRUSES" used were a "virus" having only the HA from the Tx/91 "virus" with the remaining seven genes from the 1918 "virus" (Tx/91HA:1918); a "virus" having the NA from 1918 with the remaining seven genes from the Tx/91 "virus" (1918 NA:Tx/91); and recombinant "viruses" having two 1918 (1918 HA/NA:Tx/91) or five 1918 genes (1918 HA/NA/M/NP/NS:Tx/91) with the remaining genes derived from the Tx/91 "virus"

-the HA of the 1918 "viruses" used throughout these studies WAS DERIVED FROM A/SOUTHCAROLINA/1/18 STRAIN that was shown to preferentially bind the a2,6 sialic acid (human) cellular receptor

As can be seen from these highlights, Tumpey's "virus" is nothing more than reverse-engineered recombinant "viruses" taken from multiple sources and cultured in the usual toxic conditions in both canine kidney cells and chicken embryos.

These next three sections relate to the references cited in the 2005 paper. The first two are regarding the acquisition of the "viral" RNA. Note they are not stating that they recovered any "viruses." They did not purify/isolate any particles assumed to be "virus." They took formalin-fixed, resin embedded lung tissue from corpses from 1918 and looked for RNA that they felt was "viral" based on reference sequences from other unpurified and unisolated "viruses." From there, they attempted to create sequences that they felt were most likely what the 1918 "virus" would have been made up of. The first highlights come from a paper from 1997.

REFERENCE 5:

INITIAL GENETIC CHARACTERIZATION OF THE 1918 "SPANISH" INFLUENZA VIRUS

"The Armed Forces Institute of Pathology in Washington, D.C., has autopsy material consisting of formalin-fixed paraffin-embedded tissue and hematoxylin- and eosin-stained sections from U.S. servicemen killed in the 1918 pandemic. WE RANDOMLY SELECTED 28 CASES FOR PATHOLOGICAL REVIEW. Of these, the MAJORITY DIED OF ACUTE BACTERIAL PNEUMONIA, one of the most common sequelae of the pandemic (12). Sections in these cases demonstrated acute lobar pneumonia with massive neutrophilic infiltrates. As influenza virus replication peaks within 2 days in the respiratory tract, with little virus being shed after 6 days (13, 141) WE JUDGED IT UNLIKELY THAT THESE CASES WOULD RETAIN INFLUENZA VIRUS. Several other cases, in which the victim died within 1 week after initial symptoms, DEMONSTRATED A DISTINCT HISTOLOGY NOTED BY PATHOLOGISTS PERFORMING AUTOPSIES IN 1918 (15, 16). These cases often showed massive pulmonary edema and alveolar hemorrhage with acute bronchopneumonia. ON THE ASSUMPTION THAT VIRUS MAY STILL HAVE BEEN PRESENT IN THESE CASES, we chose 14 formalin-fixed, paraffin-embedded tissue specimens representing seven such individuals for further analysis. The pathologic diagnoses noted in these cases were alveolar hemorrhage and bronchopneumonia (three cases), bronchopneumonia (three cases), and one case showing asynchrony between the disease manifestations in the left and right lungs. This case (1918 case 1) showed acute bacterial lobar pneumonia in the left lung but focal acute bronchiolitis and alveolitis in the right lung, which is indicative of a primary viral pneumonia. THIS WAS THE ONLY CASE AMONG THE 28 EXAMINED IN WHICH THE HISTOLOGIC FEATURES OF EARLY VIRAL PNEUMONIA WERE CONCLUSIVELY PRESENT."

Control amplification of reverse-transcribed fragments by polymerase chain reaction (RT-PCR) for p-actin was positive in 11 of 14 formalin-fixed, paraffin-embedded tissue samples examined. However, RNA TEMPLATES LARGER THAN 200 NUCLEOTIDES WERE NOT APPLICABLE. We therefore designed nine degenerate, consensus RT-PCR primer sets to amplify SMALL (under 200 nucleotides) FRAGMENTS OF THE GENES encoding hemagglutinin (four fragments), neuraminidase, nucleoprotein (two fragments), matrix protein 1, and matrix protein

2. TWO INFLUENZA STRAINS-ONE AVIAN [A/Duck/Alberta/35/76 (HINI)] AND ONE HUMAN [A/PR/8/34 (H1NI)L-WERE USED AS POSITIVE CONTROLS FOR EACH GENE SEGMENT TO DEMONSTRATE THAT THE PRIMERS COULD DETECT A

WIDE RANGE OF INFLUENZA STRAINS. No other influenza strains have ever been used in this laboratory. We were able to amplify and sequence ALL NINE FRAGMENTS of influenza virus RNA from 1918 case 1. Replicate RT-PCR reactions with different tissue blocks from the same case gave identical sequence results. None of the other cases selected by histologic criteria and examined by RT-PCR for influenza virus RNA were positive."

"Although the length of sequence between primers was small, phylogenetic analyses for each gene segment were possible with the use of the corresponding regions of other previously sequenced influenza viruses. Analyses were carried out with two computer software packages: Molecular Evolutionary Genetics Analysis (MEGA), version 1.01 (1 7), and Phylogenetic Analysis Using Parsimony (PAUP), version 3.1 (1 ."

doi: 10.1126/science.275.5307.1793.

In Summary (Part 2):

-they randomly selected 28 cases for pathological review

-of those, the majority died of acute bacterial pneumonia, one of the most common sequelae of the pandemic

-they judged it UNLIKELY THAT THESE CASES WOULD RETAIN INFLUENZA "VIRUS" as influenza "virus" replication peaks within 2 days in the respiratory tract, with little "virus" being shed after 6 days

-several other cases, in which the victim died within 1 week after initial symptoms, demonstrated a DISTINCT HISTOLOGY noted by pathologists performing autopsies in 1918

-on the ASSUMPTION THAT "VIRUS" MAY STILL HAVE BEEN PRESENT in these cases, they chose 14 formalin-fixed, paraffin-embedded tissue specimens representing seven such individuals for further analysis

-one case showed acute bacterial lobar pneumonia in the left lung but focal acute bronchiolitis and alveolitis in the right lung, which they claimed was INDICATIVE of a primary "viral" pneumonia

-this was the only case among the 28 examined in which the HISTOLOGIC FEATURES of early "viral" pneumonia were conclusively present so they assumed once again that "virus" must be present in the tissues

-two influenza strains-one avian [A/Duck/Alberta/35/76 (HINI)] and one human [A/PR/8/34 (H1NI)l-were used as positive controls for each gene segment to demonstrate that the primers could detect A WIDE RANGE OF INFLUENZA STRAINS

-RNA templates larger than 200 nucleotides WERE NOT AMPLIFIABLE

-therefore, they designed nine degenerate, consensus RT-PCR primer sets TO AMPLIFY SMALL (under 200 nucleotides) FRAGMENTS of the genes encoding hemagglutinin (four fragments), neuraminidase, nucleoprotein (two fragments), matrix protein 1, and matrix protein

-they state that they were able to amplify and sequence all NINE FRAGMENTS of influenza "virus" RNA from 1918 case 1

The highlights from this second paper from 1999 gives some more insight into how they reconstructed the genes said to belong to the 1918 "virus:" Note that they extract the RNA and then align to sequences stemming from human, pig, and bird "viruses" in order to claim influenza "viral" RNA. No "virus" is ever purified/isolated and only tiny fragments of RNA are created. There is nothing natural about this process.

REFERENCE 6:

ORIGIN AND EVOLUTION OF THE 1918 “SPANISH” INFLUENZA VIRUS HEMAGGLUTININ GENE

"MATERIALS AND METHODS

Case Selection.

Autopsy cases of 78 victims of the lethal fall wave of the 1918 pandemic were examined for this study. Evidence from 74 victims CONSISTED OF FORMALIN-FIXED, PARAFFIN-EMBEDDED TISSUES, stained slides, and clinical records from the files of the Armed Forces Institute of Pathology. ALL THE SAMPLES WERE SCREENED BY HISTOLOGIC ANALYSIS. The majority of individuals died of secondary acute bacterial pneumonia, the most common cause of death in the 1918 pandemic (10); MOST OF THE SAMPLES TAKEN FROM THESE INDIVIDUALS WERE NOT ANALYZED FURTHER, BECAUSE THEY WERE EXTREMELY UNLIKELY TO RETAIN INFLUENZA VIRUS (11, 12). However, 13 samples were selected by HISTOLOGIC and CLINICAL CRITERIA for further analysis. These samples were from patients who experienced acute influenza deaths after clinical courses of less than 1 week. In addition to samples taken from patients with early bronchopneumonia, samples from patients with acute massive pulmonary edema and/or hemorrhage were also selected, reflecting the unusual histopathology observed in 1918 (13). Of these 13 samples, 2 WERE POSITIVE FOR INFLUENZA RNA ON SUBSEQUENT MOLECULAR GENETIC

ANALYSIS.

Case Histories.

The first patient was a 21-year-old male stationed at Ft. Jackson, SC. He was admitted to the camp hospital on September 20, 1918 with influenza and pneumonia. He had a progressive course with cyanosis and died on September 26, 1918. During the autopsy, it was noted that he had a fatal secondary lobar bacterial pneumonia in his left lung, whereas the right lung showed only focal acute bronchiolitis and alveolitis, indicative of primary influenza pneumonia. FORMALIN-FIXED, PARAFFIN-EMBEDDED RIGHT LUNG TISSUE WAS POSITIVE FOR INFLUENZA RNA [A/South Carolina/1/18 (H1N1)] as reported (2). The second patient was a 30-year-old male stationed at Camp Upton, NY. He was admitted to the camp hospital with influenza on September 23, 1918, had a very rapid clinical course, and died from acute respiratory failure on September 26, 1918. The autopsy showed massive bilateral pulmonary edema and focal acute bronchopneumonia. FORMALIN-FIXED, PARAFFIN-EMBEDDED LUNG TISSUE WAS POSITIVE FOR INFLUENZA RNA [A/New York/1/18 (H1N1)]. RNA TEMPLATES LARGER THAN 150 NUCLEOTIDES COULD NOT BE AMPLIFIED IN THESE TWO CASES. An additional 1918 influenza case was found by examining lung tissue from four 1918 influenza victims exhumed from a mass grave in Brevig Mission on the Seward Peninsula of Alaska. Brevig Mission (called Teller Mission in 1918) suffered extremely high mortality during the influenza pandemic in November 1918. Although individual case records were not available, historical records show that influenza spread through the village in about 5 days, killing 72 people, representing about 85% of the adult population (1, 14). Victims were buried in a mass grave in permafrost. In August 1997, four of these victims were exhumed. FROZEN LUNG TISSUES WERE BIOPSIED IN SITU FROM EACH, AND TISSUES WERE PLACED IN FORMALIN, ALCOHOL FIXATIONS, AND RNAzol (Tel-Test, Friendswood, TX). Although the histologic analysis was hampered by artifacts of freezing, these tissues showed evidence of acute massive pulmonary hemorrhage and edema. ONE OF THE VICTIMS, an Inuit female (age unknown) WAS INFLUENZA RNA POSITIVE [A/Brevig Mission/1/18 (H1N1)]. In this case, RNA TEMPLATES GREATER THAN 120 NUCLEOTIDES COULD NOT BE AMPLIFIED.

RNA Extraction.

RNA lysates from the paraffin-embedded tissues were produced as described (15). RNA WAS ISOLATED FROM THE FROZEN LUNG TISSUE BY USING RNAzol (Tel-Test) according to the manufacturer’s instructions.

Reverse Transcription–PCR (RT-PCR).

RT was carried out at 37°C for 45 min in 20 µl of 1× RT buffer (GIBCO/BRL) CONTAINING 300 UNITS OF MOLONEY MURINE LEUKEMIA VIRUS REVERSE TRANSCRIPTASE, 5 µM random hexamers, 200 nM dNTP, and 10 mM DTT. RT reaction (2 µl) was added to a 20-µl PCR containing 50 mM KCl, 10 mM Tris, 2.5 mM MgCl2, 1 µM each primer, 100 nM dNTP, 1 unit of Amplitaq Gold (Perkin—Elmer), and 2 µCi of 32P-labeled dATP (3,000 Ci/mmol). The entire HA coding sequence of 1,701 nucleotides was amplified in 22 overlapping fragments, such that the sequences matching primers could be confirmed. THE PRIMERS WERE DESIGNED AS DEGENERATE H1 CONSENSUS PRIMERS BY USING ALIGNMENTS OF HUMAN, SWINE, AND AVIAN H1 HA SEQUENCES.

"PCR conditions were 9 min at 94°C; 40 CYCLES of 94°C for 30 sec, 50°C for 30 sec, 72°C for 30 sec; and 72°C for 5 min."

"ANALYSIS OF THE 1918 HA SEQUENCE PERMITS ALTERNATIVE INTERPRETATIONS AS TO ITS ORIGIN. The 1918 sequences are phylogenetically distinct from current avian strains. One possibility is that around 1918 there existed an avian strain more similar to the pandemic virus than current avian strains and that its HA entered with little modification into the human population. If avian viruses have not drifted over the past 80 years, such a strain would differ from current avian strains phylogenetically. THIS HYPOTHESIS CANNOT BE TESTED, BECAUSE AVIAN H1 INFLUENZA-VIRUS ISOLATES FROM THAT TIME DO NOT EXIST. A second possibility is that the pandemic virus had been adapting in mammals before 1918 and that it had accumulated enough changes to make its HA gene seem more mammalian by many phylogenetic criteria (e.g., parsimony and NJ). Our data and those of others (29) suggest that an entry date into the human population between 1900 and 1915 is reasonable."

"The 1918 influenza virus HA gene does not possess the cleavage site mutation seen in virulent avian influenza strains. No other known genetic changes were observed in the 1918 HA sequence that would account for the exceptional virulence of this pandemic virus. WHAT DETERMINES THE VIRULENCE OF A PARTICULAR INFLUENZA STRAIN IS QUITE COMPLEX and involves host adaptation, transmissibility, tissue tropism, and replication efficiency. THE GENETIC BASIS FOR VIRULENCE OF OTHER INFLUENZA STRAINS (FOR WHICH COMPLETE GENOMIC SEQUENCE IS AVAILABLE) CANNOT BE DETERMINED YET, but it is most likely polygenic in nature (4)."

https://www.pnas.org/content/96/4/1651

In Summary (Part 3):

-evidence from 74 victims consisted of FORMALIN-FIXED, PARAFFIN-EMBEDDED TISSUES, stained slides, and clinical records from the files of the Armed Forces Institute of Pathology

-all the samples were screened by histologic analysis

-most of the samples taken from these individuals were not analyzed further, BECAUSE THEY WERE EXTREMELY UNLIKELY TO RETAIN INFLUENZA "VIRUS"

-13 samples were selected by HISTOLOGIC and CLINICAL CRITERIA for further analysis

-2 were positive for influenza RNA on subsequent molecular genetic analysis

-Case 1: formalin-fixed, paraffin-embedded right lung tissue was positive for influenza RNA [A/South Carolina/1/18 (H1N1)]

-Case 2: formalin-fixed, paraffin-embedded lung tissue was positive for influenza RNA [A/New York/1/18 (H1N1)]

-RNA templates larger than 150 nucleotides COULD NOT BE AMPLIFIED in these two cases

-an additional 1918 influenza case was found by examining lung tissue from four 1918 influenza victims exhumed from a mass grave in Brevig Mission on the Seward Peninsula of Alaska

-frozen lung tissues were biopsied in situ from each, and TISSUES WERE PLACED IN FORMALIN, ALCOHOL FIXATIONS, AND RNAzol

-one of the victims, an Inuit female (age unknown) was influenza RNA positive [A/Brevig Mission/1/18 (H1N1)]

-in this case, RNA templates greater than 120 nucleotides COULD NOT BE AMPLIFIED

-RNA was isolated from the frozen lung tissue by using RNAzol

-RT was carried out at 37°C for 45 min in 20 µl of 1× RT buffer (GIBCO/BRL) CONTAINING 300 UNITS OF MOLONEY MURINE LEUKEMIA "VIRUS" REVERSE TRANSCRIPTASE which is a RECOMBINANT DNA polymerase that SYNTHESIZES a complementary DNA strand from single-stranded RNA, DNA, or an RNA:DNA hybrid

-the primers were designed as degenerate H1 CONSENSUS PRIMERS BY USING

ALIGNMENTS OF HUMAN, SWINE, AND AVIAN H1 HA SEQUENCES

-PCR conditions were 9 min at 94°C; 40 CYCLES of 94°C for 30 sec, 50°C for 30 sec, 72°C for 30 sec; and 72°C for 5 min

-analysis of the 1918 HA sequence permits ALTERNATIVE INTERPRETATIONS as to its origin

-the avian origin hypothesis cannot be tested, because avian H1 "influenza-virus" isolates from that time do not exist

-they state that what determines the virulence of a particular influenza strain is QUITE COMPLEX and involves host adaptation, transmissibility, tissue tropism, and replication efficiency - or in other words, they don't know how a "virus" is determined virulent beyond speculation and guesses

-THE GENETIC BASIS FOR VIRULENCE OF OTHER INFLUENZA STRAINS (for which complete genomic sequence is available) CANNOT BE DETERMINED YET, but it is most likely polygenic in nature"

Again, it can be seen that all they did was look at the 90-year-old tissue samples from a few corpses and determined that 3 of them most likely had "virus" in their tissues. They extracted RNA from the tissues and used consensus primers derived from alignments of human, pig, and bird sequences to create tiny fragments in order to claim that influenza "viral" RNA was present in the samples.

This third reference details the reverse genetics process they referenced using to create their full 1918 genome. It is nothing more than the usual cell culture tricks this time with canine kidney cells and the use of multiple "viruses' ' along with cloned cDNA's to create recombinant "viruses' ' which were used to generate sequences from.

REFERENCE 8:

SEQUENCE OF THE 1918 PANDEMIC INFLUENZA VIRUS NO STRUCTURAL GENE (NS) SEGMENT AND CHARACTERIZATION OF RECOMBINANT VIRUSES BEARING THE 1918 NS GENES

"By using the recently developed technique of generating influenza A viruses ENTIRELY FROM CLONED cDNAs, the hypothesis that the 1918 virus NS1 gene played a role in virulence was tested in a mouse model. In a BSL3+ laboratory, VIRUSES WERE GENERATED that possessed either the 1918 NS1 gene alone or the entire 1918 NS segment in a background of influenza A/WSN/33 (H1N1), A MOUSE-ADAPTED VIRUS

DERIVED FROM A HUMAN INFLUENZA STRAIN FIRST ISOLATED IN 1933."

"SYSTEMS THAT PERMIT THE GENERATION OF INFLUENZA VIRUSES FROM CLONED cDNAs ALLOW RECOMBINANT INFLUENZA VIRUSES BEARING GENES OF THE 1918 PANDEMIC VIRUS TO BE CONSTRUCTED."

"Materials and Methods

Virus Strains and Cells.

The transfectant influenza viruses, influenza A/WSN/33 (H1N1) virus (WSN) and influenza A/PR/8/34 (H1N1) virus (PR8), WERE PROPAGATED ON MADIN–DARBY CANINE KIDNEY (MDCK) CELLS (MAINTAINED IN MEM, 10% FBS). 293T CELLS WERE MAINTAINED IN DMEM, 10% FBS.

RNA Extraction, RT-PCR, and DNA Sequencing of Frozen Tissue Samples.

The 1918 case and the viral strain, A/Brevig Mission/1/18 (H1N1), used for this study were as described previously (2, 9). RNA WAS ISOLATED FROM THE FROZEN LUNG TISSUE BY USING RNAzol (Tel-Test, Friendswood, TX), following the manufacturer's instructions.

RT was carried out at 37°C for 45 min in 20 µl BY USING 300 UNITS MALONEY MURINE LEUKEMIA VIRUS-REVERSE TRANSCRIPTASE/1× RT buffer (Life Technologies, Grand Island, NY)/5 µM random hexamers/200 nM dNTPs/10 mM DTT. RT reaction (2 µl) was added to an 18-µl PCR reaction mixture containing 50 mM KCl, 10 mM Tris-HCl, 2.5 mM MgCl2, 1 µM each primer, 100 nM dNTPs, 1 unit Amplitaq Gold (Perkin–Elmer), and 2 µCi (1 Ci = 37 GBq) 32P dATP (3,000 Ci/mmol). The entire NS coding sequence of A/Brevig Mission/1/18 (H1N1) (838 nucleotides) was amplified in 13 overlapping fragments, such that the sequences corresponding to primers could be confirmed. THE PRIMERS WERE DESIGNED AS DEGENERATE NS CONSENSUS PRIMERS BY USING ALIGNMENTS OF HUMAN, SWINE, AND AVIAN NS SEQUENCES, OR AS 1918-SPECIFIC PRIMERS ONCE PARTIAL SEQUENCE WAS AVAILABLE. Primer sequences used are available on request."

https://www.pnas.org/content/98/5/2746

In Summary (Part 4):

-by using the recently developed technique of generating influenza A "viruses" ENTIRELY FROM CLONED cDNAs, the HYPOTHESIS that the 1918 "virus" NS1 gene played a role in virulence WAS TESTED IN A MOUSE MODEL

-"viruses" were GENERATED that possessed either the 1918 NS1 gene alone or the entire 1918 NS segment in a background of influenza A/WSN/33 (H1N1), A MOUSE-ADAPTED "VIRUS" DERIVED FROM A HUMAN INFLUENZA STRAIN FIRST ISOLATED IN 1933

-they claim that systems that permit the generation of influenza "viruses" from CLONED cDNAs allow RECOMBINANT influenza "viruses" bearing genes of the 1918 pandemic "virus" TO BE CONSTRUCTED

-the transfectant influenza "viruses," influenza A/WSN/33 (H1N1) "virus" (WSN) and influenza A/PR/8/34 (H1N1) "virus" (PR8), were propagated on MADIN–DARBY CANINE KIDNEY (MDCK) CELLS (maintained in MEM, 10% FBS). 293T cells were MAINTAINED IN DMEM, 10% FBS

-in other words, they used the usual cell culture tricks of creating toxic soup this time with mouse and dog DNA mixed with fetal cow blood along with the usual media ingredients and claimed "viruses" were present in these mixtures

-RNA was isolated from the frozen lung tissue by using RNAzol

-300 units Maloney murine leukemia virus-reverse transcriptase was once again used

-the primers were designed as degenerate NS CONSENSUS PRIMERS BY USING ALIGNMENTS OF HUMAN, SWINE, AND AVIAN NS SEQUENCES, or as 1918-specific primers once partial sequence was available

-in other words, the genome is a mixture of consensus sequences from humans, pigs, and birds

It is clear that this "reconstruction" of the 1918 Spanish flu "virus" is a hodgepodge of assumptions, guesswork and cell culture/sequencing tricks. They were never able to take any "virus": directly from the 90-year-old lung tissues, only RNA which they assumed was "viral" because the tiny fragments somewhat matched sequences from previous influenza genomes. The problem is these reference genomes also never come from purified/isolated "viruses" and are created using the same tricks. In order for any of them to be accurate, the purification and isolation of an actual "virus" taken directly from a human must occur first. These purified/isolated particles must be proven pathogenic in a natural way. Only once these particles are separated from everything else would it be

theoretically possible to create an accurate genome.

Until then, the 1918 Spanish flu "virus" is nothing more than a recombinant cell cultured creation from many human/animal sources stemming from unrelated RNA fragments created over years and stitched together off of human, pig, and bird reference sequences.

In other words, PURE FICTION.

(The accompanying text images details the Plasmid-Based Reverse Genetics of Influenza A "Virus" process from a manual in 2020. I'm providing it just to give some more insight into what was potentially done. I could not get the exact details of the method from 2005.

doi: 10.1007/978-1-0716-0346-8_4.)

I guess the one person rule didn't apply to cameraman James Gathany?

"As part of security and safety considerations, CDC’s Office of the Director determined that ONLY ONE PERSON WOULD BE GRANTED PERMISSION, LABORATORY ACCESS, and the tremendous responsibility of reconstructing the 1918 virus. That person was trained microbiologist Dr. Terrence Tumpey, who was approved for the project by then CDC director, Dr. Julie Gerberding."

"Dr. Tumpey’s work to reconstruct the complete 1918 virus began in the summer of 2005. To reduce risk to colleagues and the public, HE WAS REQUIRED TO WORK ON THE VIRUS ALONE AND ONLY AFTER HOURS WHEN FELLOW COLLEAGUES HAD EXITED THE LABORATORIES FOR THE DAY AND GONE HOME."

"A picture of Dr. Terrence Tumpey working in BSL3 enhanced laboratory conditions. This includes (but isn’t limited to) use of a powered air purifying respirator (PAPR), double gloves, suit, and working within a Class II biosafety cabinet (BSC). Today, Dr. Tumpey is the branch chief of the Immunology and Pathogenesis Branch in CDC’s Influenza Division. Photo credit: JAMES GATHANY - Public Health Image Library #7989."

https://www.cdc.gov/flu/pandemic-resources/reconstruction-1918-virus.html

https://docs.google.com/document/d/e/2PACX-1vRHWpdYfdF-cE54j5XSHUHBNoZUXUj8nyaQa_nLejFU19xeZmMiQaadbdbDYEIIO4zgDgRoF8ek_cre/pub

RICHARD SHOPE 1931 SWINE FLU PAPER:

I finally decided to wade into the mess that is influenza research. In determining where to start, I found that Influenza A was originally "isolated" in 1933. That seemed like a logical place to begin unraveling this mess. However, after digging a bit more, I found that the 1931 "isolation" of Swine Flu preceded that of Influenza A:

"The human influenza A virus was discovered in 1933 soon AFTER SHOPE

SUCCEEDED IN ISOLATING SWINE INFLUENZA A VIRUS IN 1931."

https://pubmed.ncbi.nlm.nih.gov/9360364/

Digging a bit deeper, I came across this paper elucidating the history a bit more. Upon reading it, something quite glaring stood out to me:

"INFLUENZA: EXPOSING THE TRUE KILLER

In the early 1930s, RICHARD SHOPE ISOLATED INFLUENZA VIRUS FROM INFECTED PIGS. Shope's finding was quickly followed by the isolation of the influenza virus from humans, PROVING THAT A VIRUS—NOT A BACTERIUM, AS WAS WIDELY BELIEVED—CAUSED INFLUENZA.

In 1892, German bacteriologist Richard Pfeiffer ISOLATED WHAT HE THOUGHT WAS THE CAUSATIVE AGENT OF INFLUENZA. The culprit, according to Pfeiffer, was a small rod-shaped bacterium that he isolated from the noses of flu-infected patients (1). He dubbed it Bacillus influenzae (or Pfeiffer's bacillus). FEW DOUBTED THE VALIDITY OF THIS DISCOVERY, in large part because bacteria had been shown to cause other human diseases, including anthrax, cholera, and plague.

The filtration question

When history's deadliest influenza pandemic began in 1918, MOST SCIENTISTS BELIEVED THAT PFEIFFER'S BACILLUS CAUSED INFLUENZA. With the lethality of this outbreak (which killed an estimated 20 to 100 million worldwide) came urgency—researchers around the world began to search for Pfeiffer's bacillus in patients, hoping to develop antisera and vaccines that would protect against infection. In many patients, BUT NOT ALL, the bacteria were found. Failures to isolate B. influenzae (now known as Haemophilus influenzae) were largely chalked up to inadequate technique, as the bacteria were notoriously difficult to culture (2).

The first potential blow to Pfeiffer's theory came from Peter Olitsky and Frederick Gates at The Rockefeller Institute. Olitsky and Gates took nasal secretions from patients infected with the 1918 flu and passed them through Berkefeld filters, which exclude bacteria. The infectious agent—which caused lung disease in rabbits—passed through the filter, SUGGESTING THAT IT WAS NOT A BACTERIUM (3, 4). ALTHOUGH THE DUO HAD PERHAPS ISOLATED THE INFLUENZA VIRUS (which they nevertheless

referred to as an atypical bacterium called Bacterium pneumosintes), OTHER RESEARCHERS COULD NOT REPRODUCE THEIR RESULTS. One of the doubters was Oswald Avery (Rockefeller Institute), who developed a culture media—chocolate agar—that optimized the growing conditions for B. influenzae and thus minimized false negative results from patient samples. THUS, THE IDEA THAT FLU WAS TRANSMITTED BY A FILTERABLE AGENT (OR VIRUS) WAS DISMISSED.

Insights from pigs

Olitsky and Gates would not be vindicated until a decade later, when Shope—A YOUNG PHYSICIAN FROM IOWA then working on hog cholera at the Rockefeller Institute—turned his attention to swine influenza.

Pig farmers in Iowa had reported two outbreaks—one in 1918 and another in 1929—of a highly contagious, influenza-like disease among their animals. THE DISEASE BORE SUCH A REMARKABLE RESEMBLANCE TO HUMAN FLU THAT IT WAS NAMED SWINE INFLUENZA. Shope and his mentor Paul Lewis took mucus and lung samples from the infected pigs and attempted to isolate the disease-causing agent. THEY QUICKLY ISOLATED A BACTERIUM THAT LOOKED EXACTLY LIKE PFEIFFER'S HUMAN BACTERIUM (and was thus called B. influenzae suis), BUT WHEN THEY INJECTED THE BACTERIA INTO PIGS, IT CAUSED NO DISEASE (5).

Shope then filtered the samples and, like Olitsky and Gates, found that the filtrate contained the infectious agent. Shope's filtrate caused a highly contagious, influenza-like disease in pigs—ALBEIT A MORE MILD ONE THAN SEEN IN NATURALLY-INFECTED PIGS. MIXING THE FILTRATE WITH THE BACTERIUM REPRODUCES THE SEVERE DISEASE. HE CONCLUDED—correctly—THAT THE FILTERABLE AGENT CAUSED THE INFECTION, WHICH THEN FACILITATED SECONDARY INFECTION WITH THE BACTERIUM (6). Shope published his results in a series of papers in The Journal of Experimental Medicine (5, 6)."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2118275/

What stood out to me wasn't the fact that until 1933 it was believed that the flu was caused by bacteria. Nor was it the fact that few "scientists" doubted bacteria as the cause. It wasn't the fact that Olitsky and Gates ended up "proving" a bacteria wasn't the cause but that it was potentially an invisible "filterable virus." It wasn't even the fact that other researchers could not reproduce the results of Olitsky and Gates thus confirming it

was not a "filterable virus" which caused the flu.

It was the fact that Richard Shope, the man given credit for "proving" that the dismissed "filterable virus" was once again considered the cause of the flu in pigs (and eventually humans), was a physician FROM IOWA!!! Naturally, being an Iowan myself, I had to read Shope's Swine Flu "proof." Consider it a matter of pride that one Iowan gets to critique the work of another nearly 100 years later. The fact that it helps to set up the Influenza A "proof" is just icing on the cake.

Below are highlights from Shope's paper with a summary at the end:

SWINE INFLUENZA III. FILTRATION EXPERIMENTS AND ETIOLOGY

"McBryde, Niles, and Moskey (1) MADE FIVE ATTEMPTS to pass the etiological agent of swine influenza through small Berkefeld or Mandler filters but WERE UNABLE TO REPRODUCE THE DISEASE BY DROPPING SUCH FILTRATES INTO THE NOSTRILS OF NORMAL HOGS. In two of these experiments unfiltered material failed to produce the disease in their control animals. Although these experiments are too few in number to be conclusive, THEY INDICATE THAT THE CAUSATIVE AGENT OF SWINE INFLUENZA IS NOT A FILTRABLE VIRUS."

EXPERIMENTAL

Since the studies in filtration reported by McBryde and his coworkers were not conclusive, the question of the filterability of the etiological agent of swine influenza has been reconsidered. THE RESULTS OF TEN FILTRATION EXPERIMENTS WITH INFECTIOUS MATERIAL FROM THE TWO STRAINS OF THE DISEASE OBTAINED IN 1928 AND THE TWO SECURED IN 1930 (2) WERE INCONSTANT AND CONFUSING. In these preliminary experiments the writer was not then cognizant of the possible etiological relationship existing between H. influenzae suis (3) and a filtrable agent to be described.

Material for filtration was prepared as follows.

DISEASED LUNG AND BRONCHIAL LYMPH NODES WERE MINCED WITH STERILE SCISSORS AND ADDED TO BRONCHIAL EXUDATE. This mixture was GROUND WITH SAND in a mortar. When it had been reduced to a pasty and fairly homogeneous consistency, a 10 to 20 per cent suspension was made by GRADUALLY ADDING

STERILE DISTILLED WATER OR INFUSION BROTH (pH 7.3). It was then SHAKEN WITH GLASS BEADS in a flask for l0 to 15 minutes and centrifuged. The supernatant fluid was removed by pipette and if more than moderately turbid it was centrifuged again. 24 hour bouillon cultures of B. prodigiosus were used to test the efficiency of all filters, and FILTRATES CULTURED IN 1 cc. AMOUNTS ON PLAIN AGAR SLANTS CONTAINING DEFIBRILLATED BLOOD were incubated at 37°C. for 48 to 72 hours before examination for growth.

Swine receiving filtered material were placed in carefully sterilized isolation units where isolation precautions were taken. IN CERTAIN EXPERIMENTS THE CONTROL ANIMAL RECEIVING UNFILTERED MATERIAL AND THOSE RECEIVING STERILE FILTRATES WERE FROM THE SAME SOURCE OR EVEN FROM THE SAME LITTER.

Of the ten preliminary filtration experiments, THREE WERE AT THE TIME INTERPRETED AS NEGATIVE, while in the remaining seven SOME EVIDENCE was obtained that the injected filtrate had contained an infectious agent. THE DISEASE INDUCED by this filtrable infectious agent, however, WAS DEFINITELY NOT TYPICAL SWINE INFLUENZA and will be referred to hereafter as "filtrate disease."

"CLINICALLY THE FILTRATE DISEASE WAS MUCH MILDER THAN SWINE INFLUENZA. In most cases there was NO ELEVATION IN TEMPERATURE, while in a few a fever temperature for 1 day was observed. This was at marked variance with the 4 to 6 day fevers seen in typical swine influenza. The usual symptoms shown by filtrate-inoculated swine were a moderate and transient apathy, some diminution in appetite for a period not exceeding 3 days, occasionally a slight cough, and, as in typical swine influenza (2), a moderate or quite marked leukopenia. THE EXTREME PROSTRATION SO COMMON IN SWINE INFLUENZA INFECTIONS WAS NOT SEEN. IN SOME INSTANCES THE DISEASE WAS SO MILD THAT IT ALMOST ESCAPED RECOGNITION ALTOGETHER. On this account and in the light of experiments to be outlined later in this paper, it seems possible that in the three preliminary experiments considered as negative, infections were actually produced but so mild in character that they escaped recognition.

The lesions exhibited at autopsy were similar in kind BUT DIFFERENT IN EXTENT, as a rule, from those encountered in typical uncomplicated swine influenza (2)."

"The filtration experiments just outlined indicated that infectious material from

experimental cases of swine influenza contains an agent capable of passage through Berkefeld filters V and N and possessing pathogenic properties when administered intranasally. H. INFLUENZAE SUIS, WHICH WAS CONSTANTLY ENCOUNTERED IN CULTURING THE RESPIRATORY TRACTS OF ANIMALS WITH TYPICAL SWINE INFLUENZA INFECTIONS (3), WAS NOT FOUND IN SIMILAR CULTURES FROM ANIMALS WITH THE MILD FILTRATE DISEASE.

ANAEROBIC CULTURES OF SEVEN FILTRATES OF SWINE INFLUENZA INFECTIOUS MATERIAL IN BLOOD BROTH AND IN 5 PERCENT SERUM BOUILLON OVER STERILE RABBIT KIDNEYS HAVE FAILED TO SHOW GROWTH. Four of the seven filtrates THUS CULTURED were tested by intranasal inoculation into swine and all were found capable of inducing the mild filtrate disease."

"Intranasal Inoculations with Mixtures of the Filtrable Agent and H. influenzae suis

Since the only constant difference bacteriologically between the mild disease induced by the filtrable agent and typical spontaneous or experimental swine influenza lies in the absence of H. influenzae suis in the filtrate-infected swine, THE COMBINATION OF THE ORGANISM AND THE FILTRABLE AGENT MAY BE ESSENTIAL FOR THE PRODUCTION OF THE NATURAL DISEASE. Experiments were conducted in which swine were inoculated intranasally with cultures of H. influenzae suis, which had been under cultivation for a long time (over 2 years in most instances), mixed with Berkefeld filtrates of infectious material from experimental cases of swine influenza. In these experiments the isolation and filtration practice outlined above was followed. The cultures of H. influenzae suis used were grown in defibrinated horse blood at the bases of plain agar slants in most instances for 24 hours. The undiluted blood culture was used in the inoculations and in all experiments the culture injected alone was identical with that mixed with filtrate before injection. The Berkefeld filtrate mixed with cultures of H. influenzae suis was identical with that injected alone in individual experiments. With the exception of the first experiment, all animals used in individual experiments were from the same source and in most instances litter mates. The results of these experiments are recorded in Table I.

ALL EIGHT OF THE SWINE INFECTED by inoculation with Berkefeld filtered infectious material or by contact with filtrate-infected swine DEVELOPED ONLY A MILD DISEASE. In some instances it was SO SLIGHT AS ALMOST TO ESCAPE RECOGNITION. None of the animals exhibited a febrile reaction. In 1 to 3 days after inoculation they appeared listless and apathetic for 2 or 3 days and there was some diminution in appetite. Those coming to autopsy showed enlarged and edematous cervical and bronchial lymph nodes, a small amount of tenacious mucoid exudate in some of the smaller bronchi, and

a scant scattered type of pulmonary atelectasis of one or more of the upper lobes of the lung.

The swine which were inoculated intranasally with pure cultures of H. influenzae suis WERE COMPLETELY NEGATIVE BOTH CLINICALLY AND AT AUTOPSY.

All the swine which received mixtures of the filtrable agent and H. influenzae suis developed a disease that was typical both clinically and at autopsy of swine influenza (4). OF THE SEVEN HOGS infected either by direct inoculation with the filtrate-culture mixture or by contact with swine so infected, THREE DEVELOPED TYPICAL SWINE INFLUENZA, and the DISEASE WAS OF ABOUT THE SAME SEVERITY AS THAT WHICH DEVELOPED IN THE CONTROL ANIMALS INOCULATED WITH UNFILTERED INFECTIOUS MATERIALS. TWO OTHERS HAD A MILD INFLUENZA, BUT IN THIS INSTANCE THE DISEASE WHICH DEVELOPED IN THE CONTROL ANIMAL WAS ALSO ATYPICALLY MILD. The remaining two swine developed exceptionally severe swine influenza and at autopsy both exhibited typical pneumonia. These two animals showed a more severe type of infection than did their controls infected by unfiltered material."

"Storage of Infectious Material

Experiments to test the keeping qualities of the agents of swine influenza have been complicated by differences in the period of survival of the two components. PIECES OF ATELECTATIC LUNG AND BRONCHIAL LYMPH NODES FROM ONE EXPERIMENTALLY INFECTED SWINE WERE STORED FOR 15 TO 33 DAYS AND FROM ANOTHER SWINE FOR 15 AND 41 DAYS IN 50 PER CENT GLYCEROL BEFORE TESTING THEM FOR INFECTIVITY. They have been found CAPABLE OF INDUCING ONLY THE MILD FILTRATE DISEASE typical in its course and at autopsy. With one exception H. INFLUENZAE SUIS HAS NOT BEEN DEMONSTRABLE IN CULTURES from the respiratory tract of swine infected with this material. With infectious material frozen and dried by Swift's method (5) THE DISEASE INDUCED BY STORED MATERIAL WAS SOMEWHAT DIFFERENT. Material that had been stored for 34 days proved capable of inducing only the filtrate type of disease when inoculated into two susceptible swine and H. INFLUENSAE SUIS WAS NOT FOUND in the respiratory tracts of these two animals at autopsy. However, another tube of this same material tested after 54 days' storage proved capable of inducing typical and rather severe swine influenza in which at autopsy H. INFLUENZAE SUIS WAS FOUND in both the bronchial exudate and the atelectatic lung. It appears that the swine influenza virus is capable of storage in a dried state or in glycerol for at least 54 or 41 days, respectively, but that the bacterial component of the mixture is less resistant to such storage. THE

IRREGULARITY IN THE RESULTS OBTAINED WITH DRIED INFECTIOUS MATERIALS MAY HAVE BEEN DUE TO FAULTY FREEZING OR DRYING OF THE PARTICULAR TUBES OF DRIED MATERIAL TESTED AFTER 34 DAYS STORAGE, FOR IT IS DIFFICULT TO UNDERSTAND WHY H. INFLUENZAE SUIS SHOULD NOT SURVIVE FREEZING AND DRYING. If it were desirable to preserve both factors the virus could be maintained in a dried state or in glycerol, while the organism could be kept under cultivation on artificial media and the two mixed before inoculation.``

SINCE THE FILTRATE-INDUCED DISEASE HAS CONSISTENTLY BEEN AT VARIANCE WITH TYPICAL SWINE INFLUENZA, IT WAS OBVIOUS THAT THE DISEASE INDUCED BY A FILTER-PASSING VIRUS COULD NOT RIGHTLY BE CONSIDERED SWINE INFLUENZA. The impression gained after consideration of a series of these mild infections was that

THE DISEASE BOTH CLINICALLY AND PATHOLOGICALLY REPRESENTED NATURAL SWINE INFLUENZA IN AN INCOMPLETE FORM.

In the preceding paper (3) it was shown that a hemophilic bacillus, H. INFLUENZAE SUIS, WAS CONSTANTLY DEMONSTRABLE in the respiratory tracts of swine ill with influenza. IT HAS BEEN CONSISTENTLY ABSENT from the respiratory tracts of swine ill WITH THE FILTRATE DISEASE. To test the possibility that swine influenza is the result of the two agents acting together, swine were inoculated intranasally with mixtures of the filtrable agent and H. influenzae suis. A disease typical of swine influenza in all clinical and pathological respects and indistinguishable from that induced by unfiltered infectious material resulted in all instances. CONTROL ANIMALS RECEIVING CULTURES OF h. INFLUENSAE SUIS ALONE DEVELOPED NO EVIDENCE OF ILLNESS AND SWINE RECEIVING THE FILTRABLE AGENT ALONE DEVELOPED THE MILD FILTRATE DISEASE. It seems permissible to interpret these experiments AS INDICATING THAT SWINE INFLUENZA IS DUE TO A FILTRABLE VIRUS AND H. INFLUENZAE SUIS ACTING TOGETHER. Their MODE OF ACTION IS UNKNOWN although two possibilities are obvious:

The first possibility is that the pathological activities of the virus are such as to create a portal of entry for h. influenzae suis and to

furnish a favorable medium in which it can multiply. UNDER SUCH AN ASSUMPTION the virus serves merely as an entering wedge for the organism and the latter determines

the clinical picture and pathology. There can be little doubt from the data presented in this and the preceding paper (3) that, in the presence of the swine influenza virus, H. INFLUENZAE SUIS POSSESSES INVASIVE POWERS WHICH IT COMPLETELY LACKS WHEN ADMINISTERED ALONE.

The second possibility is that the VIRUS IS THE IMPORTANT COMPONENT in contributing to the pathology and perhaps also to the symptoms characterizing the clinical picture, and that H. INFLUENZAE SUIS INCREASES to a marked degree the PATHOGENIC PROPERTIES OF THE VIRUS and hence the severity of the resulting disease. In this respect, the influence of h. influensae suis on the pathogenic properties of the swine influenza virus suggests the effect of certain tissue extracts on various viruses pointed out first by Duran-Reynals (6) and later amplified by Hoffman (7)."

"THE HYPOTHESIS IS NOT NEW THAT A DISEASE MAY BE INDUCED BY A BACTERIUM AND AN INVISIBLE AGENT, NOT READILY DEMONSTRABLE ALONE. It applies most directly to diseases in which the suspected bacterial agent, while readily and uniformly isolated from cases of the disease,

EITHER IS COMPLETELY INCAPABLE OF REPRODUCING THE INFECTION OR VERY RAPIDLY LOSES ITS ABILITY TO DO SO UNDER CONDITIONS OF ARTIFICIAL CULTIVATION. It is possible that such organisms do not become non-pathogenic because of rapid loss of virulence but because of the ABSENCE OF AN INVISIBLE INCITING AGENTS."

"THE CLINICAL PICTURE OF SWINE INFLUENZA, characterized by fever, anorexia, extreme prostration, leucopenia, and evidence of respiratory involvement and of muscular tenderness, IS STRIKINGLY SUGGESTIVE OF

HUMAN EPIDEMIC INFLUENZA. The onset is sudden, the course short, and convalescence usually uneventful. Death, when it occurs, is the result of an edematous type of pneumonia. The pathology of non-fatal swine influenza, characterized as it is by an exudative bronchitis and extensive pulmonary atelectasis, cannot be compared with the findings in non-fatal human influenza infections BECAUSE OF OUR LACK OF KNOWLEDGE OF THE LATTER. Probably the most significant similarity concerns the predominant bacterium encountered in the two conditions; H. INFLUENZAE SUIS IS INDISTINGUISHABLE MORPHOLOGICALLY AND CULTURALLY FROM H. INFLUENZAE. The frequency with which H. influenzae has been encountered in careful

bacteriological studies of human influenza PARALLELS the frequency of occurrence of H. influenzae suis in swine influenza, and, as in the case of the latter organism, has suggested an etiological significance. Without drawing analogy too far, THE IRREGULARITY IN THE OUTCOME OF THE FILTRATION EXPERIMENTS reported, especially by French and English investigators, IN ATTEMPTING TO DETERMINE WHETHER A FILTRABLE VIRUS CAUSES HUMAN INFLUENZA, IS VERY SIMILAR

TO THE EXPERIENCE OF THE WRITER IN THE EARLY FILTRATION EXPERIMENTS WITH SWINE INFLUENZA. The preliminary obstacles encountered in studying the nature of the etiological factors in swine influenza have had much in common with those met by investigators of human influenza. A CAREFUL INVESTIGATION WOULD SEEM WARRANTED OF THE POSSIBILITY THAT PFEIFFER'S BACILLUS AND A FILTRABLE AGENT ACTUALLY IN CONCERT TO CAUSE INFLUENZA IN MAN."

SUMMARY AND CONCLUSIONS

1. It has been possible to demonstrate, in Berkefeld filtrates of infectious material from experimental cases of swine influenza, a virus which when administered intranasally to susceptible swine induced a mild, usually afebrile illness of short duration. THE CHANGES IN THE RESPIRATORY TRACT RESEMBLED THOSE IN SWINE INFLUENZA BUT WERE USUALLY MUCH LESS EXTENSIVE. When the filtrable virus was mixed with pure cultures of h. influenzae suis and administered to swine a disease identical clinically and pathologically with swine influenza was induced. The data presented indicate that the filtrable virus of swine influenza and h. influenzae suis ACT IN CONCERT TO PRODUCE SWINE INFLUENZA AND THAT NEITHER ALONE IS CAPABLE OF INDUCING THE DISEASE."

doi: 10.1084/jem.54.3.373

In Summary:

-McBryde, Niles, and Moskey made five attempts to pass the etiological agent of swine influenza through small Berkefeld or Mandler filters but were UNABLE TO REPRODUCE THE DISEASE by dropping such filtrates into the nostrils of normal hogs

-their experiments indicated that the causative agent of swine influenza was NOT A FILTRABLE "VIRUS"

-Shope admits that the results of his ten filtration experiments with infectious material from the two strains of the disease obtained in 1928 and the two secured in 1930 were

INCONSTANT AND CONFUSING

-he prepared his filtrate by taking diseased lung and bronchial lymph nodes which were then minced with sterile scissors and added to bronchial exudate

-this mixture was ground with sand in a mortar and once it had been reduced to a pasty and fairly homogeneous consistency, a 10 to 20 per cent suspension was made by gradually adding sterile distilled water or infusion broth (pH 7.3) -it was then shaken with glass beads in a flask for l0 to 15 minutes and centrifuged

-filtrates were then cultured in 1 cc. amounts on plain agar slants containing defibrillated blood and were incubated at 37°C. for 48 to 72 hours

-Shope states that In certain experiments the control animal receiving unfiltered material and those receiving sterile filtrates were from the SAME SOURCE or even from the SAME LITTER

-of the ten preliminary filtration experiments, THREE WERE AT THE TIME INTERPRETED AS NEGATIVE, while in the remaining seven SOME EVIDENCE was obtained that the injected filtrate had contained an infectious agent

-the disease induced by this filtrable infectious agent was DEFINITELY NOT the typical swine influenza

-clinically the filtrate disease was MUCH MILDER than swine influenza

-the extreme prostration so common in swine influenzal infections WAS NOT SEEN

-in some instances the disease was SO MILD THAT IT ALMOST ESCAPED RECOGNITION altogether

-h. influenzae suis, which was constantly encountered in culturing the respiratory tracts of animals with typical swine influenza infections, WAS NOT FOUND in similar cultures from animals with the mild filtrate disease

-anaerobic cultures of seven filtrates of swine influenza infectious material in blood broth and in 5 per cent serum bouillon over sterile rabbit kidney FAILED TO SHOW GROWTH

-only FOUR OF THE SEVEN FILTRATES THUS CULTURED WERE TESTED BY INTRANASAL INOCULATION into swine and all were found capable of inducing the mild filtrate disease

-Shope states the only constant difference bacteriologically between the mild disease induced by the filtrable agent and typical spontaneous or EXPERIMENTAL swine

influenza lies in the absence of h. influenzae suis in the filtrate-infected swine

-thus he concludes the COMBINATION of the organism and the filtrable agent MAY BE ESSENTIAL for the production of the natural disease

-all eight of the swine infected by inoculation with Berkefeld filtered infectious material or by contact with filtrate-infected swine DEVELOPED ONLY A MILD DISEASE

-the swine which were inoculated intranasally with pure cultures of h. influensae suis WERE COMPLETELY NEGATIVE both clinically and at autopsy

-OF THE SEVEN HOGS INFECTED either by direct inoculation with the filtrate-culture mixture or by contact with swine so infected, THREE DEVELOPED TYPICAL SWINE INFLUENZA, and the disease was of about the same severity as that which DEVELOPED IN THE "CONTROL" ANIMALS inoculated with unfiltered infectious material

-two others had a mild influenza, but in this instance the DISEASE WHICH DEVELOPED IN THE "CONTROL" ANIMAL was also atypically mild

-the "control" animals basically were given less "purified" goo than the combination of bacteria + filtered goo given to the test animals - in other words, NOT VALID "CONTROLS"

-pieces of atelectatic lung and bronchial lymph nodes from one experimentally infected swine were stored for 15 to 33 days and from another swine for 15 and 41 days in 50 per cent glycerol before testing them for infectivity

-they were found CAPABLE OF INDUCING ONLY THE MILD FILTRATE DISEASE typical in its course and at autopsy

-with one exception h. influenzae suis was NOT DEMONSTRABLE in cultures from the respiratory tract of swine infected with this material

-with infectious material frozen and dried by Swift's method THE DISEASE INDUCED BY STORED MATERIAL WAS SOMEWHAT DIFFERENT

-he states the irregularity in the results obtained with dried infectious material MAY HAVE BEEN DUE TO FAULTY FREEZING OR DRYING of the particular tubes of dried material tested after 34 days' storage

-it was difficult for Shope to understand why h. influenzae suis could not survive freezing and drying

-Shope then admits that since the filtrate-induced disease was consistently at variance with typical swine influenza, it was obvious that the disease induced by a filter-passing virus COULD NOT RIGHTLY BE CONSIDERED SWINE INFLUENZA

-he then assumes that the disease both clinically and pathologically represented natural swine influenza IN AN INCOMPLETE FORM

-in a preceding paper, it was shown that a hemophilic bacillus, h. influenzae suis, was CONSTANTLY DEMONSTRABLE in the respiratory tracts of swine ill with influenza

-in Shope's experiments however, it was CONSISTENTLY ABSENT from the respiratory tracts of swine ill WITH THE FILTRATE DISEASE

-"control" animals receiving cultures of h. influenzae suis alone developed NO EVIDENCE OF ILLNESS and swine receiving the filtrable agent alone developed the MILD FILTRATE DISEASE

-Shope then states that it seems permissible to interpret these experiments as indicating that swine influenza is due to a filtrable virus and h. influenzae suis ACTING TOGETHER

-however, he admits that their MODE OF ACTION IS UNKNOWN

-under one ASSUMPTION, the "virus" serves merely as an entering wedge for the organism and the latter determines the clinical picture and pathology

-Shope states it is clear h. influenzae suis possesses invasive powers which it COMPLETELY LACKS WHEN ADMINISTERED ALONE

-the second possibility is that the "VIRUS" is the important component in contributing to the pathology and perhaps also to the symptoms characterizing the clinical picture, and that h. influenzae suis increases to a marked degree the pathogenic properties of the "virus" and hence the severity of the resulting disease

-Shope states the hypothesis is not new that a disease may be induced by a bacterium and AN INVISIBLE AGENT, not readily demonstrable alone

-he says this applies most directly to diseases in which the suspected bacterial agent, while readily and uniformly isolated from cases of the disease, either is COMPLETELY INCAPABLE OF REPRODUCING THE INFECTION or very rapidly loses its ability to do so UNDER CONDITIONS OF ARTIFICIAL CULTIVATION

-he concludes that it is possible that such organisms do not become non-pathogenic because of rapid loss of virulence but because of the ABSENCE OF AN INVISIBLE INCITING AGENT

-Shope states that the clinical picture of swine influenza, characterized by fever, anorexia, extreme prostration, leucopenia, and evidence of respiratory involvement and of muscular tenderness, is STRIKINGLY SUGGESTIVE of human epidemic influenza

-the pathology of non-fatal swine influenza, characterized as it is by an exudative bronchitis and extensive pulmonary atelectasis, CANNOT BE COMPARED with the findings in non-fatal human influenza infections because of A LACK OF KNOWLEDGE OF THE LATTER

-h. influenzae suis is INDISTINGUISHABLE morphologically and culturally from h. influenzae

-the frequency with which h. influenzae has been encountered in careful bacteriological studies of human influenza PARALLELS the frequency of occurrence of h. influenzae suis in swine influenza

-the irregularity in the outcome of the filtration experiments reported, especially by French and English investigators, in attempting to determine WHETHER A FILTRABLE "VIRUS" CAUSES HUMAN INFLUENZA, is very similar to the experience of Shope's own in the early filtration experiments with swine influenza

-Shope believes a careful investigation would seem warranted of the possibility that Pfeiffer's bacillus and a filtrable agent ACT IN CONCERT to cause influenza in man

-he states that the changes in the respiratory tract resembled those in swine influenza but were usually much less extensive

-Shope concludes by stating that the data presented indicate that the filtrable virus of swine influenza and h. influenzae suis ACT IN CONCERT TO PRODUCE SWINE INFLUENZA AND THAT NEITHER ALONE IS CAPABLE OF INDUCING THE DISEASE

In other words, Shope could not produce Swine Flu with the cultured bacteria they originally believed caused it. He couldn't cause Swine Flu with the invisible and assumed filterable "virus" either. It wasn't until he combined the two toxic concoctions together that he sometimes produced a similar disease. In fact, he was certain neither the bacteria nor the "virus" alone could cause disease but only when they acted together.

Keep in mind that at this point in time there was no indirect Electron Microscope nor cell culture evidence attempting to show and "prove" a "virus" was in the mixture. However, much like today, it was just assumed to be in there. No purification/isolation took place to separate a "virus." Shope created toxic soups from pieces of diseased pig atelectatic lung and bronchial lymph nodes which he ground up and mixed with various

chemicals/substances and then injected up the noses of some pigs which sometimes caused them to get sick. It is on this basis that a Swine Flu "virus" was considered "isolated" which led to the "isolation" of Influenza A a few years later.

https://docs.google.com/document/d/e/2PACX-1vTf-jh58NPVmJKgPMrRhGg-vazBd6XZC9YFjXnokk0SaqgP8Fkjl0ROa7uN76wH9fNORy6wrFFhbsdf/pub

INFLUENZA A ISOLATED IN 1933?

https://docs.google.com/document/d/e/2PACX-1vQpNj64pyuYYm_OodwZZayvwxc1GiAqhBt9RleQajvkQU11PgRz6pn5uPlo2vzTpMnMuyvIkIQrzBq0/pub

THOMAS FRANCIS JR. ISOLATES INFLUENCE A IN 1934?:

https://docs.google.com/document/d/e/2PACX-1vSBvLcEQgr-kchptgHBm6rPBFg1ETMd4L4mG3-b4DiDavR3kR0NmesH7ptZhNCcu6vkdwgPuw1nHvc1/pub

THOMAS FRANCIS JR. ISOLATES INFLUENCE A IN 1937?:

https://docs.google.com/document/d/e/2PACX-1vQfekKAToBsvgbp-8zOZsRAa8vmHcNeh8pqNNfDc4puEiJ0oUKA3axdQHSibowGtxb6OsPcoCljiPhG/pub

THOMAS FRANCIS JR. 1937 FLU REVIEW:

https://docs.google.com/document/d/e/2PACX-1vSbL7kOsvNWOWbPPAKpgLqC9Qakfp6q3hBKqaOVfPJY3aTroBdj6P0jIKGPKpQLaILmtih0fmeEODwR/pub

THOMAS FRANCIS JR. ISOLATES INFLUENCE B IN 1940?:

https://docs.google.com/document/d/e/2PACX-1vSS8DFISdQ9pNuLN4TSK_bGgG78rTL_Mhd0Rp3P_vpO3b5YW0zqBZZe1r7or8Uy26vjJu0S9COIDnkz/pub

MURICE HILLEMAN: THE MAN BEHIND THE 1957 H2N2 AND 1968 H3N2 FLU PANDEMICS:

https://docs.google.com/document/d/e/2PACX-1vRsLlcMuueA8yct-

NwJjb6kWxuvCPTKroTmspCWxJBOfPfLjomdfjkAvzr17_U4MVvUi2B2o14oXU9v/pub

HAS INFLUENZA EVER BEEN PURIFIED?
https://docs.google.com/document/d/e/2PACX-1vTabUnC0moUbyBKIe6rdTyf0F9_Tga8NxzIOZEzEnXe5TxcUXGRzkvc4JXs6zkza8dQPjMp295T_X9g/pub

CDC's Influenza Image page has no actual EM images of an Influenza "virus,"

https://docs.google.com/document/d/e/2PACX-1vQGEB_3Zw8EvrOuhKWdRuWcUFIVgZdGj2enLsc699H_75bP-DsJu1EGqpnTd_toT4rRvsc1507-vWPB/pub

WAS INFLUENZA C EVER PURIFIED/ISOLATED?
https://docs.google.com/document/d/e/2PACX-1vSnlRNi_ejG6tJKY7bpJMnrCHaV6zbHYuUKB2vfueO-aCLF3RA30ZfRMbrrs0PyyVaNOYQebrdssOXx/pub

SWINE FLU 1976:

https://docs.google.com/document/d/e/2PACX-1vSjb3qVj16929_i3mMT4UwDTKtvumTcJygqwfol6Lwp1FAEzA7IhQikO65W9vsX-3FWb88qK-1hEJrq/pub

Experiments that have been done on the common cold/flu:

https://docs.google.com/document/d/e/2PACX-1vRB6BXsffDRB3Ikq9mwSeF7uK2_SIxeGXQxG6ff-SZdIN6UDQt97ZIKRXUzfH-sUjnp1kCzk4MPWbHz/pub

ROWE'S 1953 "ADENOVIRUS" PAPER:

I was asked about the "adenovirus" yesterday and it dawned on me that I hadn't really looked into this "virus" and only had a cursory knowledge about it...so of course I had to dig a little further! According to the CDC:

"Adenoviruses are common viruses that cause a range of illness. THEY CAN CAUSE COLD-LIKE SYMPTOMS, FEVER, SORE THROAT, BRONCHITIS, PNEUMONIA, DIARRHEA, AND PINK EYE (conjunctivitis). You can get an adenovirus infection at any age. People with weakened immune systems or existing respiratory or cardiac disease are more likely than others to get very sick from an adenovirus infection."

https://www.cdc.gov/adenovirus/index.html

Hmm...from that description, "adenovirus" sure sounds a lot like a certain "Coronavirus" I've heard a lot about recently...

In any case, I wanted to track down the original paper claiming discovery of this "virus" for myself and see what kind of magnificent evidence it beholds. I haven't had any luck so far finding any papers showing purified/isolated "virus" coming directly from sick humans proven pathogenic in a natural way thus fulfilling Koch's Postulates. Maybe this would be the one!

I began my search by looking for who discovered the "adenovirus" and in what year:

"Adenoviruses were FIRST DISCOVERED IN 1953, BY ROWE and his colleagues. THESE VIRUSES WERE FIRST ISOLATED FROM ADENOID CELL CULTURE, hence

the family name of Adenoviridae. At present, 51 distinct serotypes have been recognized and recorded."

http://web.stanford.edu/group/virus/adeno/2004takahashi/webpage/second.html?

From my first search result I had a year and a name, but the next sentence took the wind right out of my sails. "Adenoviruses" were first "isolated" from CELL CULTURES. If you know anything about the cell culture process, this immediately places the samples in an unpurified state as it is mixed with numerous contaminants and foreign elements. It was definitely not a promising start in my continued quest for the holy grail of all virology papers.

I did end up finding Rowe's original 1953 paper and I have provided it in its entirety along with a summary. You can read it and make your own conclusions but I will state this:

I swear if you read one virology paper, you've read them all.

ISOLATION OF A CYTOPATHOGENIC AGENT FROM HUMAN ADENOIDS UNDERGOING SPONTANEOUS DEGENERATION IN TISSUE CULTURE.

"During the course of a study of the growth of human adenoid tissue in roller tube culture, A CHARACTERISTIC DEGENERATION has been encountered which has been found to be serially transmissible in other tissue cultures.

Methods. Adenoids were obtained during the winter and spring of 1952-53 from operations on young children. THE TISSUES WERE MINCED, WASHED 3 TIMES IN NUTRIENT MEDIA, AND CULTURED BY THE CHICKEN PLASMA CLOT TECHNIQUE. The majority of adenoids and all other tissues WERE GROWN IN A MODIFICATION OF THE BEEF AMNIOTIC FLUID MEDIUM described by Enders (1) A FEW ADENOIDS WERE CULTURED IN MEDIA THAT WERE MODIFIED FROM THOSE OF YOUNGNER et al.(2).§ Explant cultures of other tissues were prepared in the same manner; HeLa CELL CULTURES (3) WERE OBTAINED FROM MICROBIOLOGICAL ASSOCIATES, Bethesda, Md. Culture fluids were changed twice weekly, and the supernatant fluids were stored in screw-capped vials at -2O’C. Passages were made by transfer of supernatant fluid in a dilution of 10-l.

Results. During the first week of culture most adenoid cultures demonstrated sheets of epithelium, often ciliated, with a few areas of fibroblastic outgrowth. AFTER 8 TO 28 DAYS IN CULTURE, 33 of the 53 (62%) adenoids observed for this period demonstrated a characteristic rounding of the peripheral epithelium, progressing to complete

destruction of the epithelium within 7 to 10 days. THE MORPHOLOGICAL APPEARANCE OF THE ROUND CELLS WAS DISTINCTIVE, and the appearance of a few of these cells almost invariably was followed by progressive replacement of the epithelium by the same type of cell. These cells were large, round or slightly ovoid, with a smooth, distinct margin, clear peripheral cytoplasm, and a densely granular center, the nucleus obscured by the granulation.

Culture fluids of 14 adenoids demonstrating this degeneration have been TRANSFERRED TO FRESH CULTURES OF ADENOIDS, HUMAN EMBRYONIC TISSUE, or HeLa CELLS, and in 13 instances characteristic changes were produced in the recipient cultures. The changes observed in the recipient adenoid or embryonic tissues were identical with the picture of the original degeneration of the adenoids. The changes in HeLa cell cultures consisted of clumping and rounding of the cells, followed by dislodgement of the clumps from the wall of the tube. One isolation (strain No. 2) of the agent was obtained from an adenoid which degenerated on the eighth day of culture, BY PASSAGE OF THE SUPERNATANT FLUID TO CULTURES OF A SECOND ADENOID, the controls of which did not undergo spontaneous degeneration DURING 69 DAYS OF OBSERVATION AND FROM WHICH NO CYTOPATHOGENIC AGENT COULD BE ISOLATED. This strain has been carried through A TOTAL OF 17 PASSAGES, the first in adenoid, one in human embryo trachea, and the last 15 in HeLa cells, with production of the typical changes in every passage. Degeneration of the seventeenth passage tissue occurred one day after infection with fluid REPRESENTING A THEORETICAL DILUTION of the original adenoid fluid of 10-32. Fluid of the ELEVENTH PASSAGE WHEN PASSED TO CULTURES OF HUMAN FETAL TRACHEA reproduced the typical round cell degeneration after 3 days. Similarly, other strains of the agent have been carried without difficulty THROUGH SERIAL PASSAGES, and have consistently reproduced the degeneration. In both human embryo skin and HeLa cell cultures the agent has been found to reach a titer in the supernatant fluid of more than lo5 infectious units per ml when titrated in HeLa cells.

The incubation period of the cytopathogenic effects in human epithelium is usually 4 to 8 days; with higher dilutions of the inoculum the incubation period may be as long as 23 days. In cultures consisting almost entirely of human embryo fibroblasts the incubation time is longer, ranging from 8 to 15 days for the lowest dilution, to as long as 40 days with higher dilutions. Incubation time in HeLa cells varies from 2 to 20 days, depending on the concentration of the agent. THE FOLLOWING TISSUES HAVE BEEN FOUND TO SHOW CYTOPATHOGENIC EFFECTS AFTER INFECTION WITH THE AGENT: human adenoid ; human embryonic nose, pharynx, palate, tongue, trachea, skin, muscle, and pancreas ; newborn human prepuce ; HeLa cells; suckling rabbit kidney and trachea; suckling hamster trachea, lung, kidney, skin, and muscle; and chick embryo lung and skin. The effects seen in hamster and chick embryo cultures were not distinctive, being characterized by gradual death and disappearance of tissue with lysis of the plasma clot. THE FOLLOWING TISSUES HAVE SHOWN NO EVIDENCE OF CYTOPATHOGENIC CHANGES FOLLOWING INOCULATION WITH THE AGENTS: beef embryonic trachea, mouse embryo mixture, mouse glioblastoma, monkey testis,

and guinea pig trachea and kidney.

Hematoxylin-eosin stained preparations of infected human embryonic tracheal epithelium cultures have been examined by Dr. Henry Pinkerton, St. Louis University School of Medicine, who made the following description: "The normal cells take the hematoxylin stain, both nucleus and cytoplasm. The affected cells show eosinophilic swollen nuclei, with what I take to be nuclear inclusions. These are usually basophilic, but occasionally eosinophilic. There is margination and fragmentation of the nuclear chromatin (which stains deeply blue-black), often wrinkling of the nuclear membrane, and swelling of the nuclei. The cytoplasm stains deep reddish purple, and occasionally contains 10 to 12 small pale blue inclusions'. . . . Often, also, the nucleus is blended with the cytoplasm to form a granular purplish mass in which no nucleus can be recognized". Photomicrographs of stained roller tube cultures are shown in Fig. 1-3.

THE AGENT HAS NOT BEEN FOUND TO GROW ON BACTERIOLOGICAL MEDIA, including repeated cultures on thioglycollate broth, blood agar, and several types of pleuropneumonia media. Activity of the agent was destroyed by heating at 62 C for 30 minutes; the agent was filterable through a Mandler No. 14 candle with some loss in titer. No loss of activity was found after storage at 3°C for a week or after three cycles of quick-freezing and thawing. NO CLINICALLY RECOGNIZABLE DISEASE HAS BEEN PRODUCED BY THE AGENT INOCULATED BY VARIOUS ROUTES INTO EXPERIMENTAL ANIMALS, including embryonated eggs, suckling and adult mice, suckling hamsters, guinea pigs, rabbits, rhesus monkeys, and a chimpanzee.

HYPERIMMUNIZATION OF RABBITS WITH AGENT-CONTAINING TISSUE CULTURE FLUIDS RESULTED IN THE PRODUCTION OF ANTIBODIES CAPABLE OF NEUTRALIZING THE AGENT IN TISSUE CULTURE, whereas immunization with control culture fluid did not. Rabbit antisera against herpes simplex and vaccinia viruses did not neutralize the agent in tissue culture, and rabbit hyperimmune serum against strain No. 2 of the adenoid agent did not neutralize herpes simplex virus in suckling mice or vaccinia virus in tissue culture. The cytopathogenic effects of the agent were prevented or significantly delayed by addition to the cultures of human gamma globulin in a final concentration of 1:500 or some human sera in dilutions of 1 : 50 or higher, whereas other human sera showed no neutralizing capacity at dilutions of 1:25.

In this laboratory a variety of human viruses have been inoculated into human adenoid and embryo cultures and HeLa cell cultures, including herpes simplex, vaccinia, influenza A' and B, Type 1 poliomyelitis, and members of the Coxsackie A and B groups, as well as human pleuropneumonia-like organisms and PRESUMABLY VIRUS-CONTAINING MATERIALS FROM CASES OF MEASLES AND VARICELLA; IN NO INSTANCE HAS THE PICTURE PRODUCED BY THE ADENOID AGENTS BEEN PRODUCED. The pattern of destruction of adenoid epithelium by herpes simplex and poliomyelitis viruses WAS SOMEWHAT SIMILAR TO THAT OF THE ADENOID

AGENTS, but their effects on HeLa cells did not resemble that produced by the adenoid agents.

Summary. 1. From the present evidence IT APPEARS THAT AN UNIDENTIFIED, POSSIBLY NEW, TISSUE CULTURE CYTOPATHOGENIC AGENT HAS BEEN ISOLATED REPEATEDLY FROM HUMAN ADENOIDS UNDERGOING SPONTANEOUS DEGENERATION IN TISSUE CULTURE. The filterability and the inability to cultivate the agent on bacteriological media and to demonstrate organisms in stained tissue culture preparations would indicate that the agent BELONGS TO THE GROUP OF VIRUSES OR RICKETTSIAE. It is tentatively proposed to designate the agent as the "adenoid degeneration agent", abbreviated as "A.D. agent". 2. That the agent is derived from the adenoid tissue rather than from the nutrient media is indicated by the fact that some adenoids and all human embryonic tissues cultivated in the identical media and at the same time have not undergone degeneration, although they are susceptible to infection with the agent; also, repeated attempts to isolate the agents from adenoid cultures not demonstrating degeneration have been uniformly unsuccessful. 3. Further investigation is in progress to determine the relation of the agent to the adenoids and to study their possible role in human disease: particularly upper respiratory infections.

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In Summary

-during the course of a study of the growth of human adenoid tissue in roller tube culture, a CHARACTERISTIC DEGENERATION was encountered

-adenoids were obtained during the winter and spring of 1952-53 from operations on young children

-the tissues were MINCED, washed 3 times in NUTRIENT MEDIA, and CULTURED by the chicken plasma clot technic

-the majority of adenoids and all other tissues were GROWN IN A MODIFICATION OF THE BEEF AMNIOTIC FLUID MEDIUM described by Enders

-a few adenoids were CULTURED IN MEDIAthat were modified from those of Youngner et al.

-AFTER 8 TO 28 DAYS IN CULTURE, 33 of the 53 (62%) adenoids observed for this period demonstrated a CHARACTERISTIC ROUNDING of the peripheral epithelium progressing to complete destruction of the epithelium within 7 to 10 days

-the morphological appearance of the round cells was distinctive

-culture fluids of 14 adenoids demonstrating this degeneration have been TRANSFERRED TO FRESH CULTURES OF ADENOIDS, HUMAN EMBRYONIC TISSUE, or HeLa CELLS, and in 13 instances (out of how many?...they don't say... 🤷) characteristic changes were produced in the recipient cultures

-one isolation (strain No. 2) of the agent was obtained from an adenoid which degenerated on the eighth day of culture, by passage of the supernatant fluid to cultures of a second adenoid, the controls of which did not undergo spontaneous degeneration DURING 69 DAYS OF OBSERVATION AND FROM WHICH NO CYTOPATHOGENIC AGENT COULD BE ISOLATED

-this strain has been CARRIED THROUGH A TOTAL OF 17 PASSAGES, the first in adenoid, one in human embryo trachea, and the last 15 in HeLa cells, with production of the typical changes in every passage

-degeneration of the seventeenth passage tissue occurred one day after infection with fluid REPRESENTING A THEORETICAL DILUTION of the original adenoid fluid of 10-32.

-fluid of the ELEVENTH PASSAGE WHEN PASSED TO CULTURES OF HUMAN FETAL TRACHEA reproduced the typical round cell degeneration after 3 days

-other strains of the agent were carried without difficulty THROUGH SERIAL PASSAGES, and consistently reproduced the degeneration

-the following tissues were FOUND TO SHOW CYTOPATHOGENIC EFFECTS after infection with the agent: human adenoid; human embryonic nose, pharynx, palate, tongue, trachea, skin, muscle, and pancreas; newborn human prepuce; HeLa cells; suckling rabbit kidney and trachea; suckling hamster trachea, lung, kidney, skin, and muscle; and chick embryo lung and skin

-the effects seen in hamster and chick embryo cultures WERE NOT DISTINCTIVE, being characterized by gradual death and disappearance of tissue with lysis of the plasma clot

-the following tissues have shown NO EVIDENCE OF CYTOPATHOGENIC CHANGES following inoculation with the agents: beef embryonic trachea, mouse embryo mixture, mouse glioblastoma, monkey testis, and guinea pig trachea and kidney

-the agent was not found to grow on bacteriological media, including repeated cultures on thioglycollate broth, blood agar, and several types of pleuropneumonia media

-NO CLINICALLY RECOGNIZABLE DISEASE HAS BEEN PRODUCED BY THE AGENT INOCULATED BY VARIOUS ROUTES INTO EXPERIMENTAL ANIMALS, including embryonated eggs, suckling and adult mice, suckling hamsters, guinea pigs,

rabbits, rhesus monkeys, and a chimpanzee

(*note many of the tissues from these animals showed CPE yet they could still not produce disease in any of them...*)

-hyperimmunization of rabbits with AGENT-CONTAINING TISSUE CULTURE FLUIDS resulted in the production of antibodies capable of neutralizing the agent in tissue culture *(if they were unable to produce disease in rabbits, what were the antibodies doing there...?)*

-in their laboratory, a variety of human "viruses" were said to have been inoculated into human adenoid and embryo cultures and HeLa cell cultures, including herpes simplex, vaccinia, influenza A' and B, Type 1 poliomyelitis, and members of the Coxsackie A and B groups, as well as human pleuropneumonia-like organisms and PRESUMABLY "VIRUS-CONTAINING" MATERIALS from cases of measles and varicella; IN NO INSTANCE HAS THE PICTURE PRODUCED BY THE ADENOID AGENTS BEEN PRODUCED

-in other words, **they are basing their disease-less producing "virus" on pictures of patterns they had never seen before which they created during tissue culture experiments...**

-but then they admit that the pattern of destruction of adenoid epithelium by herpes simplex and poliomyelitis "viruses" WAS SOMEWHAT SIMILAR to that of the adenoid agents, yet it wasn't the "same" as their effects on HeLa cells did not resemble that produced by the adenoid agents ...

-from their present evidence they claim that an unidentified, POSSIBLY NEW, tissue culture cytopathogenic agent has been "isolated" repeatedly from human adenoids UNDERGOING SPONTANEOUS DEGENERATION in tissue culture

-they claim it should belong to the "virus" or rickettsiae group as it was "filterable" but could not be grown in bacterial culture...in other words, it was INVISIBLE

-they believe the agent was derived from the adenoid tissue RATHER THAN FROM THE NUTRIENT MEDIA because SOME ADENOIDS and all human embryonic tissues cultivated in the identical media and at the same time have not undergone degeneration, although they are susceptible to infection with the agent

-and also, repeated attempts to "isolate" the agents from adenoid cultures not demonstrating degeneration have been uniformly unsuccessful...**even though they never actually isolated anything from cultures showing degeneration either...**

-they conclude that further investigation is in progress to determine the relation of the "agent" to the adenoids and to study their possible role in human disease: PARTICULARLY UPPER RESPIRATORY INFECTIONS, even though there was ZERO

EVIDENCE that this "agent" anything other than not-so-distinct patterns in tissue culture

It is very obvious that the researchers tried really hard to make the case that they had discovered and "isolated" a new "virus" yet the evidence they present shows just the opposite. Not only were they unable to show purified/isolated "virus" taken directly from the adenoids of sick patients (as there is no indication the children were even sick to begin with), they were unable to PRODUCE ANY CLINICAL DISEASE with their "agent." All they can say is that sometimes, through different types of tissue/cell cultures experiments and numerous culture passages between them, they saw some sort of pattern in the cultures after waiting days/weeks/months which depended on the conditions of the experiments. Even then they admit that herpes simplex and polio produced similar patterns in cultures. In the end, the "adenovirus," just like every other "virus" before and after it, is one big NOTHINGBURGER.

https://docs.google.com/document/d/e/2PACX-1vSKunR3Nc9jIPBgfyjsm4h2SCqNmw9YjHaM9Xu1kxhUInQ9hgXLdUsipyTN1x9KxZQbt7etfGbkhMgT/pub?fbclid=IwAR1J5y4P5tOqnULCXBH8w755hUee3hqJQubr2g95EpflOoZ71CqIAGPLi7c

KAPIKIAN'S 1972 "NOROVIRUS" PAPER:

One of the hang-ups Virologists, Microbiologists, Germ Theory defenders, etc. have when challenged with providing proof of the purification/isolation of any "virus"directly from a human (not cell cultured goo) along with proof of pathogenicity is the lack of this proof in any of the original "virus" papers. They can not provide this evidence as it simply does not exist. They try to wiggle around this citing various INDIRECT studies involving cell culture experiments, genomes, antibodies, animal studies, etc. suggesting "virus" but they can not share DIRECT scientific proof these particles claimed to be "viruses" actually exist and cause disease.

However, recently it seems they have come up with what they feel is the holy grail of evidence fulfilling the request for proof of purified/isolated "viruses" coming directly from humans and proven pathogenic:

Enter the "Norovirus."

This lovely figment of the imagination stems entirely from the study of particles found in human feces. They claim this evidence meets the requirement for coming directly from a human as it was only found in the poop juice of the "infected" and can not be cultured. While they are technically correct that the poop comes from humans, they always seem to forget the part about PURIFICATION (free of foreign materials; anything that contaminates, pollutes, debases, adulterates) and ISOLATION (separated from everything else). In the original 1972 study, not only were the methods used mostly undefined, they admit to adding phosphate buffered saline to the samples as well as serum from "infected" patients.

Regarding pathogenicity, it would seem fairly obvious that drinking the poop juice from anyone will most likely make one sick irregardless of any assumed "virus." However even then, not everyone who drank the poop juice became ill and to date, animal models recreating the disease are considered unsatisfactory.

In any case, here is a brief summary about the "Norovirus" and the many limitations still existing nearly 40 years after it's supposed discovery:

"The Norwalk virus agent (the original prototype virus is referred to as Norwalk virus in this review) was originally visualized by using immunoelectron microscopy (1), revealing 27-nm virus-like particles (Fig. 1). EFFORTS TO CULTIVATE THE PATHOGEN IN CELL CULTURE AND TO DEVELOP AN ANIMAL MODEL WERE UNSUCCESSFUL (8); therefore, the evolving literature focused on describing the physical characteristics of this small, round-structured virus in clinical specimens AND ON THE SEROLOGIC RESPONSE TO INFECTION (9, 10)."

"ALTHOUGH NO ANIMAL MODEL TO DATE HAS BEEN ENTIRELY SATISFACTORY, it has been demonstrated that chimpanzees can be successfully infected with GI.1 norovirus (269), while gnotobiotic pigs (270) and gnotobiotic calves (271) can be successfully infected with GII.4 norovirus."

"WHILE THE INABILITY TO CULTURE THE VIRUS IN VITRO PRECLUDES THE ABILITY TO DETECT NEUTRALIZING ANTIBODY BY CLASSICAL METHODS, the prevention of binding of norovirus-derived VLPs or P particles to HBGA (blocking antibody) is believed to be an accurate SURROGATE of neutralization (324, 325)."

"Several advances into understanding the relationship among the viral strain, the host human blood group antigen type, and disease susceptibility have recently been elucidated, BUT THIS WORK HAS NOT YET BEEN EXTENDED TO CLINICAL PRACTICE. The interplay of norovirus and host immunity STILL POSES MANY UNANSWERED QUESTIONS. Areas of future research may overcome technical limitations, SUCH AS THE INABILITY TO CULTIVATE NOROVIRUS IN VITRO, AND MAY ELUCIDATE A WAY TO DIRECTLY MEASURE NEUTRALIZING ANTIBODIES, which could pave the way for vaccine development."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4284304/

From this source from 2015, it is apparent that the "Norovirus" can not be cultured, does not have a suitable animal model, and that neutralizing antibodies can not be directly detected. In fact, they claim that the inability to culture the "virus" precludes (i.e. prevent from happening, make impossible) the ability to detect netralizing antibodies. So what does that say about the original 1972 evidence claiming the detection of "Norovirus" when it was based almost entirely on antibody detection?

Another source also highlights these glaring problems:

"The challenges in norovirus research are to DEVELOP MODELS IN WHICH TO STUDY THE VIRUSES, TO DEVELOP METHODS TO MORE EASILY DETECT THE VIRUSES, and to develop ways to treat and prevent norovirus infection.

A MAJOR HINDRANCE TO NOROVIRUS RESEARCH HAS BEEN THE LACK OF A SYSTEM IN WHICH TO GROW THE VIRUS. Scientists like to have a CELL CULTURE SYSTEM and a SMALL ANIMAL MODEL SYSTEM in which they can study details of how viruses cause illness and use these systems to test antiviral agents."

https://www.bcm.edu/departments/molecular-virology-and-microbiology/emerging-infections-and-biodefense/specific-agents/norovirus

So beyond the lack of a purified/isolated "virus," there is no cell culture system nor any animal model and challenges include developing models/methods to study and detect it. One would think these things would be figured out and readily available had the "Norovirus" been properly purified/isolated in 1972... (?)

Before delving into the 1972 paper, here are a few insights from 2000 by the lead author himself, Albert Z. Kapikian:

"The search for a viral agent was based on the rationale that (1) THE ETIOLOGY of most episodes of infectious gastroenteritis among pediatric and adult populations WAS UNKNOWN [2, 3]; (2) IT WAS ASSUMED THAT VIRUSES WERE IMPORTANT in these outbreaks because bacteria were associated etiologically only infrequently"

"Although the study specimens that were tested were derived from individuals with nonbacterial gastroenteritis, THERE WAS NO PRACTICAL WAY OF KNOWING WHETHER THEY CONTAINED INFECTIOUS MATERIAL THAT WAS CAPABLE OF PRODUCING DISEASE. IT WAS POSSIBLE THAT THE INABILITY TO DETECT A VIRUS RESULTED FROM THE ABSENCE OF AN INFECTIOUS AGENT IN THE TEST SPECIMEN."

"THE POWER OF THIS TECHNIQUE WAS SHOWN CLEARLY IN THESE RHINOVIRUS

STUDIES, in which a relatively low-titered tissue-culture suspension of rhinovirus 1A was reacted with a specific goat serum or a control (PBS). The mixture was then centrifuged, and the pellet was reconstituted with distilled water and stained with phosphotungstic acid [27]. Examination of the control preparation revealed scattered, randomly distributed, 27-nm particles, SOME OF WHICH COULD NOT BE IDENTIFIED CONCLUSIVELY AS A VIRUS (figure 1). However, in the virus-serum preparation, the 27-nm rhinovirus particles were no longer randomly distributed but appeared in the form of large and small aggregates coated with antibodies and standing out clearly from the background, leaving no doubt that they were virus particles."

"Following incubation of the stool filtrate WITH A VOLUNTEER'S CONVALESCENT SERUM AND FURTHER PREPARATION FOR ELECTRON MICROSCOPY, glistening aggregates of nonenveloped, antibody-coated 27-nm, VIRUS-LIKE PARTICLES, WHICH RESEMBLED RHINOVIRUSES, were visualized."

"THE VISUALIZATION OF VIRUS-LIKE PARTICLES was very promising, but it was clear that further studies were needed to determine the significance of this finding."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7110248/

It is clear from Kapikian's words that they went in believing a "virus" was responsible for the non-bacterial cases of gastrointestinal disease and that they had no way of determining whether this was true or not as they were unable to culture any "virus." So he set out to use the same IEM technique he had utilized previously to find 27 mm particles in tissue culture he claimed to be "Rhinoviruses" in order to find 27 mm particles in stool samples from the Norwalk outbreak which...resembled "Rhinoviruses..." .....

I provided highlights from the original 1972 study with a brief overview of the antibody methods used. For space, I edited out some of the antibody segments so if you desire to torture yourself reading useless experimental data that they admitted in 2015 can not be done for unculturable "viruses," click on the link below the highlights for more:

VISUALIZATION BY IMMUNE ELECTRON MICROSCOPY OF A 27-nm PARTICLE ASSOCIATED WITH ACUTE INFECTIOUS NONBACTERIAL GASTROENTERITIS

"A 27-nm particle was observed by immune electron microscopy in an infectious stool filtrate derived from an outbreak in Norwalk, Ohio, of acute infectious nonbacterial gastroenteritis. BOTH EXPERIMENTALLY AND NATURALLY INFECTED INDIVIDUALS DEVELOPED SEROLOGICAL EVIDENCE OF INFECTION; this along with other evidence suggested that the particle was the etiological agent of Norwalk gastroenteritis."

"IN SPITE OF INTENSIVE EFFORTS, AN ETIOLOGICAL AGENT HAS NOT BEEN FOUND FOR ACUTE INFECTIOUS NONBACTERIAL GASTROENTERITIS -a usually self-limited disease characterized by a spectrum of clinical symptoms which may include vomiting, diarrhea, abdominal pain, or a combination thereof (5, 10, 16). This syndrome affects a broad segment of the population and was the second most common disease experienced in a 10-year family study (8, 9). THE DISEASE, which has been given various descriptive names, WAS TRANSMITTED TO VOLUNTEERS IN THE 1940's AND 1950's AND AGAIN MORE RECENTLY IN 1971 AND 1972, BUT ALL ATTEMPTS TO DEFINITIVELY CULTIVATE AND CHARACTERIZE AN ETIOLOGICAL AGENT IN VITRO HAVE FAILED (5, 6, 11-17, 19, 21, 22). In the 1971 study, A FILTRATE FROM A RECTAL SWAB SPECIMEN from an adult who developed a secondary case of acute nonbacterial gastroenteritis during an outbreak in Norwalk, Ohio, induced the typical illness IN TWO OF THREE VOLUNTEERS; it was SERIALLY PASSAGED TWO ADDITIONAL TIMES IN VOLUNTEERS and again induced the typical illness (1, 5, 11, 12). Characterization studies in volunteers revealed that the infectious agent in Norwalk outbreak-derived filtrates was less than 66 nm in diameter and PROBABLY LESS THAN 36 nm and, in addition, was not inactivated by ether, acid, or heating at 60C for 30 min (11).

IN AN ATTEMPT TO DETECT THESE FASTIDIOUS, PRESUMABLY VIRAL, GASTROENTERITIS AGENTS, we adapted the technique of immune electron microscopy to the study of stool filtrates derived from the Norwalk outbreak. Previously, this method had been employed in serological or antigenic studies, or both, of various viruses, and was used for the successful observation of rubella virus (3, 4).

Recently, this technique had facilitated the detection of Australia antigen and permitted the observation of rhinoviruses in semi purified suspensions (18, 23). Furthermore, immune electron microscopy has been employed successfully for the detection of an antigenic inner component of the Dane particle associated with hepatitis virus B and enabled the detection of a new coronavirus strain (2, 7, A. Z. Kapikian, H. D. James, Jr., S. J. Kelly, and A. L. Vaughn, Infect. Immunity 7, 1973, in press). THE PRESENT STUDIES WHICH RESULTED IN THE DETECTION OF SMALL "PICORNA OR PARVOVIRUS-LIKE'' PARTICLES TO WHICH CERTAIN VOLUNTEERS AND NATURALLY INFECTED INDIVIDUALS DEVELOPED SIGNIFICANT ANTIBODY INCREASES are described below.

The 2% second human passage stool filtrate (8Fiia) used in these immune electron microscopy studies was DERIVED FROM A STOOL SPECIMEN OF A VOLUNTEER WHO DEVELOPED GASTROENTERITIS AFTER ORAL ADMINISTRATION OF A STOOL FILTRATE DERIVED FROM ONE OF THE TWO VOLUNTEERS WHO BECAME ILL AFTER RECEIVING THE ORIGINAL INOCULUM FROM THE NORWALK OUTBREAK (1, 11, 12). The 8FMa pool, which had been filtered through a 1,200- and a 450-nm membrane filter (Millipore Corp.) and PREPARED BY PREVIOUSLY DESCRIBED METHODS, WAS KNOWN TO CONTAIN AN INFECTIOUS AGENT; IT HAD INDUCED GASTROENTERITIS IN 6 OF 10 VOLUNTEERS, BUT EXTENSIVE ATTEMPTS TO RECOVER OR DETECT AN ETIOLOGICAL AGENT BY CONVENTIONAL TECHNIQUES WERE UNSUCCESSFUL (11, 12; R. Dolin et al., unpublished studies). Therefore, WE EXAMINED THIS FILTRATE FOR THE PRESENCE OF VIRUS PARTICLES BY IMMUNE ELECTRON MICROSCOPY UTILIZING INACTIVATED CONVALESCENT SERUM FROM EXPERIMENTALLY INFECTED VOLUNTEERS AS THE SOURCE OF SPECIFIC ANTIBODY as previously described in our coronavirus studies (A. Kapikian et al., Infect. Immunity 7, 1973, in press). THIS APPROACH WAS TAKEN IN THE HOPE THAT VIRUS PARTICLES WOULD APPEAR IN THE FORM OF AGGREGATES, thereby enabling the observation of a small virus, possibly present in low titer. The serum-stool filtrate mixtures (and at various times, 0.85% phosphate-buffered [pH 7.4] saline [PBS]-stool filtrate mixtures) were incubated at room temperature for 1 hr routinely. PBS WAS THEN ADDED, IF NECESSARY, TO MAKE A FINAL PRE-CENTRIFUGATION VOLUME OF 1.0 ml FOR EACH MIXTURE. The mixtures were then centrifuged at 17,000 rev/min for 90 min in a Sorvall RC2B centrifuge with an SS34 fixed-angle rotor. The supernatant fluid was carefully discarded; the pellet or sediment was suspended with a few drops of distilled water, stained with 3% phosphotungstic acid (PTA), pH 7.2, placed on a 400-

mesh Formvar-carbon coated grid, with the excess fluid being removed with the edge of a filter paper disc, and the grid examined at a magnification of 40,000 with a Siemens Elmiskop 1A electron microscope (3, 18). In the initial experiment, REACTION OF 0.4 mnl OF THE 8Fiia STOOL FILTRATE WITH 0.1 ml OF A 1:10 DILUTION OF CONVALESCENT SERUM FROM VOLUNTEER A who developed the typical illness after challenge with an 8Fii stool filtrate (see Table 1) RESULTED IN THE APPEARANCE OF AGGREGATES similar to the one shown in Fig. 1. THE PARTICLES WHICH WERE HEAVILY COATED WITH ANTIBODY WERE NOT RANDOMLY DISTRIBUTED BUT WERE PRESENT AS GROUPS WHICH STOOD OUT CLEARLY FROM THE SURROUNDING MATTER AND APPEARED TO RESEMBLE THE PICORNA OR PARVOVIRUSES.

IN STOOL FILTRATE-PBS CONTROL PREPARATIONS, OCCASIONAL PARTICLES OR GROUPS OF PARTICLES WITHOUT APPARENT ANTIBODY WERE SEEN, and Fig. 2 shows a particularly favorable orientation of six such particles. THE SIGNIFICANCE OF THESE PARTICLES WOULD HAVE BEEN DIFFICULT TO EVALUATE WITHOUT THE PREVIOUS EXPERIENCE ACQUIRED FROM EXAMINING SIMILAR, BUT HEAVILY COATED, PARTICLES WHICH HAD BEEN AGGREGATED BY ANTIBODY. These particles appeared to have cubic symmetry and there was a suggestion of surface substructure, but a definite pattern could not be ascertained (Fig. 2). THEY MEASURED APPROXIMATELY 27 nm IN THEIR SHORTEST DIAMETER AND 32 nm IN THEIR LONGEST AND AGAIN RESEMBLED BOTH THE PICORNAVIRUSES AND THE PARVOVIRUSES MORPHOLOGICALLY.

These studies were then extended to include both pre challenge and convalescent sera in an attempt to detect serological evidence of infection by immune electron microscopy. A 0.2-ml amount of a 1:5 dilution of inactivated serum was mixed with 0.8 ml of the 8Fiia stool filtrate since in preliminary studies 0.1 ml of a higher dilution of serum plus 0.4 ml of the 8Fiia stool filtrate resulted in variable staining with PTA. In these serological studies, the grids were read without prior knowledge of the specimen being examined in order to eliminate the possibility of biased interpretation. Routinely, five squares on each grid were examined in a median time of approximately 1 hr, and the preparation was then rated for the quantity of antibody as follows: 0 = no aggregates (3 OR MORE PARTICLES IN A GROUP WERE CONSIDERED TO CONSTITUTE AN AGGREGATE); 1+ = glistening aggregates, lightly covered with antibody; 2+ = moderately glistening aggregates, moderately covered with antibody; 3+ non glistening aggregates, heavily coated with antibody; 4+ = non glistening aggregates so heavily

coated with antibody that they were almost obscured. A 1 + difference was considered to be a significant change in the amount of antibody present. An example of an aggregate scored as 1 + is shown in Fig. 3, and another scored as 4+ is shown in Fig. 4."

"Although all four volunteers who developed illness after challenge with the 8FMia stool filtrate developed serological evidence of infection, IT WAS POSSIBLE THAT THE OBSERVED PARTICLES MIGHT REPRESENT A VIRUS NOT RELATED TO THE ETIOLOGICAL AGENT OF THE NORWALK OUTBREAK; IT WAS CONCEIVABLE THAT AN ADVENTUROUS VIRUS COULD EITHER HAVE BEEN PRESENT IN THE STOOL OF THE PATIENT FROM THE ORIGINAL NORWALK OUTBREAK OR COULD HAVE BEEN ACQUIRED DURING PASSAGE THROUGH VOLUNTEERS."

"It was noteworthy that each of the 13 individuals demonstrated the presence of antibody in pre-, acute-phase, or early sera, SUGGESTING THAT INFECTION WITH THE AGENT DERIVED FROM THE NORWALK OUTBREAK, OR A RELATED AGENT (OR AGENTS), WAS QUITE COMMON. Possibly, the agents of nonbacterial gastroenteritis may resemble certain respiratory viruses in their capacity to reinfect with facility.

We have presented data SUGGESTING that the 27-nm particle was the etiological agent of Norwalk gastroenteritis. ALTHOUGH IT IS CONCEIVABLE THAT THE 27-nm PARTICLE INDUCED INFECTION WHICH WAS NOT RELATED TO THE DISEASE, it is unlikely. In any case, ADDITIONAL LABORATORY AND EPIDEMIOLOGICAL STUDIES ARE NEEDED TO CONFIRM THE POSTULATED ETIOLOGICAL RELATIONSHIP."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC356579/pdf/jvirol00275-0197.pdf

In Summary;

-they claim both experimentally and naturally infected individuals developed SEROLOGICAL EVIDENCE of infection yet, beyond being theoretical, antibody responses would be impossible to determine without purifying/isolating the particles believed to be a "virus" first

-disregarding that they start off presuming an infectious agent, in spite of intensive efforts, AN ETIOLOGICAL AGENT HAD NOT BEEN FOUND for acute infectious nonbacterial gastroenteritis

-the disease was said to be transmitted to volunteers in the 1940's and 1950's and again more recently in 1971 and 1972, but ALL ATTEMPTS TO DEFINITIVELY CULTIVATE AND CHARACTERIZE AN ETIOLOGICAL AGENT IN VITRO FAILED

-a filtrate from a RECTAL SWAB SPECIMEN from an adult who developed a secondary case of acute nonbacterial gastroenteritis during an outbreak in Norwalk, Ohio, induced the typical illness in TWO OF THREE volunteers (small sample size and only 66% "success")

-this was SERIALLY PASSAGED two additional times in volunteers and again induced the typical illness

-In an attempt to detect what they PRESUME is "VIRAL," gastroenteritis agents, they adapted the technique of immune electron microscopy to the study of stool filtrates

-they present studies which resulted in the detection of small "PICORNA OR PARVOVIRUS-LIKE" PARTICLES to which CERTAIN (not all) volunteers and naturally infected individuals developed "significant antibody increases" which, yet again, would be impossible to determine without first purifying/isolating a "virus"

-the 2% SECOND HUMAN PASSAGE STOOL FILTRATE (8Fiia) used in these immune electron microscopy studies was derived from a stool specimen of a volunteer who developed gastroenteritis AFTER ORAL ADMINISTRATION OF A STOOL FILTRATE derived from one of the two volunteers who became ill after receiving the original inoculum from the Norwalk outbreak

-in other words, a person drank another person's poop juice and got an upset stomach...mind-blowing work here...

-of course, what is in the poop juice mixture is hidden behind PREVIOUSLY DESCRIBED METHODS

-they claim the poop juice was known to contain an infectious agent as it had induced

gastroenteritis in 6 of 10 volunteers (so again, around 60% "success")

-however, even though they "knew" an infectious agent was in the poop juice, EXTENSIVE ATTEMPTS to recover or detect an etiological agent by conventional techniques WERE UNSUCCESSFUL

-they examined the filtrate for the presence of "virus" particles by immune electron microscopy utilizing inactivated convalescent serum from experimentally infected volunteers as the source of "specific antibody"

-this approach was taken IN THE HOPE THAT "VIRUS" PARTICLES WOULD APPEAR IN THE FORM OF AGGREGATES

-PBS WAS ADDED, if necessary, to make a final pre-centrifugation volume of 1.0 ml for each mixture

-reaction of 0.4 mnl of the 8Fiia stool filtrate WITH 0.1 ml OF A 1:10 DILUTION OF CONVALESCENT SERUM FROM VOLUNTEER A RESULTED IN THE APPEARANCE OF AGGREGATES similar to the one shown in Fig. 1

-in other words, the aggregates of "virus" particles were only found by EM when serum from other volunteers were added to the poop juice

-the particles WHICH WERE HEAVILY COATED WITH ANTIBODY were not randomly distributed but were present as groups which stood out clearly FROM THE SURROUNDING MATTER (i.e. not purified/isolated) and appeared to resemble the "picorna or parvoviruses"

-however, in stool filtrate-PBS control preparations, occasional particles or groups of particles WITHOUT APPARENT ANTIBODY were seen

-the significance of these particles would have been difficult to evaluate without the previous experience acquired from examining SIMILAR, BUT HEAVILY COATED, PARTICLES which had been aggregated by antibody

-they measured approximately 27 nm in their shortest diameter and 32 nm in their longest and AGAIN RESEMBLED BOTH THE "PICORNAVIRUSES" AND THE "PARVOVIRUSES" MORPHOLOGICALLY

-in other words, they found the exact same particles when not using serum with no

antibody response...

-3 OR MORE PARTICLES in a group were considered to constitute an aggregate (nothing SUBJECTIVE about that...)

-Rating scale:

A) 1+ = glistening aggregates, lightly covered with antibody

B) 2+ = moderately glistening aggregates, moderately covered with antibody

C) 3+ non glistening aggregates, heavily coated with antibody

D) 4+ = non glistening aggregates so heavily coated with antibody that THEY WERE ALMOST OBSCURED

-they claim it was noteworthy that each of the 13 individuals demonstrated the presence of antibody in pre-, acute-phase, or early sera, suggesting that infection with the agent derived from the Norwalk outbreak, OR A RELATED AGENT (OR AGENTS), was quite common (which seems to also suggest that the antibody response isn't SPECIFIC if it could relate to AGENTS OTHER THAN "NOROVIRUS"......?)

-they admit it was possible that the OBSERVED PARTICLES MIGHT REPRESENT A "VIRUS" NOT RELATED to the etiological agent of the Norwalk outbreak

-it was conceivable that an adventitious "virus" could either have been present in the stool of the patient from the original Norwalk outbreak or could have been acquired during passage through volunteers

-they conclude that they presented data SUGGESTING that the 27-nm particle was the etiological agent of Norwalk gastroenteritis

-they again admit that it is conceivable that the 27-nm particle induced infection WHICH WAS NOT RELATED TO THE DISEASE

-as always, additional laboratory and epidemiological studies ARE NEEDED TO CONFIRM THE POSTULATED ETIOLOGICAL RELATIONSHIP

This paper essentially amounts to using EM to pick random particles from poop which resemble preconceived ideas of what a "virus" should look like and seeing if they clump together when serum is added to them. They then infer the meaning behind the subjective analysis of the clumping based on a scale they created. Meanwhile, no purified/isolated "virus" is ever shown nor are these unpurified/unisolated particles ever proven pathogenic. It all boils down to one completely subjective shitty experiment (pun somewhat intended).

Beyond this "stunning" evidence based on random particles in EM with antibodies which are impossible to determine for unpurified/unisolated "viruses," is the fact that there are apparently many (30-40%) asymptomatic (i.e. HEALTHY) cases of "Norovirus." Don't just take my word for it:

"Assuming that all norovirus outbreaks (n = 55) were the result of random sampling from an identical distribution and ignoring genogroup and genotype specificities, THE ASYMPTOMATIC RATIO WAS ESTIMATED AT 32.1% (95% confidence interval [CI], 27.7–36.7). Although not significant, separate estimation of the asymptomatic ratio of the GII.4 genotype appeared to be greater than other genotypes and WAS ESTIMATED AT 40.7% (95% CI, 32.8–49.0)."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6111106/

"Asymptomatic" is considered if a person is a carrier for a disease or infection but experiences no symptoms. There was no consensus among the studies on this definition. SOME STUDIES DEFINED "ASYMPTOMATIC" AS HEALTHY PERSONS WITH NO SYMPTOMS OF GASTROENTERITIS (diarrhea, vomiting, or fever, etc.). Others included people without symptoms of gastroenteritis for at least 1 week prior and more than 3 weeks after the day of stool collection. Finally, in some studies, NOROVIRUS WAS DETECTED IN NON DIARRHEAL STOOL SPECIMENS COLLECTED FROM HEALTHY PERSONS, but it was unknown if they had vomiting or other symptoms."

https://www.thelancet.com/journals/eclinm/article/PIIS2589-5370(18)30026-9/fulltext

So with all of this taken into consideration, how does the evidence for "Norovirus" stack up? Let's look to Koch's Postulates, the basic logical requirements needed to prove a pathogen causes disease, to find out:

1. The pathogen must be found in diseased but not healthy individuals

FAIL: It is estimated anywhere from 30-40% asymptomatic healthy cases

2. The pathogen must be cultured from the diseased individual in a pure form

FAIL: It can not be cultured at all in any form

3. Inoculation of a healthy individual with the cultured pathogen must recreate the disease

FAIL: Not everyone came down with symptoms of disease and no satisfactory animal model exists recreating the disease

4. The pathogen must be re-isolated from the inoculated, diseased individual and matched identically to the original pathogen

FAIL: It was never purified/isolated originally nor were any purified/isolated particles ever shown to be pathogenic

It would seem that the "Norovirus" evidence is not the bases loaded grand slam people thought it was. It is more akin to bottom of the ninth, down by 10 runs with 2 outs, and the weakest batter is at bat with no balls and 2 strikes.

Swing and a miss.

You're out!

Game over.

https://docs.google.com/document/d/e/2PACX-1vR7I4KMhC43wImtkbJmRKphrazeY4t7r5x_hoo5qU1hPIq9z2H4hwrEaHEgpHaeUY-Z85Ha4zIwVijL/pub?fbclid=IwAR2PO0BBkv1TpWcmqSArD_JXAZJ64rFjBNOpSx1UaAu9VAcXl07AH_PrQBc

This is the evidence for Hepatitis E (HEV) from 1983

This is the evidence for Hepatitis E (HEV) from 1983. This is the fraud of Virology for all to see. Here is the abstract with some photos from the article as I could not copy/paste highlights:

"Typical acute hepatitis was reproduced in a human volunteer immune to hepatitis A virus (HAV) AFTER ORAL ADMINISTRATION OF POOLED STOOL EXTRACTS FROM PRESUMED CASES of epidemic non-A, non-B hepatitis. Markers of hepatitis B infection, anti-HAV IgM, and increase in total anti-HAV level were not detectable in the volunteer's sera during the course of infection. Spherical 27- to 30-nm virus-like particles were visualized by immune electron microscopy (IEM) in stool samples collected during preclinical and early postclinical phases. These particles banded in CsCl at a buoyant density of 1.35 g/cm3. They reacted in the IEM test with sera from individuals who had experienced two non-B hepatitis episodes but did not react with sera from routine anti-

HAV IgM-positive hepatitis patients. INTRAVENOUS INOCULATION OF CYNOMOLGUS MONKEYS WITH THE VIRUS-CONTAINING STOOL EXTRACT resulted in histopathologically and enzymatically confirmed hepatitis, excretion of virus-like particles, and antibody response to them."

https://pubmed.ncbi.nlm.nih.gov/6409836/

Link to study (thanks Jordan Grant):

https://sci-hub.do/10.1159/000149370

Here you will see that the "proof" for Hepatitis E is feeding a single volunteer pooled poop juice from 9 patients PRESUMED to be sick with Hepatitis. They also intravenously injected the poop juice into monkeys.

Note:

-the presumption of Hepatitis illness in the patients the samples were taken from

-the poop juice is only PARTIALLY PURIFIED.

-the volunteer did not develop any symptoms until day 36

-No CPE was observed in the cell culture and they appeared NONPATHOGENIC

-the particles shared the same characteristics as those associated with Hepatitis A

"Science"

Https://docs.google.com/document/d/e/2PACX-1vRBYVrxqCRZIPbincQyQYYHKVRA8dHoOdR6bs2KvAhyVsiN1cRQcRNeVKzezW0NlLQFbM2DTtwRNkqD/pub

THE VIROLOGY STUDIES BEHIND THE HPV VACCINE

The Troubling Truth Behind HPV Vaccines : Prepare to be Outraged

OCTOBER 27, 2018 BY KENDALL NELSON

ADVERSE REACTIONS UNDERREPORTED

The first two HPV vaccines to go to market were Merck's Gardasil vaccine in 2006 and GlaxoSmithKline's (GSK's) Cervarix in 2009. (Both are still marketed in other countries but are no longer in use in the U.S., having been replaced by Merck's Gardasil-9 vaccine in 2017.) HPV vaccines were problematic since their introduction, despite the statement on the CDC's website that "HPV vaccination gives your child safe, effective, and long-lasting protection against HPV cancers."2 Moreover, statistics show that Gaby is far from an anomaly: to date, over fifty-eight thousand adverse reactions—including four hundred twenty-seven deaths—have been reported after HPV vaccine injections in the U.S. alone.3 What makes these numbers even more shocking is the U.S. Food and Drug Administration's (FDA's) estimate that less than 1 percent of all vaccine-related adverse reactions are ever reported.4

Part of the problem is that many doctors don't even know that there is a government system for reporting adverse events, called the Vaccine Adverse Event Reporting System (VAERS).5 For those who do, the system is complicated and time-consuming to use.6 Another barrier to reporting adverse reactions is what doctors are taught in medical school—that vaccines are so safe, they may never encounter a vaccine reaction during their entire career. Therefore, doctors often do not realize that medical conditions arising after vaccination could be vaccine-related injuries.

A 2016 study out of Canada highlighted the under-reporting of vaccine injuries. The study looked at over one hundred ninety-five thousand girls who had received HPV vaccines. Within forty-two days of HPV vaccination, the girls experienced over twenty thousand emergency room visits (n=19,351) or hospitalizations (n=958). However, only one hundred and ninety-eight adverse events were reported.7

HIDING AND DENYING THE DAMAGE

With statistics like these, one would think that the pharmaceutical companies that manufacture HPV vaccines and the authorities responsible for protecting public health by ensuring vaccine safety and efficacy would acknowledge that there is a problem, but instead of reevaluating HPV vaccines or pulling them off the market, these entities continually dismiss the onslaught of injuries as "coincidental" or "psychosomatic."8 HPV-vaccine-associated injuries include (but are not limited to) muscle pain and weakness; encephalopathy (brain inflammation); rheumatoid arthritis; Guillain-Barré syndrome (GBS); multiple sclerosis; amyotrophic lateral sclerosis (ALS); lupus; POTS; chronic fatigue syndrome (CFS); primary ovarian failure (POV); strokes; seizures; facial paralysis; and sudden cardiac death.9 Tragically, many adolescents have been accused of "faking" their illnesses right up until their deaths.

Some efforts to minimize the evidence of serious adverse reactions to HPV vaccines may go so far as to constitute criminal activity. In 2016, Dr. Sin Hang Lee, a scientist and doctor, wrote an open letter of complaint to Dr. Margaret Chan, at the time the director-general of the World Health Organization (WHO). Dr. Lee's letter alleged scientific misconduct and cover-up of HPV vaccine dangers by global health officials.10 The source of information for Dr. Lee's letter was a trail of emails and other communications between global health officials obtained via an Official Information Act request in New Zealand. The communications provided evidence that the same officials who were busy reassuring the public that HPV vaccines were safe knew that Gardasil and Cervarix were more likely than other vaccines to cause a potentially dangerous inflammatory response.

Specifically, WHO officials knew that the vaccines trigger the release of cytokines or proteins called tumor necrosis factors (TNFs), which can cause cell death.11 The release of TNFs can also result in a wide range of reactions such as tumor regression, septic shock (a serious whole-body inflammatory response that can result in dangerously low blood pressure and death) and cachexia (a wasting syndrome where the person loses weight, becomes fatigued and experiences muscle atrophy).12

THE RUSH TO MARKET

Perhaps the grossest example of FDA misconduct of all time is the fact that Gardasil was fast-tracked.13 The time period from clinical trial to recommending the vaccine was only four years, even though most vaccines take an average of three years to develop and five to ten more for universal acceptance. Fast-tracking is a process meant to "facilitate the development of drugs

which treat a serious or life-threatening condition."14 It is a misuse of fast-tracking to apply it toward the licensure of a vaccine designed to eliminate a sexually transmitted virus with which the majority of sexually active men and women are infected at one point or another—a virus that 90 percent of infected individuals clear naturally from the body within two years.15

In addition to the potentially fraudulent fast-tracking of Gardasil, the vaccine was only studied in twelve hundred girls under the age of sixteen before its recommendation for universal use in all eleven- to twelve-year-old girls. No studies looked at Gardasil's use in children with preexisting health problems or its use in combination with the other vaccines routinely given to American adolescents.16 Similarly, Cervarix, which was licensed in the U.S. in 2009, was studied for less than six years in fewer than twelve hundred healthy girls under the age of fifteen.

Typically, trials of new drugs compare one group that is given the drug against a "control" group that is given an inert (inactive) placebo, most often a saline solution. However, the clinical trials for Gardasil and Cervarix did not use a legitimate placebo in each of their control groups.17 Instead of receiving a saline solution, participants in several of the Gardasil control groups received aluminum in the form of a neurotoxic adjuvant present in all HPV vaccines. In the case of Cervarix, control group participants were given hepatitis A vaccine or other childhood vaccines—capable of causing adverse reactions—in lieu of a true placebo. Did this result in fraudulent conclusions? One might ask, how is it possible to detect adverse reactions properly without a legitimate control group?16

CORPORATE TRACK RECORD

If you think Merck can be trusted with your daughter's or son's well-being, just look at its corporate history of engaging in criminal fraud with regard to other pharmaceutical products. For example, Merck made a "hit list" to "destroy," "neutralize" and "discredit" doctors who criticized the company's disastrous drug, Vioxx.18 Ultimately, Merck entered a guilty plea and agreed to pay a fine of nine hundred fifty million dollars.19 (This, of course, was not much of a fine considering that Gardasil accounted for more than two billion dollars in revenues in 2016 alone.20) Former Merck scientists have accused Merck in federal court of vaccine research fraud regarding the efficacy of its measles, mumps and rubella (MMR) vaccine.21

When we interviewed Dr. Diane Harper, one of the world's leading HPV experts and principal investigator for Merck's Gardasil and GSK's Cervarix clinical trials, she raised concerns about

both vaccines and described Merck's advertising campaign as "egregious and aggressive." Dr. Russell Blaylock, a retired neurosurgeon and health freedom advocate, has gone so far as to say that Merck's widely aired One Less campaign was a "complete fraud." Blaylock proclaimed, "It has never been shown that (Gardasil) prevents cervical cancer."22 According to Harper, "The concept that our daughters are cancer deaths waiting to happen is just not accurate," yet Merck has not been shy about insinuating just that.

Another fact important to understand, again explained by Harper, is that there are no data showing that HPV vaccines remain effective beyond five years, while a full fifteen years of immunity coverage are necessary to prevent cervical cancer. In Harper's view, the moment Merck gained FDA approval for Gardasil, the company stopped studying the vaccine, performing no long-term safety monitoring.

WHICH IS RISKIER?

What are the cervical cancer facts? According to the CDC and the National Institutes of Health (NIH), of the nearly 1.6 million diagnosed cancer cases (all cancers) and more than five hundred fifty thousand cancer deaths that occur in the U.S. annually, less than 3 percent involve chronic HPV-infection-associated cervical or other genital cancers in women and men.23 For the period from 2003 through 2007, the incidence rate for cervical cancer was 8.1 cases per hundred thousand women per year in the U.S. (versus upwards of forty per hundred thousand in high-incidence countries) and the mortality rate was 2.4 deaths per hundred thousand women per year (compared to fifteen or more per hundred thousand in high-mortality countries).24 While it may be true that some women who are chronically infected with HPV for many years and who do not promptly identify and treat precancerous cervical lesions may go on to develop cervical cancer and possibly die, it is also important to know that after Pap test screening became a routine part of health care for American women in the 1950s, cervical cancer cases in the U.S. dropped 74 percent—and the CDC recommends continued Pap tests whether women get the HPV vaccine or not.25

A study by researchers at the University of Texas looked at HPV vaccination data from 2007–2012. The results showed that young women twenty to twenty-six years of age who received the four-strain Gardasil vaccine were actually more likely than non-HPV-vaccinated women to be infected with high-risk nonvaccine strains of HPV ten years later.26 The implications of these results are sobering, suggesting that while the vaccine may have reduced infection with the four targeted HPV strains, "other, possibly more pathogenic, HPV viruses

moved in to fill the void"; in other words, the vaccine "exposed the girls who took it to greater risk for HPV infection than those that did not take the vaccine."27

MISLEADING MARKETING

Since 2017, Merck's Gardasil-9 has taken the place of both Gardasil and Cervarix in the U.S. The CDC currently recommends Gardasil-9 for both females and males ages nine through twenty-six, administered using a two-dose or three-dose schedule and costing an average of two hundred ten dollars per shot. Merck is marketing Gardasil-9 as an "improvement" over Gardasil, claiming it will prevent 80 percent of all vulvar, cervical and anal cancers28 (up from the 65 percent for Gardasil). Unlike its predecessor, which targeted four strains of HPV, Gardasil-9 targets nine of the more than one hundred fifty known strains of HPV, most of which are harmless. Gardasil-9 also targets genital warts. However, the "new and improved" version of Gardasil is no prize. What Merck does not advertise is the fact that Gardasil-9 contains more than double the toxic aluminum content of the original vaccine and has no fewer reported side effects.

Merck followed its initial One Less campaign with its I Chose advertising campaign in 2008, which featured a variety of young women explaining why they decided to get vaccinated, ending with one woman explaining that her dreams don't include cervical cancer. Then, a decade after Gardasil's introduction, Merck shifted from One Less and I Chose to attempts to shame parents into getting their children vaccinated, playing on parents' basic instinct to protect their children. (One could easily label the 2016 campaign as the Who Knew? campaign, with both boys and girls asking their parents in the television commercials, "Did you know—Mom, Dad?")

Up until that point, the vaccine had not been heavily promoted to boys and young men, despite FDA approval for males in 2009. All that changed in 2016 when Merck began targeting all eleven- to twelve-year-olds, female or male. Oddly, not until 2018 did a Merck advertisement even mention how one contracts HPV (through intimate sexual contact). The newest Versed ad campaign aims to educate youth by telling them to "get smart about HPV" and get "vocal."

Despite the huge amounts of money spent on HPV vaccine advertising, consumers in the U.S. apparently are not taking the bait. HPV vaccines have had a persistently low adoption rate. According to the CDC, as of 2016, fewer than half of seventeen-year-olds (49.5 percent of girls and 37.5 percent of boys) were up to date with the recommended HPV vaccine series,29 falling far short of the health agency's 2020 goal for 80 percent of both girls and boys to be HPV-

vaccinated.30

It's not just U.S. citizens who are getting wise to HPV vaccination dangers. Several countries—including Japan,31 France32 and India33—have stopped recommending HPV vaccines and/or have filed lawsuits on behalf of HPV-vaccine-injured families. In Japan, Gardasil has become such a scandal that the country's uptake rate is currently under 1 percent.34 In many European Union (EU) countries, HPV vaccine coverage rates remain "lower than expected," and some EU countries make individuals who want the pricy vaccine pay for it themselves.35 In Ireland, a group of parents with Gardasil-injured children is formally known as "Regret."36

FERTILITY AT RISK

In 2016, concurrent with the "Who Knew" campaign, Merck suffered a major blow as the American College of Pediatricians (ACPeds) sounded an alarm by releasing a statement expressing concerns about a potential connection between HPV vaccines and premature ovarian failure (POF) in adolescent girls.37 Since the licensure of HPV vaccines, reports to VAERS include forty-eight cases of ovarian damage, two hundred fifty-six cases of spontaneous abortion, one hundred seventy-two cases of amenorrhea and one hundred seventy-two cases of irregular menstruation believed to be caused by HPV vaccination in the U.S.38 That this is cause for concern is supported by a June 2018 study in the Journal of Toxicology and Environmental Health that looked at a database of more than eight million American women and found a 25 percent increase in childlessness associated with HPV vaccination.39 According to data from the CDC, more than 12 percent of American women—one in eight—have trouble conceiving and bearing a child.40

Other research has implicated aluminum in fertility problems. Dr. Christopher Exley, an aluminum expert at Keele University in England, examined sixty-two semen samples and found "unequivocal evidence" of high concentrations of aluminum, especially in the semen of men with low sperm counts.41 Another toxic ingredient found in Merck's Gardasil vaccines is polysorbate 80, which has been associated with a myriad of health problems and has proven to cause ovarian toxicity in rats.42 Polysorbate 80 was used along with aluminum in some of Merck's bogus "placebo" control groups in prelicensure studies.38

EXTENDING THE VACCINE'S REACH

Despite all of the problems with HPV vaccines, U.S. politicians are increasingly trying to mandate HPV vaccines for school admission. In 2007, Governor Rick Perry of Texas signed an executive order that required HPV vaccination for all eleven- to twelve-year-old schoolgirls. Why? The CDC says it's important to vaccinate people before they become sexually active, but perhaps Perry's order had more to do with the fact that his former chief of staff was the leading lobbyist for Merck.43 Fortunately, the Texas state legislature overturned Perry's order. Even so, Perry launched an unfortunate trend. Today, children in Rhode Island, Virginia and the District of Columbia must be vaccinated against HPV to go to school (unless they take a religious or philosophical exemption), and in California, minors do not need parental consent to get HPV vaccines. In its statement communicating concern about primary ovarian failure, ACPeds expressed opposition to HPV vaccine mandates, saying, "The College is opposed to any legislation which requires HPV vaccination for school attendance."37

In fact, mandating any vaccine is unethical—whether for students, parent volunteers, health care workers or any other person—especially because vaccine manufacturers are virtually exempt from liability in the U.S. The National Childhood Vaccine Injury Act (NCVIA) of 1986 made it almost impossible to sue pharmaceutical companies or those who administer vaccines if a person becomes vaccine-injured. Instead, that person must appeal to the government-run National Vaccine Injury Compensation Program (NVICP), which has a terrible reputation for financially compensating those injured or killed by vaccines. That said, the program has paid almost six million dollars to forty-nine Americans after the U.S. Court of Federal Claims found that Gardasil had injured the individuals.44

As of June 2018, we have something new to worry about: Merck has received an FDA "priority review" to expand Gardasil to women and men aged twenty-seven to forty-five years.45 Merck is pushing for expansion to this age group despite the fact that most adults have already been exposed to HPV by those ages. Merck itself writes, "Gardasil-9 has not been demonstrated to provide protection against diseases from vaccine HPV types to which a person has previously been exposed through sexual activity."46

THE WORST VACCINE

I've been in the vaccine awareness community for some time now. I've made a movie about the vaccine controversy; I've worked hard to educate people on the risks involved with vaccines; and I've fought against several hundred bad pieces of vaccine legislation over the past three years. In my experience, the HPV vaccine is the worst vaccine on the market. The truth is that HPV

vaccines have injured and killed far more children than ever would have gone on to develop HPV-associated cancers without the vaccine.

For many, HPV vaccines are reminiscent of the thalidomide scandal of the 1960s, when doctors prescribed the drug to pregnant women to alleviate morning sickness. Unfortunately, thalidomide caused phocomelia (malformation of the limbs), affecting thousands of children worldwide and often resulting in death.

Dr. Bernard Dalbergue (former physician at Merck) predicted in 2014 that the Gardasil vaccine would become “the greatest medical scandal of all time.”47 Dr. Russell Blaylock likewise has concluded that the harm from HPV vaccines far exceeds any claimed benefits. According to Blaylock, “The general public is woefully unaware of the fact that vitamin B12, folic acid, vitamin C, curcumin (turmeric), quercetin and many other natural nutrients and vitamins naturally prevent HPV and cervical cancer.”22 Attention to health and nutrition can address many of the factors that increase the risk of developing HPV-related cancers, which include smoking; long-term oral contraceptive use; a weakened immune system; co-infection with chlamydia or HIV; poor nutrition; deficiencies of vitamins C and B, carotenes and folate; heavy drinking; and chronic inflammation.

When women face only a 0.6 percent risk of cervical cancer and men face a 0.2 percent risk of rare anal and penile cancers, it seems irrational to continue using a vaccine with so many complications, let alone mandate the vaccine or expand the age groups covered by HPV vaccine recommendations. These vaccines have been plagued by controversy since their inception, causing more injury than any other vaccine in history. Despite an undeniable litany of adverse effects that includes death, the vaccines continue to be administered to millions of people without their fully informed consent. In addition, the HPV vaccines may well be worthless for their stated purpose—their heavy marketing as “cancer prevention” proceeds despite the fact that no long-term studies have ever been done to prove their efficacy. Blaylock says—and I agree—that “the entire vaccine program is based upon nonsense, fear and concocted fairy tales.” No amount of Merck’s clever advertising will convince me otherwise. Only unbiased, credible science could change my mind, and so far, that kind of science has not been done.

References

ARE AT THE BOTTOM OF THIS PAGE;

https://www.westonaprice.org/health-topics/the-troubling-truth-behind-hpv-vaccines-prepare-to-be-outraged/?fbclid=IwAR243sm-NADalz4vOsvIrgGskdY6togVYmsqSvxIzNYQCus0RC-TfEIlrXg

HPV MADNESS:

Looking back at the original papers, it was clear that HPV was nothing more than random particles found in grounded up wart tissue. There was no attempt at purification/isolation of any "virus" nor was there any proof of pathogenicity. They claimed they were "virus-like" particles due to how similar they looked to plant "viruses." The later HPV-16 and HPV-18 "discoveries' ' linked to cervical cancer were just as bad as they amounted to nothing more than hybridized cloned DNA fragments that had little to no genetic relationship to the previous HPV.

So what exactly is HPV? How many versions are there? What effect does it supposedly have on the body besides warts and cancer? How do they determine how many cancers are caused by this "virus?" Let's take a look.

According to Cancer.net:

"THERE ARE MORE THAN 150 TYPES OR "STRAINS" OF HPV. Most HPV infections DO NOT CAUSE SYMPTOMS OR HEALTH PROBLEMS, SO YOU MAY NOT KNOW IF YOU HAVE THE VIRUS. However, some types of HPV cause cancer or abnormal growths that can turn into cancer. These growths are called precancerous lesions."

"What types of cancer are caused by HPV?

Research LINKS these types of cancer with HPV:

Cervical cancer. HPV causes NEARLY all cervical cancers. About 70% of HPV-related cervical cancer is caused by HPV-16 or HPV-18. However, MOST GENITAL HPV INFECTIONS WILL NOT CAUSE CANCER. Smoking can also raise the risk of cervical cancer in women with HPV.

Oral cancer. HPV can cause cancer of the mouth and tongue. It can also cause cancer of the

oropharynx. This is the middle part of the throat, from the tonsils to the tip of the voice box. These HPV-related cancers are rising.

Other cancers. HPV is also LINKED to less common cancers. They include anal, penile, vaginal, and vulvar cancers."

https://www.cancer.net/navigating-cancer-care/prevention-and-healthy-living/hpv-and-cancer

Notice that there are over 150 strains of HPV most of which do not cause symptoms or health problems whatsoever and many people will never know they even have it. Even if you do get genital HPV, most will never cause cancer. They also say that research LINKS HPV to certain cancers. The research does not PROVE this link.

Did you notice that they state HPV causes NEARLY all, but not all, cases of cervical cancer? It also does not cause symptoms of disease in the vast majority who are found to have it. If HPV does not cause disease in everyone nor cause all cases of cervical cancer, then it immediately fails Koch's first Postulate for proving pathogenicity:

1. the microorganism or other pathogen MUST BE PRESENT IN ALL CASES of the disease but SHOULD NOT BE FOUND IN HEALTHY ORGANISMS

What are the other causes of cervical cancer?

"But HPV is NOT THE ONLY CAUSE of cervical cancer. MOST WOMEN WITH HPV DON'T GET CERVICAL CANCER, and OTHER RISK FACTORS, like smoking and HIV infection, INFLUENCE WHICH WOMEN exposed to HPV are more likely to DEVELOP CERVICAL CANCER."

https://www.cancer.org/cancer/cervical-cancer/causes-risks-prevention/what-causes.html

"IT ISN'T CLEAR WHAT CAUSES CERVICAL CANCER, but it's certain that HPV plays a role. HPV is very common, and most people with the virus never develop cancer. THIS MEANS OTHER FACTORS — SUCH AS YOUR ENVIRONMENT OR YOUR LIFESTYLE CHOICES — ALSO DETERMINE WHETHER YOU'LL DEVELOP CERVICAL CANCER."

https://www.mayoclinic.org/diseases-conditions/cervical-cancer/symptoms-causes/syc-20352501

The "OTHER FACTORS:"

"Family History of Cervical Cancer

Age - Sexual and Reproductive History - Socioeconomic Status - Smoking - HIV Infection - In Utero DES Exposure - Long-term use of oral contraceptives"

https://www.cancerquest.org/patients/cancer-type/cervical-cancer

If HPV is the "virus" which supposedly causes cervical cancer but it doesn't cause cancer without other factors such as smoking, environment, and lifestyle choices, can it really be considered THE cause of cervical cancer?

"The truth is that HAVING HPV DOES NOT MEAN YOU HAVE OR WILL GET CERVICAL CANCER. MOST WOMEN WILL BE EXPOSED TO HPV AT SOME POINT IN THEIR LIVES, and for most women, HPV infections will go away on their own without causing any problems."

https://www.mountsinai.org/care/cancer/services/gynecologic/conditions/cervical/myths-facts

So HPV is not the only cause of cervical cancer and MOST women who "get" HPV never get cervical cancer. There are also those who do get cervical cancer but do not test positive for HPV called HPV-negative cases:

"Since 2017, the annual screening report in Belgium SUGGESTS THAT 15% OF THE CERVICAL CANCERS WERE HPV NEGATIVE."

"THE FACT THAT THERE ARE HPV NEGATIVE CANCERS should not undermine all idea's regarding primary HPV screening."

"Despite the fact that we believe that almost all cervical cancers are due to a HPV infection, WE DO NOT ALWAYS FIND HPV IN WOMEN WITH CERVICAL CANCER."

"First of all, there are cervical cancers independent from HPV infection, THESE ARE THE TRUE HPV NEGATIVE CANCERS."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6516188/

"HPV-NEGATIVE CERVICAL CANCER

Conversely, another area of cervical cancer that is less discussed BUT HAS BEEN DOCUMENTED, IS HPV-NEGATIVE DISEASE.

In a study of Belgium women with cervical cancer prior to 2000, 13% WERE FOUND TO HAVE HPV-NEGATIVE DISEASE, while an additional trial REPORTED 7.1% OF WOMEN WITH HPV-NEGATIVE CERVICAL CANCER between 2001 and 2008. Globally, the percentage of HPV-negative cervical cancer cases RANGES FROM 7% TO 11%."

"However, the idea that HPV-negative cervical cancer is possible, especially in a disease that is mainly driven by HPV positivity, is not a unanimous opinion. Maurie Markman, MD, editor-in-chief, OncologyLive®, and physician and president of Medicine and Science at Cancer Treatment Centers of America, said that the issue surrounding HPV-negative disease is that THE HPV MAY SIMPLY NOT BE FOUND THROUGH TESTING, BUT IS PRESENT.

“If you're talking about the cervical cancer that we speak about, you can have the HPV found after very carefully searching, [EVEN] IF IT'S 1% [OF HPV POSITIVITY] OR LESS,” Markman said. “SOME OF THIS IS DEFINITIONAL. THE FACT THAT YOU DON'T FIND IT DOESN'T MEAN IT ISN'T THERE.”

Furthermore, THE POSSIBILITY OF NOT HAVING HPV DETECTED IN A DISEASE should not deter individuals from undergoing HPV vaccination nor regular cervical cancer screening, he emphasized.

“WE CAN CERTAINLY SHOW THAT 70% TO 80% OF HIV-POSITIVE CERVICAL CANCERS ARE DUE TO THE 2 MAJOR TYPES: HPV 16 and 18. There are another 20% to 30% of cervical cancers that are DUE TO OTHER [HPV] TYPES, AND THERE ARE DOZENS OF HPV TYPES. Therefore, it's not at all clear that the issue with lack of preventing cervical cancer has anything to do with being HPV negative. IT'S JUST THERE ARE TYPES OF HPV THAT WE MAY NOT BE INCLUDING IN THE VACCINE.”

https://www.onclive.com/view/hpv-negative-cervical-cancer-myth-or-an-area-to-therapeutically-tackle-

In the previous study, it is suggested that 15% of all cervical cancers are HPV-negative. According to this article, anywhere from 7-11% of cervical cancers are not related to HPV. Even the two major types only account for 70-80%. The other 20-30%? Well, those are just one of the over

150 strains of HPV that exist. Don't concern yourself with those numbers (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) as they aren't even included in the vaccines. Remember, according to Maurie Markman, these HPV-Negative cases are just "definitional." Just because they don't find HPV doesn't mean it's not there. Also remember, most people have or will get HPV but the vast majority will never get sick nor even know they have it. If they do have one of the major types, they most likely won't get cancer. However, if they do happen to get cancer, it was definitely HPV...even if it was not detected. ☺

Confused? Let's see if the WHO can clear things up. According to the WHO:

"What is HPV?

Human papillomavirus (HPV) is the most common viral infection of the reproductive tract. MOST SEXUALLY ACTIVE WOMEN AND MEN WILL BE INFECTED AT SOME POINT IN THEIR LIVES AND SOME MAY BE REPEATEDLY INFECTED.

The peak time for acquiring infection for both women and men is shortly after becoming sexually active. HPV is sexually transmitted, but penetrative sex is not required for transmission. Skin-to-skin genital contact is a well-recognized mode of transmission.

THERE ARE MANY TYPES OF HPV, AND MANY DO NOT CAUSE PROBLEMS. HPV infections usually clear up without any intervention within a few months after acquisition, and about 90% clear within 2 years. A SMALL PROPORTION OF INFECTIONS WITH CERTAIN TYPES OF HPV CAN PERSIST AND PROGRESS TO CERVICAL CANCER.

How HPV infection leads to cervical cancer

ALTHOUGH MOST HPV INFECTIONS CLEAR UP ON THEIR OWN AND MOST PRE-CANCEROUS LESIONS RESOLVE SPONTANEOUSLY, there is a risk for all women that HPV infection may become chronic and pre-cancerous lesions progress to invasive cervical cancer.

IT TAKES 15 TO 20 YEARS FOR CERVICAL CANCER TO DEVELOP in women with normal immune systems. It can take only 5 to 10 years in women with weakened immune systems, such as those with untreated HIV infection."

https://www.who.int/news-room/fact-sheets/detail/human-papillomavirus-(hpv)-and-cervical-cancer

So according to the WHO, we once again see nearly everyone "gets" or has HPV. There are many different types and most do not cause health problems. Only a small proportion ever lead to health problems. But watch out, if that "virus" which is harmless to the vast majority decides to strike, you will feel those effects in 15-20 years.

How do they know that HPV will lead to cancer in 15-20 years? What long-term studies are there showing a person becoming infected with HPV which then follows them for the next 15-20 years to see if they develop cancer? Why would it take the "virus" 15-20 years to cause illness in a person? Why is it harmless to the vast majority? Do the so-called "antibodies" just ignore the HPV floating freely in the body? I'm pretty positive they don't care to even think about these questions let alone attempt to answer them.

The more I dig into HPV, the more it sounds like another imaginary "virus" which likes to hide inside the body for 10+ years until it decides to strike. It seems fitting that the man who "discovered" the HPV/cancer link shared the Nobel Prize with the French "discoverers" of HIV.

They obviously love to link HPV to cancer even though they claim the vast majority of people infected will remain "asymptomatic" and never know they had it, but how do they determine the percentages on how many cancers are actually caused by HPV? For that we turn to the CDC.

According to the CDC:

"Number of HPV-Associated Cancer Cases per Year

AN HPV-ASSOCIATED CANCER is a specific cellular type of cancer that is DIAGNOSED IN A PART OF THE BODY WHERE HPV IS OFTEN FOUND. These parts of the body include the cervix, vagina, vulva, penis, anus, rectum, and oropharynx (back of the throat, including the base of the tongue and tonsils).1 2 These cellular types include carcinoma external icon of the cervix and squamous cell carcinoma external icon of the vagina, vulva, penis, anus, rectum, and oropharynx. RESEARCHERS USE CANCER REGISTRY DATA TO ESTIMATE THE NUMBER OF HPV-ASSOCIATED CANCERS IN THE UNITED STATES BY LOOKING AT CANCER IN PARTS OF THE BODY AND CANCER CELL TYPES THAT ARE MORE LIKELY TO BE CAUSED BY HPV. CANCER REGISTRIES DO NOT ROUTINELY COLLECT DATA ON WHETHER HPV IS IN THE CANCER TISSUE. CDC studies3 4 have reported the number of HPV-associated cancer cases per year, and these studies have more information on how HPV-associated numbers were calculated.

Number of HPV-Attributable Cancer Cases per Year

AN HPV-ATTRIBUTABLE CANCER IS A CANCER THAT IS PROBABLY CAUSED BY HPV. HPV causes nearly all cervical cancers and many cancers of the vagina, vulva, penis, anus, rectum, and oropharynx. A CDC study5 used population-based data from cancer tissue TO ESTIMATE THE PERCENTAGE OF THESE CANCERS THAT ARE PROBABLY CAUSED BY HPV. Since rectal cancer was not included in the CDC genotyping study, the percentage of anal cancer caused by HPV was used because recent studies have shown that the HPV-associated types of anal and rectal squamous cell carcinomas are similar.2

To find the number of HPV-attributable cancers, MULTIPLY THE NUMBER OF HPV-ASSOCIATED CANCERS BY THE PERCENTAGE OF THESE CANCERS THAT ARE PROBABLY CAUSED BY HPV. For example, about 7,083 people are diagnosed with anal cancer each year, and about 91% of anal cancers ARE THOUGHT TO BE CAUSED BY HPV. 91% of 7,083 is about 6,500, as shown in the table below."

https://www.cdc.gov/cancer/hpv/statistics/cases.htm

So we have HPV-ASSOCIATED cancers and HPV-ATTRIBUTABLE cancers. For "Associated," these are just cancers which occur where HPV is typically found. They do not know whether HPV was present in the tissue or not as they DO NOT COLLECT THIS DATA. They just lump together any cancers which occur where they determine HPV hangs out and create a nice little estimate. Nothing suspicious about that.

For "Attributed," these are cancers PROBABLY caused by HPV. Just as with "Associated" there is no need for certainty. Rest assured, HPV PROBABLY caused these cancers. To figure out how many HPV-Attributed cancers there are, you just take your HPV data-less and totally "accurate" Associated estimate and multiply that by the percentage you think are cases where HPV PROBABLY caused it. Viola! You have your percentage of cancers PROBABLY caused by HPV.

Makes sense, right?

Just for further clarity, also from the CDC:

"HPV-ASSOCIATED CANCERS ARE ESTIMATED by examining cancer in parts of the body and cancer cell types THAT ARE MORE LIKELY TO BE CAUSED BY HPV. Cancer registries DO NOT COLLECT DATA ON THE PRESENCE OR ABSENCE OF HPV IN CANCER TISSUE AT THE TIME OF DIAGNOSIS.

In general, HPV IS THOUGHT TO BE RESPONSIBLE for more than 90% of anal and cervical

cancers, about 70% of vaginal and vulvar cancers, and more than 60% of penile cancers.

Oropharyngeal cancers traditionally have been caused by tobacco and alcohol, but recent studies show that about 70% of cancers of the oropharynx MAY BE LINKED to HPV. Many cancers of the oropharynx MAY BE CAUSED by a combination of tobacco, alcohol, and HPV."

https://www.cdc.gov/cancer/hpv/statistics/

For this "link" to cancers "probably" caused by random DNA fragments never properly purified/isolated nor ever proven pathogenic by fulfilling Koch's Postulates, we have untold numbers of children's lives being ruined while they are injected with extremely toxic vaccines:

"Additionally, the following postmarketing adverse experiences have been spontaneously reported for GARDASIL:

-BLOOD AND LYMPHATIC SYSTEM DISORDERS: Autoimmune hemolytic anemia, idiopathic thrombocytopenic purpura, lymphadenopathy.

-RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS: Pulmonary embolus.

-GASTROINTESTINAL DISORDERS: Pancreatitis.

-GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS: Asthenia, chills, DEATH, malaise.

-IMMUNE SYSTEM DISORDERS: Autoimmune diseases, hypersensitivity reactions including

-anaphylactic/anaphylactoid reactions, bronchospasm.

-MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS: Arthralgia, myalgia.

-NERVOUS SYSTEM DISORDERS: Acute disseminated encephalomyelitis, Guillain-Barré syndrome, motor neuron disease, paralysis, seizures, transverse myelitis.

-INFECTIONS AND INFESTATIONS: Cellulitis.

-VASCULAR DISORDERS: Deep venous thrombosis."

https://www.fda.gov/media/90064/download

In Summary:

-there are 150+ strains of HPV

-most people will "get" HPV in their lifetime

-the vast majority never experience any symptoms and will never know they have HPV

-just because one has HPV doesn't mean they will get cancer

-other factors (smoking, environment, lifestyle) are needed for cancer to occur

-there are many cases of cervical cancer where HPV is not detected

-it takes 15-20 years for HPV to "cause" cervical cancer

-HPV cancer estimates are based on PROBABLE cases as they do NOT COLLECT DATA on whether the cancer tissue has HPV or not

-the Gardasil vaccine lists DEATH as an adverse event among numerous other life-destroying conditions in addition to the regular side effects

It's time to end this madness.

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RICHARD SHOPE 1933 RABBIT SPV PAPER: PRECURSOR TO HPV

Unfortunately for Iowa and humanity, Richard Shope was not only responsible for the Swine Flu "virus," he was also the one to "discover" the papillomavirus in rabbits which eventually led to the "discovery" of the human papillomavirus and it's inevitable association with cervical cancer. His work paved the way for toxic vaccines for both the flu and HPV. Sadly, he is celebrated for his research:

"Virologist Richard Shope helped discover the cause of the 1918 flu and LAY THE GROUNDWORK FOR LIFE-SAVING VACCINATIONS."

"As people throughout the world roll up their sleeves to receive COVID-19 vaccinations, they can thank an alumnus of the State University of Iowa's College of Medicine for HIS CRITICAL ROLE IN MAKING SUCH TREATMENT POSSIBLE."

"His 1931 findings alone were significant, AS WERE LATER CONTRIBUTIONS ON PAPILLOMAVIRUSES. In 1957 he received the esteemed Albert Lasker Clinical Medical Research Award, and in 1963 he was awarded an honorary doctorate degree by the UI."

https://magazine.foriowa.org/story.php?ed=true&storyid=2058

As can be seen, Shope's work is bragged about in Iowa. He was also credited in a 2009 New York Times article with helping to pave the way for the HPV vaccine. Amusingly, the article speaks of many "pseudoscientific myths" which linked herpes, promiscuity, Jewish women not eating bacon, and foreskin smegma as causes of cervical cancer but left out the fact that Shope's work and the HPV link to cancer is yet another in a long line of pseudoscientific myths:

"But each step forward to those techniques was a triumph of hard science over the PSEUDOSCIENTIFIC MYTHS that for centuries surrounded the disease.

The first was posited by a doctor in Florence in 1842. He noticed that PROSTITUTES AND MARRIED WOMEN DIED OF CERVICAL CANCER, BUT NUNS ALMOST NEVER DID. Though he might have discerned that it was sexually transmitted, he was thrown off by another fact: nuns often died of breast cancer. His conclusion was that nuns' corsets were dangerously tight.

One may laugh, but prominent American scientists made a similar error in the 1970's, noting that many women with cervical cancer had a history of genital herpes. INSTEAD OF REALIZING THAT IT WAS A COINCIDENCE, THEY ERRONEOUSLY CONCLUDED THAT THE HERPES VIRUS WAS THE CAUSE. And they were closer to the mark than 1950's researchers, who had BLAMED SMEGMA, which builds up under the foreskin of men who do not wash.

RESEARCH THAT COULD HAVE LED THEM IN THE RIGHT DIRECTION WAS DONE IN THE 1930's BY DR. RICHARD SHOPE OF THE ROCKEFELLER UNIVERSITY, who on a hunting trip heard a friend describe seeing rabbits with "horns," which were actually large warts.

Dr. Shope asked his friend to send some of the horns. He then ground them up, filtered them through porcelain that let only tiny virus-size particles through, and injected the filtrate into other rabbits, which grew horns in turn."

"Dr. Shope's work showed the cause was a virus, but it was not until the 1980's that DNA amplification allowed a German researcher, Dr. Harald zur Hausen, to pin down papilloma as the cause."

https://www.nytimes.com/2006/08/29/health/29hpv.html

Below are highlights from Shope's 1933 paper along with a summary (my apologies in advance: it's a long post):

INFECTIOUS PAPILLOMATOSIS OF RABBITS

"Our attention was recently called to a disease occurring in wild cottontail rabbits in northwestern Iowa. Rabbits shot there by hunters were said to have numerous horn-like protuberances on the skin over various parts of their bodies. The animals were referred to popularly as "horned" or "warty" rabbits.

Warts from a naturally occurring case of the disease in Iowa were obtained and sent to the laboratory in sterile 50 PERCENT GLYCEROL.

These GLYCERINATED WARTS FURNISHED US OUR ORIGINAL MATERIAL FOR INVESTIGATION. A little later, in a shipment of a dozen wild cottontail rabbits from southern Kansas, three were found to be affected with the same wart-like disease. To date, out of 75 wild cottontail rabbits received from Kansas eleven have been found to carry one or more warts. These eleven animals serve as the basis for our description of the naturally occurring disease.

Description of the Naturally Occurring Disease

In wild cottontail rabbits THE PRESENCE OF WARTS HAS CAUSED NO APPARENT DISCOMFORT in our experience AND INDUCED NO DEMONSTRABLE EVIDENCE OF GENERALIZED ILLNESS. Most of the animals were sacrificed, shortly after their arrival, for pathological material, but four, kept under observation for 7 weeks or longer, AT NO TIME APPEARED ILL AND WERE IN GOOD PHYSICAL CONDITION WHEN FINALLY KILLED. The number of warts on individual animals in our series varied from one to ten in all cases except one. The exceptional animal was almost literally covered with warts and these, when removed at autopsy, were sufficient to fill a 200 cc. flask."

"Experimental Transmission

No difficulty has been encountered in transmitting the condition to either domestic or wild cottontail rabbits when materials from naturally occurring cases have been employed. The method used is, in brief, as follows:

Either freshly removed warts or those that have BEEN STORED IN 50 PERCENT GLYCEROL at refrigerator temperature ARE GROUND TO A FINE PASTE WITH STERILE SAND AND PHYSIOLOGICAL SALINE in a mortar. MORE PHYSIOLOGICAL SALINE IS ADDED TO MAKE A 3 TO 5

PERCENT FINAL SUSPENSION. Such a suspension is then centrifuged and THE SUPERNATANT FLUID, WHICH IS ONLY SLIGHTLY TURBID, IS REMOVED AND USED FOR INOCULATION. Suspensions prepared in this way remain infectious for at least a month when kept at refrigerator temperature.

INOCULATION BY SCARIFICATION was regularly performed in these experiments. Rabbits to be inoculated were shaved on the abdomen or sides and LIGHTLY SCARIFIED EITHER BY NEEDLE OR BY RUBBING THEM SHAVEN SKIN WITH A MODERATELY COARSE GRADE OF STERILIZED SANDPAPER. To obtain DISCRETE WARTS the former method was employed, while SCARIFICATION WITH SANDPAPER WAS USED WHEN A CONFLUENT AND MASSIVE GROWTH OF WARTS WAS DESIRED. The scratches were made only deep enough to cause a barely perceptible oozing of blood-tinged fluid. A small amount of the infectious suspension was immediately applied by dropping it from a syringe, and this fluid was rubbed well into the scarifications by means of a spatula or the flat handle of a scalpel. The area thus inoculated was allowed to become almost dry before the animal was released and put into its cage."

"IN SPITE of the great size of many of our experimentally produced wart masses, the ANIMALS SHOWED NO LOSS IN WEIGHT AND THE ENTIRE COURSE OF THE DISEASE WAS FREE FROM ANY GENERAL CLINICAL EVIDENCE OF ILLNESS. In their gross appearance the experimental warts in both domestic and wild rabbits have been identical with those seen in the naturally occurring disease. Photographs of experimentally produced warts are given in Figs. 2 to 4.

Experimental warts, as well as those occurring naturally, appear to remain stationary when they reach 1 to 1.5 cm. in height. One of our rabbits, however, at present, 6 months after inoculation, is carrying a large wart mass which in places is 3 cm. in height. WITH TWO EXCEPTIONS, we have seen no warts retrogress in animals infected in the usual way. In the exceptional animals, one a wild and the other a domestic rabbit, warts developed slowly after an unusually long incubation period. They reached a maximum height of only 2 to 3 ram. between 30 and 40 days after inoculation and in 50 days had COMPLETELY DISAPPEARED. BOTH OF THESE ANIMALS WERE INOCULATED WITH THE SAME INFECTIOUS SUSPENSION AND WERE THE OILY ONES SO INOCULATED. In no animal in which growth of warts took place in the usual fashion and in which the lesions reached a thickness of 1 cm. or more have we seen any evidence of retrogression. To date we have had experimentally infected animals under observation for 6 months only. While there has been no evidence of retrogression of the papillomata except in the cases mentioned, there has also, so far, been NO EVIDENCE THAT THE LESIONS OF PROLONGED STANDING ARE ACQUIRING MALIGNANT PROPERTIES. Animals are being held under observation to determine what the ultimate fate of the papillomata will be."

"Filterability of the Wart-Inducing Agent

WARTS TO BE USED AS A SOURCE OF INFECTION in the filtration experiments WERE REMOVED FROM THE 50 PERCENT GLYCEROL IN WHICH THEY HAD BEEN STORED AND WERE WASHED IN THREE CHANGES OF STERILE PHYSIOLOGICAL SALINE. They were then MINCED WITH STERILE SCISSORS, GROUND IN A MORTAR WITH STERILE SAND, and SUSPENDED IN SUFFICIENT PHYSIOLOGICAL SALINE TO MAKE AN APPROXIMATELY 5 PERCENT SUSPENSION. Suspensions thus prepared were cleared by centrifugation. The decanted supernatant fluid was usually almost water-clear with only a faint opalescence, and for this reason was rapidly filtrable. 1 cc. OF A BROTH CULTURE OF B. PRODIGIOSUS WAS ADDED TO EACH 15 to 20 cc. OF FLUID just before it was passed through Seitz or Berkefeld filters. The resulting filtrates were tested for sterility in 1.5 cc. amounts.

All filtrates recorded were bacteriologically sterile.

The results of the filtration experiments are summarized in Table I. Warts produced by filtrates, recorded in Table I as positive, were as extensive and characteristic as those in the CONTROL ANIMALS WHICH HAD BEEN INOCULATED WITH UNFILTERED SUSPENSIONS. Furthermore, WHEN DOMESTIC RABBITS WERE USED AS THE TEST ANIMALS, filtration, especially through Berkefeld V or N candles, instead of prolonging the incubation period as might be expected because of some POSSIBLE REMOVAL OF THE FILTRABLE AGENT by absorption on the filter surface, USUALLY HAD EITHER NO EFFECT or shortened the period. In wild rabbits, from the limited data at hand, it would seem that filtration resulted in a slight prolongation of the incubation period. From the data recorded in Table I it can be concluded that the etiological agent causing warts in rabbits readily passes Berkefeld filters of V, N, or W porosity but does not regularly pass a Seitz filter when two pads are employed. Filtration through a Seitz filter, using one pad, allowed not only the virus to pass but also B. prodigiosus.

NO EXTENSIVE ATTEMPTS TO CULTIVATE VISIBLE MICROBIAL FORMS FROM FILTRATES OF PROVEN INFECTIVITY WERE MADE. However, during the investigation active filtrates have been cultured repeatedly in plain and blood broth and on plain and blood agar and such cultures have remained sterile both as regards the test organism, B. prodigiosus, or any other visible bacterial form. While no special media have been employed in these attempts to demonstrate the bacteriological sterility of active filtrates, the results obtained using the media mentioned above, considered with the fact that sections of actively growing warts or films of active unfiltered infectious suspensions have FAILED TO REVEAL THE PRESENCE OF ANY CONSTANT PERCEIVABLE MICROBIAL FORM, would seem clearly to indicate that NO VISIBLE ORGANIZED

AGENT IS ECOLOGICALLY ESSENTIAL TO THE WART PRODUCTION."

"Routes of Infection

ONLY THE METHOD OF INOCULATION BY SCARIFICATION HAS YIELDED CONSTANT RESULTS IN OUR HANDS. Inoculation intravenously with infectious Berkefeld filtrates, after first abrading an area of the skin of the abdomen with a sterile needle, led to infection of the abraded areas in two out of four cases. Of the two positive animals, one, a wild rabbit, developed only a single wart; while the other, a domestic rabbit, developed four warts on the abraded area and two on the back of the neck. The incubation period in both of these cases was over three times as long as that of the control animals infected by scarification. At autopsy, ALL FOUR INTRAVENOUSLY INOCULATED ANIMALS WERE FREE FROM VISCERAL PATHOLOGY ASCRIBABLE TO THE WART-INDUCING AGENT. Inoculations of either wild or domestic rabbits intraperitoneally, subcutaneously, intratesticular, or intracerebrally, with filtrates of proven infectivity on scarification, HAVE YIELDED ENTIRELY NEGATIVE CLINICAL AND PATHOLOGICAL RESULTS. ABOUT 50 PERCENT OF THE INTRADERMAL INOCULATIONS RESULTED IN WART FORMATION although in these instances the WARTS APPEARED NOT AT THE POINT WHERE THE INOCULUM HAD BEEN DEPOSITED BUT AT THE POINT WHERE THE NEEDLE HAD PIERCED THE EPIDERMIS and where some of the inoculum had leaked from the needle tract. The incubation period of warts produced in this way was always longer than when infection had been accomplished by scarification.``

"Attempts to Transmit the Wart-Producing Agent in Series through Rabbits

In all, twenty-six domestic and wild rabbits have been inoculated in the usual way with suspensions prepared from experimentally engendered domestic rabbit warts ranging in age from 1 to 116 days. Not only did ALL SUCH INOCULATIONS YIELD NEGATIVE RESULTS but the animals, when subsequently tested, were found to be still fully susceptible to infection with the wart-producing agent from wild rabbit papillomata. On the other hand, either naturally occurring or experimentally produced warts from wild rabbits proved readily transmissible to either wild or domestic rabbits. Warts from nine naturally occurring cases of the disease in wild rabbits have been tested and all found to be infectious for both wild and domestic rabbits. In like manner, experimentally produced warts from nine wild rabbits have been tested for infectivity. EIGHT OF THESE PROVED INFECTIOUS for either domestic or wild rabbits WHILE THE WARTS FROM ONE PROVED TO BE NON-TRANSMISSIBLE.

WE HAVE NOT YET ATTEMPTED TO PASS THE WART-PRODUCING AGENT THROUGH A LONG SERIES OF WILD RABBITS but in the course of obtaining fresh infectious material it has at present

reached its third serial passage. IN SPITE OF THE FACT THAT THE AGENT CANNOT BE PROPAGATED IN SERIES THROUGH DOMESTIC RABBITS, it is PROBABLE that it can be passed indefinitely in series through wild rabbits and that any of these serial wild rabbit passages can be used in infecting domestic rabbits.

NO ATTEMPT HAS SO FAR BEEN MADE TO TRANSMIT THE DOMESTIC RABBIT WARTS BY MEANS OF TISSUE GRAFTS, although in a small number of experiments FRESHLY PREPARED CELL-CONTAINING SUSPENSIONS OF YOUNG ACTIVELY GROWING PAPILLOMATA FROM DOMESTIC RABBITS HAVE YIELDED NEGATIVE RESULTS WHEN INOCULATED INTRACUTANEOUSLY OR SUBCUTANEOUSLY INTO DOMESTIC RABBITS. Instead, it has seemed best to study the rabbit papillomata first as an infectious process caused by a filtrable agent and to determine, if possible, WHY THIS AGENT SHOULD BE READILY TRANSMISSIBLE IN SERIES WHEN INDUCING WARTS IN WILD RABBITS AND NON-TRANSMISSIBLE WHEN INDUCING SIMILAR GROWTHS IN DOMESTIC RABBITS.

That the degree of maturity of the warts in domestic rabbits at the time that attempts were made to transmit them in series was not a determining factor is indicated by the fact that WARTS TAKEN AT INTERVALS OF 6 TO 8 DAYS, FROM THEIR FIRST APPEARANCE UNTIL THEY WERE 116 DAYS OLD, YIELDED NO SUCCESSFUL INFECTIONS.

Domestic rabbit warts GLYCERINATED FOR VARYING PERIODS of time were repeatedly tested for infectivity TO DETERMINE WHETHER OR NOT GLYCEROL STORAGE HAS AN ACTIVATING EFFECT ON THE AGENT AS IT DOES ON HERPES VIRUS of low activity (3-5). The results of these experiments were all negative.

In a series of experiments conducted before the presence of neutralizing antibodies in the blood serum of wart-bearing animals had been demonstrated, it was found that when an inactive domestic rabbit wart suspension was mixed with an equal part of a suspension prepared from wild rabbit warts of known infectivity, THE RESULTING MIXTURE WAS EITHER COMPLETELY NON-INFECTIOUS OR THE INCUBATION PERIOD WAS PROLONGED AND THE RESULTING WARTS FEW IN NUMBER AS COMPARED WITH CONTROL ANIMALS. This suggested the presence in warts from domestic rabbits of an inhibitory substance similar to that found by Sittenfield, Johnson, and Jobling (6) and Murphy, Helmer, Claude, and Sturm (7) in fowl tumors. In the light of subsequent experiments in which the sera of wart-bearing rabbits were found to neutralize partially or completely the wart-producing agent, it seems possible that the inhibitory properties observed in non-infectious domestic rabbit wart suspensions might in reality have

been due to contained humoral antibodies.

A point of argument against this belief is that, while humoral antibodies were demonstrable in the sera from both infected wild and domestic rabbits, only the domestic rabbit warts possessed demonstrable inhibitory properties. WE HAVE AS YET MADE NO SYSTEMATIC ATTEMPT TO RENDER EXPERIMENTAL DOMESTIC RABBIT WARTS INFECTIOUS BY REMOVAL OF A HYPOTHETICAL INHIBITORY SUBSTANCES. We have tried, however, to infect rabbits with inactive experimental domestic rabbit wart suspensions that had been heated to 60°C. for 30 minutes in the hope that that temperature might inactivate the possible inhibitor without affecting the wart-producing agent, with suspensions prepared from domestic rabbit wart cells that had been washed repeatedly and sufficiently to remove all freely soluble humoral antibody, and with Berkefeld filtrates of inactive wart sus-pensions. ALL THREE OF THESE PROCEDURES YIELDED COMPLETELY NEGATIVE RESULTS. Both the Iowa and the Kansas strain of the disease were used in these attempts to transmit warts in series through domestic rabbits."

"The NON-TRANSMISSIBILITY of the agent in series through one of its demonstrably susceptible hosts, the domestic rabbit, IS NOT A CHARACTERISTIC OF MOST OF THE KNOWN VIRUS DISEASES."

ANOTHER PROPERTY OF THE WART-PRODUCING AGENT THAT IS UNUSUAL AMONG VIRUSES CAUSING DISEASES IN ANIMALS IS ITS RESISTANCE TO HEAT.

Suspended in 0.9 percent NaC1 solution it proved capable of withstanding a temperature of 65° C. for 30 minutes in sealed ampoules without apparent damage to its wart-producing properties. Virus heated to 67°C. for 30 minutes, while still active, produced, in our limited number of experiments, warts which either developed scantily or retrogressed after a few days' growth. WE ARE AWARE OF NO OTHER ANIMAL VIRUS WHICH WILL WITHSTAND SO HIGH A TEMPERATURE IN THE MOIST STATE; most are completely inactivated at much lower temperatures. However, among the plant viruses, which are on the whole as susceptible as animal viruses to the effects of heat, there are several which withstand heating to 65°C. or more (10). The virus of tobacco mosaic is an example of a typical plant virus that is relatively heat resistant (11). For this reason IT DOES NOT SEEM NECESSARY TO CONSIDER SERIOUSLY THE POSSIBILITY THAT THE UNUSUAL HEAT RESISTANCE OF THE WART-PRODUCING AGENT ELIMINATES IT FROM CLASSIFICATION AS A VIRUS.``

"The other extreme is exemplified by THE PAPILLOMATA INDUCED IN DOMESTIC RABBITS WHICH, WHILE INITIATED BY THE SAME VIRUS, HAVE SO FAR RESISTED TRANSMISSION EITHER TO DOMESTIC OR WILD RABBITS. These are thus analogous to many of the tumors of mammals

which cannot be transmitted in series by the usual methods of transplantation. No objection to the eligibility of the domestic rabbit warts for consideration as neoplastic processes could be raised on the grounds that a causative agent distinct from the proliferating cells can be discriminated. A study of this epithelial new growth in domestic rabbits without knowledge of its causation would probably lead an investigator to classify it as one of that large group of so called "spontaneous" mammalian tumors THAT ARE NON-TRANSMISSIBLE. IT WOULD NOT EVEN BE SUSPECTED THAT THE PAPILLOMATA HAD BEEN CAUSED BY A FILTRABLE VIRUS OF WILD RABBIT ORIGIN."

"Rabbits carrying experimentally produced papillomata are partially or completely immune to reinfection and, furthermore, their sera partially or completely neutralize the causative virus. The disease is transmissible in series through wild rabbits and virus of wild rabbit origin is readily transmissible to domestic rabbits, producing in this species papillomata identical in appearance with those found in wild rabbits. HOWEVER, THE CONDITION IS NOT TRANSMISSIBLE IN SERIES THROUGH DOMESTIC RABBITS."

doi: 10.1084/jem.58.5.607.

In Summary:

-warts from a naturally occurring case of the disease in Iowa were obtained and sent to the laboratory in sterile 50 PERCENT GLYCEROL

-these GLYCERINATED warts furnished the original material for investigation

(A little detour on glycerol toxicity:

"The concentration- and temperature-dependence of GLYCEROL TOXICITY was determined by exposing the cells to a range of glycerol concentrations at two temperatures, 21°C and 37°C. TO DECOUPLE TOXICITY AND OSMOTIC DAMAGE, THE CELLS WERE BROUGHT TO THE PEAK GLYCEROL CONCENTRATION using multiple steps as necessary (see S1 Supporting Information). Fig 2 depicts the time-dependent cell yields for glycerol exposures at 21°C and 37°C. In general, CELL YIELD DECREASED AS GLYCEROL EXPOSURE TIME INCREASED, AS GLYCEROL CONCENTRATION INCREASED AND AS TEMPERATURE INCREASED. Indeed, statistical analysis by 3-way ANOVA revealed that all of these factors (i.e., exposure time, concentration and temperature) HAD STATISTICALLY SIGNIFICANT EFFECTS ON THE CELL YIELD ($p < 0.0001$). An exponential decay model was fit to the data to determine a cytotoxicity rate constant k for each glycerol concentration and temperature. Although the data has high variability and deviates from the exponential decay model in some cases, THE RESULTS SHOW THAT THE RATE OF CELL

DEATH INCREASES WITH BOTH GLYCEROL CONCENTRATION AND TEMPERATURE."

"THE TOXICITY RATE IS HIGHER AT 37°C than 21°C, and THE TOXICITY RATE INCREASES WITH GLYCEROL CONCENTRATION."

https://journals.plos.org/plosone/article?id=10.1371%2Fjournal.pone.0142828

"The effect of glycerol on proliferation of BHK, CHO, HBL, MCF-7, and human glioma cells was studied. CELL PROLIFERATION WAS SIGNIFICANTLY DECREASED IN ALL THE CELLS LINES AT GLYCEROL CONCENTRATIONS OF 2–4% in the culture medium. The inhibition was dose-dependent, complete suppression of proliferation occurring at a glycerol concentration of 4% for the MCF-7 cell line and 6–8% for the BHK, CHO and human glioma cells. The viability of the cells was not significantly affected until higher concentrations of glycerol (12%+) were present. Recovery studies with BHK cells indicated that replacement of the glycerol medium with glycerol-free medium resulted in full recovery following exposure to 4% glycerol and only partial recovery (65%) of proliferation rate following exposure to 10–12% glycerol. It is concluded that GLYCEROL, a substance that is normally present in tissues, CAN SERVE AS A POTENT INHIBITOR OF CELL PROLIFERATION."

https://www.sciencedirect.com/.../abs/pii/002432059190275G

"Guinea pigs given more than 5 ml of a 50% glycerol solution daily by stomach tube DIED WITH ACUTELY TOXIC SYMPTOMS. Rabbits tolerate 10 ml daily."

https://doi.org/10.3181%2F00379727-111-27875

All of this is to show that the warts used to "infect" the rabbits were already doused in 50% glycerol which is toxic to cells and animals)

-the presence of warts caused NO APPARENT DISCOMFORT in and induced NO DEMONSTRABLE EVIDENCE OF GENERALIZED ILLNESS

-four rabbits, kept under observation for 7 weeks or longer, AT NO TIME APPEARED ILL and were

in good physical condition when finally killed

-the removed warts were prepared for inoculation:

1. Either freshly removed warts or those that have been stored in 50 PERCENT GLYCEROL at refrigerator temperature ARE GROUND TO A FINE PASTE WITH STERILE SAND AND PHYSIOLOGICAL SALINE in a mortar

2. MORE PHYSIOLOGICAL SALINE IS ADDED to make a 3 to 5 per cent final suspension

3. Such a suspension is then centrifuged and the supernatant fluid, WHICH IS ONLY SLIGHTLY TURBID (i.e. deficient in clarity or purity), is removed and used for inoculation

(Another quick detour on physiological saline:

"DISASTROUS PHYSIOLOGICAL EFFECTS OF SALINE ON THE CELL MEMBRANE demonstrated in cardiac cells"

"SALINE IS NOT AN IDEAL STORAGE SOLUTION. It has a low pH, no buffering capacity, and lacks other ions and nutrients."

"Storing myocytes in saline for only 2 h resulted in EXCESSIVE CELL DEATH."

"SALINE IS DISASTROUS for the function of the heart muscle and leads to depolarization, sustained contraction and unexcitable tissue. Saline should not be used as a storage medium, EVEN FOR SHORT PERIODS OF TIME."

https://pubmed.ncbi.nlm.nih.gov/15513314/

"Over the years, the name has morphed into what is more commonly called “normal saline” or “physiological saline” DESPITE NO ADDITIONAL EVIDENCE OR RATIONALE for the relabeling. THE IMPLIED NORMALCY AND PHYSIOLOGICAL PROPERTY have perpetuated indiscriminate use of saline in medical practice."

"Despite its name, SALINE IS NEITHER “NORMAL” NOR “PHYSIOLOGICAL”. Compared to human serum, saline has a nearly 10% higher Na concentration and 50% higher Cl concentration."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4794509/

These are from studies on human tissues but they make the point clear that "physiological" saline is not an inert substance that these ground up warts are being subjected to which is then put into the rabbits.)

-inoculation by SCARIFICATION was regularly performed in these experiments

-scarification was done by needle or by rubbing the shaven skin with a moderately coarse grade of sterilized SANDPAPER

-to obtain discrete warts the needle method was utilized while scarification with sandpaper was used when they wanted a confluent and massive growth of warts

-in spite of the great size of many of the EXPERIMENTALLY PRODUCED wart masses, the animals showed NO LOSS IN WEIGHT and the entire course of the disease was FREE FROM ANY GENERAL CLINICAL EVIDENCE OF ILLNESS

-they did not see any warts retrogress in animals infected in the usual way except for two cases

-both of these animals were inoculated with the SAME INFECTIOUS SUSPENSION and were the ONLY ONES SO INOCULATED (thus showing the type of experimental reaction is determined by how the material for inoculations are prepared)

-there was NO EVIDENCE that the lesions of prolonged standing acquired MALIGNANT properties

-warts to be used as a source of infection in the filtration experiments were REMOVED FROM THE 50 PERCENT GLYCEROL in which they had been stored and were WASHED IN THREE CHANGES OF STERILE PHYSIOLOGICAL SALINE

-they were then minced with sterile scissors, ground in a mortar with sterile sand, and suspended in sufficient PHYSIOLOGICAL SALINE TO MAKE AN APPROXIMATELY 5 PERCENT SUSPENSION

-1 cc. of a BROTH CULTURE OF B. PRODIGIOSUS WAS ADDED TO EACH 15 to 20 cc. OF FLUID just before it was passed through Seitz or Berkefeld filters

-no extensive attempts to cultivate visible microbial forms from filtrates of proven infectivity were made

-sections of actively growing warts or films of active unfiltered infectious suspensions FAILED TO REVEAL THE PRESENCE OF ANY CONSTANT PERCEIVABLE MICROBIAL FORM, which indicated to them that NO VISIBLE ORGANIZED AGENT IS ECOLOGICALLY ESSENTIAL TO THE WART PRODUCTION

-in other words, because they could not see another microbe, it wasn't there and had no role in wart production...unlike the "virus" which they also could not see but ASSUMED was there and responsible 🤷

-only the method of inoculation by SCARIFICATION yielded constant results

-all four INTRAVENOUSLY (in the veins) inoculated animals were FREE FROM VISCERAL PATHOLOGY ascribable to the wart-inducing agent

-inoculations of either wild or domestic rabbits intraperitoneally (in the stomach), subcutaneously (under the fat layer of skin), intratesticular (in the testicles), or intracerebrally (in the brain), with filtrates of proven infectivity on scarification, YIELDED ENTIRELY NEGATIVE CLINICAL AND PATHOLOGICAL RESULTS

-about 50 percent of the intradermal (under the thin layer of skin) inoculations resulted in wart formation although in these instances the warts appeared not at the point where the inoculum had been deposited but AT THE POINT WHERE THE NEEDLE HAD PIERCED THE EPIDERMIS and where some of the inoculum had leaked from the needle tract

-all inoculations from warts produced by domestic rabbits YIELDED NEGATIVE RESULTS in both domestic and wild rabbits

-however, the animals were found to be still fully susceptible to infection with the wart-producing agent from wild rabbit papillomata

-In other words, their experiments showed wart material said to contain the "virus" from domestic rabbits was non-infectious while wart material from wild rabbits containing the same "virus" was somehow infectious...

-experimentally produced warts from nine wild rabbits were tested for infectivity and eight of these proved to be infectious for either domestic or wild rabbits WHILE THE WARTS FROM ONE PROVED TO BE NON-TRANSMISSIBLE

-they never attempted passing the wart-producing agent through a long series of wild rabbits

-he admits that the "virus" cannot be propagated in series through domestic rabbits -however, he still assumes that it is PROBABLE that the "virus" can be passed indefinitely in series through wild rabbits and that any of these serial wild rabbit passages can be used to infect domestic rabbits

-no attempt was made to transmit the domestic rabbit warts by means of tissue grafts

-freshly prepared cell-containing suspensions of young actively growing papillomata from domestic rabbits yielded NEGATIVE RESULTS when inoculated intracutaneously or subcutaneously into domestic rabbits

-he could not determine why the "virus" was readily transmissible in series when inducing warts in wild rabbits yet non-transmissible when inducing similar growths in domestic rabbits

-warts taken at intervals of 6 to 8 days, from their first appearance until they were 116 days old, YIELDED NO SUCCESSFUL INFECTIONS

-domestic rabbit warts GLYCERINATED FOR VARYING PERIODS of time were repeatedly tested for infectivity TO DETERMINE WHETHER OR NOT GLYCEROL STORAGE HAS AN ACTIVATING EFFECT ON THE AGENT AS IT DOES ON HERPES VIRUS of low activity

-in other words, they assume that glycerol itself has no toxic effects on the animals and that it just "activates" the "virus"

-they made no systematic attempt to render experimental domestic rabbit warts infectious by removal OF A HYPOTHETICAL inhibitory substance (they just assume hypothetical substances may be present)

-they tried to infect rabbits with inactive experimental domestic rabbit wart suspensions that had been heated to 60°C. for 30 minutes yet all three of the procedures yielded COMPLETELY NEGATIVE RESULTS

-both the Iowa and the Kansas strain of the disease were used in these attempts to transmit warts in series through domestic rabbits

-they admit that the NON-TRANSMISSIBILITY of the "virus" in series through one of its demonstrably susceptible hosts, the domestic rabbit, IS NOT A CHARACTERISTIC OF MOST OF THE KNOWN "VIRUS" DISEASES

-they state that another property of the wart-producing agent that is unusual among "viruses" causing diseases in animals is its resistance to heat

-they admit they are aware of no other

animal "virus" which will withstand so high a temperature in the moist state

-they do state some plant "viruses" can withstand heat and for this reason alone, they conclude that it DID NOT SEEM NECESSARY TO CONSIDER SERIOUSLY THE POSSIBILITY that the unusual heat resistance of the wart-producing agent ELIMINATES IT FROM CLASSIFICATION AS A "VIRUS"

-they state the papillomata induced in domestic rabbits which, WHILE INITIATED BY THE SAME "VIRUS," RESISTED TRANSMISSION either to domestic or wild rabbits

-they hypothesize that a study of the epithelial new growth in domestic rabbits without knowledge of its causation would probably lead an investigator to classify it as one of that large

group of so called "spontaneous" mammalian tumors that are NON-TRANSMISSIBLE

-they believe it would NOT EVEN BE SUSPECTED that the papillomata had been CAUSED BY A FILTRABLE "VIRUS" of wild rabbit origin

There you have it. To "prove" a "virus" caused warts in rabbits, Shope and co. minced and ground up glycerinated rabbit warts with sterile sand and added heaping amounts of physiological saline solution along with culture broth of B. Prodigiosus. They then tried every which way to infect the rabbits. They injected these rabbits directly in their veins, in their stomachs, in the fat layers of their skin, in their testicles, and in their brains and all attempts ended in negative results. They could only achieve results about 50% of the time when they inoculated the rabbits directly under their skin yet reactions only occur at the site of injection. They could not transmit infection from domestic rabbits to other domestic rabbits or to wild rabbits. They only succeeded when using wart material from wild rabbits. The only way they ever had success with this material was through scarification by using needles and sandpaper directly on the shaved skin of the rabbits. If they wanted a small amount of warts, the needle was used. If they wanted many warts, sandpaper was used. Never once did they consider the method of scarification as a potential cause of the warts, even in the face of numerous negative results attempting to inject an invisible "virus."

It is truly amazing the lengths these "scientists" will go to in order to attempt to "prove" their assumptions correct. Because of Shope's work with rabbits, we got the Shope Papillomavirus. This paved the way for researchers to do the same ridiculous method of grinding up human wart tissue and comparing it under EM to plant "viruses" and rabbit warts in order to create the human papillomavirus (HPV). Shope's work led to the disastrous HPV and flu vaccines that have ruined countless lives. We have all of this suffering thanks to the shoddy pseudoscientific work of one man.

(Images below are not from the 1933 study.)

https://docs.google.com/document/d/e/2PACX-1vRkd5VH0SOhYfUtpoKsXSt_1FUyGqfHTY2yuojHByzHz5tZRrvQY_d6j0KoEu4zt4m4opGR7cCYnDMl/pub

HPV: HUMAN OR ANIMAL?

In Virology, in order to determine whether particles believed to be "viruses" are actually pathogenic, they must demonstrate pathogenicity in a suitable host. This is done through shockingly cruel animal models of experimentation due to the obvious ethical concerns regarding experimenting on human Guinea pigs (please disregard the human Guinea pig experiments currently going on with the "Covid" jabs).

Normally, they go through trial and error until they find the perfect animal host which, upon injection with unpurified toxic cell culture soup said to contain the "virus," sometimes comes down with something that kinda sorta somewhat resembles the human disease if looked at by tilting your head at the right angle. This is how they "prove" pathogenicity and then study how the "virus" works on these animal hosts.

With HPV, however, there are a few problems. For one, unlike other "viruses," they simply can't grow it in the cell cultures they normally use, thus they can not shoot up the helpless animals with cultured HPV goo to determine pathogenicity. The other problem is that even if they could grow HPV in culture, it is supposedly a "virus" that only infects humans thus they can not infect these animals with it either. They also can not find enough "virus" in humans so they have no way of testing "real HPV."

So they came up with some, shall we say, "creative" ways to get around these problems. Below are highlights from a 2009 paper that details these "solutions" but in order to fully grasp what they did, a few definitions may help:

1. "VIRUS-LIKE" PARTICLES: molecules that closely resemble "viruses," but are non-infectious because they contain no "viral" genetic material (i.e. not "real viruses")

2. PSEUDOVIRUSES: synthetic chimeras that consist of a surrogate "viral" core, derived from a parent "virus," and an envelope glycoprotein on its surface derived from a heterologous "virus"

(i.e. not "real virus")

3. TRANSGENIC MICE: a genetically modified mouse or genetically engineered mouse model; a mouse that has had its genome altered through the use of genetic engineering techniques (i.e. not a "real" mouse)

So without further ado, I present their "solutions:"

ANIMAL MODELS FOR HUMAN PAPILLOMAVIRUS-ASSOCIATED CERVICAL PATHOGENESIS

"HPV IS STRICTLY SPECIES AND TISSUE-RESTRICTED AND CANNOT BE PROPAGATED IN VITRO, which has retarded the development of in-vivo models for HPV infection. MUCH OF OUR UNDERSTANDING ON HPV, its life cycle, its oncogenicity and its synergy with cofactors WAS FIRST ESTABLISHED IN VITRO OR BY ANIMAL PAPILLOMAVIRUSES IN VIVO. However, KEY DIFFERENCES BETWEEN SUCH SYSTEMS AND NATURAL HPV INFECTION HAVE LIMITED THEIR USE IN ADDRESSING CERTAIN IMPORTANT MECHANISMS OF HPV-ASSOCIATED CARCINOGENESIS. With the development of sophisticated molecular techniques, the direct study of HPV in vivo has become less problematic. ALTHOUGH SOME UNCERTAINTY REMAINS, the animal model system for HPV-associated cervical disease has been maintained as the most scientifically and economically powerful in-vivo model.

So far, ANIMAL MODEL SYSTEMS, BASED ON HPV VIRUS-LIKE PARTICLES, PSEUDOVIRUSES, TRANSGENIC MICE, HPV-TRANSFORMED CERVICAL CELL LINES, have been invaluable in the recognition of the natural history of HPV infection, the synergy between HPV and its cofactors, as well as evaluating the efficacy and safety of new prophylactic and therapeutic modalities for HPV-associated cervical precancers and invasive cancers."

"Human papillomavirus is strictly species and tissue-restricted, and EVEN IN EXPERIMENTAL SETTINGS, IT DOES NOT INFECT ANY OTHER HOST THAN HUMANS. THE TITER OF INFECTIOUS HPV VIRIONS ISOLATED FROM NATURALLY OCCURRING HUMAN LESIONS IS EXTRAORDINARILY LOW. Meanwhile, HPV CANNOT BE PROPAGATED IN ROUTINE CELL CULTURES. These characteristics have retarded the development of in-vivo models for HPV infection. Thus, MUCH OF OUR UNDERSTANDING ON HPV, its life cycle, ITS ONCOGENICITY and its synergy with genetic and environmental co-factors WAS FIRST ESTABLISHED BY ANIMAL PAPILLOMAVIRUSES (reviewed in [4]), which possess considerable homology to HPV."

"Many pathological mechanisms of cervical carcinogenesis may be evaluated in vitro or by animal papillomaviruses in vivo. However, KEY DIFFERENCES BETWEEN SUCH SYSTEMS AND NATURAL HPV INFECTION HAVE LIMITED THEIR USE IN ADDRESSING CERTAIN IMPORTANT

EVENTS IN HPV-ASSOCIATED CARCINOGENESIS. THE BIOLOGICAL BEHAVIOR OF HPV IS ESSENTIALLY SOMEWHAT DIFFERENT FROM THAT OF ANIMAL PAPILLOMAVIRUSES; meanwhile, in-vitro systems are usually UNABLE TO CLARIFY IMPORTANT IN-VIVO PHENOMENA, such as cell cycle control, signal cascade, and immune regulation. Thus, animal models for HPV-associated cervical pathogenesis have been employed to provide unique insights into analyzing HPV transmission, persistence, vaccination, and what is more important, the investigation of cervical carcinogenesis."

"To date, there are NO STUDIES indicating that HPV VIRIONS produced in vitro CAN INFECT KERATINOCYTES and initiate a reproductive life cycle. The NON AVAILABILITY OF EFFICIENT TECHNIQUES TO PRODUCE HIGH-TITER HPV VIRIONS has hindered insight into the life cycle of HPV. However, highly efficient systems TO PRODUCE HPV VIRUS-LIKE PARTICLES (VLPs) AND PSEUDOVIRUSES HAVE BEEN EMPLOYED AS A SUBSTITUTE for the study of its life cycle [13]. Thus, the mechanisms that regulate the binding and entry of HPV into cells are beginning to be elucidated."

"Recombinant L1 or L1 and L2 can be synthesized in a variety of expression systems to produce self-assembled VLPs, which possess morphological characteristics indistinguishable from authentic HPV virions and are still able to enter target cells. VLPs REPRESENT NON-INFECTIOUS HPV CAPSIDS WITHOUT VIRAL GENOME and are capable of inducing an immune response. ON THE BASIS OF VLPs TO ELICIT PROTECTIVE IMMUNITY AGAINST INFECTIOUS VIRAL CHALLENGES IN ANIMAL MODELS [14], TWO L1 VLP-based VACCINES HAVE BEEN LICENSED FOR THE PREVENTION OF HPV INFECTION IN HUMANS SINCE 2006 (reviewed in [15])."

"In contrast to VLPs, PSEUDOVIRUS is composed of HPV structural proteins L1 and L2 and carries one or more encapsidated reporter genes (so-called PSEUDOGENOME) OTHER THAN THE HPV GENOME. The MODIFIED HUMAN EMBRYONIC KIDNEY CELL LINE, 293TTor 293FT cells, is co-transfected with plasmids expressing codon-modified L1, L2 and are porter plasmid, resulting in the self-assembly of the reporter plasmid into the L1/L2 capsids TO GENERATE INFECTIOUS PSEUDOVIRUSES."

"HUMAN PAPILLOMAVIRUS CANNOT BE PROPAGATED IN CELL CULTURE. Therefore, PSEUDOVIRUSES HAVE BEEN USED INSTEAD OF AUTHENTIC HPV VIRIONS TO STUDY THE MOLECULAR BIOLOGY OF HPV AND EVALUATE THE PROTECTIVE EFFICACY OF NEUTRALIZING ANTIBODIES DERIVED FROM VLPs [25]. It has been demonstrated in vitro that lactoferricin [26] and sulfated polysaccharides [27,28], such as heparin, cellulose sulfate, dextran sulfate and carrageenan, can block the transmission of pseudoviruses by binding the viral capsid or heparan sulfate on the cell surface. However, SUCH IN-VITRO SYSTEMS FAIL TO FULLY REPRESENT SOME ASPECTS OF HPV INFECTION OF KERATINOCYTES IN VIVO."

"ALTHOUGH SOME UNCERTAINTY PERSISTS, PSEUDOVIRUSES ARE THOUGHT TO VIRTUALLY MIMIC THE HPV GENITAL TRANSMISSION PHASE, which are not species-restricted [27,29]. Thus,

a PSEUDOVIRUS-BASED cervico-vaginal challenge mouse model WAS RECENTLY CONSTRUCTED TO IMITATE the establishment phase of HPV infection in vivo [30]."

"Since the introduction of transgenic technologies in the early 1990s, an extremely valuable ANIMAL MODEL SYSTEM FOR HPV-INDUCED CARCINOGENESIS HAS BEEN ACQUIRED THROUGH HPV TRANSGENIC MICE, to explore the contribution of oncogenes regulating cervical neoplasia."

"It has been found that CHRONIC ESTROGEN EXPOSURE specifically induced a multistage neoplasia in the cervix and vagina in all of K14-HPV mice and developed invasive cancers in 60% of the treated transgenic mice, closely mimicking cervical cancer progression in humans [37]. Using estrogen titration, K14-HPV16 mice treated with 0.05 mg/60-day 17b-estradiol developed cervical neoplasia solely at the transformation zone without any

other reproductive tract site, WHICH CLOSELY IMITATES CLINICAL CERVICAL CARCINOGENESIS IN WOMEN [38]. Thus, CHRONIC ESTROGEN EXPOSURE IS A LEADING CO-FACTOR OF HPV ONCOGENES FOR CERVICAL NEOPLASIA IN TRANSGENIC MICE. In addition, K14-HPV16 mice developed tumors with increased efficiency when induced with chemical carcinogens, such as 7,12-dimethylbenz(a) anthracene and 12-O-tetradecanoylphorbol-13-acetate [33,39]. GENETIC BACKGROUND IS ALSO SIGNIFICANT IN THE DETERMINATION OF SUSCEPTIBILITY TO INVASIVE CANCERS, which is in keeping with the effect of HLA genotype in the human disease."

"Although K14E6 and K14E7 mice developed tumors in the skin, NO SPONTANEOUS REPRODUCTIVE MALIGNANCIES AROSE WITHOUT EXOGENOUS ESTROGEN TREATMENT [33,40]."

"These results indicated the NECESSITY OF

ESTROGEN in the initiation, maintenance and progression of cervical cancer in combination with HPV oncogenes."

"Furthermore, HUMAN CERVICAL CANCER CELL LINES, such as HPV 16 (CaSki and SiHa), HPV 18 (HeLa) and HPV 68 (ME-180)-transformed cell lines, CAN ALSO SUBCUTANEOUSLY BE TRANSPLANTED IN THE IMMUNODEFICIENT NUDE MICE, to investigate the biological behavior of cervical cancer and anticancer treatments [60–62]. HOWEVER, THE CONCLUSIONS DRAWN FROM THESE ANIMAL MODELS ARE UNABLE TO REFLECT THE INTERACTIONS BETWEEN TUMOR

CELLS AND THE HUMAN IMMUNE SYSTEM. Thus, the humanized-severe combined immunodeficient (hu-SCID) mouse model, produced by engrafting human peripheral blood lymphocytes, lymphoid tissues or bone marrow cells from healthy adult female volunteers into SCID mice, has been DESIGNED TO MIMIC THE HUMAN IMMUNE SYSTEM and induce specific antitumor immunity [63]."

"CONCLUSION

Human papillomavirus is strictly species and tissue-restricted and cannot be propagated in vitro, WHICH HAS HAMPERED OUR RECOGNITION OF HPV AS A PATHOGEN. Nevertheless, the animal model system for HPV associated pathogenesis in the cervix has been important in the recognition of the natural history of HPV infection, the synergy between HPV and its cofactors (genetic and environmental), as well as evaluating the efficacy and safety of new prophylactic and therapeutic modalities for cervical cancers. ALTHOUGH SOME UNCERTAINTY REMAINS, ANIMAL MODEL SYSTEMS WILL STILL LEAD THE WAY AND CONTRIBUTE SIGNIFICANTLY TO UNSOLVED ISSUES ON HPV-ASSOCIATED CERVICAL PATHOGENESIS in the future.``

http://dx.doi.org/10.1097/MRM.0b013e328331ad65

In Summary:

-HPV is strictly species and tissue-restricted and CANNOT BE PROPAGATED IN VITRO

-they state that much of our understanding on HPV, its life cycle, its ONCOGENICITY (ability to form tumors) and its synergy with co-factors was first established in vitro or BY ANIMAL PAPILLOMAVIRUSES in vivo

-key DIFFERENCES between such systems and natural HPV infection have limited their use in addressing certain important mechanisms of HPV-associated carcinogenesis

-animal model systems are based on HPV virus-like particles, pseudoviruses, transgenic mice, and HPV-transformed cervical cell lines

-even in experimental settings, HPV does not infect any other host than humans

-the titer (or amount) of infectious HPV virions isolated from naturally occurring human lesions is EXTRAORDINARILY LOW

-they admit that the biological behavior of HPV is ESSENTIALLY SOMEWHAT DIFFERENT FROM THAT OF ANIMAL PAPILLOMAVIRUSES (and we use them to make conclusions about humans

why again?)

-in-vitro systems are usually UNABLE TO CLARIFY important in-vivo (within a living organism) phenomena, such as cell cycle control, signal cascade, and immune regulation

-there are NO STUDIES indicating that HPV virions produced in vitro (in the lab) can infect keratinocytes and initiate a reproductive life cycle

-the nonavailability of efficient techniques to produce high-titer (amounts) of HPV virions HAS HINDERED INSIGHT into the life cycle of HPV

-thus they turned to producing HPV "VIRUS-LIKE" particles (VLPs) and "PSEUDOVIRUSES" to substitute for the study of its life cycle

-VLPs represent non-infectious HPV capsids without viral genome - in other words, they are imitations of the already fake "virus"

-ON THE BASIS OF VLPs to elicit protective immunity against infectious viral challenge in animal models, two L1 VLP-BASED VACCINES have been licensed for the prevention of HPV infection in humans since 2006

-"pseudoviruses" are full of "PSEUODGENOMES" which means the more fake "virus" is full of an even more fake genome

-the MODIFIED HUMAN EMBRYONIC KIDNEY CELL LINE, 293TTor 293FT cells, is co-transfected with plasmids expressing codon-modified L1, L2 and are porter plasmid, resulting in the self-assembly of the reporter plasmid into the L1/L2 capsids TO GENERATE INFECTIOUS PSEUDOVIRUSES

-Human papillomavirus cannot be propagated in cell culture

-therefore, pseudoviruses have been used INSTEAD OF AUTHENTIC HPV VIRIONS to study the molecular biology of HPV and EVALUATE the protective efficacy of neutralizing antibodies derived from VLPs

-in other words, they use the fake "infectious virus" to test the fake antibodies derived from the fake noninfectious "virus-like" particles

-they admit such in-vitro (in the lab) systems fail to fully represent some aspects of HPV infection of keratinocytes in vivo (within a living organism)

-they also admit that even though some uncertainty persists, pseudoviruses are THOUGHT TO VIRTUALLY MIMIC the HPV genital transmission phase

-a pseudovirus-based cervico-vaginal challenge mouse model was recently CONSTRUCTED

TO IMITATE the establishment phase of HPV infection in vivo

-they have also turned to transgenic (genetically altered) mice as an animal model system for HPV-induced carcinogenesis

-It was found that CHRONIC ESTROGEN EXPOSURE specifically induced a multistage neoplasia in the cervix and vagina in all of K14-HPV mice and DEVELOPED INVASIVE CANCERS IN 60% OF THE TREATED TRANSGENIC MICE, closely MIMICKING cervical cancer progression in humans

-thus, they concluded exposing genetically altered mice to CHRONIC ESTROGEN EXPOSURE is a LEADING CO-FACTOR of HPV oncogenes for cervical neoplasia rather than THE factor

-they concluded this even knowing NO spontaneous reproductive malignancies arose WITHOUT EXOGENOUS ESTROGEN TREATMENT

-they also state the NECESSITY OF ESTROGEN in the initiation, maintenance and progression of cervical cancer but add that it is in combination with HPV oncogenes

-they also take HUMAN CERVICAL CANCER CELL LINES, such as HPV 16 (CaSki and SiHa), HPV 18 (HeLa) and HPV 68 (ME-180)-transformed cell lines, and then subcutaneously TRANSPLANT INTO THE IMMUNODEFICIENT NUDE MICE to investigate the biological behavior of cervical cancer and anticancer treatments

-in other words, they use human cervical cancer cells (not HPV) to create cervical cancer in IMMUNODEFICIENT mice to study it

-they admit that the conclusions drawn from these animal models are UNABLE TO REFLECT the interactions between tumor cells and the human immune system

-the humanized-severe combined immunodeficient (hu-SCID) mouse model is produced by engrafting human peripheral blood lymphocytes, lymphoid tissues or bone marrow cells from healthy adult female volunteers into SCID mice, and this has been DESIGNED TO MIMIC THE HUMAN IMMUNE SYSTEM and induce specific antitumor immunity

-they state that human papillomavirus is strictly species and tissue-restricted and cannot be propagated in vitro, WHICH HAS HAMPERED THE RECOGNITION OF HPV AS A PATHOGEN

-they conclude by stating that although some UNCERTAINTY REMAINS, animal model systems will still lead the way and contribute significantly to unsolved issues on HPV-associated cervical pathogenesis

As can be seen from this paper, everything we know about HUMAN papillomaviruses actually comes from ANIMAL papillomaviruses. They have just equated what they "know" about the animal version to humans. However, knowing that this was not enough due to key differences between animal and human systems, the biological behavior of the "virus," and the modes of HPV infection, they decided to synthetically create "viruses" to inject into genetically modified mice to mimic HPV infection. They also utilized "pseudoviruses" for their mimicry purposes. Then they cranked up the estrogen levels into these mice and upon producing tumors, state that they were successful with their synthetic "virus" while relegating the unnaturally high estrogen levels to a "cofactor." Keep in mind that these synthetic VLP's are used in the vaccines as well as to judge their efficacy based on challenge trials using "pseudoviruses" and not HPV.

Creating synthetic "virus-like" particles to inject into genetically engineered mice in order to "prove" the existence/pathogenicity/mechanisms of hybridized cloned DNA fragments based off of grounded up "virus-like" particles taken from warts.

This is Virology.

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FOR MORE INFO ON HPV VAX www.whale.to/vaccines/gardasil_h.html

PAPERS ON CORONAVIRUS'

Breakdown of Coronavirus Papers:

D.A TYRRELL 1965 "CORONAVIRUS" DISCOVERY:

The highlights below are from a 1965 paper which is considered the first evidence of human "coronaviruses." Notice the various assumptions that are made as well as the extent to which they go to create the effect that they are looking for.

CULTIVATION OF A NOVEL TYPE OF COMMON-COLD VIRUS IN ORGAN CULTURES

"In recent years it has become evident that the common cold and similar minor upper respiratory diseases are due to infection with viruses belonging to a number of different groups, including adenoviruses, myxoviruses-such as the influenza, para-influenza, and respiratory syncytial viruses-enteroviruses, and rhinoviruses. When tests adequate to detect all these are used a virus or a 83-haemolytic streptococcus can be isolated from about one-third of patients suffering from colds and related diseases (Working Party, 1965). The failures might occur because no virus or bacteria were present in the respiratory secretions tested, but in one study (Kendall et al., 1962)

TWO OUT OF FOUR SUCH SPECIMENS WHICH APPARENTLY CONTAINED NO VIRUS WERE ADMINISTERED TO VOLUNTEERS AND PRODUCED COLDS ; IT IS THEREFORE LIKELY THAT SOME FAILURES ARE DUE TO THE PRESENCE OF VIRUSES WHICH CANNOT BE CULTIVATED BY PRESENT METHODS.

In the past four years efforts have therefore been made to discover something of the nature of such viruses AND TO DEVISE METHODS OF CULTIVATING THEM IN THE LABORATORY. Some success has been achieved and is reported in this paper."

"Materials and Methods

New Viruses.-The primary sources of these were NASAL WASHINGS IN PHOSPHATE-BUFFERED

SALINE which were collected at the height of a cold, MIXED WITH AN EQUAL VOLUME OF BACTERIOOGICAL NUTRIENT BROTH, and stored at -70' C.

OTHER VIRUSES WERE PROPAGATED IN CHICK EMBRYOS OR IN TISSUE CULTURES as appropriate, and were handled by standard methods.

ORGAN CULTURES WERE PREPARED MAINLY FROM THE TRACHEA OF 14- TO 22-WEEK-OLD HUMAN EMBRYOS obtained at hysterotomy from cases in which there was no clinical suspicion of an infection in the mother or foetus. Four to six tissue fragments were planted with the ciliated surface uppermost on the scratched surface of a 6-cm. plastic Petri dish. (Falcon), AND 1.25 ml. OF MEDIUM 199 (Glaxo) CONTAINING 0.035 g. of SODIUM BICARBONATE PER LITRE WAS ADDED. The dish was incubated at 33' C. in a humidified box, and the medium was changed daily for two days. CULTURES WERE THEN INOCULATED BY DRIPPING 0.3 ml. OF INOCULUM ON TO THE FRAGMENTS ; THERE AFTER THE MEDIUM REMOVED EACH DAY WAS MIXED WITH BROTH and stored at - 700 C. After about 10 days some cultures were fixed in Bouin's solution, embedded, sectioned, and stained with haematoxylin and eosin."

"Results

Most work has been done with a nasal swab and washing number B814, OBTAINED 'FROM A BOY WITH A TYPICAL COMMON COLD IN 1960 (Kendall et al., 1962). FURTHER INFECTIOUS SECRETIONS WERE OBTAINED FROM VOLUNTEERS WHO DEVELOPED COLDS AFTER INTRANASAL INOCULATION OF THE ORIGINAL SPECIMEN. In this way THREE SERIAL PASSAGES OF THE COLD-PRODUCING AGENT WERE MADE IN MAN, AND IT WAS CONCLUDED THAT IT MUST BE SELF-PROPAGATING. In over 20 experiments washings were tested by inoculation into a variety of test systems for known viruses."

"Further tests with a limited number of techniques showed that there was NO EVIDENCE THAT THE COLD-PRODUCING AGENT WAS PROPAGATED EVEN FOR A FEW DAYS TO A SUFFICIENT EXTENT TO PRODUCE THE SMALL AMOUNT OF VIRUS USUALLY NEEDED TO CAUSE A COLD IN A VOLUNTEER (Table II)."

"We therefore attempted to determine by experiments in volunteers a few basic properties which would confirm that we were indeed dealing with a virus. These experiments, also shown in Table II, indicate that the infectivity of B814 can pass a bacteria-tight filter, is inactivated by ether, and CAN INDUCE COLDS IN VOLUNTEERS GIVEN SUFFICIENT ANTIBIOTICS TO CURE A FULLY DEVELOPED INFECTION with the Eaton agent (M. pneumoniae). These results showed

that B814 is a virus, not a mycoplasma, and that it is not an adenovirus, enterovirus, or rhinovirus because it is ether-labile. ANOTHER UNCULTIVABLE AGENT PRODUCED COLDS IN TWO OUT OF SIX VOLUNTEERS AFTER ETHER TREATMENT. This was the agent recovered from the subject H. G. P. on 26 July 1957 (Tyrrell and Bynoe, 1961). IT WAS CONCLUDED THAT THERE MUST BE AT LEAST TWO BIOLOGICALLY DIFFERENT VIRUSES AMONG THESE "UNCULTIVABLE" VIRUSES."

"The experiments done so far are summarized in Table III. THERE WAS SOME TROUBLE WITH BACTERIAL AND FUNGAL CONTAMINATION AT FIRST, but later the technique outlined under " Methods " was found to be trouble-free. It was regularly possible to produce colds in volunteers WHO WERE GIVEN CULTURE FLUIDS from the first or later passages. FOR SEVERAL REASONS IT IS BELIEVED THAT THE B814 VIRUS WAS MULTIPLYING AND CAUSING THESE COLDS. Firstly, no colds were produced by fluids from " dummy " cultures containing no tissue and inoculated one or two days before with nasal washings. Similarly, no colds were produced by fluids from numerous uninoculated parallel cultures set up from the same embryos, changes at the same time in the same cabinet, and using the same medium as those used for the virus-infected cultures; no colds were produced by medium from inoculated cultures in which ferret trachea was used instead of human tissue. On the other hand, COLDS WERE PRODUCED WITH FLUIDS COMING FROM CULTURES WHICH HAD BEEN CHANGED UP TO EIGHT TIMES AFTER INOCULATION, INVOLVING A LAPSE OF AT LEAST A WEEK IN CULTURE and a probable dilution of the order of 108 of the original inoculum.

COLDS WERE ALSO PRODUCED AFTER FOUR SERIAL PASSAGES IN WHICH THE FLUIDS COLLECTED DAILY BETWEEN ONE (SOMETIMES THREE) AND 10 DAYS AFTER INOCULATION WERE POOLED AND USED TO INOCULATE FURTHER BATCHES OF CULTURES. This method of serial passage was adopted in order to ensure that some infectious virus was passed, because in some experiments with rhinoviruses and respiratory syncytial virus the viruses had been shed into the medium for only a few days and at rather unpredictable intervals after the inoculation of the cultures. FLUID FOR THIS FOURTH SERIAL PASSAGE WAS ALSO INOCULATED AFTER OVERNIGHT TREATMENT WITH ETHER; IT CAUSED COLDS IN NONE OF SIX VOLUNTEERS. Another aliquot was filtered through a membrane of A.P.D. 0.59 4u and PRODUCED COLDS IN THREE OUT OF SIX VOLUNTEERS WHO WERE TREATED WITH DEMETHYLCHLORTETRACYCLINE. It was concluded that these colds WERE DUE TO THE PRESENCE OF AN ETHER-LABILE VIRUS, as were those produced by the washings used to initiate the serial passage. SIMILAR CULTURE FLUIDS FAILED TO CAUSE DISEASE when inoculated intramuscularly and intranasally into adult white mice, intracerebrally and intraperitoneally into suckling white mice, and intracerebrally and intranasally into guinea-pigs; AND NO VIRUS WAS ISOLATED BY AMNIOTIC INOCULATION OF 10-

DAY-OLD CHICK EMBRYOS."

"In both groups the illness was a typical common cold. FEVER WAS RARE, but there was often considerable malaise, AND THE NOSE OFTEN STREAMED WITH WATERY SECRETION-ONE VOLUNTEER USED 120 PAPER HANDKERCHIEFS IN ONE DAY-BUT THERE WAS LITTLE COUGH AND NO SPUTUM, and on the average the disease cleared up in less than a week.

"Preliminary Attempts to Propagate More Otherwise Uncultivable Viruses in Organ Cultures

A number of other nasal washings were collected from patients with colds and tested for respiratory viruses. FROM CERTAIN OF THESE NO VIRUS WAS RECOVERED, AND SO THEY WERE INOCULATED INTO ORGAN CULTURES. Some results obtained are outlined in Table VI. THIS SHOWS THAT THE VIRUSES CONTAINED IN THESE SPECIMENS WERE APPARENTLY PROPAGATED IN ORGAN CULTURES, BECAUSE CULTURE FLUIDS CAUSED COLDS. The organ- culture fluids were tested further by inoculating them into a range of tissue cultures. In the case of the M.T. strain the virus apparently grew, as judged by inoculation of volunteers, but was obviously different from B814, because tests in volunteers showed that it was ether-stable. When organ-culture fluids were inoculated into other cultures they produced a CYTOPATHIC EFFECT WHICH RESEMBLED THAT DUE TO RHINOVIRUSES.

The specimens from another organ-culture experiment with strain G.T. were titrated in human diploid cells and the data are plotted on Fig. 1, which shows that a rhinovirus had grown freely and could be readily detected after three passages in organ culture. THE ORIGINAL NASAL WASHING HAD PRODUCED SOME DOUBTFUL CYTOPATHIC EFFECT WHEN TESTED IN FOUR BATCHES OF HUMAN-EMBRYO-KIDNEY CELLS, AND A MORE DEFINITE EFFECT IN ONE STRAIN OF HUMAN-EMBRYO FIBROBLASTS, ALTHOUGH THE LATTER WAS NOT SUCCESSFULLY PASSAGED. Finally, the virus F.T. was apparently a rhinovirus that FAILED TO PRODUCE ANY CYTOPATHIC EFFECT IN THE FORM IN WHICH IT WAS PRESENT IN THE NASAL SECRETIONS AND IN THE FIRST PASSAGE IN ORGAN CULTURES, ALTHOUGH IT COULD PRODUCE COLDS. It nevertheless multiplied in organ culture, and became adapted so that it rapidly damaged the cilia (see below), and also was able to produce a cytopathic effect in cells of a human fibroblast strain.

We report these experiments in order to illustrate that organ cultures may be of value in cultivating and recognizing other "difficult" respiratory viruses, and also to emphasize that BECAUSE A VIRUS IS GROWN IN ORGAN CULTURES IT IS NOT SAFE TO CONCLUDE THAT A NEW

TYPE OF VIRUS HAS BEEN CULTIVATED."

"Discussion

THESE EXPERIMENTS SEEM TO SHOW THAT ORGAN CULTURES OF HUMAN TRACHEAL EPITHELIUM CAN SUPPORT THE GROWTH OF AT LEAST ONE RESPIRATORY VIRUS WHICH WE HAVE BEEN UNABLE TO GROW BY ANY OTHER LABORATORY TECHNIQUE. AFTER CONSIDERABLE INITIAL DOUBTS we now believe that the B814 strain is a virus virtually unrelated to any other known virus of the human respiratory tract, although, since it is ether-labile, it may be a myxovirus. It is disappointing that so far no satisfactory serological test is available, but there are many possibilities still to be explored for instance, we have not yet tried to use the fluorescent antibody technique with sections of organ cultures, because model experiments with cultures infected with influenza virus were not encouraging. Further, WE ARE TESTING IN ORGAN CULTURE SPECIMENS FROM OTHER PATIENTS WITH RESPIRATORY DISEASES IN ORDER TO FIND OUT HOW FREQUENTLY HITHERTO UNRECOGNIZED VIRUSES CAN BE ISOLATED. These results will be reported later, but it seems likely that some of these may be fastidious rhinoviruses. However, it also seems possible that organ cultures of other tissues might be useful in propagating other viruses which have not so far been grown in dedifferentiated cells-for example, the viruses of molluscum contagiosum, and gastro-enteritis. IN ORDER TO INCREASE THE CHANCES OF SUCCESS IN SUCH EXPERIMENTS IT IS ALSO NECESSARY TO LOOK FOR FURTHER IMPROVEMENTS IN THE TECHNIQUE OF ORGAN CULTURE, IN PARTICULAR A SUBSTITUTE FOR HUMAN FOETAL CELLS AND IMPROVEMENTS IN THE MEDIUM AND OTHER CONDITIONS USED."

"Summary

Volunteers developed colds after the intranasal inoculation of secretions derived from a boy with a common cold. Colds developed, although the secretions were passed through a filter of A.P.D. 0.59 ju AND THE VOLUNTEERS WERE TREATED WITH DEMETHYLCHLORTETRACYCLINE. No colds developed if the washings were treated with ether.

THE VIRUS THUS DEMONSTRATED WOULD NOT GROW IN TISSUE CULTURES AND EGGS WHICH WOULD SUPPORT THE MULTIPLICATION OF KNOWN VIRUSES OF THE UPPER RESPIRATORY TRACT. It multiplied and was serially propagated in organ cultures of human foetal tracheal epithelium. The colds produced by washings and tissue cultures WERE CLINICALLY SIMILAR, and in the aggregate distinct, from those produced by M rhinoviruses.

Sera of infected volunteers were tested by haemagglutination-inhibition and complement-fixation tests; a small proportion showed slight rises against influenza C and Sendai viruses.

Infection of organ cultures with B814 WAS DETECTED WITH DIFFICULTY by a decline in ciliary activity and by degenerative changes in sections of the tissue, but there was a tenfold to a hundredfold reduction in titre on challenge of the cultures with other viruses-virus interference.

Other viruses distinct from B814 have been recognized and similarly cultivated, INCLUDING UNCHARACTERIZED ETHER-STABLE VIRUSES WHICH MAY BE RHINOVIRUSES."

doi: 10.1136/bmj.1.5448.1467.

In summary:

-it is assumed from the start that even if specimens contain no known "virus" and still produce colds (2 out of 4), there must be an unknown "virus" yet to be discovered

-the last 4 years were spent looking to come up with lab techniques in order to create these "viruses"

-nasal washings were mixed with phosphate-buffered saline soulution and a bacterological nutrient broth

-organ cultures were derived from 14-22 week-old fetus trachea

-MEDIUM 199 with sodium bicarbonate was added to the tissue

(Quick side note on what is in Medium 199: "The liquid growth medium on top is known as Medium 199, a salt solution that includes vitamins, amino acids, and FETAL BOVINE SERUM, plus sucrose, phosphate, glutamate, NEOMYCIN, and recombinant human albumin")

-medium was removed daily and mixed with broth

-most of the material used came from a 9 year old boy with a common cold in 1960

-additional secretions were collected from volunteers who were intranasally inoculated with the original mixture and developed colds -3 serial passages were done this way which led them to

conclude it was self-propagating

-techniques used to cultivate the "virus" showed no evidence enough was created in order to produce colds

-they determined it was not bacterial as it passed through bacteria filters and produced colds in those given antibiotics but not in those given ether

-another "uncultivable virus" produced colds in 2 out of 6 volunteers so they claimed there were at least two new biologically different "viruses" present

-bacterial and fungal contamination was a problem

-colds were produced when cultures were changed up to 8 times for at least a week

-colds were produced after cultures were changed and pooled together and cultured

-volunteers given fluids containing ether produced no colds while 3 out of 6 given fluids containing antibiotics produced colds which led them to believe it is an ether-labile "virus"

-similar culture fluids failed to produce disease in animals nor culture "virus" in chick embryos

-colds produced rarely had a fever nor any cough/sputum just malaise and lots of handkerchiefs

-no "viruses" were collected from certain nasal washings but since the culture fluids caused colds, "viruses" were assumed to be present

-original sample failed to produce cytopathic effect as did a presumed rhinovirus

-they admit just because something is grown in culture does not mean it is a new "virus"

-they conclude that after CONSIDERABLE INITIAL DOUBTS, their results SEEM TO SHOW a new "virus" was cultured in fetal organs

D.A. TYRRELL'S 1966 CORONAVIRUS PAPER:

This is the second paper considered as preliminary evidence for the existence of human "coronaviruses." Notice that once again, there are numerous assumptions being made such as: if culture fluid makes one sick through intranasal inoculation, then there must be an unknown "virus" present in the mixture. There are also no accompanying EM images showing any "crown-like" particles nor any "viruses" whatsoever.

CULTIVATION OF VIRUSES FROM A HIGH PROPORTION OF PATIENTS WITH COLDS

"With present methods of tissue culture and testing, it is USUALLY POSSIBLE TO CULTIVATE A VIRUS FROM ABOUT A QUARTER TO A THIRD OF ADULT PATIENTS WITH COMMON COLDS. Organ cultures of human foetal tracheal or nasal epithelium have been shown to support the growth of representative strains of all known respiratory viruses.l 2 These cultures have also been used to cultivate some APPARENTLY " NEW " VIRUSES-namely (a) 2 rhinoviruses which will produce a cytopathic effect in human diploid-cell strains only after passage in organ culture 3 (b) a rhinovirus which will not grow at all in such strains 4; and (c) AN ETHER-LABILE VIRUS APPARENTLY UNRELATED TO ANY OF THE KNOWN ETHER-LABILE VIRUSES OF THE HUMAN RESPIRATORY TRACT.3 Using organ cultures we have recently attempted to cultivate viruses from a series of patients with colds and similar acute respiratory illnesses.

METHOD

The specimens consisted of nasal washings collected at irregular intervals between 1961 and 1964, and more regularly since then from 21 adults and 2 children shortly after the onset of illness. The patients were laboratory staff and their families and members of the general public in whom spontaneous colds developed while they were staying at the Unit. Specimens were not collected when it seemed likely on epidemiological grounds that the infection had been caused by a virus that had already been collected. We also tested four pools of washings taken from

volunteers INFECTED WITH STRAINS OF VIRUS WHICH WE HAD REPEATEDLY FAILED TO CULTIVATE. THE WASHINGS WERE MADE WITH ISOTONIC PHOSPHATE-BUFFERED SALINE, pH 7-1, AND WERE MIXED WITH AN EQUAL VOLUME OF NUTRIENT BROTH and stored at - 70 °C until tested.

All specimens were tested in " standard " tissue cultures of monkey kidneys, HeLa cells, human diploid cell strains, and human foetal kidney.5 The specimens were also inoculated into organ cultures 1; medium was collected daily from these between the second and tenth day and stored with broth as for nasal washings. THE FLUIDS WERE THEN COMBINED, AND THE POOL WAS RETESTED IN STANDARD CULTURES; if negative, it was inoculated intranasally into 6 or more volunteers. IF 1 OR MORE VOLUNTEERS GOT COLDS THE SPECIMEN WAS TAKEN TO CONTAIN A VIRUS. In most cases two passages were made in organ cultures.

RESULTS

The results obtained so far are outlined in the accompanying table. The rate of virus isolation in standard cultures was about that expected, and the viruses were of various types with rhinoviruses predominating. The rhinoviruses were identified by their biological properties, but were not serotyped. All the viruses recognised by direct inoculation of standard cultures were also recognised by testing the media of organ cultures inoculated with the same washing. However, additional rhinoviruses were cultivated in organ cultures and were then readily recognised by their effect on diploid cells, although the original specimens were negative when tested in these cells. SOME OF THESE VIRUSES WERE DIFFICULT TO PROPAGATE AND IDENTIFY, because they grew to low titres in human diploid fibroblast cells.

In addition, SIX FURTHER COLD-PRODUCING AGENTS WERE DETECTED BECAUSE ORGAN CULTURE FLUIDS CAUSED COLDS IN VOLUNTEERS. The tests are incomplete, but no reduction in ciliary activity was noted in cultures in which these agents were growing. Altogether, therefore, twenty-five viruses or other agents were recovered from 33 specimens (75%) and nineteen of these (57%) were recognised in the laboratory. OF THE 8 SPECIMENS FROM WHICH NO VIRUS WAS ISOLATED, 6 were still available and were inoculated into volunteers. 3 OF THESE PRODUCED COLDS; HENCE IT IS CLEAR THAT THERE ARE A FEW COLD-PRODUCING AGENTS WHICH CANNOT AT PRESENT BE PROPAGATED IN ORGAN CULTURES. We conclude, nevertheless, that these organ cultures were an efficient method of propagating viruses from patients' colds. The combination of organ culture with diploid fibroblast cell cultures revealed the presence of nine more rhinoviruses than could be found by standard techniques. BUT WE OBVIOUSLY NEED TO DEVISE SIMPLE METHODS OF DETECTING SOME OF THE OTHER NEW AGENTS IN ORGAN CULTURE MEDIUM AND THEN TO CHARACTERISE THEM. SOME AT LEAST MAY RESEMBLE THE

B814 VIRUS DESCRIBED EARLIER.3

DISCUSSION

AGAINST THE POSSIBILITY THAT THE VIRUSES MIGHT ORIGINATE FROM THE ORGAN CULTURES is the finding that a cold developed in only 1 out of 113 volunteers who were given fluids from uninoculated cultures made from embryos used in these experiments and that no viruses were isolated by inoculating the same fluids into " standard " tissue cultures. Washings collected from 11 persons who had recovered from their colds were also tested; a rhinovirus was isolated in organ culture from 1 woman who had carried a similar virus two weeks before, when she had a cold; and one culture fluid caused a cold in a volunteer. THUS THERE WERE TWO APPARENT ISOLATIONS FROM 11 SPECIMENS; THIS IS A SIGNIFICANTLY LOWER RATE THAN THE RATE OBTAINED BY ALL METHODS FROM ALL PATIENTS WITH COLDS ($p < 0'01$) and the rate from the 23 patients from whom viruses were not isolated by standard methods ($p < 0-05$). These figures and the typical clinical picture produced in volunteers show that the agents isolated are the genuine cause of the colds.

SUMMARY

A VIRUS OR UNCHARACTERISED COLD-PRODUCING AGENT WAS CULTIVATED FROM 25 OF 33 NASAL WASHINGS by inoculating them into organ cultures of human-embryo nasal or tracheal epithelium."

doi: 10.1016/s0140-6736(66)92364-6.

In summary:

-they are only able to cultivate "viruses" from 1/4 to 1/3 of patients with colds

-cultures were used to cultivate "apparently new viruses"

-nasal washings were collected from patients assumed to have a "virus" which they had repeatedly been unable to successfully cultivate

-nasal washings were combined with isotonic phosphate buffered saline and an undefined nutrient broth

-specimens were tested in monkey kidney cells, HeLa cells, human diploid cells, and human fetal kidney cells as well as organ tissues

-fluids from these were checked regularly and then POOLED TOGETHER

-if tested negative, these fluids were intranasally inoculated into volunteers and IF JUST 1 PERSON produced cold-like symptoms, a "virus" was assumed to be in the fluids

-they admit to difficulty propagating and identifying "viruses"

-6 "viruses" were "identified" in organ culture fluid as they determined the fluid caused some volunteers to become sick

-they admit they were unable to "isolate viruses" from 8 specimens, but 3 of them caused symptoms when inoculated into volunteers so they concluded there were "viruses" present that could not be cultivated in organ tissues

-they admit that they need to devise ways to detect these "new agents" in organ tissue culture so they can characterize them as they assume some may resemble the B814 "virus" from the 1965 paper

-they assume that the "virus" did not originate from the organ tissue culture as only 1 of 113

volunteers produced symptoms from uninoculated culture fluid in embryos

-however, there were 2 out of 11 apparent "virus" isolations from volunteers who had colds (1 was a "rhinovirus" and another unidentified)

-they conclude that a "virus" or an "uncharacterized cold-producing agent" was cultivated from 25 of 33 nasal washing cultures

Keep in mind, all of these results pertain to cell and tissue cultures. These are the exact opposite of purification/isolation of any "virus." They are starving cells and mixing together nasal washings with various chemicals/nutrients along with foreign animal/human DNA and looking for a specific outcome called the cytopathogenic effect. However, this effect can be created from the culturing conditions as well as numerous contaminants. No "virus" is necessary and any "virus" found is based on nothing but assumptions as none were ever EM imaged, characterized, nor proven pathogenic.

CORONAVIRUS 229E:

In 1966, Dorothy Hamre "isolated" what she termed specimen 229E from tissue cultures using specimens from college students. She noticed what she said was distinct cytopathic changes from known "viruses" which led them to conclude it was a new respiratory "virus." It is considered one of the main causes of the common cold.

One of the issues with this study is the use of complement fixation as proof of a novel "virus." What is the Complement Fixation test?

"Complement fixation test is one of the serological tests used in virology to identify the presence of SPECIFIC ANTIBODIES or ANTIGENS. In this context, complements are proteins in series form within the patient's serum which act in response to an antibody antigen complex."

Article Source: http://EzineArticles.com/6693407

The problem with this method is that it is not sensitive nor is it specific. A purified/isolated "virus" must be discovered first so that the specific antibodies/antigens are known before they can be used to find it. This is just one of many issues with this paper. Highlights below:

A NEW VIRUS ISOLATED FROM THE HUMAN RESPIRATORY TRACT.

"In the winter of 1962, five agents were

ISOLATED IN SECONDARY HUMAN KIDNEY TISSUE CULTURES WHICH PRESENTED A CYTOPATHIC EFFECT (CPE) QUITE DISTINCT FROM THAT CUSTOMARILY PRODUCED BY KNOWN VIRUSES ASSOCIATED WITH RESPIRATORY ILLNESSES. This report presents the evidence for CONSIDERING THESE AGENTS as strains of a new respiratory virus, POSSIBLY ASSOCIATED WITH MILD UPPER RESPIRATORY ILLNESSES of man."

"Results. Isolation and growth in tissue culture. This virus was recovered from 5 specimens, 4 obtained from individuals with minor upper respiratory illnesses AND ONE FROM A WELL INDIVIDUAL during the winter of 1962

(Table I). ALL OF THESE SPECIMENS YIELDED VIRUS ONLY AFTER A SECOND BLIND PASSAGE IN HUMAN KIDNEY CELLS. NO VIRUS WAS RECOVERED IN THE SECONDARY MONKEY KIDNEY CULTURES OR H.Ep. 2 CELL CULTURES INOCULATED WITH THESE SPECIMENS. These viruses produced CPE in human diploid cell strains and these cultures, HEL(1) or WI-38(4), were used for all further experiments. The CPE in HEL or WI-38 WAS SLOW, WITH FIRST CHANGES NOTED AFTER 6 DAYS' INCUBATION at 33°C on roller drums.

The cell monolayer became “stringy” in appearance but this developed generally rather than focally. Inclusion bodies were not found in cells stained by H and E. Many small vacuoles in the cytoplasm of cells were the first changes noted in stained cells."

"Characterization of the new virus, 229E. Henz Agglutination tests. High titered pools of 229E grown in WI-38 cell cultures were inoculated into secondary monkey kidney cultures and H.Ep. 2 cell cultures and 2 blind passages carried out. THERE WAS NO EVIDENCE OF CPE IN EITHER CELL CULTURE NOR WAS THERE HEMADSORPTION OF GUINEA PIG RED BLOOD CELLS ON THE

MONKEY KIDNEY CULTURES. WI-38 cell cultures infected with this virus ALSO DID NOT HEMASORB GUINEA PIG CELLS. High titer pools of 229E virus were tested for hemagglutinin with both guinea pig and chicken red blood cells at 4OC room temperature and 37°C. NO hemagglutinin was demonstrated. Plaques were produced by 229E virus on WI-38 cultures under a methylcellulose overlay after 7 days' incubation at 33°C."

"Cultures for Mycoplasma. CELL CULTURES

EMPLOYED IN OUR LABORATORIES FOR ISOLATION OF VIRUSES FREQUENTLY CONTAIN MYCOPLASMA. After treatment of WI-38 cultures with 50 pgJml of AUREOMYCIN, no Mycoplasma could be detected by culture either anaerobically or aerobically on PPLO agar 'plates, nor were they isolated from pools of 229E virus grown in such WI-38 cultures."

"Serologic tests. The 229E virus was tested for neutralization by antisera for some of the known myxoviruses shown in Table III. None of the antisera neutralized 229E virus. Recently 229E CF antigen prepared in our laboratories was tested by Dr. Robert Chanock of NIAID WITH THE ANTISERA PREPARED IN HIS LABORATORY TO THE KNOWN MYXOVIRUSES. These were ferret antisera for respiratory syncytial virus and measles; guinea pig antisera for parainfluenza 1 (HA-2 and Sendai) , parainfluenza 2 (CA and SVS), parainfluenza 3 (HA-1 and SF-4), parainfluenza 4, mumps, influenza A, influenza B, influenza C, and NDV. Homologous titers ranged from 1:40

to 1 : 1280. THERE WERE NO CROSS REACTIONS BY COMPLEMENT FIXATION TEST WITH THESE SERA AND 229E ANTIGEN.

Antibody in human sera. The results of neutralization tests and complement fixation tests on the sera of students from whom 229E virus was isolated are summarized in Table TV. A 4-fold or greater rise in neutralizing antibody was detected in sera from all of the students from whom the virus had been isolated, and 4 out of 5 showed a rise by CF test. HOWEVER, CF TITERS WITH ONE EXCEPTION (243E,F) WERE LOW."

"Discussion. The ether sensitive RNA virus described above was isolated during the second year of a 5-year study of URIs among medical students at the University of Chicago. NO FURTHER ISOLATIONS WERE MADE IN THE SUCCEEDING YEARS. However, human kidney cultures were replaced by WI-38 cultures for virus isolation the fourth and fifth years of the study. Although

this virus could be adapted to grow in WI-38 cells, limited experience with reisolation indicated that WI-38 cultures might not be optimal for isolation.

The WD diploid cell was apparently more sensitive. Unfortunately, this cell strain has not been recovered from frozen storage at the Wistar Institute."

"Four of the five isolations were made from specimens from URIs SUGGESTING THAT THIS VIRUS MAY BE ETIOLOGICALLY ASSOCIATED WITH THESE ILLNESSES."

"Summary. A new ether sensitive RNA

virus was isolated during surveillance osf URI among medical students in the winter of 1962. This virus is ANTIGENICALLY UNRELATED to all known human myxoviruses."

https://doi.org/10.3181%2F00379727-121-30734

In summary:

-5 agents were "isolated" from human kidney tissue culture and produced CPE in these cultures which was considered distinct

-they concluded that these agents may possibly be a new "virus" associated with upper respiratory infections

-"virus" was isolated from 4 sick individuals and one HEALTHY individual

-"virus" was only "isolated" from the second blind passage in human kidney cells

-no "virus" was isolated from either monkey kidney cells nor in H.Ep 2 cells

-CPE observed was slow and first noted on day 6

-there was no evidence of CPE in the monkey kidney cells nor the H.Ep. 2 cells nor was there any hemadsorption of Guinea pig red blood cells in the monkey kidney nor the WI-38 cultures

-they admit that cell cultures in their labs are frequently contaminated with mycoplasma

-AUREOMYCIN was used in WI-38 cultures to control mycoplasma

-they used complement fixation tests to say this "virus" was anti genetically different from known "viruses"

-CF titers were low

-the "virus" was "isolated" during the 2nd year of a 5 year study

-no other "isolations" were made in the succeeding 3 years

-because 4 of the 5 specimens were associated with URI's, they assume the "virus" is etiologically similar to these illnesses

-they conclude that the "virus" is anti genetically unrelated to all known "myxoviruses" even though the CF test used to determine this is not sensitive nor specific and the specific antigen must be known beforehand which is impossible if the "virus" has never been properly purified/isolated

As is seemingly always the case with these "virus" papers, assumptions are made based on small sample sizes/inaccurate tests and conflicting evidence is ignored in order to claim a new "virus" has been discovered. Beyond the fact cell cultures are already impure, there were no attempts at "purifying" any "virus" from these cell culture soups in any way. There was no mention beyond the antibiotic used as to what was done to the samples nor the cell culture. There were no attempts at getting EM images of the supposedly newly discovered "virus." There were no attempts to prove pathogenicity of their "isolate."

Just more of the same ol' shoddy pseudoscience regularly found in Virology.

D.A. TYRRELL'S 1967 CORONAVIRUS PAPER:

This paper by D.A. Tyrrell in 1967 is supposedly the first to show the "Coronavirus" structure. What is interesting is that within the first paragraph, they admit that the images come from unpurified samples. They cite two sources for why they believe purification is not necessary. One is a paper involving crystallization of two plant "viruses" and the other is the co-authors own study from 1963 which admits this:

"First, it is a sensitive and rapid method of establishing the presence or absence of virus in any preparation.

Second, since the material receives the MINIMAL AMOUNT OF HANDLING, VIRUSES RETAIN STRUCTURAL DETAILS THAT MAY BE LOST IN LENGTHY PURIFICATION PROCEDURES.

Third, a virus can be EXAMINED IN RELATION TO OTHER PARTICLES AROUND IT and to the constituents of the cell in which it is contained."

doi: 10.1083/jcb.16.3.616.

According to the co-authors' own study, purification may destroy structural details and they want to examine these unknown, uncharacterized particles in relation to other particles. This raises the question, if they have never seen these "viruses" nor characterized them before, how would they know which ones are the particles they are looking for verses those that are around it?

Right off the bat, this paper isn't off to a good start and in all honesty, the conclusions and images presented are able to be disregarded based off the lack of purification/isolation alone. However, there are some interesting admittances in the study as well:

THE MORPHOLOGY OF THREE PREVIOUSLY UNCHARACTERIZED HUMAN RESPIRATORY VIRUSES THAT GROW IN ORGAN CULTURE

"INTRODUCTION

Organ cultures of respiratory epithelium provide a practical and sensitive means of propagating human respiratory viruses. Some viruses can at present only be grown in this way (Tyrrell & Bynoe, 1965, 1966; Hoorn & Tyrrell, 1966). Unfortunately, several such viruses can only be detected by inoculating volunteers. It was therefore decided to try to detect these viruses and characterize them morphologically by the electron-microscope technique of negative staining. ORIGINALLY NEGATIVE STAINING WAS APPLIED TO PURIFIED PREPARATIONS OF VIRUS PARTICLES (Brenner &Horne, 1959), BUT IT WAS LATER SHOWN THAT CRUDE PREPARATIONS OF WHOLE CELLS COULD ALSO BE USED TO STUDY CELL-ASSOCIATED VIRUSES (Horne& Nagington, 1959; Almeida & Howatson, 1963; Parsons, 1963).

In the present instance, suspensions obtained by mild treatment of the organ cultures in a glass homogenizer have been used for microscopy and THREE UNCHARACTERIZED RESPIRATORY VIRUSES have been seen. Two of these were of a morphological type not previously associated with human disease.

METHODS

Organ culture. NASAL EPITHELIUM AND TRACHEA WERE DISSECTED FROM HUMAN EMBRYOS OF 14 TO 24 WEEKS GESTATION and planted on scratched areas of plastic Petri dishes 60 mm. diameter AA grade (Esco Rubber Co.). THE NASAL EPITHELIUM WAS SUPPLIED WITH 2 ml. OF EAGLE'S MEDIUM CONTAINING 0-2 ~o (w/v) BOVINE PLASMA ALBUMIN; THE TRACHEA RECEIVED 1.25 ml. The dishes were incubated at 33 ° in sealed humidified plastic boxes in an atmosphere of 5 % (v/v) carbon dioxide in air. AFTER 2 DAYS'

INCUBATION, THE MEDIUM WAS CHANGED and the cultures inoculated by dropping 0.2 ml. of virus suspension on to the tissue. The cultures were then incubated for a further 4 days, when the medium was removed and, where possible, titrated for virus in roller tube tissue cultures incubated at 33 ° .

Electron microscopy. The tissue fragments were treated very lightly in a loosely fitting glass homogenizer of the TenBroeck type with a few drops of distilled water. MOST OF THE TISSUE FRAGMENT REMAINED INTACT AND MAINLY THE SUPERFICIAL, VIRUS-INFECTED CELLS WERE DETACHED. A drop of this cell suspension was mixed with an equal quantity of 3 % (w/v) phosphotungstic acid adjusted to pH 6.0 with potassium hydroxide. A small amount of this mixture was then placed on a carbon+ formvar coated grid, excess fluid removed with filter paper and the specimen examined immediately in a Philips 200 electron microscope. If this procedure could not be carried out within a few hours of harvesting the tissue fragments, they were frozen at -70 ° until convenient.

Several known viruses were examined to establish the practicability of the method which was used then on three uncharacterized viruses that cause human upper respiratory disease. These are (a) strain 229 E of Hamre & Procknow (1966); (b) strain B 814 (Tyrrell & Bynoe, 1965); (c) strain LAKEY (Tyrrell & Bynoe, 1966).

RESULTS

VIRUS PARTICLES OR VIRAL COMPONENTS WERE DETECTED IN ALMOST ALL THE CULTURES INOCULATED WITH KNOWN VIRUSES, and in no instance in an uninoculated control. An additional control was provided by examining cultures that had been inoculated with herpes simplex and vaccinia viruses and then not incubated but held at 4 °. NO VIRUS PARTICLES WERE SEEN IN THESE PREPARATIONS.

Each of the three uncharacterized human viruses revealed virus particles or viral components associated with the negatively stained cellular fragments. Strain 229 E contained particles (P1. 1, figs. 1, 2) resembling closely the particles of avian infectious bronchitis (Berry et al. 1964). The particles are pleomorphic in form and although varying somewhat in size have an average diameter of about 800 to 1200/~. The surface of the particles is covered with a distinct layer of projections roughly 200 A, long. These projections seem to have a narrow stalk just within the limit of resolution of the microscope and a 'head' roughly 100 A across.

Similar particles were found in organ cultures infected with the second uncharacterized strain, B 814, which cannot at present be grown in tissue cultures (Tyrrell & Bynoe, 1965). The PARTICLES FROM THIS STRAIN (P1. 1, figs. 3, 4) WERE INDISTINGUISHABLE BOTH FROM THOSE OF 229 E

(P1. 1, figs. 1, 2) AND OF AVIAN INFECTIOUS BRONCHITIS.

The third unknown strain, LAKEY, had been obtained from the nasal washings from a patient with a cold (Tyrrell & Bynoe, 1966). It had been passed twice in organ cultures and the medium from these cultures produced colds in volunteers. On one occasion a very poor hemadsorption had been seen in a few outlying cells of a roller tube culture of rhesus monkey kidney inoculated 10 days previously with culture medium. ALTHOUGH THIS OBSERVATION COULD NOT BE REPEATED it was a clue suggesting that this might be some type of myxovirus. The electron-microscope preparation showed a great deal of helical material with a diameter of 180 A which was indistinguishable from the internal component of viruses such as the parainfluenza group (PI. 2, figs. 5, 6). Until adsorption to and elution from red cells has been definitely established it is not possible to say that it is a myxovirus, but the morphology found does establish that the virus belongs to the subgroup of compound viruses having a morphology like that of Newcastle disease virus (Waterson & Almeida, 1966).

DISCUSSION

The procedure that we have used for identifying viruses grown in organ culture is both simple and speedy. ANY ATTEMPT AT A CONVENTIONAL PURIFICATION PROCEDURE FROM THE TISSUE FRAGMENTS WOULD BE DIFFICULT AND INEFFICIENT SINCE THE AMOUNT OF MATERIAL AVAILABLE IS SO SMALL AND THE PROPORTION OF INFECTED CELLS IS SO LOW. When the culture

is handled in the way described, WE BELIEVE THAT THE CELLS THAT GO INTO SUSPENSION AND ARE USED FOR ELECTRON MICROSCOPY ARE MAINLY THOSE THAT ARE INFECTED WITH VIRUS. Virus particles were identified even when the titre in the supernatant was low.

Probably the most interesting finding from these experiments was that two human respiratory viruses, 229 E and B 814. ARE MORPHOLOGICALLY IDENTICAL WITH AVIAN INFECTIOUS BRONCHITIS. THEIR BIOLOGICAL PROPERTIES, AS FAR AS THEY ARE KNOWN, ARE CONSISTENT WITH THIS. Both the human viruses are ether sensitive as is avian infectious bronchitis 229 E, have a similar size by filtration and multiply in the presence of an inhibitor of DNA synthesis. It will be interesting to compare the serology of the morphologically similar human and chicken viruses."

doi 10.1099/0022-1317-1-2-175.

In summary:

-the study uses unpurified crude preparations to image "virus" particles

-all 3 "viruses" were uncharacterized

-nasal epithelium and trachea from 14-24 week old embryos were used for cultures

-both were supplied with Eagle's Medium containing Bovine Plasma Albumin

-Medium was changed after 2 days incubation along with inoculation of "virus"

-most tissue fragments remained intact and the mainly the assumed "virus-infected" cells became detached

-'virus" particles or components were detected in ALMOST all of the "known viruses"

-they used an unincubated culture of herpes simplex and vaccinia as "controls" and no "virus" particles were produced

-B814, 229E, and avian infectious bronchitis are indistinguishable from each other

-they admit that purification would have been difficult and inefficient due to the small amount of material and low proportion of infected cells

-they BELIEVE that the cells under EM are the ones mainly infected with "virus"

-they conclude from these unpurified samples that B814, 229E, and avian infectious bronchitis are morphologically identical and that the biological properties, or at least the ones that are KNOWN, are consistent with this conclusion

When you read one "virus" paper, you are pretty much reading them all. They always consist of unpurified particles and assumptions that are not backed up by the evidence.

McINTOSH 1967 PRELIMINARY CORONAVIRUS PAPER:

The second strain of "Coronavirus" that was discovered was OC43 by Kevin McIntosh in 1967. Below are highlights from his preliminary paper before naming specimen OC43 in a future paper a few months later. Once again, it gives some solid insight into the madness of Virology in regards to grotesque cell culture experiments, unpurified materials, and ridiculous assumptions.

RECOVERY IN TRACHEAL ORGAN CULTURES OF NOVEL VIRUSES FROM PATIENTS WITH RESPIRATORY DISEASE

Despite recent advances in tissue culture methodology, ONLY 20 TO 35 PERCENT OF ADULTS WITH ACUTE UPPER RESPIRATORY TRACT ILLNESS YIELD VIRUSES WHICH CAN BE CULTIVATED BY THE STANDARD VIRUS RECOVERY TECHNIQUES.3' 4, 12 Recently, an increase in virus isolation from patients with common colds was achieved by Tyrrell and Bynoe through the use of human embryonic tracheal and nasal organ cultures.9' 20 VIRUSES WERE DETECTED IN HARVESTS FROM ORGAN CULTURES EITHER BY THE INDUCTION OF HEMADSORPTION OR CYTOPATHIC EFFECT (CPE) IN SUBINOCULATED TISSUE CULTURE OR BY THE PRODUCTION OF COLDS IN VOLUNTEERS. One virus, strain B814, which was recovered by these techniques and produced colds in volunteers, was of particular interest since it was ether-labile and grew only in human ciliated epithelium. Subsequently, Tyrrell found that this virus resembled in morphology the avian infectious bronchitis virus (IBV), a medium-sized virus which appears to be distinct from the myxoviruses.2, 18 Another newly recognized, medium-sized, ether-labile virus, strain 229E, was recovered from students with colds by Hamre and Procknow using standard tissue culture techniques.5

During a survey of acute upper respiratory illness which was carried out in our laboratory, and in which the standard tissue culture techniques were used, we noted a sharp decrease in the rate of virus isolation during the winter of 1965-66, similar to that reported by others.3' 4 12 Stimulated by the findings of Tyrrell and Bynoe, WE USED THE ORGAN CULTURE TECHNIQUE TO STUDY SPECIMENS, FROM THIS AND A PREVIOUS SURVEY,12 WHICH FAILED TO YIELD AGENTS IN STANDARD TISSUE CULTURES. From 23 such specimens 8 agents were recovered, including 6 with an unusual-morphology which resembled that of IBV. The recovery and properties of these

6 viruses form the basis of this report.

Materials and Methods.-Collection of specimens and virus isolation attempts in tissue culture:

Specimens were obtained from employees of the National Institutes of Health with acute upper respiratory illness on or before the fourth day of illness.12 Two-tenths ml of freshly collected nasopharyngeal wash fluid was inoculated into each of two tubes of the following tissue cultures: HEp-2, primary human embryonic kidney, rhesus monkey kidney, and human diploid cell strains (HDCS) WI-26, WI-38, and AT-39.13 The remaining fluid was stored at -60C. Tissue cultures were obtained from commercial sources, maintained as previously described,' and were incubated at 330C on roller drums. The tubes were observed for cytopathic effect twice weekly. Monkey kidney cultures were tested for hemadsorption at 5- to 7-day intervals, and a single blind passage of most human embryonic kidney cultures was made at 21 days. ONLY THOSE NASOPHARYNGEAL WASHINGS IN WHICH VIRUS COULD NOT BE DETECTED BY THESE METHODS WERE EXAMINED BY ORGAN CULTURE TECHNIQUES. Acute phase sera were drawn at the time the washings were collected and convalescent phase sera approximately 3 weeks thereafter.

Organ cultures: Human embryonic tracheal organ cultures were prepared and maintained by a modification of the method of Hoorn and Tyrrell.6,7 TRACHEAS WERE OBTAINED FROM FETUSES SPONTANEOUSLY ABORTED AT 5-9 MONTHS' GESTATIONAL AGE. The tissue was excised en bloc by sterile technique and IMMEDIATELY STORED IN COLD HANK'S BALANCED SALT SOLUTION WITH 10% FETAL CALF SERUM, 250 u/ml PENICILLIN, and 250 ,g/rnl STREPTOMYCIN. Within 2 to 48 hr the tracheas were trimmed, washed, and cut into fragments 2-3 mm square. A set of four fragments was placed mucosal side up in a plastic Petri dish previously scratched lightly with the point of a scalpel. THE FRAGMENTS WERE PARTIALLY COVERED WITH 1.25 ml of LEIBOVITZ MEDIUM,9 SUPPLEMENTED WITH 0.2% BOVINE ALBUMIN, 0.1 mM GLUTAMINE, 250 u/ml PENICILLIN, and 250 ,sg/ml STREPTOMYCIN. The pH of the medium was adjusted to 7.4 with a few drops of 1 N NaOH. The beating of cilia was observed through a dissecting microscope by incident illumination. Only those fragments initially showing strong ciliary beating were used.

After inoculation organ culture plates were incubated at 33 C on a rocker platform moving at one full cycle every 2-3 min. DAILY OR EVERY OTHER DAY, MEDIUM WAS REMOVED FROM THE PLATES AND STORED at -60'C, AND THE CULTURES WERE REFED. Control organ cultures retained full ciliary activity for a variable period up to 3 weeks, the longest interval tested. To perform subpassages, stored harvests from the second through the tenth day of incubation WERE

THAWED AND COMBINED, and 0.2-0.5 ml OF THIS POOL WAS INOCULATED INTO FRESH ORGAN CULTURE PLATES.

Viruses: Dr. Dorothy Hamre kindly supplied strain 229E,1 which had been purified by the terminal dilution technique in HDCS (WI-38). Chick-embryo-grown avian infectious bronchitis virus, Beaudette strain (66579), was kindly supplied by Dr. Harold DeVolt.

Electron microscopy: POOLED FLUIDS FROM INOCULATED OR CONTROL ORGAN CULTURES were clarified by low-speed centrifugation at 2000 rpm for 10 min in the PR-2 International centrifuge, and centrifuged onto a cushion of 60% sucrose at 111,000 X g for 90 min in the SW-39 rotor of a Spinco model L ultracentrifuge. A drop of material from the sucrose-medium interface was prepared by the pseudoreplication technique of Sharp"6 as modified by Smith and Melnick,'7 negatively stained with 2% phosphotungstic acid (PTA) at pH 5.0 or 7.0 and mounted on copper grids. Suspensions of virus 229E grown in HDCS (WI-38) were clarified as above and concentrated by centrifugation at 18,500 rpm (54,000 X g) in the SW-39 rotor for 90 min. The pellet was resuspended in a small volume of 1% ammonium acetate, stained with PTA at pH 5.0, and spread on formvar coated grids.

Allantoic fluid suspensions of IBV were clarified by centrifugation as above and then dialyzed against distilled water at 4°C for 5 hr. A drop of the unconcentrated sample was then negatively stained with PTA at pH 5.0 or 7.0 and spread on a coated grid."

"Results.-Recovery of agents from organ culture: Table 1 presents a summary of our experience with common cold patients whose nasopharyngeal washings were studied in organ culture. Two methods were used to detect the presence of viruses in these specimens. FIRST, INOCULATED ORGAN CULTURES WERE OBSERVED FOR AN EFFECT ON CILIARY ACTIVITY. SECOND, HARVESTS OF THIRD OR FOURTH PASSAGE CULTURE MEDIUM WERE EXAMINED BY ELECTRON MICROSCOPY FOR VIRUS PARTICLES.

By these means 8 agents were detected in tracheal organ cultures inoculated with specimens from 23 patients. THE PRESENCE OF TWO OF THESE COULD BE DETECTED ONLY BY THEIR CILIARY IMMOBILIZING EFFECT (CIE); VIRUS PARTICLES WERE NOT VISUALIZED IN ORGAN CULTURE HARVESTS, and both were ether-resistant. Possibly these agents are fastidious

rhinoviruses which grow only in organ culture.8 SIX OTHER AGENTS WERE DETECTED BY ELECTRON MICROSCOPY OF ORGAN CULTURE HARVESTS. These viruses possessed a similar and characteristic morphology; because of their resemblance to IBV,2 we have tentatively designated them "IBV-like" viruses. FOUR OF THESE AGENTS PRODUCED CIE.

Characteristics of "IBV-like" viruses in organ and tissue culture: (i) Ciliary immobilizing effect: Table 2 summarizes the behavior of the six agents in organ culture. Except for isolate 664, CIE WAS NEVER OBSERVED BEFORE THE THIRD PASSAGE, but once detected was usually seen during later passages of the virus. Specimen 664 produced CIE on the second passage, BUT THIS EFFECT WAS NOT OBSERVED AGAIN DURING REPETITION OF THE SECOND PASSAGE OR DURING TWO SUBSEQUENT PASSAGES.

Following inoculation of cultures, the time at which CIE was first observed (hereafter referred to as the CIE interval) was never less than four days and never more than ten days. The usual CIE interval was eight days, and did not vary by more than two days among different dishes derived from the same embryo. However, WHEN A VIRUS SUSPENSION WAS INOCULATED ONTO CULTURES DERIVED FROM DIFFERENT EMBRYOS, A DIFFERENCE IN CIE INTERVAL OF AS MUCH AS FOUR DAYS WAS OBSERVED. Because of this variation each comparative test was carried out with tissue derived from a single embryo.

(ii) Detection of "IBV-like" viruses by electron microscopy: As is shown in Table 2,

CHARACTERISTIC PARTICLES WERE NOT SEEN IN THE FIRST AND SECOND PASSAGES OF SPECIMEN 501 BUT APPEARED IN MODERATE NUMBERS IN THE THIRD. Once observed during the third or fourth passage, particles were consistently seen during later passages of each isolate so tested. Control harvests from uninoculated organ cultures were frequently examined, and in no instance were "IBV-like" particles seen."

Attempts to grow "IBV-like" viruses in tissue cultures, eggs, and ferret organ culture: ATTEMPTS TO GROW THESE VIRUSES IN TISSUE CULTURES WERE UNSUCCESSFUL. These tests included (a) observation for CPE and hemadsorption in numerous cell lines and strains of human and nonhuman origin, (b) attempts to visualize immunofluorescent antigen in HDCS cells, (c) search for evidence of interference with ECHO 11 virus, (d) electron microscopic examination of tissue culture harvests, and (e) back passage of tissue culture harvests into tracheal organ culture with examination for CIE and virus particles. Replication of "IBV-like" viruses was likewise not

detected in embryonated eggs or in ferret tracheal organ culture.

Reisolation of "IBV-like" viruses: Three attempts were made to reisolate "IBV-like" agents from the original nasopharyngeal washings, and all were successful. In each of the three reisolation series "IBV-like" particles were seen by electron microscopy in third passage harvests. With each reisolation attempt a parallel passage series was initiated USING 0.2 ml OF MEDIUM AS INOCULUM INSTEAD OF NASOPHARYNGEAL WASHING. This control series and the reisolation series itself were carried out by identical techniques in organ cultures derived from the same embryos. Concentrated harvests from infected and control cultures were coded and examined "blind." In no instance were "IBV-like" agents seen in control material.

Electron microscopic appearance: Figures 1 and 2 show the appearance of an "IBV-like" agent, isolate 501, after six passages in organ culture. Comparison with 229E (Fig. 3) and IBV (Fig. 4) shows their remarkable similarity. An electron micrograph of influenza A2 is also shown for comparison (Fig. 5).

All "IBV-like" viruses, 229E, and IBV itself show the following characteristics: (1) an over-all diameter of 160 mu with a variation of 440 mMi; (2) a moderate pleomorphism with resultant elliptical, round, or tear-drop shapes but no filamentous or "tailed" forms; (3) characteristic spikes 20 my long, usually club- or pear-shaped, narrow at the base and 10 mju wide at the outer edge, spaced widely apart and distributed fairly uniformly about the circumference of the particle."

Discussion. Evidence from this study for the human origin of the "IBV-like" agents is provided by the successful reisolation of viruses with characteristic morphology in each of the three attempts made. This evidence is strengthened by our failure to detect such virus particles in control of uninfected preparations which were passaged parallel to the infected material in cultures derived from the same embryos. In addition, two of the individuals who yielded "IBV-like" viruses appeared to develop a rise in neutralizing antibodies during convalescence.

HOWEVER, THE DATA REPORTED HERE FURNISH NO EVIDENCE THAT THE VIRUSES RECOVERED WERE THE CAUSE OF ILLNESS, FOR INDIVIDUALS WITHOUT RESPIRATORY DISEASE WERE NOT STUDIED DURING THE SOME TIME PERIOD. In this regard it is of interest that strain 229E was

recovered from FIVE INDIVIDUALS all of whom developed fourfold or greater rises in neutralizing antibody and FOUR OF WHOM HAD AN UPPER RESPIRATORY ILLNESS AT THE TIME the virus was isolated.5 M\oreover, strain B814 was shown to cause colds in volunteers who received organ-culture-grown virus.'9 IT APPEARS LIKELY, THEREFORE, THAT ON FURTHER STUDY THE "IBV-like" VIRUSES ISOLATED IN THIS STUDY WILL BE SHOWN TO CAUSE ILLNESS IN MAN.

Our experience with the recovery of "IBV-like" agents during the winter of 1965-66 provides GROUNDS FOR SPECULATION AS TO THE POSSIBLE ROLE of this group ill acute upper respiratory illness. In September, October, and November of 1965, 21 of 50 specimens tested by standard tissue culture techniques yielded recognizable agents. During this period ten specimens were studied in organ culture and no "IBV-like" viruses were recovered. During the subsequent three-month period, when only four agents were isolated by tissue culture techniques from 60 specimens tested, "IBV-lilke" viruses were recovered in organ culture from five of nine specimens studied. IF THESE VIRUSES ARE SHOWN ULTIMATELY TO CAUSE RESPIRATORY TRACT DISEASE IN MAN, THEN IT IS POSSIBLE THAT THEY ARE IMPORTANT PATHOGENS DURING THOSE PERIODS in mid-winter when rhinovirus prevalence declines.3' 4, 12

The limited supply of human embryonic tracheas restricts rapid expansion of our understanding of the "IBV-like" viruses. To facilitate their investigation, it will be necessary to adapt them to growth in tissue culture. It is clear, however, that ciliated human embryonic organ culture represents a sensitive system for the recovery of agents from patients with respiratory disease.20 With the addition of the electron microscope for examination of concentrated harvests, ORGAN CULTURE COULD PROVE TO BE A USEFUL TOOL IN THE SEARCH FOR NEW AGENTS IN MANY DIFFERENT DISEASES.

The causative organism of avian infectious bronchitis'4 is a filterable, ether-labile,10 RNA-containing and medium-sized2 virus and was initially assigned to the myxovirus group. Berry et al.2 examined IBV by the negative staining technique and pointed out the contrast between its morphology and that of the myxoviruses. A HUMAN COUNTERPART OF IBV WAS, HOWEVER, NOT RECOGNIZED UNTIL TYRRELL'8 DREW ATTENTION TO THE RESEMBLANCES BETWEEN STRAINS B814 AND THE AVIAN PATHOGEN. It appears now that a new group of viruses is emerging with members which infect the respiratory tract of birds and man. One member of the group, strain 229E, grows and produces CPE in tissue culture; strain B814 and the viruses reported here can only be propagated in the laboratory iin human ciliated respiratory tract organ culture. The group resembles the myxo- and paramyxoviruses in size, ether lability, and, where tested, nucleic acid type; however, it differs in morphology. In place of the closely spaced,

narrow surface projections carried by the myxo- and paramyxoviruses those of the "IBV-like" viruses are club-shaped and more widely spaced.

Summary.-In a study of acute upper respiratory tract disease in adults, NASO-PHARYNGEAL WASHINGS WHICH FAILED TO YIELD VIRUSES BY STANDARD TISSUE CULTURE TECHNIQUES WERE EXAMINED IN HUMAN EMBRYONIC TRACHEAL ORGAN CULTURES. FROM 2 SPECIMENS, 8 AGENTS WERE RECOVERED, 2 of which appeared to be ether-stable and WERE DETECTABLE ONLY BY THEIR CILIARY IMMOBILIZING EFFECT IN ORGAN CULTURE. The remaining 6 were detected when organ culture harvests were examined by electron microscopy. These viruses exhibited an unusual morphology closely resembling that of avian infectious bronchitis virus (IBV) and two other ether-labile agents recovered from man: strain 229E, described by Hamre and Procknow, and strain B814, an organ-culture-propagated virus described by Tyrrell. Five of the six "IBV-like" viruses were examined and found to be inactivated by ether. This group, for which IBV is the morphologic prototype, appears to be distinct from the myxoviruses. The implications of these findings are discussed in relation to acute upper respiratory tract disease of undetermined etiology."

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In Summary:

-only 20 to 35 percent of patients with URI's yield "viruses" which can be recovered by standard techniques

-INDIRECT evidence such as hemadsorption, CPE, or colds in volunteers was used as evidence a "virus" is in a culture

-they used embryonic organ cultures to find (create) "viruses" that could not be found (created) using the standard tissue culture techniques

-tracheas from 5-9 month old aborted fetuses were collected and stored in Hanks Salt Solution along with 10% fetal bovine serum as well as penicillin and streptomycin

-fragments were partially covered in Leibovitz Medium, bovine albumin, glutamine, penicillin, and streptomycin

-Medium was removed and replaced either daily or every other day

-for subpassages, samples collected from the 2nd through tenth day of incubation were

collected and POOLED TOGETHER in a fresh plate

-two methods were used to determine a "virus" was present: checking for ciliary immobilizing effect (CIE) or looking for "virus" particles under EM

-8 agents were recovered from organ cultures for which 2 could only be determined by CIE as no particles could be seen in EM. The other 6 were determined by EM images and only 4 produced CIE in cell culture.

-CIE was never regularly observed before the third passage

-attempts to grow IBV-like particles in tissue cultures were unsuccessful

-medium was used as a control for uninoculated cultures rather than nasopharyngeal washings from healthy volunteers

-they admit that the conclusion can not be made that the "viruses" collected in this study were the cause of any disease as they did not study any healthy volunteers

-they mention Hamre's 229E study where 4 of 5 volunteers where "virus" was found had URI's yet ignore that one was HEALTHY

-they believe it appears likely that these "IBV-like" particles will be shown to cause disease in man in FUTURE studies

-their "evidence" provides grounds for speculation on the possible role of these particles they claim as "viruses" and IF they are shown to be pathogenic in FUTURE studies, they claim it is possible these particles played a role in previous pathogenic periods

-they claim organ culture could be useful for discovering new "viruses"

-human strains of Avian Bronchitis were never found until Tyrrell pointed out that the particles he found look a lot like those found in Chickens

There are numerous assumptions and conclusions that are drawn by the authors of this study which are left to be decided by future studies. They even state that the particles may not be pathogenic as they never studied healthy volunteers. As usual, the claims of "virus" are based on INDIRECT evidence from unpurified cell culture soup never proven pathogenic. In other words, par for the course in Virology.

Maybe it's all about how they color them...?

Images on the left are claimed to be Influenza. Images on the right are claimed to be "Coronavirus."

McINTOSH 1967 CORONAVIRUS OC43:

After his preliminary paper on "IBV-like" particles, McIntosh went on to adapt the growth of his "virus" in suckling mouse brains and then to tissue cultures. Instead of realizing that the particles he created were most likely coming from the cultures of mice brains themselves, he came to the conclusion that his "IBV-like" particles were closely related to the morphologically identical Hepatitis "virus" in mice (MHV). Thus we have what is considered the second major "Coronavirus:" OC43. Below are some highlights from his paper:

GROWTH IN SUCKLING-MOUSE BRAIN OF "IBV-LIKE" VIRUSES FROM PATIENTS WITH UPPER RESPIRATORY TRACT DISEASE

"With the use of human embryonic tracheal organ culture,9 six strains of a medium-sized (120-160 mgt) virus bearing a close morphologic resemblance to avian infectious bronchitis virus (JBV) were recovered in this laboratory from patients with colds.13 THESE VIRUSES WERE DETECTED WHEN ORGAN CULTURE FLUID HARVESTS WERE CONCENTRATED TEN TIMES AND EXAMINED WITH THE ELECTRON MICROSCOPE. In negatively stained preparations the viruses appeared round and moderately pleomorphic with widely spaced, club-shaped surface projections. The tentative designation of "IBV-like" viruses was given to members of this group, whose morphology clearly differed from that of the myxoviruses. The "IBV-like" viruses were shown to be inactivated by ether, but their growth was not inhibited by 5-bromodeoxyuridine.12 Serologic studies in organ culture suggested that the patients from whom the viruses were recovered developed a neutralizing antibody response. HOWEVER, FURTHER STUDY OF THESE VIRUSES WAS IMPEDED BY THE LACK OF A TISSUE CULTURE OR

EXPERIMENTAL ANIMAL SYSTEM WHICH WOULD SUPPORT THEIR GROWTH. For this reason, and because we found that MOUSE HEPATITIS VIRUS (MHV), another RNA-containing ether-labile virus, WAS MORPHOLOGICALLY IDENTICAL TO MEMBERS OF THE "IBV-like" VIRUS GROUP IN NEGATIVELY STAINED PREPARATIONS,2 newborn mice were inoculated with the "IBV-like" virus strains. ENCEPHALITIS OCCURRED IN MICE INOCULATED WITH TWO OF THE SIX STRAINS. The preliminary results of these studies are reported here.

Growth of "IBV-Like" Viruses in Suckling-Mouse Brain.-"IBV-like" viruses were originally recovered by INOCULATING THROAT WASHINGS FROM PATIENTS WITH COLDS ONTO HUMAN EMBRYONIC TRACHEAL ORGAN CULTURES. Suspensions used for mouse inoculation were prepared from MATERIAL PASSAGED FOUR TO FIVE TIMES IN ORGAN CULTURE and contained "IBV-like" virus particles when examined by electron microscopy after tenfold concentration. Pregnant Swiss mice of the CD-1 strain were obtained from Charles River 1\louse Farms, Inc., Wilmington, :\Mass. Several years previously, the mice from this colony had been shown to be free of _IHV contamination."6 DURING OUR STUDIES WE WERE UNABLE TO DEMONSTRATE THE PRESENCE OF MHV

ANTIBODY OR INFECTION IN MICE AT THE TIME THEY WERE RECEIVED IN THE LABORATORY. The mice were kept in battery jars with a tightly fitting pad of 3/8-inch glass-fiber material inserted in the lid. Strict isolation precautions were observed, and the mice were housed in a room used only for these experiments.

Suckling mouse passages were performed either by COMBINED INTRACRANIAL (IC) AND INTRAPERITONEAL (IP) INOCULATION OR BY THE IC ROUTE ALONE. On first passage, two of the six strains, designated OC38 and OC43, CAUSED A DISEASE WHICH BEGAN 11-15 DAYS AFTER INOCULATION AND WAS CHARACTERIZED BY GENERALIZED TREMORS, RIGIDITY, AND LETHARGY. Two of 8 mice inoculated with OC38, and 3 OF 16 MICE INOCULATED WITH OC43 WERE AFFECTED. At the same time 16 control uninoculated mice remained well. On serial subpassage of brain suspensions, the syndrome developed after progressively shorter incubation periods. AT THE FOURTH PASSAGE ALL INOCULATED MICE DIED, and in that and later passages the period between inoculation and death was stabilized at 48-60 hours. Control sub passages were carried out in parallel with the infected passage series. NONE OF THE CONTROL MICE DEVELOPED AN ENCEPHALITIC SYNDROME.``

"ENCEPHALITIS WAS INDUCED BY IC INOCULATION OF MICE FIVE DAYS OF AGE OR YOUNGER,

BUT WEANING MICE INOCULATED BY EITHER THE IC OR IP ROUTE DID NOT DEVELOP SIGNS OF ILLNESS. ASYMPTOMATIC INFECTION PROBABLY OCCURRED IN THE LATTER GROUP, since serum drawn three weeks following inoculation contained both neutralizing and COMPLEMENT-FIXING (CF) antibodies.

TENFOLD CONCENTRATED SEVENTH-PASSAGE MOUSE BRAIN SUSPENSION contained "IBV-like" virus particles when examined by electron microscopy using the negative staining technique. Moreover, mouse-adapted virus at all passage levels appeared to replicate in human embryonic tracheal organ cultures, as indicated by electron microscopic examination of culture harvests. Adaptation of one human organ culture-grown virus strain, OC43, to suckling-mouse brain was attempted a second time and successfully repeated. However, this adaptation differed from the first in that mice developed encephalitic signs only after two blind passages. Virus recovered from the brains of affected third passage mice was identical to the originally adapted virus strain in its serological reactivity."

"In those tests in which a CF antibody rise was not demonstrated a 25-50 per cent fixation of complement by convalescent sera was observed. HOWEVER, SUCH REACTIONS ARE NOT CONSIDERED POSITIVE BY THE ARBITRARY CRITERIA USED IN OUR LABORATORY.

"Differentiation of "IBV-Like" Viruses Propagated in Mouse Brain from Mouse

Hepatitis Virus.-In view of the extensive prevalence of MHV in mouse colonies,1 the mouse-adapted "IBV-like" VIRUS STRAINS MUST BE CLEARLY DIFFERENTIATED FROM KNOWN STRAINS OF MHV. As mentioned previously, the adaptation of organ-culture-grown "IBV-like" viruses to suckling mice could be repeated, with the recovery from mouse brain suspensions of virus which reacted with specific hyperimmune serum. Mice in all control passages failed to yield virus or to develop encephalitis. High-titer immune sera to both "IBV-like" virus strains failed at low dilutions to fix complement with MHV antigens.``

"Additional evidence that one of the mouse-adapted viruses (strain OC43) was serologically similar to its organ-culture-passaged counterpart was provided by a neutralization test in organ culture, performed by a technique reported previously.'3 The suspension of virus strain OC43 used in this test had been passaged previously only in human embryonic tracheal organ culture. Its growth in organ culture, AS DETERMINED BY THE APPEARANCE IN THE CULTURE MEDIUM OF PARTICLES WITH CHARACTERISTIC ELECTRON MICROSCOPIC MORPHOLOGY, was inhibited by a

1:640 dilution of mouse antiserum prepared against mouse-adapted virus strain OC43 (homologous titer 1:2560 in suckling mice), but not by a 1:40 dilution (the lowest tested) of MHV polyvalent mouse antiserum (homologous titer 1: 2560 in roller tube cultures)."

"Summary.-Six viruses recovered from patients with upper respiratory tract disease and BEARING A CLOSE MORPHOLOGIC RESEMBLANCE TO BOTH INFECTIOUS BRONCHITIS VIRUS (IBV) AND MOUSE HEPATITIS VIRUS (MHV) were inoculated into suckling mice. TWO OF THE SIX "IBV-like" STRAINS GREW IN MICE AND CAUSED AN ENCEPHALITIC SYNDROME. Brain suspensions from affected mice fixed complement with homologous human convalescent sera and specific mouse immune sera. In neutralization and complement-fixation tests the two strains were shown to be identical with each other and distinct from IBV and strain 229E (another human respiratory virus morphologically similar to IBV). However, THEY SHOWED A CONSISTENT ONE-WAY SEROLOGIC RELATIONSHIP WITH SEVERAL STRAINS OF MIHV. The evidence that these mouse-adapted "IBV-like" viruses are distinct from known strains of MHV was presented. In a preliminary seroepidemiologic survey, approximately one third of patients with common colds during the winter of 1965-1966 developed complement-fixing antibodies for the "IBV-like" virus antigens."

doi: 10.1073/pnas.58.6.2268.

In Summary:

-the "viruses" found and examined under EM came from unpurified organ culture fluid

-they picked particles that fit what they were looking for morphologically yet could not produce these same particles in tissue cultures or experimental animal systems

-they decided to use newborn suckling mice to culture their "virus" in as the MHV found in mice looked morphologically identical to the particles they were looking for

-2 of the 6 strains of "IBV-like" particles caused encephalitis (not a symptom of "Coronaviruses) in the mice

-throat washings from patients with colds were inoculated onto human embryo tracheal cultures and were passaged 4 to 5 times

-the mice were assumed to be free of MHV as they had no antibody response and showed no symptoms

-inoculations in mice were either done by intracranial (brain) and intraperitoneal (stomach)

injections or just intracranial alone

-2 strains (OC38 and OC43) were considered to have caused disease 11-15 days after inoculation due to the presence of tremors, rigidity, and lethargy in the mice

-only 2 of 8 mice inoculated with OC38 and 3 of 16 mice inoculated with OC43 were affected

-after 4 passages of the mice brain suspensions, all inoculated mice died

-none of the control mice developed encephalitis (again, not a common symptom of "Coronaviruses" but definitely a symptom of BRAIN INJECTIONS)

-the mice 5 days or younger developed encephalitis after IC injection while the older weaning mice injected by IC or IP did not

-however, they believe ASYMPTOMATIC infection occurred as the weaning mice had antibodies three weeks later

-they used complement fixation tests (which can not be used for novel "viruses" as antibodies/antigens would be unknown)

-they admit their criteria for these tests and what constitutes a positive result are ARBITRARY

-they admit their "IBV-like" strains must be clearly differentiated from the morphologically identical MHV strains in mice

-they differentiated them by complement fixation tests and the "controls" of uninoculated mice not developing encephalitis nor producing "virus"

-they determined OC43 grown in mice was the same "virus" grown in human organ cultures due to it's similar appearance in EM as well as by serologic neutralization tests (which, again, would be impossible to determine with novel "viruses")

-their conclusion is that the particles they grew in these mice brain cultures were morphologically identical to IBV and MHV but were somehow neither of them and were different "viruses" even though they shared a serological relationship with several MHV strains

The grotesque experiments on mice described above along with the intracranial injections and wild leaps in logic are eerily reminiscent of the Polio experiments. These researchers used an animal that they knew could produce similar particles to the ones they were looking for in order to claim they had a new "virus" even though there was no evidence to support these conclusions. It is nothing but the same Virology tricks of creating particles in unpurified organ/tissue cultures from humans and animals, injecting these unpurified substances

unnaturally into the brains of animals, and then saying "Eureka" when brain inflammation occurs.

"Science."

(Image below is said to be "Coronavirus OC43" yet it did not come from this "study" as no EM images were presented)

NAMING THE "CORONAVIRUSES:"

In 1968, a group of 8 virologists sent word to Nature that they had all discovered similar (or the same) particles from tissue and organ cultures in humans which resembled those in chickens and mice. They used several indirect methods such as complement fixation, neutralization tests, hemadsorption, serology tests, etc. in order to detect these "viruses" as they never properly purified/isolated any of these particles nor proved them pathogenic. They used human embryonic tracheal cultures mixed with animal blood, antibiotics, "nutrients," and who knows what else in order to find and pick similar looking particles in EM images from unpurified samples containing many different kinds of unknown particles. In the case of OC43, they cultured their "virus" in suckling mice brain knowing full well that they could find similar particles in the mice which had already been claimed as Mouse Hepatitis Virus (MHV) but still decided what was in their cultures was a new human "Coronavirus."

Below is the announcement as it appeared in Nature in 1968:

"VIROLOGY

Coronaviruses

A NEW GROUP OF VIRUSES WITH THE NAME OF CORONAVIRUSES HAS BEEN RECOGNIZED BY AN INFORMAL GROUP OF VIROLOGISTS who have sent their conclusions to Nature. (They are J. D. Almeida; D. M. Berry; C. H. Cunningham; D. Hamre; M. S. Hofstad; L. Mallucci; K. McIntosh; D. A. J. Tyrrell.) They point out that with negative staining, avian infectious bronchitis virus has a characteristic electron microscopic appearance resembling, but distinct from, that of myxoviruses. PARTICLES ARE MORE OR LESS ROUNDED IN PROFILE; ALTHOUGH THERE IS A CERTAIN AMOUNT OF POLYMORPHISM, there is also a characteristic "fringe" of projections 200 A long, which are rounded or petal shaped, rather than sharp or pointed, as in the myxoviruses.

THIS APPEARANCE, RECALLING THE SOLAR CORONA, IS SHARED BY MOUSE HEPATITIS VIRUS AND SEVERAL VIRUSES RECENTLY RECOVERED FROM MAN, NAMELY STRAIN B814, 229E AND SEVERAL OTHERS. These viruses also share a number of other properties as indicated in the table. (Anyone interested in the data on which the table is based may obtain a short bibliography on application to Dr D. A. J. Tyrrell at the Common Cold Research Unit, Salisbury, Wiltshire.)

Some other relevant properties should be mentioned. THERE IS AN ANTIGENIC RELATIONSHIP BETWEEN THE HUMAN AND MURINE STRAINS, but none has been detected between avian strains and the others. A haemagglutinin has been detected by certain workers using avian infectious bronchitis virus and also antigens separable from the virus particle, but these have so far not been recorded for the human or murine strains.

IN THE OPINION OF THE EIGHT VIROLOGISTS THESE VIRUSES ARE MEMBERS OF A PREVIOUSLY UNRECOGNIZED GROUP WHICH THEY SUGGEST SHOULD BE CALLED THE CORONAVIRUS, to recall the characteristic appearance by which these viruses are identified in the electron microscope.

These suggestions have been received by members of the Myxovirus Study Group (chairman, Professor A. P. Watm'son) under the International Committee for the Nomenclature of Viruses (ICNV). The suggestions were found acceptable and are now to be considered by the Vertebrate Virus Committee of the ICNV."

Almeida JD, Berry DM, Cunningham CH, Hamre D, Hofstad MS, Mallucci L, McIntosh K, Tyrrell DAJ. Virology: Coronaviruses. Nature 1968; 220(5168): 650.

It is because of these 8 virologists and their unpurified particles, which they decided were the cause of common colds without direct proof, that we are currently dealing with "Coronaviruses" now. Three strains were originally identified (B814 in 1965, 229E in 1966, and OC43 in 1967) yet only two (229E, OC43) stood the test of time and were considered the main human "Coronaviruses" for the next 30+ years. B814, the first human strain identified, just up and disappeared. Then in 2003, at the dawn of molecular virology and after 30+ years of apparent hibernation, the "Coronavirus" decided to re-emerge with several new human strains.

"CORONAVIRUS" DISCOVERY Papers:
https://docs.google.com/document/d/e/2PACX-1vTCTJaZOpHk-OtFNIspUMha6AsXQVVSoEggOkpoCcFOmhyetd9JNUay4L_OWQR6QyeP7vGhE1L-Q7SN/pub

SARS-COV-1

J.S.M. PEIRIS 2003 SARS-COV-1 PAPER:

On November 16, 2002, the first case of atypical pneumonia was reported in Guangdong province in the southern part of China. It wasn't until nearly 4 months later on March 12th, 2003 that the WHO announced a global alert about a severe pneumonia affecting parts of Asia. On March 24th, 2003, a CDC laboratory analysis suggested that this "new" respiratory disease was caused by a "Coronavirus." On April 16th, 2003, 5 months after the first reported case of this atypical pneumonia in China, the WHO issued a press release stating that "SARS-COV-1" was

the official cause of SARS. Below are highlights one of the papers cited as evidence for this new "Coronavirus:"

CORONAVIRUS AS A POSSIBLE CAUSE OF SEVERE ACUTE RESPIRATORY SYNDROME

"We collected nasopharyngeal aspirates and serum samples from all patients. Paired acute and convalescent sera and faeces were available from some patients. A LUNG-BIOPSY TISSUE SAMPLE FROM ONE PATIENT WAS PROCESSED FOR VIRAL CULTURE AND REVERSE-TRANSCRIPTASE PCR (RT-PCR) and for routine histopathological examination and electron microscopy. WE USED AS CONTROLS nasopharyngeal aspirates, and faeces and sera submitted for microbiological

investigation OF OTHER DISEASES from patients whose identities were masked."

"The nasopharyngeal aspirate was assessed by rapid immunofluorescence antigen detection for influenza A and B, parainfluenza types 1, 2, and 3, respiratory syncytial virus and adenovirus,3 AND WAS CULTURED FOR CONVENTIONAL RESPIRATORY PATHOGENS on Mardin Darby Canine Kidney, LLC-Mk2, RDE, Hep-2 and MRC-5 cells.4 Subsequently, fetal rhesus kidney (FRhK-4) and A-549 cells were added to the panel of cell lines used."

"AFTER CULTURE AND GENETIC SEQUENCING OF A CORONAVIRUS FROM TWO PATIENTS, we developed an RT-PCR to detect the coronavirus sequence from nasopharyngeal aspiration samples."

"CORONAVIRUS-INFECTED FETAL RHESUS KIDNEY CELLS were fixed in acetone and used in an indirect immunofluorescence assay TO DETECT A SEROLOGICAL RESPONSE TO THE VIRUS."

"Random RT-PCR assay

TO FIND OUT THE GENETIC SEQUENCE INFORMATION OF AN UNKNOWN RNA VIRUS, WE DID A RANDOM RT-PCR ASSAY. Total RNA from virus-infected and virus-uninfected fetal rhesus kidney cells were isolated. The RNA samples were reverse transcribed with primer 5--GCCGGAGCTCTGCAGAATTCNNNNNN-3-, where N=A, T, C, or G, and cDNA was amplified by a primer 5--GCCGGAGCTCTGCAGAATTC-3-. Unique PCR products (in size) in the infected cell preparation were cloned and sequenced, and the GENETIC HOMOLOGY COMPARED WITH

THOSE IN GENBANK."

"Routine microbiological investigation for known viruses and bacteria by culture, antigen detection, and PCR was negative IN MOST CASES. Blood culture was positive for ESCHERICHIA COLI in one man aged 74 years admitted to intensive care. The finding was attributed to a hospital-acquired urinary-tract infection. KLEBSIELLA PNEUMONIAE AND HAEMOPHILUS INFLUENZA WERE ISOLATED FROM THE SPUTUM SAMPLES OF TWO OTHER PATIENTS ON ADMISSION."

VIRUSES WERE ISOLATED ON FETAL RHESUS KIDNEY CELLS FROM THE LUNG BIOPSY AND NASOPHARYNGEAL ASPIRATE, RESPECTIVELY, OF THESE TWO PATIENTS. The initial cytopathic effect noted was the appearance of rounded refractile cells appearing 2–4 days after inoculation.

THE CYTOPATHIC EFFECT DID NOT PROGRESS IN THE INITIAL CULTURE TUBES BUT ON SUBSEQUENT PASSAGE, and appeared in 24 h. The two virus isolates did not react with the routine panel of reagents used to identify virus isolates, including those to influenza A, B, parainfluenza types 1, 2, and 3, adenovirus, and respiratory syncytial virus (DAKO, Glostrup, Denmark). They also did not react in RT-PCR assays for influenza A and human metapneumovirus, or in PCR assays for mycoplasma. The virus was ether sensitive, which shows that it was an enveloped virus. ELECTRON MICROSCOPY OF NEGATIVE STAINED (3% potassium phospho-tungstate, pH 7·0) ultracentrifuged CELL-CULTURE EXTRACTS showed the presence of pleomorphic enveloped virus particles of around 80–90 nm (range 70–130 nm) in diameter with surface morphology compatible with a coronavirus (figure 1). Thin-section electron microscopy of infected cells revealed virus particles of 55–90 nm diameter within smooth walled vesicles in the cytoplasm (figure 2, B). Virus particles were also seen at the cell surface. The overall findings were compatible with coronavirus infection in the cells.

A thin-section electron micrograph of the lung biopsy sample from the 53-year-old male contained 60–90 nm viral particles in the cytoplasm of desquamated cells. THESE VIRAL PARTICLES WERE SIMILAR IN SIZE AND MORPHOLOGIC TO THOSE OBSERVED IN THE CELL CULTURED VIRUS ISOLATES FROM BOTH PATIENTS (figure 2, A).

"The RT-PCR PRODUCTS GENERATED IN A RANDOM PRIMER RT-PCR ASSAY were analysed, and unique bands found in the virus-infected samples were cloned and sequenced. Of 30 clones examined, ONE CONTAINING 646 bp OF UNKNOWN ORIGIN WAS IDENTIFIED. Sequence analysis of this DNA fragment SUGGESTED THIS SEQUENCE HAD A WEAK HOMOLOGY TO VIRUSES OF THE FAMILY OF CORONAVIRIDAE. DEDUCED AMINOACID SEQUENCE (215 amino acids) from this unknown sequence, however, HAD THE HIGHEST HOMOLOGY (57%) TO THE RNA POLYMERASE OF BOVINE CORONAVIRUS AND MURINE HEPATITIS VIRUS, confirming that this virus belongs to the family of Coronaviridae. Phylogenetic analysis of the protein sequences showed that this virus, although most closely related to the group II coronaviruses, was a distinct virus (figure 3).

BASED ON THE 646 bp SEQUENCE OF THE ISOLATE, SPECIFIC PRIMERS FOR DETECTING THE NEW VIRUS WERE DESIGNED FOR RT-PCR DETECTION of this human pneumonia-associated coronavirus genome in clinical samples. OF THE 44 NASOPHARYNGEAL SAMPLES AVAILABLE FROM THE 50 SARS PATIENTS, 22 HAD EVIDENCE OF HUMAN PNEUMONIA-ASSOCIATED CORONAVIRUS RNA. Viral RNA was detectable in ten of 18 faecal samples tested. The specificity of the RT-PCR reaction was confirmed by sequencing selected positive RT-PCR-amplified products. None of 40 nasopharyngeal and faecal samples from patients with unrelated diseases were reactive on RT-PCR."

"IF SEROPOSITIVITY TO HUMAN PNEUMONIA-ASSOCIATED CORONAVIRUS IN ONE SERUM SAMPLE OR VIRAL RNA DETECTION IN THE NASOPHARYNGEAL ASPIRATES OR STOOLS IS DEEMED EVIDENCE OF INFECTION WITH THE CORONAVIRUS, 45 of the 50 patients have evidence of infection. Of the five patients with no virological evidence of coronavirus infection, only one had a serum sample tested more than 14 days after onset of clinical disease."

"Discussion

The outbreak of SARS is unusual in several ways, especially in the appearance of clusters of patients with pneumonia in health-care workers and family contacts. In this series of patients, investigations for conventional pathogens of atypical pneumonia proved negative. However, a virus belonging to the family Coronaviridae was isolated from the lung biopsy and nasopharyngeal aspirate of TWO SARS PATIENTS and other patients with SARS had a SEROLOGICAL RESPONSE to this virus."

"Phylogenetically, human pneumonia-associated coronavirus was not closely related to any

known human or animal coronavirus or torovirus. WE BASED OUR ANALYSIS ON A 646 bp FRAGMENT OF THE POLYMERASE GENE which showed that the virus belongs to antigenic group 2 of the coronaviruses, along with murine hepatitis virus and bovine coronavirus."

"MOST PATIENTS who had clinically defined SARS had EITHER SEROLOGICAL OR RT-PCR EVIDENCE OF INFECTION by this virus."

"No evidence of human-metapneumovirus infection, by RT-PCR or rising antibody titre, was detected in any of our patients and no other pathogen was CONSISTENTLY detected. IT IS THEREFORE HIGHLY LIKELY THAT THIS CORONAVIRUS IS EITHER THE CAUSE OF SARS OR A NECESSARY PREREQUISITE FOR DISEASE PROGRESSION. WHETHER OTHER MICROBIAL OR NON-MICROBIAL COFACTORS PLAY A PART IN PROGRESSION OF THE DISEASES REMAINS TO BE INVESTIGATED."

"The EPIDEMIOLOGICAL DATA at present SEEM TO SUGGEST that the virus is spread by droplets or by direct and indirect contact, although airborne spread cannot be ruled out. The finding of infectious virus in the respiratory tract supports this contention. Preliminary evidence also suggests that the virus may be shed in the faeces. HOWEVER, DETECTION OF VIRAL RNA DOES NOT PROVE THAT THE VIRUS IS VIABLE OR TRANSMISSIBLE. If a viable virus is detectable in the faeces, this is potentially an additional route of transmission."

"We have provided evidence that a virus in the coronavirus family is the causal agent of SARS. HOWEVER IT REMAINS POSSIBLE THAT OTHER VIRUSES ACT AS OPPORTUNISTIC SECONDARY INVADERS TO INCREASE THE DISEASE PROGRESSION, A HYPOTHESIS THAT NEEDS TO BE INVESTIGATED FURTHER."

doi: 10.1016/s0140-6736(03)13077-2.

In Summary:

-a lung biopsy was only carried out on one patient

-they used samples from patients with OTHER DISEASES as a control

-they used numerous cell lines (Mardin Darby Canine Kidney, LLC-Mk2, RDE, Hep-2 and MRC-5 cells as well as fetal rhesus monkey and A-549 cells) to culture respiratory "viruses"

-after cell cultures and genetic sequences from TWO patients, they created an RT-PCR test to detect "virus" in others

-unpurified fetal rhesus Monkey kidney cell culture was used to determine a serological response

-to determine the genetic sequence for an unknown "virus," they did a RANDOM RT-PCR assay

-there were instances of other "pathogens" found in SARS cases such as E. Coli, Klebsiella Pneumoniae, and Haemophilus Influenzae

-"virus" was "isolated" (i.e. cultured) in fetal rhesus monkey kidney cells from the lung biopsy and nasopharyngeal aspirate from TWO patients

-CPE did not occur in the first passage but in subsequent ones

-EM images came from the unpurified rhesus monkey kidney cell culture supernatant

-they determined particles in the lung biopsy were similar in size and morphology to those that they found in the culture fluid and they decided they must be the same thing

-of 30 cloned samples from the random primer RT-PCR assay, ONE showed a 646 bp fragment of unknown origin

-this sequence had a WEAK HOMOLOGY to the "Coronaviridae" family

-DEDUCED amino acid sequence had the highest homology (only 57%) to "bovine coronavirus" and "murine Hepatitis virus"

-specific PCR primers were designed based on this 646 bp fragment from one cloned sample from one patient to detect the "virus" in others

-of the 44 patient samples tested, only 22 were positive for this fragment

-they ponder that if the INDIRECT methods of detecting seropositivity in ONE serum sample or detecting "viral" RNA in nasopharyngeal aspirates or feces can be used as evidence of infection, only then would 45 of the 50 patients be considered positive for the assumed "virus"

-again, "virus" was only "isolated" (i.e. cultured) from TWO patients while the rest were deemed

positive due to serological tests

-their analysis was solely based on the unknown 646 bp fragment of one cloned sample (out of 30)

-they state that no other pathogen was CONSISTENTLY detected so it must be this newly discovered "virus" which was never purified/isolated in this study nor proven pathogenic

-however, they state while it is "highly likely" that "SARS-COV-1" is EITHER the cause of SARS or a PREREQUISITE for more serious disease, ruling out other microbial or non-microbial co-factors had yet to be investigated

-they admit that finding "viral" RNA does not prove that the "virus" is viable or transmissible

-they end by stating a "Coronavirus" is the causal agent of SARS yet there could be other "viruses" acting as secondary invaders

In other words, the same small sample size, the same unpurified cell culture soup, the same unpurified EM images, the same useless serological tests, and the same assumptions/conclusions but this time with the further indirect PCR and genomic data added in to keep things fresh.

Sadly, still no properly purified/isolated "virus" coming directly from the samples of sick patients, still no proven pathogenicity, and still no proper controls.

Koch's Postulates

Next time anyone says that Koch's Postulates are outdated or that it is not necessary to fulfill them in order to prove a "virus" causes disease, they should read this press release from the WHO during the 2003 SARS "pandemic" which states otherwise:

"CONCLUSIVE IDENTIFICATION OF A CAUSATIVE MUST MEET ALL CRITERIA IN THE SO-CALLED "KOCH'S POSTULATE." The additional experiments NEEDED TO FULFILL THESE CRITERIA are currently under way at a laboratory in the Netherlands."

https://www.who.int/csr/don/2003_03_27b/en/

"WHO says coronavirus causes SARS

Relating to the earlier post about the WHO stating Koch's Postulates must be satisfied in order to prove a "virus" is the cause of disease:

"WHO says coronavirus causes SARS

THE ORGANIZATION CLAIMED THAT ITS COLLABORATING LABORATORIES HAVE CONCLUDED THE CORONAVIRUS MEETS ALL FOUR OF "KOCH'S POSTULATES" for a causative agent: it must be found in all cases of the disease, it must be isolated from the host and grown in pure culture, it must reproduce the original disease when introduced into a susceptible host, and it must be found in the experimental host so infected."

"ONE QUESTION STILL OPEN IS WHETHER THIS CORONAVIRUS IS TRULY THE ONLY VIRUS

NEEDED TO CREATE THE DISEASE'S MOST ACUTE SYMPTOMS IN HUMANS. But now the virus is fully sequenced, laboratories like Frank Plummer's at the National Microbiology Laboratory WHICH ONLY DETECTED MINUTE AMOUNTS OF CORONAVIRUS, IN ABOUT HALF THE CASES OF SARS, AND SOME VIRUS IN PEOPLE WITHOUT SARS, will soon have much more effective PCR probes to search with."

https://genomebiology.biomedcentral.com/.../gb-spotlight...

So according to the WHO, a new "Coronavirus" was claimed as the cause of SARS by fulfilling Koch's postulates yet there was still the question of if the "new virus" was the ONLY CAUSE.

Also, it was found by PCR in minute quantities in ONLY HALF of the SARS cases and also found in PEOPLE WITHOUT SARS.

I'm starting to seriously doubt that the WHO truly understands Koch's Postulates

Based on this, it immediately fails Koch's first Postulate:

1. The microorganism must be found IN ABUNDANCE IN ALL ORGANISMS SUFFERING FROM THE DISEASE, but should NOT BE FOUND IN HEALTHY ORGANISMS.

THOMAS RIVERS 1937 REVISION OF KOCH'S POSTULATES:

By 1937, it was clear that virologists were unable to satisfy any of Koch's Postulates in order to prove invisible particles assumed to be "viruses" existed and could cause disease. Even Robert Koch himself had difficulty with his own Postulates which led him to wiggle around some of them in attempts to prove pathogenicity of certain bacteria. Instead of accepting that the Postulates, as originally stated, worked and disproved the Germ Theory, virologists looked to various indirect immunological methods to prove their claims.

This led to Thomas River's own attempts to water down Koch's Postulates by revising them to allow virologists even more wiggle room and expanding the 4 Postulates to 6. Unfortunately for all of Virology, Koch trapped them in a logical prison that they can not free themselves from. If they deny Koch's original Postulates, they are denying logic itself. Highlights from River's attempted revision below:

VIRUSES AND KOCH'S POSTULATES

"Even at the present time, THE CAUSE OF CERTAIN DISEASES IS SAID BY SOME INDIVIDUALS TO BE UNKNOWN OR UNDISCOVERED, because no cultivable bacteria or visible protozoan parasite of etiological significance has been demonstrated in them. For instance, A FEW YEARS AGO COWIE MADE THE STATEMENT IN A SCIENTIFIC PAPER THAT THE ETIOLOGICAL AGENT OF POLIOMYELITIS IS UNKNOWN, and in the recent book, An American Doctor's Odyssey, HEISER REMARKED THAT "THE MICROBE WHICH CAUSES SMALLPOX HAS NEVER BEEN DISCOVERED."

"Microorganisms were known to exist long before their relation to disease was appreciated. After the discovery of this relation

IT WAS NOT UNCOMMON FOR MORE THAN ONE KIND OF ORGANISM TO BE ACCREDITED WITH THE ABILITY OF PRODUCING THE SAME MALADY."

"The above conditions laid down for the proof of the etiological relation of a microorganism to a disease constitute what are now known as Koch's postulates. His dictum has had a profound influence on workers investigating infectious maladies and FOR MANY YEARS AN INFECTIOUS AGENT WAS NOT ACCEPTED AS THE CAUSE OF A DISEASE UNLESS THE POSTULATES HAD BEEN SATISFIED. With the development of the science of immunology, however, immunological reactions added much to the knowledge of the specific relation of microbes to disease, and now IT IS POSSIBLE TO BRING EXCELLENT EVIDENCE THAT AN ORGANISM IS THE CAUSE OF A MALADY WITHOUT THE COMPLETE SATISFACTION OF THE POSTULATES. In spite of this fact, THERE ARE CERTAIN WORKERS WHO STILL REFUSE TO AGREE THAT THE CAUSE OF AN INFECTIOUS DISEASE HAS BEEN DISCOVERED UNLESS ALL THE CONDITIONS ORIGINALLY LAID DOWN BY KOCH HAVE BEEN MET. This is particularly true regarding the viral maladies, the etiological agents of which

have not been cultivated on ordinary lifeless media."

"IT IS OBVIOUS THAT KOCH'S POSTULATES HAVE NOT BEEN SATISFIED IN VIRAL DISEASES. Moreover, it is equally evident that proof of the etiological significance of viruses has been obtained without their satisfaction. Such a statement, however, does not imply that certain conditions do not have to be met before the specific relation of a virus to a disease is established. The conditions are: (a) A specific virus must be found associated with a disease with a degree of regularity. (b) The virus must be shown to occur in the sick individual not as an incidental or accidental finding but as the cause of the disease under investigation.

IN MANY RESPECTS THE CONDITIONS JUST STATED FOR VIRAL MALADIES ARE SIMILAR TO THOSE OF KOCH for the proof of the specific relation of bacteria to disease. Nevertheless, THERE ARE CERTAIN DIFFERENCES. In the first place, IT IS NOT OBLIGATORY TO DEMONSTRATE THE PRESENCE OF A VIRUS IN EVERY CASE OF THE DISEASE PRODUCED BY IT. Secondly, the EXISTENCE OF VIRUS CARRIERS is recognized. Finally, IT IS NOT ESSENTIAL THAT A VIRUS BE GROWN ON LIFELESS MEDIA OR IN MODIFIED TISSUES CULTURES.

How does one go about proving that a virus is the cause of a disease? Viruses, regardless of whether they are parasites OR THE FABRICATIONS OF AUTOCATALYTIC PROCESSES, are intimately associated with host cells and, therefore, should always be found at the proper time in specific lesions. In addition, viruses, as is the case with bacteria, may be found also in the blood stream, not necessarily multiplying there but appearing frequently only as a phenomenon of overflow from lesions in the tissues. With these facts in mind, tissues with lesions, exudate from such lesions, and blood are collected aseptically and inoculated into a susceptible experimental host of the same or different species. THE MATERIAL SHOULD BE FREE FROM ORDINARY MICROBES; IF NOT, THE MICROBES SHOULD BE KILLED OR REMOVED IN A PROPER MANNER, e.g., BY FILTRATION. If the inoculated animals become sick or die in a characteristic manner, and, if the disease in them can be transmitted from animal to animal by MEANS OF INOCULATIONS WITH BLOOD OR EMULSIONS OF INVOLVED TISSUES free from ordinary microbes or rickettsiae, ONE IS FAIRLY CONFIDENT THAT THE MALADY IN THE EXPERIMENTAL ANIMALS IS INDUCED BY A VIRUS. On the other hand, SUCH FINDINGS DO NOT NECESSARILY INDICATE THAT THE ACTIVE AGENT WAS PRESENT IN THE ORIGINAL MATERIAL USED FOR INOCULATION OF EXPERIMENTAL HOSTS.

When a natural disease under investigation exhibits characteristic features, e.g., paralysis or

intracellular inclusions, they are sought for in the experimental malady. IF ONE FINDS THEM,

ONE IS ENCOURAGED, BUT PROOF IS STILL LACKING THAT THE VIRUS OPERATING IN THE EXPERIMENTAL HOSTS WAS PRESENT IN THE MATERIAL TAKEN FROM THE INDIVIDUAL WITH THE NATURAL INFECTION. Not infrequently several viruses produce the same clinical and pathological pictures, and at times the same virus does not induce similar changes in different hosts. Consequently, regardless of the disease picture produced in the experimental animals, ONE IS STILL FACED WITH THE PROBLEM OF DEMONSTRATING THAT THE VIRUS CAUSING IT WAS PRESENT IN THE MATERIAL USED FOR INOCULATION OF THE FIRST GROUP OF ANIMALS."

"If a virus is the actual cause of a disease, immune substances are usually absent from the patients' serum at the onset of illness

and make their appearance during the period of recovery. HOWEVER, THIS IS NOT UNIVERSALLY TRUE, INASMUCH AS RECOVERY SOMETIMES TAKES PLACE WITHOUT THE DEVELOPMENT OF ANTIBODIES, AND OCCASIONALLY AN INDIVIDUAL POSSESSING ANTIBODIES AGAINST A VIRUS SUCCUMBS TO A DISEASE CAUSED BY IT.

ALTHOUGH THE ABSENCE OF ANTIBODIES FOR A VIRUS AT THE ONSET OF AN ILLNESS AND THEIR APPEARANCE LATER in the course of the disease or during convalescence CONSTITUTE HIGHLY SUGGESTIVE EVIDENCE that the virus is responsible for the malady, THEY ALONE SHOULD NOT BE ACCEPTED AS INCONTROVERTIBLE PROOF THAT SUCH IS THE CASE."

"To summarize, IT CAN BE SAID THAT THE CAUSE OF VIRAL DISEASES IS KNOWN AND THAT KOCH'S POSTULATES AS PROPOSED BY HIM DO NOT HAVE TO BE FULFILLED IN ORDER TO PROVE THAT A VIRUS IS THE CAUSE OF A DISEASE. However, THE SPIRIT OF HIS RULES OF PROOF STILL HOLDS in that a worker must demonstrate that a virus is not only associated with a disease but that it is actually the cause. THE METHODS OF DOING THIS ARE DIFFERENT FROM THE ONES USED BY KOCH BUT ARE EQUALLY EFFICIENT. At the present time, this is accomplished by the production with A DEGREE OF REGULARITY of a transmissible infection in susceptible experimental hosts by means of inoculation of material, free from ordinary microbes or rickettsiae, obtained from patients with the natural disease, and by the demonstration THROUGH THE USE OF PROPER CONTROLS and immunological studies described above THAT

THE VIRUS WAS NEITHER FORTUITOUSLY PRESENT IN THE PATIENTS NOR ACCIDENTALLY PICKED UP IN THE EXPERIMENTAL ANIMALS."

https://jb.asm.org/content/jb/33/1/1.full.pdf

In Summary:

-there were people in the field who disagreed that "viruses" were the true cause of disease

-Cowie made the claim that the agent responsible for Polio had yet to be discovered

-Heiser remarked that a microbe causing Smallpox had never been discovered

-it was not uncommon for more than one microbe/organism to be claimed as the cause of the same disease

-for many years, it was accepted that Koch's Postulates must be fulfilled in order to prove an infectious agent causes disease

-Rivers states it is possible to provide evidence an agent causes disease without fulfilling all of Koch's Postulates yet admits many are unwilling to accept this evidence without the Postulates being fulfilled

-Rivers admits that it is obvious Koch's Postulates have not been satisfied for viral diseases

-Rivers proposes his own revisions and states while they are similar to Koch's Postulates, they are different

-Rivers allows for the "virus" not to be found in every case of disease

-Rivers introduces the concept of "virus" carriers

-Rivers states that "viruses" do not need to be grown in culture

-Rivers admits that "viruses" could be fabrications of an autocatalytic process

-he says that the sample must be free of other microbes and if not, they should be removed or killed by filtration

-he believes that if an animal becomes sick or dies from inoculation of blood/emulsified tissues and then their blood/emulsified tissues produces disease/death in further inoculated animals, one can be FAIRLY CONFIDENT a "virus" is present

-however, he says that this does not necessarily mean the agent that produced disease/death in the inoculated animals was the same as what was in the original sample

-Rivers admits that even if they can reproduce the characteristics of a disease by experimentation in a lab, that still is not proof that what caused disease in the experiment was in the original sample from the sick patient or animal

-he states it is a problem to show that what caused illness in subsequent animal inoculations was in the original inoculated animal

-part of Rivers revisions stem from using immune response experiments as proof yet he admits that the presence/absence of antibodies is not universal and that people can recover without antibodies and can succumb to disease even with antibodies

-he admits the the presence/absence of antibodies alone is not incontrovertible proof of a "virus"

-Rivers concludes that "viruses" have been proven and that Koch's Postulates do not need to be fulfilled as originally proposed by him

-Rivers states that his revisions still hold the spirit of Koch's Postulates

-Rivers admits his Postulates are different than Koch's based on the methods used

These are just a few highlights and I highly recommend reading the full 12 pages as there are many interesting admissions I unfortunately had to leave out. It is clear that Rivers revisions of Koch's Postulates are watered-down to make life easier for virologists to skirt around logic. Anyone claiming that they fulfilled Koch's Postulates by using the criteria laid down by Rivers are outright lying and being intentionally fraudulent...which in all honesty, sums up Virology to a T.

DROSTEN 2003 SARS-COV-1 PAPER:

Christian Drosten, a German Virologist who helped to create the problematic SARS-COV-2 PCR test without ever actually seeing a "virus" whilst having his paper and test quickly and suspiciously peer-reviewed and approved in just 24 hours, was also heavily involved in the original SARS-COV-1 "discovery" process. Below are some highlights from this paper:

IDENTIFICATION OF A NOVEL CORONAVIRUS IN PATIENTS WITH SEVERE ACUTE RESPIRATORY SYNDROME

"Methods

Clinical specimens from patients with SARS were SEARCHED FOR UNKNOWN VIRUSES WITH THE USE OF CELL CULTURES AND MOLECULAR TECHNIQUES.

Results

A novel coronavirus was identified in patients with SARS. THE VIRUS WAS ISOLATED IN CELL CULTURE, and a sequence 300 nucleotides in length was obtained by a polymerase-chain reaction (PCR)–based RANDOM-AMPLIFICATION PROCEDURE. Genetic characterization indicated that the virus is ONLY DISTANTLY RELATED TO KNOWN CORONAVIRUSES (IDENTICAL IN 50 TO 60 PERCENT OF THE NUCLEOTIDE SEQUENCES). On the basis of the obtained sequence, conventional and real-time PCR assays for specific and sensitive detection of the novel virus were established. Virus was detected in a variety of clinical specimens from patients with SARS but not in controls. High concentrations of viral RNA of up to 100 million molecules per milliliter were found in sputum. Viral RNA was also detected at extremely low concentrations in plasma during the acute phase and in feces during the late convalescent phase. Infected patients showed seroconversion on the Vero cells in which the virus was isolated.

Conclusions

The novel coronavirus MIGHT HAVE A ROLE in causing SARS."

"Microbiologic testing for common pathogens A large number of tests for known respiratory pathogens were performed with specimens from all three patients in Frankfurt. The test results were negative, except as follows.

PARAMYXOVIRUS-LIKE PARTICLES WERE SEEN IN THROAT SWABS AND SPUTUM SAMPLES FROM THE INDEX PATIENT BY ELECTRON MICROSCOPY. The particles were scarce. However, several PCR tests specific for virus species of the family Paramyxoviridae were negative (including tests for human metapneumovirus), as were PCR assays based on primers designed to react broadly with all members of that family.

C. pneumoniae was not detected by PCR or antigen ELISA in sputum of the index patient from day 9. However, on day 11, ELECTRON MICROSCOPY OF CELLS IN A BRONCHOALVEOLAR-LAVAGE SPECIMEN FROM THE INDEX PATIENT SHOWED A SEVERE INTRACELLULAR BACTERIAL INFECTION, and the bronchoalveolar-lavage cells reacted in immunofluorescence analyses with a monoclonal antibody DIRECTED AGAINST C. PNEUMONIAE. Consistent with this finding, there was an increase by a factor of four in the C. pneumoniae IgA titer in the index patient between day 10 and day 13."

"Isolation and characterization of a novel coronavirus

AFTER SIX DAYS OF INCUBATION (on March 21), A CYTOPATHIC EFFECT WAS SEEN ON VERO-CELL CULTURES INOCULATED WITH SPUTUM OBTAINED FROM THE INDEX PATIENT on day 7. Twenty-four hours after a single passage, nucleic acids were purified from the supernatant. RANDOM AMPLIFICATION WAS PERFORMED WITH 15 DIFFERENT PCRs under low-stringency conditions. WE HAD PREVIOUSLY SHOWN THAT THIS METHOD IS ABLE TO DETECT UNKNOWN PATHOGENS GROWING IN CELL CULTURE (UNPUBLISHED DATA). To detect RNA viruses, an initial reverse-transcription step was included."

"The principal finding of the study is the identification of a novel coronavirus in patients with SARS. IT APPEARS THAT PATIENTS WITH SARS ARE ACUTELY INFECTED WITH THIS VIRUS, SINCE

THEY HAVE VIRUS-SPECIFIC IgG SEROCONVERSION. The high rate of positivity among patients WITH PROBABLE CASES during an outbreak of SARS in Hanoi, in conjunction with the complete negativity among all healthy contacts of patients affected by the same outbreak, provides evidence OF AN ASSOCIATION BETWEEN THE DISEASE AND THE PRESENCE OF THIS NOVEL VIRUS. The involvement of a coronavirus in a respiratory disease would not be without precedent: the two human coronaviruses are known to cause mild respiratory illness.9 ONE SHOULD BEAR IN MIND, HOWEVER, THAT IN THE PAST, VIRUSES HAVE BEEN INITIALLY ISOLATED FROM PATIENTS WITH A SPECIFIC DISEASE BUT SUBSEQUENT INVESTIGATIONS REVEALED NO ACTUAL ASSOCIATION AT ALL.10,11 Thus, LARGER STUDIES WITH APPROPRIATE CONTROL GROUPS ARE NEEDED TO VERIFY OR ELIMINATE OUR HYPOTHESIS ABOUT THE CAUSE OF SARS. The assays that have been established provide an excellent tool for such studies. It should also be taken into account that antigen that was present in primary cultures was used to detect the antibody response, and IT REMAINS TO BE FIRMLY ESTABLISHED THAT THIS RESPONSE IS INDEED DIRECTED AGAINST THE NOVEL CORONAVIRUS RATHER THAN AGAINST AN UNKNOWN AGENT THAT MIGHT HAVE BEEN ISOLATED SIMULTANEOUSLY. This possibility could be tested with the use of plaque-purified virus or recombinant proteins as antigen.

By testing for a broad range of known pathogens, WE ALSO OBTAINED EVIDENCE FOR INFECTION WITH PARAMYXOVIRUSES AND C. PNEUMONIAE. Paramyxoviruses —

In particular, human metapneumovirus, which was previously implicated in SARS12 — could be largely ruled out by further investigation. INFECTION WITH CHLAMYDIA WAS CONFIRMED IN SEVERAL ASSAYS. However, chlamydia was not found in other patients with SARS.3 Hence, IT REMAINS UNCLEAR WHETHER THESE PATHOGENS HAVE A ROLE AS CAUSATIVE FACTORS OR COFACTORS IN SARS."

doi: 10.1056/NEJMoa030747

In Summary:

-the aim of study was to detect unknown "viruses" by way of indirect methods such as cell culture and molecular techniques

-they claim the "virus" was "isolated" in cell culture which is the exact opposite of isolation due to the adding/mixing of various foreign animal DNA, chemicals, nutrients, antibiotics, etc. with the sample

-they utilized a RANDOM-amplification PCR procedure to detect sequence of the "virus" in cell culture fluids

-the "virus" was considered to be DISTANTLY related to known "Coronaviruses" (only 50-60% identical in nucleotide sequences)

-they created their own PCR tests to detect parts of this sequence in SARS patients

-they determine that the "virus" MIGHT HAVE A ROLE in causing SARS

-Paramyxovirus-like particles and C. Pneumoniae were also found in samples

-CPE was seen after 7 days of incubation

-random amplification of culture fluid by PCR was performed to detect a "novel virus"

-they state that this method was shown to be able to detect unknown pathogens in UNPUBLISHED RESEARCH

(Quick side note. This is from a study done in 2017, fourteen years after Drosten's paper stated they could detect unknown pathogens by PCR. This paper, along with examples from previous studies, states it is impossible:

"However, EXTRACTION OF VIRAL RNA DIRECTLY FROM CULTURE OFTEN YIELDS VIRAL RNA WITH HIGH HOST RNA BACKGROUND (Marston et al. 2013)."

"Similarly, Cowan et al. (2005) noted that PCR-based enrichment techniques where A PRIORI KNOWLEDGE OF TARGET SEQUENCES IS REQUIRED FOR PCR PRIMER DESIGN RENDER THE ENRICHMENT STRATEGIES INEFFECTIVE IN THE CHARACTERIZATION OF NOVEL VIRUSES. It follows that the SAME SEQUENCE AMBIGUITY IN VIRAL GENOMES WOULD POSE A PROBLEM FOR USING CUSTOMIZED SEQUENCING ADAPTERS DURING DIRECT RNA SEQUENCING."

https://amb-express.springeropen.com/articles/10.1186/s13568-019-0772-y

Maybe this is why Drosten's data proving his method went UNPUBLISHED...but I digress...)

-they assume patients with SARS are infected due to "virus-specific" seroconversion which is impossible to determine without a purified/isolated "virus"

-they admit that "viruses" have been "isolated" in the past which were believed to be the cause of disease and turned out not to be so

-they admit LARGER studies with APPROPRIATE CONTROLS need to be done in order to verify or eliminate their HYPOTHESIS

-they admit that there needs to be confirmation that the antibody response needs to be confirmed as being due to this new "coronavirus" rather than some unknown agent which may have been "isolated" along with it

-since they also "isolated" Paramyxoviruses, C. Pneumoniae, and Chlamydia from SARS patients, it remains unclear whether these also play a role in SARS

Note that once again there was no properly purified/isolated "virus" taken directly from a sick patient nor proven pathogenic. There were no accompanying EM images as they used an indirect PCR method to sequence an unknown "virus." The only evidence any new "virus" exists at all from this paper comes from random letters in a computer database.

(Below image is not from this study.)

POUTANEN 2003 SARS-COV-1 PAPER:

This is one of the four papers used as evidence that SARS-COV-1 fulfilled Koch's Postulates. It fails miserably as there is no properly purified/isolated "virus" taken directly from a sick patient

nor is there any proof of pathogenicity in this paper. On top of that, they do not determine if it is a new "coronavirus," a metapneumovirus, both of them, or some other unknown pathogen causing SARS. Highlights from the study below:

IDENTIFICATION OF SEVERE ACUTE RESPIRATORY SYNDROME IN CANADA

"The results of laboratory investigations were negative or not clinically significant EXCEPT FOR THE AMPLIFICATION OF HUMAN METAPNEUMOVIRUS FROM RESPIRATORY SPECIMENS FROM FIVE OF NINE PATIENTS and the isolation and amplification of a novel coronavirus from five of nine patients. IN FOUR CASES BOTH PATHOGENS WERE ISOLATED."

"CONCLUSIONS

SARS is a condition associated with substantial morbidity and mortality. It appears to be of viral origin, with patterns suggesting droplet or contact transmission. THE ROLE OF HUMAN METAPNEUMOVIRUS, A NOVEL CORONAVIRUS, OR BOTH REQUIRES FURTHER INVESTIGATION."

"Further virologic studies were completed on all respiratory specimens received from 9 of the 10 patients. THESE INCLUDED VIRAL CULTURES (INCLUDING INOCULATION ONTO CELL CULTURE AND INTO EMBRYONATED HEN EGGS AND INTRACEREBRAL INOCULATION OF SUCKLING MICE), immune electron microscopy of nasopharyngeal swabs and bronchoalveolar fluids with serum obtained during the convalescent phase from Patient 10, RT-PCR for conserved portions of the polymerase gene of RNA viruses, and nested RT-PCR with genus-specific degenerative primers for paramyxoviruses and bunyaviruses. Results for all of these tests have been negative, with two exceptions. HUMAN METAPNEUMOVIRUS WAS AMPLIFIED by nested RT-PCR from bronchoalveolar-lavage fluid and nasopharyngeal swabs FROM FIVE OF NINE PATIENTS WITH SARS AND FROM A NASOPHARYNGEAL SWAB FROM AN ASYMPTOMATIC CONTACT OF ONE OF THE PATIENTS in Toronto (Patient 3) with use of the following primer pair: 5'CTTTGGACTTAATGACAGATG3' and 5'GTCTTCCTGTGCTAACTTTG3'.4 For confirmation of these positive findings, the amplicons were sequenced and found to be unique, ruling out the possibility of cross-contamination in the laboratory.

In addition, A NOVEL CORONAVIRUS WAS ISOLATED FROM VERO CELL CULTURES INOCULATED WITH RESPIRATORY SPECIMENS FROM FIVE OF NINE PATIENTS WITH SARS. FOUR OF THESE PATIENTS HAD SPECIMENS FROM WHICH METAPNEUMOVIRUS WAS ALSO IDENTIFIED. A

cytopathic effect on the Vero cell cultures was noted on day 6 of incubation. On the basis of collaboration with investigators in Hong Kong and at the Centers for Disease Control and Prevention (CDC) in Atlanta, who reported isolating a novel coronavirus from patients with SARS in other areas of the world, RT-PCR was completed targeting conserved regions of the coronavirus polymerase gene using the following primer pair: 5'CAGAGCCATGCCTAACATG3' and 5'AATGTTTACGCAGGTAAGCG3'. A novel coronavirus identical to that reported by the CDC2 was amplified from all five cultures. In addition, nested RT-PCR using the same primers plus 5'TGTTAAACCAGGTGGAAC3' and 5'CCTGTGTTGTAGATTGCG3' amplified the coronavirus directly from bronchoalveolar-lavage fluid FROM THREE OF NINE PATIENTS TESTED, ALL OF WHOM ALSO HAD CORONAVIRUS ISOLATED FROM CELL CULTURE as described above."

"At a different laboratory, a coronavirus was also identified independently by amplification directly from bronchoalveolar-lavage fluid FROM THREE OF SIX PATIENTS TESTED. All three of these patients had coronavirus ISOLATED FROM CELL CULTURE and amplification as described above."

"FURTHER STUDIES ARE CURRENTLY BEING COMPLETED TO HELP DETERMINE WHETHER THE HUMAN METAPNEUMOVIRUS AND A NOVEL CORONAVIRUS, EITHER ALONE OR IN COMBINATION, ARE THE CAUSE OF SARS OR WHETHER OTHER THUS FAR UNDETECTED PATHOGENS ARE POSSIBLY RESPONSIBLE. The possibility that coinfection of either virus with another agent may be responsible for SARS cannot be excluded."

"Epidemiologic investigations and laboratory studies SUGGEST THAT MOST PATIENTS with disease meeting the definition of SARS in both Toronto and Vancouver CAN BE LINKED TO A COMMON SOURCE AND TO COMMON POTENTIAL CAUSATIVE AGENTS. On the basis of preliminary investigations, it appears that this syndrome MAY BE DUE IN PART TO THE NEWLY DESCRIBED RESPIRATORY VIRAL PATHOGEN, HUMAN METAPNEUMOVIRUS,6 TO A NOVEL CORONAVIRUS, OR BOTH.

EVIDENCE OF THE ROLE OF HUMAN METAPNEUMOVIRUS INCLUDES ITS AMPLIFICATION FROM RESPIRATORY SPECIMENS FROM FIVE OF NINE CANADIAN PATIENTS WITH SARS AND ONE ASYMPTOMATIC CONTACT AND THE IDENTIFICATIONS OF A METAPNEUMOVIRUS FROM RESPIRATORY SPECIMENS FROM OTHER NON-CANADIAN PATIENTS WITH SARS (Tam J, Department of Microbiology, Chinese University of Hong Kong: personal communication). In addition, THE RANGE OF CLINICAL FINDINGS, from asymptomatic disease to severe pneumonia

and death, IS SIMILAR TO THAT DESCRIBED IN HUMAN METAPNEUMOVIRUS INFECTION.7 On the other hand, the severity with which the Canadian cases of SARS presented and the high attack rate of SARS among close contacts have not been described in patients with human metapneumovirus infection, suggesting that human metapneumovirus alone may not be responsible for SARS, that a genetic variant of the human metapneumovirus is potentially responsible, or that human metapneumovirus is not related to SARS but is an incidental finding. Indeed, we know little about the prevalence of asymptomatic carriage of human metapneumovirus, and such information would be helpful in interpreting the meaning of our amplification of this virus in patients meeting the criteria for SARS.8,9

THE NOVEL CORONAVIRUS IDENTIFIED IN FIVE OF NINE CANADIAN CASES MAY ALSO BE A POSSIBLE CAUSATIVE AGENT OF SARS. Further evidence includes its identification by other investigators around the world from specimens from other patients with SARS and reports of positive immunofluorescence antibody tests in serum from patients from whom the coronavirus was isolated.2 In addition, known human coronaviruses are recognized to cause respiratory infection, albeit typically less severe than that described in the Canadian patients with SARS.10 Finally, coronaviruses are known to infect both animals and humans, and it is logical to consider that the emergence of a new disease may be related to the emergence of a novel coronavirus that originated with a limited range of animal hosts and evolved to involve an altered range that now includes humans. ALTHOUGH ONE CAN SPECULATE ABOUT THE POSSIBLE ROLES OF BOTH CORONAVIRUSES AND HUMAN METAPNEUMOVIRUS IN SARS, IT IS CURRENTLY NOT CLEAR WHAT ROLE, IF ANY, EITHER OF THESE VIRUSES HAS IN CAUSING SARS. FURTHER COLLABORATIVE INVESTIGATIONS ARE NEEDED."

"THE MECHANISM OF TRANSMISSION OF THE AGENT OR AGENTS CAUSING SARS IS NOT YET UNDERSTOOD."

https://www.nejm.org/doi/full/10.1056/NEJMoa030634

In Summary:

-a metapneumovirus was "isolated" in 5 out of 9 SARS patients as well as from one asymptomatic contact

-"SARS-COV-1" was also only "isolated" from 5 out of 9 patients

-in 4 out of 9 cases, both the metapneumovirus and "SARS-COV-1 were "isolated"

-they concluded that the metapneumovirus, the new "Coronavirus," OR BOTH need to be investigated further

-cell cultures and inoculation into embryonated hen eggs and intracerebral inoculation into suckling mice brains were carried out

-the "novel coronavirus" was cultured in Vero cells yet the materials used beyond that are undefined

-RT-PCR was only able to detect "SARS-COV-1" in 3 out of 9 patients

-at an independent lab, only 3 of 6 patients had "SARS-COV-1 RNA detected by PCR

-they state further studies are under way to determine if it is the metapneumovirus, "SARS-COV-1," a combo of the 2, or some other unknown pathogen causing SARS

-coinfection with either "virus" and another unknown agent could not be excluded

-evidence SUGGESTS that most patients with SARS could be linked to a common source and to common POTENTIAL causative agents

-beyond this study, metapneumoviruses were "isolated" from non-Canadians with SARS

-the range in clinical symptoms in SARS is similar to metapneumovirus infection

-they determine that it is not clear what role, IF ANY, that the "new Coronavirus" or the metapneumovirus may have in relation to SARS

-they state further collaborative investigations are needed

-the method of transmission for the agent or agents causing SARS is not yet understood

It is clear upon reading this study that not only do they not prove "SARS-COV-1" as the cause of SARS, they make an even better case that it could be metapneumovirus. Even then, they state that they can not conclude whether it is the new "Coronavirus," the metapneumovirus, both, neither, or some as of yet undiscovered agent that could be the cause of SARS. If there is one

thing made clear from this study, they were unable to determine anything at all.

KSIAZEK 2003 SARS-COV-1 PAPER:

This is another of the four papers used to say that Koch's Postulates, or at least some of them, were fulfilled for "SARS-COV-1." Once again, they fail at even fulfilling the first two Postulates. The "virus" is not found in all cases of SARS nor is it ever properly purified/isolated directly from a sick host nor proven pathogenic. They use the same unpurified cell cultures and do not disclose the materials used during the culturing process.This study does try to implement many indirect serological and molecular techniques to prove a causative relationship between a novel "Coronavirus" and SARS, but these techniques (such as RT-PCR, immunohistochemical and immunofluorescence staining, as well as Indirect fluorescence antibody tests and enzyme-linked immunosorbent assays) are not requirements of Koch's Postulates nor are they reliable measures to identify a "novel virus." Highlights from the study below:

A NOVEL CORONAVIRUS ASSOCIATED WITH SEVERE ACUTE RESPIRATORY SYNDROME

"RESULTS

None of the previously described respiratory pathogens were consistently identified. However, a novel coronavirus was isolated from patients who met the case definition of SARS. CYTOPATHOLOGICAL FEATURES WERE NOTED IN VERO E6 CELLS INOCULATED WITH A THROAT-SWAB SPECIMEN. Electron-microscopical examination revealed ultrastructural features characteristic of coronaviruses. Immunohistochemical and immunofluorescence staining revealed reactivity with group I coronavirus polyclonal antibodies. CONSENSUS CORONAVIRUS PRIMERS DESIGNED TO AMPLIFY A FRAGMENT OF THE POLYMERASE GENE by reverse transcription–polymerase chain reaction (RT-PCR) were used to obtain a sequence that clearly identified the isolate as a unique coronavirus ONLY DISTANTLY RELATED TO PREVIOUSLY SEQUENCED CORONAVIRUSES. With specific diagnostic RT-PCR primers we identified several identical nucleotide sequences in 12 patients from several locations, a finding consistent with a point-source outbreak. Indirect fluorescence antibody tests and enzyme-linked immunosorbent assays made with the new isolate have been used to demonstrate a virus-specific serologic response. This virus may never before have circulated in the U.S. population."

"Methods

GENERAL APPROACH

THE NONSPECIFIC NATURE OF THE CLINICAL PRESENTATION OF PATIENTS WITH SARS and the urgency of finding a cause required that clinical specimens be tested rapidly for a broad range of viral, bacterial, chlamydial, and rickettsial agents (the CDC case definition of SARS is available as Supplementary Appendix 1 with the full text of this article at http://www.nejm.org). Laboratory testing focused foremost on known respiratory pathogens, especially those that might specifically target the lower respiratory tract through the progression of disease. A combination of traditional methods was applied, INCLUDING VIRUS ISOLATION IN SUCKLING MICE AND CELL CULTURE, electron microscopy, histopathological examination, serologic analysis, and general and specialized bacterial culture techniques. The molecular techniques of polymerase chain reaction (PCR), reverse-transcription PCR (RT-PCR), and real-time PCR were used. Priority was given to testing for the following agents: yersinia, mycoplasma, chlamydia, legionella, Coxiella burnetii, spotted fever and typhus group rickettsiae, influenza viruses A and B, Paramyxovirinae and Pneumovirinae subfamily viruses (specifically, human respiratory syncytial virus and human metapneumovirus), Mastadenoviridae, Herpetoviridae, Picornaviridae, Old and New World hantaviruses, and Old World arenaviruses."

"ISOLATION OF VIRUS

TO IDENTIFY VIRUSES ASSOCIATED WITH SARS, WE INOCULATED A VARIETY OF CLINICAL SPECIMENS (blood, serum, material from oropharyngeal swabs or washings, material from nasopharyngeal swabs, and tissues of major organs collected at autopsy) ONTO A NUMBER OF CONTINUOUS CELL LINES, including Vero E6, NCI-H292, MDCK, LLC-MK2, and B95-8 cells, AND INTO SUCKLING ICR MICE BY THE INTRACRANIAL AND INTRAPERITONEAL ROUTES. All cultures were observed daily for cytopathic effect. MAINTENANCE MEDIUM WAS REPLENISHED AT DAY 7, and cultures were terminated 14 days after inoculation. ANY CULTURES EXHIBITING IDENTIFIABLE CYTOPATHIC EFFECT WERE SUBJECTED TO SEVERAL PROCEDURES TO IDENTIFY THE CAUSE OF THE EFFECT. Suckling mice were observed daily for 14 days, and WE FURTHER TESTED ANY SICK OR DEAD MICE BY PREPARING A BRAIN SUSPENSION THAT WAS FILTERED AND SUBCULTURED. Mice that remained well after 14 days were euthanized, and their test results were recorded as negative. TISSUE-CULTURE SAMPLES SHOWING CYTOPATHIC EFFECT WERE PREPARED FOR ELECTRON-MICROSCOPICAL EXAMINATION. Negative-stain electron-microscopical specimens were prepared by drying culture supernatant, mixed 1:1 with 2.5 percent paraformaldehyde, onto Formvar Carbon-coated grids and staining with 2 percent methylamine tungstate. Thin-section electron-microscopical specimens were prepared by fixing

a washed cell pellet with 2.5 percent glutaraldehyde and embedding it in epoxy resin. FOR RT-PCR ASSAYS, CELL-CULTURE SUPERNATANTS WERE PLACED IN LYSIS BUFFER. In addition, a master seed was prepared from the remaining culture supernatant and cells by freeze-thawing the culture flask, clarifying the thawed contents by centrifugation at 1000×g,and dispensing the supernatant into aliquots stored in gas phase over liquid nitrogen. The master seed was subcultured into 850-cm2roller bottles of Vero E6 cells for the preparation of formalin-fixed positive control cells for immunohistochemical analysis, mixed with normal E6 cells, and gamma-irradiated for preparation of spot slides for indirect fluorescence antibody tests or extracted with detergent and gamma-irradiated for use as an enzyme-linked immunosorbent assay (ELISA) antigen for antibody tests."

"We used the following antibody and tissue controls: SERUM SPECIMENS FROM NON INFECTED ANIMALS, VARIOUS CORONAVIRUS-INFECTED CELL CULTURES AND ANIMAL TISSUES, NONINFECTED CELL CULTURES, and NORMAL HUMAN AND ANIMAL TISSUES."

"TWO CELL LINES, VERO E6 CELLS AND NCI-H292 CELLS, inoculated with oropharyngeal specimens from Patient 16 (a 46-year-old male physician with an epidemiologic link to a hospital with multiple patients with SARS) initially showed cytopathic effect (Table 1). Blood, nasopharyngeal, and throat-swab specimens were collected on March 12, day 1 after onset. At that time, the patient's physical examination was normal except for fever and shortness of breath. During the course of the disease, his status worsened, and he died. A RHINOVIRUS WAS ISOLATED FROM THE INOCULATED NCI-H292 CELLS. Further study suggested that this virus was not associated with patients with SARS, so it will not be discussed here."

"EXAMINATION OF CYTOPATHIC-EFFECT–POSITIVE VERO E6 CELLS by thin-section electron microscopy revealed CHARACTERISTIC CORONAVIRUS PARTICLES within the cisternae of the rough endoplasmic reticulum and in vesicles (Figure 2A).14,15 EXTRACELLULAR PARTICLES WERE FOUND IN LARGE CLUSTERS and adhering to the surface of the plasma membrane. Negative-stain electron microscopy identified coronavirus particles, 80 to 140 nm in diameter, with 20-to-40-nm complex surface projections surrounding the periphery (Figure 2B). Hemagglutinin esterase-type glycoprotein projections were not seen."

"Lung tissues were obtained at autopsy from three patients and by open-lung biopsy in one patient, 14 to 19 days after the onset of SARS. Confirmatory laboratory evidence of infection with coronavirus was available for two patients (Patients 6 and 17) and included PCR

amplification of coronavirus nucleic acids from tissues, viral isolation from bronchoalveolar-lavage fluid, or detection of serum antibodies reactive with coronavirus (Table 1). FOR TWO PATIENTS, NO SAMPLES WERE AVAILABLE FOR MOLECULAR, CELL-CULTURE, OR SEROLOGIC ANALYSIS; HOWEVER, THE CONDITION OF BOTH PATIENTS MET THE CDC DEFINITION OF PROBABLE SARS, and both had strong epidemiologic links with laboratory-confirmed cases of SARS."

"NO OBVIOUS INTRANUCLEAR OR INTRACYTOPLASMIC VIRAL INCLUSIONS WERE IDENTIFIED (Figure 4B), AND ELECTRON-MICROSCOPICAL EXAMINATION OF A LIMITED NUMBER OF THESE SYNCYTIAL CELLS REVEALED NO CORONAVIRUS PARTICLES. NO DEFINITIVE IMMUNOSTAINING WAS IDENTIFIED IN TISSUES FROM SARS PATIENTS with the use of a battery of immunohistochemical stains reactive with coronaviruses from antigenic groups I, II, and III."

"EVALUATION OF VERO E6 CELLS INFECTED WITH CORONAVIRUS ISOLATED FROM A PATIENT WITH SARS revealed viral cytopathic effect that included occasional multinucleated syncytial cells BUT NO OBVIOUS VIRAL INCLUSIONS (Figure 4C)."

"PATIENTS

Nineteen patients with SARS have been identified as infected with the new coronavirus BY VIRUS ISOLATION, RT-PCR, OR SEROLOGIC TESTS; all have direct or indirect links to the SARS outbreak in Hong Kong or Guangdong Province, China (Table 1). WE WERE ABLE TO AMPLIFY BY RT-PCR AND OBTAIN THE VIRUS SEQUENCE from clinical specimens or virus isolates FROM 12 OF THESE PATIENTS. All 12 sequences were identical to those of the first isolate as noted above. FOR FOUR CONVALESCENT PATIENTS, INFECTION WAS DEFECTED SEROLOGICALLY ALONE; FOR NINE PATIENTS IT WAS DETECTED BY RT-PCR ALONE; FOR THREE BY VIRUS ISOLATION AND RT-PCR; FOR TWO BY VIRUS ISOLATION, RT-PCR, AND SEROLOGIC ANALYSIS; AND FOR ONE BY RT-PCR AND SEROLOGIC ANALYSIS. We found none of the coronavirus-infected patients to be infected with human metapneumovirus. In only one patient was both SARS coronavirus and another respiratory virus detected; Patient 16 had both SARS coronavirus and a rhinovirus. A VARIETY OF RESPIRATORY PATHOGENS WERE ALSO IDENTIFIED BY RT-PCR IN OTHER PATIENTS WHOSE SAMPLES WERE SUBMITTED FOR SARS TESTING, INCLUDING 5 WITH HUMAN METAPNEUMOVIRUS (sequencing showed that each was distinct) AND 12 WITH RHINOVIRUSES (sequencing showed that each was distinct). None of the patients who were positive for human

metapneumovirus had pneumonia.``

"Discussion

The isolation of a novel coronavirus from the respiratory secretions of a patient with SARS and the subsequent demonstration of this virus or a serologic response to this virus in others with SARS DEMONSTRATE AN ETIOLOGIC ASSOCIATION between this virus and SARS."

"The novel human coronavirus identified in this study shares antigenic features with various group I coronaviruses, but genetic comparisons suggest it is distinct from group I coronaviruses and from coronaviruses in groups II and III. The factor or factors responsible for this apparent dichotomy remain to be elucidated; however, CORRELATION BETWEEN ANTIGENIC AND GENETIC CHARACTERISTICS OF THESE VIRUSES IS OCCASIONALLY UNCLEAR, AND THE PLACEMENT OF SOME OTHER HUMAN CORONAVIRUSES WITHIN SPECIFIC ANTIGENIC GROUPS HAS NOT ALWAYS BEEN WELL DEFINED.18-20"

"THE IDENTIFICATION OF THIS NOVEL CORONAVIRUS RELIED ON CLASSIC TISSUE-CULTURE ISOLATION TO AMPLIFY THE PATHOGEN AND THEN ON ELECTRON-MICROSCOPICAL STUDIES TO IDENTIFY THE TYPE OF VIRUS, a member of the family Coronaviridae, and molecular studies to confirm the identity of the virus, characterize its unique nature, AND HELP LINK IT TO THE DISEASE. The discovery of this new virus underscores the importance of versatile techniques such as virus isolation and electron microscopy in identifying etiologic pathogens. As with previous outbreak investigations, ELECTRON MICROSCOPY PROVED TO BE A RAPID TECHNIQUE THAT DID NOT REQUIRE SPECIFIC REAGENTS FOR OR PRIOR KNOWLEDGE OF A PARTICULAR AGENT BUT THAT COULD NEVERTHELESS CATEGORIZE A PATHOGEN ON THE BASIS OF ITS APPEARANCE AND MORPHOGENESIS.21-24"

"WE WERE NOT, HOWEVER, ABLE TO DEMONSTRATE CORONAVIRUS ANTIGENS IN PATIENT TISSUES BY HISTOLOGIC AND IMMUNOHISTOCHEMICAL METHODS OR TO DEMONSTRATE A DIRECT INVOLVEMENT IN THE PATHOLOGIC PROCESS. NEITHER WERE WE ABLE TO DEMONSTRATE SARS-ASSOCIATED CORONAVIRUS INFECTION IN ALL SUSPECTED PATIENTS WITH SARS."

"POSSIBLE REASONS FOR THE INABILITY TO DEMONSTRATE INFECTION IN SOME PATIENTS WITH

SUSPECTED SARS include the lack of sufficient sensitivity of the assays to detect the pathogen and the immune response and the timing and type of specimens tested. For example, we have not yet received convalescent-phase serum specimens from many patients with suspected SARS and have not serologically ruled out infection in many such patients. In addition, we are just beginning to study the type and timing of clinical specimens most likely to support a diagnosis of infection with this new virus. We have made rapid progress in developing our diagnostic assays and are continuing to improve them for the detection of this virus or an immune response to it. IN ADDITION, THE CASE DEFINITION OF SARS IS VERY BROAD AND MOST LIKELY INCLUDES OTHER INFECTIOUS DISEASES. WE ARE ALSO CONTINUING TO TEST FOR OTHER INFECTIOUS AGENTS THAT MIGHT BE ASSOCIATED WITH SARS, including those that might contribute to the severity of disease or increase the efficiency of viral transmission. Further clinical analysis of patients with SARS in whom there is laboratory confirmation of infection with the new coronavirus might help refine the case definition further."

"THE ISOLATION AND GROWTH OF A HUMAN-DERIVED CORONAVIRUS IN VERO E6 CELLS WERE UNEXPECTED. THE ONLY HUMAN OR ANIMAL CORONAVIRUS THAT HAS BEEN SHOWN TO GROW IN VERO CELLS IS PORCINE EPIDEMIC DIARRHEA VIRUS (isolated in China from pigs), AND IT REQUIRES THE ADDITION OF TRYPSIN TO CULTURE MEDIUM FOR GROWTH IN VERO E6 CELLS.25However, like the sequences of the other known coronaviruses, the sequences for porcine epidemic diarrhea virus are distinct from those of SARS-associated coronavirus, indicating that porcine epidemic diarrhea virus is not the parent virus to this new coronavirus. Because of the death of Dr. Carlo Urbani during the investigation, we propose that our first isolate be named the Urbani strain of SARS-associated coronavirus."

"THE PRIMARY HISTOPATHOLOGICAL LESIONS seen in the lungs of four patients we studied ARE CONSISTENT WITH A NONSPECIFIC RESPONSE TO ACUTE LUNG INJURY THAT CAN BE CAUSED BY INFECTIOUS AGENTS, TRAUMA, DRUGS, OR TOXIC CHEMICALS."

https://www.nejm.org/doi/full/10.1056/nejmoa030781

In Summary:

-throat-swab specimens were inoculated onto Vero E6 cells and cultured until cytopathogenic effects was observed

-CONSENSUS "Coronavirus" primers were designed for a fragment of the polymerase gene and

the sequence was found to be distantly related to other "Coronaviruses"

-SARS had a nonspecific clinical representation necessitating them to look at various possible agents for the cause

-cell cultures and inoculations in suckling mice were utilized to find the "virus"

-a variety of clinical specimens and numerous continuous cell lines were used to culture "viruses" and were inoculated intracranially (brain) and intraperitoneally (stomach) into suckling mice

-cultures were checked daily for CPE and medium was replenished on day 7

-if they noticed IDENTIFIABLE CPE, they went searching for a CAUSE

-any sick or dead suckling mice had their brains put into a suspension and then filtered/subcultured

-unpurified tissue-cultures showing CPE were examined by EM

-unpurified cell culture supernatant was put into lysis buffer for RT-PCR assays

-for antibody and tissue culture controls, they used serum from noninfected animals, various "Coronavirus-infected" cell cultures and animal tissues, "non infected" cell cultures, and normal human and animal tissues - however, there is no mention of taking samples from healthy humans for controls

-a rhinovirus was "isolated" from the unpurified cell culture from one patient but it was deemed unimportant

-unpurified Vero cell cultures which showed CPE were checked by EM for "Coronavirus-like" particles

-many extracellular particles in large groupings were found and assumed to be "Coronaviruses"

-two patients were deemed SARS cases even without molecular, cell culture, or serologic evidence as they met the CDC definition for PROBABLE SARS

-no intranuclear or intracytoplasmic viral particles were found

-EM examination of syncytial cells revealed no "Coronavirus" particles

-no definitive immunostaining was identified in SARS tissues

-evaluation of Vero cell culture revealed no obvious viral inclusions

-only 12 of 19 patients yielded PCR sequences of the new "Coronavirus"

-"SARS-COV-1" infection was determined by either cell culture, RT-PCR, and/or serologic tests

-a variety of respiratory pathogens were identified by RT-PCR in SARS specimens such as 5 metapneumovirus and 12 rhinoviruses

-they state that their cell culture and serologic evidence provides an etiologic ASSOCIATION between the "virus" and SARS

-they admit correlation between antigenic and genetic characteristics of "Coronaviruses" are unclear and placement of human "Coronaviruses" in antigenic groups is not well-defined

-they identified the "virus" through tissue-culture to amplify the "virus," EM imaging of unpurified cell culture supernatant to find the "virus" they were looking for, and molecular techniques to confirm its identity (sounds familiar...)

-they admit EM does not need reagents nor prior knowledge of a "virus" to find one as they rely on appearance and morphogenesis to determine it is there within the unpurified cell culture

-they admit they were unable to demonstrate "Coronavirus" antigens in patient tissues by histologic or immunohistochemical methods nor prove direct involvement in the pathologic process

-they were also UNABLE to demonstrate "SARS-COV-1" infection IN ALL SARS PATIENTS

-they admit that the case definition for SARS is very broad and encompasses many other diseases

-they state they are continuing to look for other infectious agents that may be the cause of SARS

-they state that growing a human "Coronavirus" in Vero cells was unexpected yet admit a pig "Coronavirus" has been grown this way when trypsin is added to the media

-the lung trauma seen in SARS patients is consistent with lung injury that can be caused by

trauma, drugs, or toxic chemicals

As stated earlier, they fail to provide evidence of a purified/isolated "virus" taken directly from a sick patient which is then proven pathogenic. At least they admit they were unable to show "SARS-COV-1" in all SARS patients thus failing Koch's first Postulate invalidating this whole study as proof that the Postulates have been fulfilled. They also admit they are continuing to look for other infectious agents as the possible cause of SARS, again disqualifying this study as proof.

FOUCHIER 2003 SARS-COV-1 KOCH'S POSTULATES FULFILLED(?):

"It is obvious that KOCH'S POSTULATES HAVE NOT BEEN SATISFIED in viral diseases."

-Thomas Rivers 1937

During the 2003 SARS "epidemic," the WHO regularly announced updates about their search for the causative agent of what was claimed to be a new disease. On March 27th, 2003, they admitted that the criteria that needed to be fulfilled in order to prove that there was a new "virus" causing a new disease was Koch's Postulates: four logic based rules proposed by Robert Koch that have been the burden of proof since the late 1800's:

"CONCLUSIVE IDENTIFICATION OF A CAUSATIVE MUST MEET ALL CRITERIA IN THE SO-CALLED “KOCH'S POSTULATE.” The additional experiments NEEDED TO FULFILL THESE CRITERIA are currently under way at a laboratory in the Netherlands."

-WHO 2003

https://www.who.int/csr/don/2003_03_27b/en/

On April 15th, 2003, the WHO stated that the experiments were done and that the criteria for satisfying Koch's Postulates had been met thus proving that the "new" disease SARS was caused by a new "Coronavirus:"

"Scientists had been almost certain the new form of coronavirus first isolated from sick patients March 21 by the University of Hong Kong was the cause of SARS. BUT THEY COULD NOT SAY FOR SURE UNTIL THEY HAD SATISFIED WHAT IS KNOWN AS KOCH'S POSTULATES _ FOUR SCIENTIFIC TESTS THAT VERIFY WHETHER A VIRUS CAUSES A CERTAIN DISEASE.

"THE KOCH'S POSTULATES HAVE BEEN FULFILLED, SO WE CAN NOW SAY FOR CERTAIN THAT THE NEW CORONAVIRUS IS THE CAUSE OF SARS," said Dr. Klaus Stohr, a World Health Organization virologist who is coordinating the scientists' work.

https://www.mrt.com/news/article/Scientists-Confirm-Virus-As-Cause-of-SARS-7816916.php

Ron Fouchier, the lead researcher for the 2003 SARS paper claiming fulfillment of Koch's Postulates, stated this in 2012 while speaking about MERS:

"For starters, we'll find out whether animals get sick from this virus. YOU CAN ISOLATE A VIRUS FROM A PATIENT, BUT THAT DOES NOT MEAN THEY DIED FROM IT; TO SHOW THAT IT CAUSES DISEASE YOU NEED TO FULFILL KOCH'S POSTULATES. THAT'S WHAT WE DID FOR SARS, and it's what we hope to do here; we've applied for emergency ethical approval. The most obvious animal species to put this virus in are mice, ferrets, and perhaps monkeys. We've got to see what we can get approval for."

https://www.sciencemag.org/news/2012/09/ron-fouchier-new-coronavirus-we-need-fulfill-kochs-postulates

According to the WHO and lead researcher Ron Fouchier, all of Koch's Postulates had been met and the newly identified "SARS-COV-1" was the true cause of SARS. However, did they really satisfy Koch's Postulates for SARS?

KOCH'S POSTULATES FULFILLED FOR SARS VIRUS?

"ACCORDING TO KOCH'S POSTULATES, AS MODIFIED BY RIVERS FOR VIRAL DISEASES, SIX CRITERIA ARE REQUIRED to establish a virus as the cause of a disease1. THE FIRST THREE CRITERIA — isolation of virus from diseased hosts, cultivation in host cells, and proof of filterability — HAVE BEEN MET FOR SCV BY SEVERAL GROUPS 2,3,4,5. Moreover, of 96 individuals complying with the World Health Organization's definition of SARS6 in Hong Kong, 86 (90%) yielded laboratory evidence of SCV infection.

WE HAVE TESTED FOR THE THREE REMAINING CRITERIA: production of comparable disease in the original host species or a related one, re-isolation of the virus, and detection of a specific immune response to the virus. WE INOCULATED TWO MACAQUES WITH VERO-CELL-CULTURED SCV ISOLATED FROM A FATAL SARS CASE, and monitored their clinical signs, virus excretion and antibody response. THE ANIMALS WERE KILLED SIX DAYS POST-INOCULATION (d.p.i.), and we then carried out gross and histopathological examinations of them.

Both SCV-inoculated macaques became lethargic from 3 d.p.i. onwards and developed a temporary skin rash, and ONE SUFFERED RESPIRATORY DISTRESS from 4 d.p.i. onwards. The macaques excreted virus from the nose and throat at 2–6 d.p.i., as shown by polymerase chain reaction with reverse transcription (RT-PCR) and by virus isolation (see supplementary information). The isolated virus was identical to that inoculated, as shown by negative-contrast electron microscopy (Fig. 1a) and RT-PCR analysis. Seroconversion to SCV, as determined BY INDIRECT IMMUNOFLUORESCENCE ASSAY USING INFECTED VERO CELLS, was demonstrated in two other SCV-infected macaques at 16 d.p.i.. The virus was also isolated from the faeces of one of these animals (see supplementary information).

At gross necropsy, ONE MACAQUE HAD SEVERE MULTIFOCAL PULMONARY CONSOLIDATION, and SCV infection was detected in lung tissue by RT-PCR and virus isolation. Histologically, both macaques had interstitial pneumonia of differing severity. The one with gross lesions had diffuse alveolar damage, marked by necrosis of alveolar and bronchiolar epithelium and flooding of alveolar lumina with proteinaceous fluid, admixed with fibrin, erythrocytes, alveolar macrophages and neutrophils (Fig. 1b). Occasional multinucleated cells (syncytia) were present in the lumen of bronchioles and alveoli (Fig. 1c). These lesions are indistinguishable from those in biopsied lung tissue and in autopsy material from SARS patients5, including the presence of syncytia in alveolar lumina4.

SCV thus fulfils all of Koch's postulates as the primary aetiological agent of SARS. THIS DOES NOT EXCLUDE THE POSSIBILITY THAT OTHER PATHOGENS, including human metapneumovirus (hMPV) and Chlamydia pneumoniae, MAY HAVE EXACERBATED THE DISEASE IN SOME SARS PATIENTS. However, these were not present in SCV-inoculated macaques (RESULTS NOT SHOWN), were not found consistently in SARS patients, and do not usually cause the lesions associated with SARS. Moreover, lesions in macaques infected experimentally with hMPV isolated from a non-SARS individual7 were limited to mild suppurative rhinitis and minimal erosion in conducting airways, and disease was not exacerbated in two SCV-infected macaques subsequently inoculated with hMPV (RESULTS NOT SHOWN)."

https://www.nature.com/articles/423240a

In Summary:

-the researchers did not attempt to satisfy Koch's Postulates but instead Rivers modified and watered-down version of them:

https://m.facebook.com/story.php?story_fbid=10158168879148576&id=502548575

-thus from the very first paragraph, they invalidate their own fulfillment claim as Rivers 6 Postulates are admittedly different from Koch's 4 Postulates

-they then state that the first 3 RIVERS criteria were met by other researchers and referenced the four papers below:

PEIRIS:

https://m.facebook.com/story.php?story_fbid=10158162232388576&id=502548575

DROSTEN:

https://m.facebook.com/story.php?story_fbid=10158164423513576&id=502548575

POUTANEN:

https://m.facebook.com/story.php?story_fbid=10158166281663576&id=502548575

KSIAZEK:

https://m.facebook.com/story.php?story_fbid=10158168700128576&id=502548575

-Not a single one of these papers could meet the first requirement of Koch's Postulates as they could not find their new "Coronavirus" in every case of the disease.

-None of the papers properly purified/isolated any "virus" directly from a sick patient but instead took samples and cultured them in foreign animal cells likely containing added fetal bovine serum, antibiotics, chemicals, nutrients, etc.

-Not a single paper gave detailed methods on how they cultured their "viruses" nor attempted to purify and separate the assumed "virus" particles by ultracentrifugation nor filtration even from their cell culture soup.

-Every one of the papers admitted to other potential pathogens isolated from SARS cases that could possibly be the causative agent for disease or act as a cofactor in disease progression

-the researchers then state that they satisfied the last 3 RIVERS criteria themselves

-they used unpurified Vero cell culture supernatant and inoculated two macaques while only observing them for 6 days

-only one of the macaques developed respiratory distress

-the two macaques had different levels of lung damage at autopsy with one severe and the other one not so

-both macaques were lethargic and developed a temporary skin rash, neither of which are main symptoms of SARS:

"SYMPTOMS OF SARS

In general, SARS begins with a HIGH FEVER (temperature greater than 100.4°F [>38.0°C]). Other symptoms may include HEADACHE, an overall FEELING OF DISCOMFORT, and BODY ACHES. Some people also have MILD RESPIRATORY SYMPTOMS at the outset. About 10 percent to 20 percent of patients have DIARRHEA. After 2 to 7 days, SARS patients may develop a DRY COUGH. Most patients develop PNEUMONIA."

https://www.cdc.gov/sars/about/fs-sars.html

There can be no claim by this paper as to the fulfillment of Koch's Postulates as they did not even attempt to fulfill them. The WHO, Ron Fouchier, and the other researchers blatantly lied.

Instead, they attempted Rivers criteria and even failed at fulfilling his watered-down version as they:

1. did not isolate a "virus" from a diseased host 2. did not cultivate a "virus" in host cells but instead used monkey kidney cells

3. did not provide any proof of filterability

4. did not produce the same disease in an animal host

5. did not re-isolate a "virus" from the animals

6. did not prove that the immune response was specific

This paper and the disease associated with it are the shining example of the fraud currently being perpetrated on us by Virology today.

For the best insight into the fraud presented in this and other papers, I highly recommend taking 30 minutes out of your day and watching Dr. Andrew Kaufman "The Rooster in the River of Rats:"

https://m.youtube.com/watch?v=-Wp_PwYFMyM

VAN DER HOEK 2004 CORONAVIRUS HCoV-NL63:

These newer "Coronavirus" discoveries highlight the fatal flaws currently dominating Virology: the over reliance on molecular tests and data as proof of "virus" discovery. In this paper, they create their own technique, the VIDISCA method, that they claim can sequence the genome of an unknown "virus." What they don't tell you is that this sequence comes directly from an unpurified monkey kidney cell culture supernatant that was mixed with the nasopharyngeal aspirate from a 7-month old baby. It does not come directly from the sample of a sick patient that has been properly purified/isolated. They claim discovery of a new "Coronavirus" based solely the sequence as there are NO EM IMAGES of any new "Coronavirus," just letters in a database.

Bear with me as this is a long one. Highlights below:

IDENTIFICATION OF A NEW HUMAN CORONAVIRUS

"Three human coronaviruses are known to exist: human coronavirus 229E (HCoV-229E), HCoV-OC43 and severe acute respiratory syndrome (SARS)-associated coronavirus (SARS-CoV). Here we report the identification of a fourth human coronavirus, HCoV-NL63, USING A NEW METHOD OF VIRUS DISCOVERY. The virus was isolated from a 7-month-old child SUFFERING FROM BRONCHIOLITIS AND CONJUNCTIVITIS. The complete genome sequence indicates that this virus is not a recombinant, but rather a new group 1 coronavirus. The in vitro host cell range of HCoV-NL63 is notable BECAUSE IT REPLICATES ON TERTIARY MONKEY KIDNEY CELLS AND THE MONKEY KIDNEY LLC-MK2 CELL LINE. The viral genome contains distinctive features, including a

unique N-terminal fragment within the spike protein. Screening of clinical specimens from individuals suffering from respiratory illness identified seven additional HCoV-NL63-infected individuals, indicating that the virus was widely spread within the human population."

"To date, THERE IS STILL A VARIETY OF HUMAN DISEASES WITH UNKNOWN ETIOLOGY. A VIRAL ORIGIN HAS BEEN SUGGESTED FOR MANY OF THESE DISEASES, EMPHASIZING THE IMPORTANCE OF A CONTINUOUS SEARCH FOR NEW VIRUSES 1,2,3. Major difficulties are encountered, however, when searching for new viruses. First, SOME VIRUSES DO NOT REPLICATE IN VITRO, at least not in the cells that are commonly used in viral diagnostics. Second, FOR THOSE VIRUSES THAT DO NOT REPLICATE IN VITRO AND CAUSE A CYTOPATHIC EFFECT (CPE), THE SUBSEQUENT VIRUS IDENTIFICATION METHODS MAY FAIL. ANTIBODIES RAISED AGAINST KNOWN VIRUSES MAY NOT RECOGNIZE THE CULTURED VIRUS, AND VIRUS-SPECIFIC PCR METHODS MAY NOT AMPLIFY THE NEW VIRAL GENOME. To solve both problems, we developed a new method for virus discovery based on the cDNA-amplified restriction fragment–length polymorphism technique (cDNA-AFLP4). Here we report the identification of a new coronavirus using this method of Virus-Discovery-cDNA-AFLP (VIDISCA)."

"The new coronavirus that we present here was isolated from a child suffering from bronchiolitis and conjunctivitis. THIS WAS NOT AN ISOLATED CASE, AS WE IDENTIFIED THE VIRUS IN CLINICAL SPECIMENS FROM SEVEN ADDITIONAL INDIVIDUALS, both infants and adults, during the last winter season. We also resolved the complete sequence of the viral genome, which revealed several unique features."

"Results

Virus isolation from a child with acute respiratory disease

In January 2003, a 7-month-old child was admitted to the hospital with coryza, conjunctivitis and fever. Chest radiography revealed typical features of bronchiolitis. A nasopharyngeal aspirate specimen was collected 5 d after the onset of disease (sample NL63). Diagnostic tests for respiratory syncytial virus, adenovirus, influenza viruses A and B, parainfluenza virus types 1, 2 and 3, rhinovirus, enterovirus, HCoV-229E and HCoV-OC43 yielded negative results. THE CLINICAL SAMPLE WAS SUBSEQUENTLY INOCULATED ONTO HUMAN FETAL LUNG FIBROBLASTS, TERTIARY MONKEY KIDNEY CELLS (Cynomolgus monkey) AND HeLa CELLS. CPE WAS DETECTED EXTENSIVELY ON TERTIARY MONKEY KIDNEY CELLS, and was first noted 8 d after inoculation. The CPE was diffuse, with a refractive appearance in the affected cells followed by cell detachment. MORE PRONOUNCED CPE WAS OBSERVED UPON PASSAGE ONTO THE MONKEY

KIDNEY CELL LINE LLC-MK2, with overall cell rounding and moderate cell enlargement (Supplementary Fig. 1 online). Additional subcultures on human fetal lung fibroblasts, rhabdomyosarcoma cells and Vero cells remained negative for CPE."

"Virus discovery by the VIDISCA method

IDENTIFICATION OF UNKNOWN PATHOGENS USING MOLECULAR BIOLOGY TOOLS IS DIFFICULT BECAUSE THE TARGET SEQUENCE IS NOT KNOWN, SO GENOME-SPECIFIC PCR PRIMERS CANNOT BE DESIGNED. To overcome this problem, we developed the VIDISCA method based on the cDNA-AFLP technique4. The advantage of VIDISCA is that prior knowledge of the sequence is not required, as the presence of restriction enzyme sites is sufficient to guarantee PCR amplification. The input sample can be either blood plasma or serum, or culture supernatant. Whereas cDNA-AFLP starts with isolated mRNA, VIDISCA begins with a treatment to selectively enrich viral nucleic acid, including a centrifugation step to remove residual cells and mitochondria (Fig. 1a). A DNase treatment is also used to remove interfering chromosomal and mitochondrial DNA from degraded cells (viral nucleic acid is protected within the viral particle). Finally, by choosing frequently cutting restriction enzymes, the method can be fine-tuned such that most viruses will be amplified. We were able to amplify viral nucleic acids in EDTA-treated plasma from a person with hepatitis B viral infection, and from a person with an acute parvovirus B19 infection (Fig. 1b). The technique can also detect HIV-1 in cell culture, demonstrating its capacity to identify both RNA and DNA viruses (Fig. 1b).

THE SUPERNATANT OF THE CPE-POSITIVE LLC-MK2 CULTURE NL63 WAS ANALYZED BY VIDISCA. THE SUPERNATANT OF UNINFECTED CELLS WAS USED AS A NEGATIVE CONTROL. After the second PCR amplification step, unique and prominent DNA fragments were present in the test sample but not in the control (1 of 16 selective PCR reactions is shown in Fig. 1c). THESE FRAGMENTS WERE CLONED AND SEQUENCED. Thirteen of 16 fragments showed sequence similarity to members of the coronavirus family, but significant sequence divergence with known coronaviruses was apparent in all fragments, indicating that we had identified a new coronavirus. The sequences of the 13 VIDISCA fragments are provided in Supplementary Figure 2 online."

"Detection of HCoV-NL63 in patient specimens

To show that HCoV-NL63 originated from the nasopharyngeal aspirate of the child, WE DESIGNED A DIAGNOSTIC RT-PCR THAT SPECIFICALLY DETECTS HCoV-NL63. THIS TEST CONFIRMED THE PRESENCE OF HCoV-NL63 IN THE CLINICAL SAMPLE. The sequence of the RT-

PCR product of the 1b gene was identical to that of the virus identified upon in vitro passage in LLC-MK2 cells (DATA NOT SHOWN).

Having confirmed that the cultured coronavirus originated from the child, the question remained as to whether this was an isolated clinical case, or whether HCoV-NL63 is circulating in humans. To address this question, WE USED TWO DIAGNOSTIC RT-PCR ASSAYS TO EXAMINE RESPIRATORY SPECIMENS OF HOSPITALIZED INDIVIDUALS AND THOSE VISITING THE OUTPATIENT CLINIC between December 2002 and August 2003 (Fig. 2). WE IDENTIFIED SEVEN ADDITIONAL INDIVIDUALS CARRYING HCoV-NL63 (Table 1). SEQUENCE ANALYSIS OF THE PCR PRODUCTS INDICATED THEY PRESENCE OF A FEW CHARACTERISTIC POINT MUTATIONS IN SEVERAL SAMPLES, SUGGESTING THAT SEVERAL VIRUSES WITH DIFFERENT MOLECULAR MARKERS MAY BE CIRCULATING (Fig. 3 and Supplementary Fig. 3 online). At least five of the HCoV-NL63-positive individuals suffered from respiratory tract illness; the clinical data of two individuals was not available. Including the index case, five of the patients were children less than 1 year old, and three patients were adults. Two adults were likely to be immunosuppressed, as one of them was a bone marrow transplant recipient and the other an HIV-positive patient suffering from AIDS, with very low CD4+ cell counts (Table 1). No clinical data was available for the third adult. ONE PATIENT WAS CONNECTED WITH RESPIRATORY SYNCYTIAL VIRUS (no. 72), AND THE HIV-INFECTED PATIENT (no. 466) CARRIED PNEUMOCYSTIS CARINI. No other respiratory agent was found in the other patients, suggesting that the respiratory symptoms were caused by HCoV-NL63. All positive samples were collected during the last winter season, with a detection frequency of 7% in January 2003. None of the 306 samples collected in the spring and summer of 2003 contained HCoV-NL63 ($P < 0.01$ by two-tailed t test)."

"We next aligned the sequence of HCoV-NL63 with the complete genomes of other coronaviruses. The percentage nucleotide identity was determined for each gene and is listed in Table 2. All genes except the M gene shared the highest identity with HCoV-229E. To confirm that HCoV-NL63 is a new member of the group 1 coronaviruses, we conducted phylogenetic analysis using the nucleotide sequence of the 1a, 1b, S, M and N genes (Fig. 4b). For each gene analyzed, HCoV-NL63 clustered with the group 1 coronaviruses. The 1a, 1b and S genes of HCoV-NL63 are most closely related to those of HCoV-229E. However, further inspection revealed a subcluster of HCoV-NL63, HCoV-229E and PEDV. PHYLOGENETIC ANALYSIS COULD NOT BE PERFORMED FOR THE ORF3 AND E GENES BECAUSE THE REGIONS WERE TOO VARIABLE OR TOO SMALL FOR ANALYSIS, respectively. Bootscan analysis by the Simplot software version 2.5 (ref. 28) found no signs of recombination (data not shown)."

"OUR DATA INDICATE THAT HCoV-NL63 CAUSES ACUTE RESPIRATORY DISEASE IN CHILDREN BELOW THE AGE OF 1 YEAR, AND IN IMMUNOCOMPROMISED ADULTS. To date, NO KNOWN VIRAL PATHOGEN CAN BE IDENTIFIED IN A SUBSTANTIAL PORTION OF RESPIRATORY DISEASE CASES IN HUMANS (20–30%; ref. 38). Several assays have been used to diagnose coronavirus infections. Traditionally, an antibody test is implemented to measure a rise in titers of antibodies to the human coronaviruses HCoV-229E or HCoV-OC43 (ref. 12). ANTIBODIES TO HCoV-NL63 MIGHT CROSS-REACT WITH HCoV-229E, given that these viruses are members of the same serotype. If this were the case, HCoV-NL63 INFECTIONS MIGHT HAVE BEEN MISDIAGNOSED AS HCoV-229E. Molecular biology tools such as RT-PCR assays 39,40 were designed to selectively detect the human coronaviruses HCoV-229E and HCoV-OC43, but these assays will not detect HCoV-NL63. EVEN THE RT-PCR ASSAY THAT WAS DESIGNED TO AMPLIFY ALL KNOWN CORONAVIRUSES 40 IS NOT ABLE TO AMPLIFY HCoV-NL63 BECAUSE OF SEVERAL MISMATCHES WITH THE PRIMER SEQUENCES. The availability of the complete HCoV-NL63 genome sequence means that these diagnostic assays can be substantially improved."

"FUTURE EXPERIMENTS WITH MORE SENSITIVE DIAGNOSTIC TOOLS SHOULD YIELD A MORE ACCURATE PICTURE OF THE PREVALENCE OF THIS VIRUS AND ITS ASSOCIATION WITH RESPIRATORY DISEASE."

METHODS (found in Supplementary Material)

"THE ORIGINAL NASOPHARYNGEAL ASPIRATE WAS INOCULATED ONTO A VARIETY OF CELLS. The cultures were kept in a rollerdrome at 34°C and inspected by eye every 3 to 4 days. MAINTENANCE MEDIUM WAS REPLENISHED EVERY 3 TO 4 DAYS. TWO DIFFERENT TYPES OF MEDIUM WERE USED: Optimem 1 (Invitrogen, Breda, The Netherlands) without bovine fetal serum was used for the tMK cells, and MEM Hanks' /Earle's medium (Invitrogen, Breda, The Netherlands) WITH 3% BOVINE FETAL SERUM FOR THE REMAINING CELL TYPES."

https://www.nature.com/articles/nm1024#MOESM5

In Summary (Part 1):

-the researchers created their own method known as VIDISCA to sequence a new "Coronavirus

-the "virus" came from a 7-month-old infant suffering bronchitis and conjunctivitis

-the "virus" was "isolated" from the unpurified mixture of the infants nasopharyngeal aspirate

and tertiary monkey kidney cells

-they admit that the cause of many respiratory diseases remain unknown and that "viruses" are the presumed cause thus the need to hunt for new "viruses"

-they state that there are two main problems searching for new "viruses:"

1. Some "viruses" do not culture in cells in vitro

2. For these "viruses" that do not replicate nor cause CPE, "virus" identification methods such as antibody and PCR may fail

-cells lines such as human fetal lung fibroblasts, tertiary monkey kidney, and HeLa cells were used for culturing

-tertiary monkey cells saw the most extensive CPE out of all and even more CPE was observed once it was passaged into the LLC-MK2 monkey kidney cells

-they admit that using molecular tools to find unknown "viruses" is difficult since the target sequence is unknown and genome-specific PCR primers can not be designed

-the supernatant of LLC-MK2 was examined by VIDISCA and uninoculated cell cultures were used as a "control"

-they developed their own PCR test to detect the sequence they created from the supernatant of the LLC-MK2 cell culture in clinical samples

-they then used their own PCR to test clinical samples from 2002-2003 to find 7 more "positive" cases in order to show that their "discovery" was not an isolated incident

-several characteristic mutations were found in the 7 samples suggesting multiple "viruses" with different molecular markers were in circulation

-2 of the 7 cases were immunocompromised adults: one a bone-marrow transplant recipient and the other an HIV patient with pneumocystis carinii

-their data shows that NL63 affects infants under 1 and immunocompromised adults

-antibodies for HL63 might cross-react with 229E

-PCR primer used to identify all "Coronaviruses" can not detect NL63 due to several mismatches with the primer sequence

-they leave it to future experiments with better technology to get a more accurate idea as to NL63's prevalence and association with respiratory disease

-the NP swab was inoculated on many cells and media was replenished every 3-4 days

-two different media were used during the culturing process

As can be seen, these researchers discovered nothing more than letters in a database. They created a sequence using their own VIDISCA program from unpurified cell culture supernatant mixed with a sample from an infant.They fabricated cases by combing through old clinical samples utilizing their own "diagnostic" PCR. There aren't even any accompanying EM images of this new "Coronavirus."

Everything relating to NL63 depends on how accurate their VIDISCA method is and whether or not it can actually sequence unknown "viruses." This is from the same researchers describing the limitations of their VIDISCA method in 2011, seven years after their "discovery" of HCoV-NL63 using this method:

"Virus discovery cDNA-AFLP (VIDISCA) is

a virus discovery method based on recognition of restriction enzyme cleavage sites, ligation of adaptors and subsequent amplification by PCR. However, DIRECT DISCOVERY OF UNKNOWN PATHOGENS IN NASOPHARYNGEAL SWABS IS DIFFICULT DUE TO THE HIGH CONCENTRATION OF RIBOSOMAL RNA (rRNA) THAT ACTS AS COMPETITOR."

"THESE CONDITIONS ARE GENERALLY ONLY

MET WHEN VIRUS CULTURE SUPERNATANT IS USED. IN CLINICAL RESPIRATORY SAMPLES LIKE NASOPHARYNGEAL SWABS IN UNIVERSAL TRANSPORT MEDIUM (UTM) VARIOUS AMOUNTS OF COMPETITOR RNA/DNA FROM DISRUPTED CELLS/BACTERIA CAN BE PRESENT. Ribosomal RNA,

which is ~80% of the total cellular RNA, is one of the biggest problems due to its high copy number and its stability within ribosomes. IN PARTICULAR RNA VIRUSES ARE DIFFICULT TO DISCOVER SINCE IN THESE CASES A REVERSE TRANSCRIPTION IS NEEDED, WHICH WILL ENABLE rRNA TO ACT AS COMPETITION NUCLEIC ACID SEQUENCES."

"RESPIRATORY SAMPLES CONTAIN NON-VIRAL NUCLEIC ACIDS THAT INTERFERE IN VIRUS DISCOVERY TECHNIQUES LIKE VIDISCA. It is relatively easy to decrease the influence of background bacterial or human DNA and mRNA by centrifugation and DNase/RNase treatment, BUT RIBOSOMAL RNA (rRNA) IS DIFFICULT TO ELIMINATE BECAUSE THE RIBOSOMAL PROTEINS PROTECTS THE rRNA INSIDE THE RIBOSOMES."

"Sequence independent amplification methods, such as VIDISCA and random-PCR, can identify viral sequences without prior knowledge of a viral genome. Unfortunately, THE DETECTION OF UNKNOWN VIRAL PATHOGENS IN RESPIRATORY CLINICAL MATERIAL IS DIFFICULT with these sequence independent virus discovery methods BECAUSE OF LOW VIRAL LOAD AND HIGH BACKGROUND NUCLEIC ACIDS IN THESE SAMPLES. During the last years sequence independent virus discovery techniques WERE MOSTLY USED WITH VIRUS CULTURE SUPERNATANT, as they contain high concentrations of viral genomes [6,12], or to discover previously unknown DNA viruses [13-15]. SO FAR NO STUDY HAS BEEN ABLE TO IDENTIFY NOVEL HUMAN RESPIRATORY RNA VIRUSES WITH SEQUENCE INDEPENDENTLY AMPLIFICATION TECHNIQUES. Thus sequence independent amplification techniques like VIDISCA HAVE TO BE OPTIMIZED TO ALLOW DISCOVERY WITHOUT REQUIRING A CULTURE AMPLIFICATION STEP."

"WE ALSO OBSERVED THE PRESENCE OF UNKNOWN SEQUENCES WITHIN OUR DATA SET. IT COULD BE THAT THESE SEQUENCES ARE DERIVED FROM YET UNKNOWN VIRUSES, OR IT COULD BE THAT THE SEQUENCES ARE PART OF A GENOMIC SEQUENCE FROM A KNOWN ORGANISM, e.g. a bacterium of which not the complete genomic sequence is present in the Genbank databases. THUS CARE SHOULD BE TAKEN TO ASSIGN SEQUENCES AS POTENTIALLY VIRAL, SINCE SO MANY ORGANISMS HAVE NOT BEEN FULLY SEQUENCED."

https://journals.plos.org/plosone/article?id=10.1371%2Fjournal.pone.0016118

In Summary (Part 2):

-direct discovery of unknown pathogens in NP swabs is difficult due to ribosomal RNA acting as a

competitor

-conditions are only met when "virus" culture supernatant is used

-transport media used to carry NP swabs contains various DNA/RNA from disrupted cells/bacteria

-RNA "viruses" are difficult as reverse-transcriptase is needed which enables rRNA to act as competition nucleic acid sequences

-respiratory samples contain "non-viral" nucleic acid which interferes with VIDISCA

-ribosomal RNA is difficult to eliminate

-discovering unknown "viruses" from respiratory samples is difficult due to low "viral load" and high nucleic acid background in samples

-so far, NO STUDY has been able to identify novel human respiratory RNA "viruses" using sequence independently amplification techniques such as VIDISCA

-VIDISCA would have to be optimized to discover novel "viruses" without cell culture

-they observed unknown sequences in their data that could be unknown "viruses" or from genome sequences of known organisms

-sequences should be labelled "POTENTIALLY VIRAL' since so many sequences remain unknown for most organisms

Reading this review of their own VIDISCA system seven years after they "discovered" NL63 doesn't make the technology nor their finding sound all that accurate, does it?

But wait, there's more!

Here is some additional insight into the VIDISCA methods used:

"The authors state that “the identification of unknown pathogens using molecular biology tools is difficult because the target sequence is not known so that PCR-specific initiators cannot be designed“. WHAT THEY USED IS A TOOL THEY DEVELOPED THEMSELVES CALLED VIDISCA which,

they claim, does not require prior knowledge of the sequence!

Is that possible?

Let's see how it works: FIRST THE CULTURE IS PREPARED AND IT IS ASSUMED THAT A VIRUS IS PRESENT DUE TO THE EVIDENCE OF "CYTOPATHIC EFFECT". The novelty introduced by this method is that "restriction enzymes" are added, enzymes that cut the nucleic acid molecules at certain locations and always by the same length.

In this way, if after the action of these enzymes they observe many fragments of DNA or RNA that are the same or very similar, THEY DEDUCE THAT IT COMES FROM A VIRUS, since the host genome would present random cuts, while the virus genome presents a large number of copies that are the same due to the replication of the virus.

And is such a deduction correct? Of course not!

THIS ASSUMPTION (which adds to the previous assumption that there is a virus) DOES NOT TAKE INTO ACCOUNT THAT THERE ARE "VIRUS-LIKE PARTICLES", "RETROVIRUS-LIKE PARTICLES", "ENDOGENOUS RETROVIRUSES", "EXOSOMES", "EXTRACELLULAR" PARTICLES AND EVEN MITOCHONDRIAL DNA.

In denial, THERE ARE A MULTITUDE OF PARTICLES THAT POSSESS THE SAME REPRODUCTIVE CHARACTERISTICS IN LARGE QUANTITIES AS "VIRUSES" AND THEREFORE CAN FALSIFY RESULTS BY PRODUCING LARGE NUMBERS OF IDENTICAL COPIES when cut by enzymes as recognised in an article on the VIDISCA technique entitled Enhanced bioinformatic proSling of VIDISCA libraries for virus detection and Discovery. It was published in volume 263 of Virus Research on April 2, 2019, and its authors-Cormac M. Kinsella et al.-recognise that "NO REDUNDANCY IS EXPECTED IN THE VIDISCA INSERT FROM THE HOST BACKGROUND NUCLEIC ACID EXCEPT IN THE CASE OF 'VIRUS-LIKE' CHARACTERISTICS, i.e., high copy numbers as in mitochondrial DNA."

https://principia-scientific.com/confirmed-pcr-tests-cannot-detect-sars-cov-2-cause-of-covid19

Once again for emphasis:

"NO REDUNDANCY IS EXPECTED IN THE VIDISCA INSERT FROM THE HOST BACKGROUND

NUCLEIC ACID EXCEPT IN THE CASE OF "VIRUS-LIKE" CHARACTERISTICS"

Creating their own technology which can not do what they state it can in order to claim the indirect discovery of a new "virus" with nothing physical backing it up, just a bunch of A's, C's, T's, and G's in a computer database. This is the SCAM called Molecular Virology.

WOO 2005 CORONAVIRUS HKU1 PAPER:

Over the years, I have read a lot of bad "virus" papers. This one for the "discovery" of HKU1 may just take the top spot on that list...which is no easy task. I won't spoil it here and will let the highlights speak for themselves.

CHARACTERIZATION AND COMPLETE GENOME SEQUENCE OF A NOVEL CORONAVIRUS, CORONAVIRUS HKU1, FROM PATIENTS WITH PNEUMONIA

"DESPITE EXTENSIVE LABORATORY INVESTIGATIONS in patients with respiratory tract infections, NO MICROBIOLOGICAL CAUSES CAN BE IDENTIFIED IN A SIGNIFICANT PROPORTION OF PATIENTS."

"Here we report the discovery of another novel coronavirus, coronavirus HKU1 (CoV-HKU1), FROM A SEVENTY ONE-YEAR-OLD MAN WITH PNEUMONIA who had just returned from Shenzhen, China. Quantitative reverse transcription-PCR showed that the amount of CoV-HKU1 RNA was 8.5 to 9.6 × 106 copies per ml in his nasopharyngeal aspirates (NPAs) during the first week of the illness and dropped progressively to undetectable levels in subsequent weeks. He developed increasing serum levels of specific antibodies against the recombinant nucleocapsid protein of CoV-HKU1, with immunoglobulin M (IgM) titers of 1:20, 1:40, and 1:80 and IgG titers of <1:1,000, 1:2,000, and 1:8,000 in the first, second and fourth weeks of the illness, respectively. ISOLATION OF THE VIRUS BY USING VARIOUS CELL LINES, MIXED NEURON-GLIA CULTURE, AND INTRACEREBRAL INOCULATION OF SUCKLING MICE WAS UNSUCCESSFUL."

"SINCE NO MICROBIOLOGICAL CAUSE CAN BE IDENTIFIED FOR A SIGNIFICANT PROPORTION OF

PATIENTS WITH RESPIRATORY TRACT INFECTIONS (18, 29), research has been conducted to identify novel agents."

"In this study, we report the discovery of a novel group 2 coronavirus IN THE NASOPHARYNGEAL ASPIRATES (NPAs) OF PATIENTS WITH PNEUMONIA. The complete genome of the coronavirus was sequenced and analyzed. Based on the findings of this study, we propose that this new virus be designated coronavirus HKU1 (CoV-HKU1)."

"RNA extraction.Viral RNA WAS EXTRACTED FROM THE NPA, URINE, AND FECAL SPECIMENS by using the QIAamp Viral RNA Mini kit (QIAgen, Hilden, Germany). The RNA pellet was resuspended in 10 µl of DNase-free, RNase-free double-distilled water and was used as the template for RT-PCR."

"Complete genome sequencing and genome analysis.The complete genome of CoV-HKU1 was amplified and sequenced BY USING THE RNA EXTRACTED FROM THE NPAs AS A TEMPLATE. The RNA was converted to cDNA by a combined RANDOM-PRIMING and oligo(dT) priming strategy."

"SEQUENCES WERE ASSEMBLED AND MANUALLY EDITED TO PRODUCE A FINAL SEQUENCE OF THE VIRAL GENOME. The nucleotide sequence of the genome and the deduced amino acid sequences of the open reading frames (ORFs) WERE COMPARED TO THOSE OF OTHER CORONAVIRUSES. Phylogenetic tree construction was performed by using the PileUp method with GrowTree (Genetics Computer Group, Inc.). Prediction of signal peptides and their cleavage sites was performed by using SignalP (21). Protein family analysis was performed by using PFAM and InterProScan (1, 2). Prediction of transmembrane domains was performed by using TMpred and TMHMM (11, 32). PHDhtm WAS ALSO USED WHEN THERE WAS DISAGREEMENT BETWEEN THE RESULTS OBTAINED BY USING TMpred AND TMHMM (3). Potential N-glycosylation sites were predicted by using ScanProsite (7)."

"RESULTS

Index patient and microbiological tests.A 71-year-old Chinese man was admitted to hospital in January 2004 because of fever and productive cough with purulent sputum for 2 days. HE HAD A HISTORY OF PULMONARY TUBERCULOSIS more than 40 years ago complicated by cicatrization of the right upper lobe and bronchiectasis with chronic Pseudomonas aeruginosa colonization of airways. HE WAS A CHRONIC SMOKER AND ALSO HAD CHRONIC OBSTRUCTIVE AIRWAY DISEASE, HYPERLIPIDEMIA, AND ASYMPTOMATIC ABDOMINAL AORTIC ANEURYSM. He had just returned from Shenzhen, China, 3 days before admission. A chest radiograph showed patchy infiltrates over the left lower zone. NPA for direct antigen detection of respiratory viruses, RT-PCR of influenza A virus, human metapneumovirus, and SARS-CoV, and viral cultures were negative.

AFTER THE VIRUS WAS DETERMINED TO BE A CORONAVIRUS, THE NPAs WERE INOCULATED INTO RD (human rhabdomyosarcoma), I13.35 (murine macrophage), L929 (murine fibroblast), HRT-18 (colorectal adenocarcinoma), AND B95a (marmoset B-lymblastoid) CELL LINES AND MIXED NEURON-GLIA CULTURE. NO CYTOPATHIC EFFECT WAS OBSERVED. QUANTITATIVE RT-PCR, USING THE CULTURE SUPERNATANTS AND CELL LYSATES TO MONITOR THE PRESENCE OF VIRAL REPLICATION, ALSO SHOWED NEGATIVE RESULTS. MOREOVER, INTRACEREBRALLY INOCULATED SUCKLING MICE REMAINED HEALTHY AFTER 14 days. SPUTUM WAS NEGATIVE FOR BACTERIAL AND MYCOBACTERIAL PATHOGENS. Paired sera for antibodies against Mycoplasma, Chlamydia, Legionella, and SARS-CoV were negative. His symptoms improved, and he was discharged after 5 days of hospitalization.``

"DISCUSSION

We report the characterization and complete genome sequence of a novel coronavirus detected in the NPAs of patients with pneumonia."

"THE FACT THAT THE PRESENT VIRUS COULD NOT BE RECOVERED FROM CELL CULTURES COULD BE RELATED TO THE LACK OF A SUSCEPTIBLE CELL LINE FOR CoV-HKU1 OR THE INHERENTLY LOW RECOVERY RATE OF SOME CORONAVIRUSES. Many decades after the recognition of HCoV-229E and HCoV-OC43, the other non-SARS human respiratory coronaviruses known to cause pneumonia at low frequencies (27, 35, 40), THERE ARE STILL ONLY A FEW PRIMARY VIRUS ISOLATES AVAILABLE, and organ culture is required for primary isolation of HCoV-OC43. In our experience, SARS-CoV CAN BE RECOVERED ONLY FROM LESS THAN 20% OF PATIENTS with serologically and RT-PCR-documented SARS-CoV pneumonia. After the discovery of CoV-HKU1 in the index patient, we conducted a preliminary study on 400 NPAs that were collected last year during the SARS period. Among these 400 NPAs, CoV-HKU1 WAS DEFECTED IN ONE SPECIMEN, with a viral load comparable to that of the index patient. THESE RESULTS SUGGESTED THAT CoV-HKU1 IS NOT ONLY AN INCIDENTAL FINDING IN AN ISOLATED PATIENT BUT A PREVIOUSLY UNRECOGNIZED CORONAVIRUS ASSOCIATED WITH PNEUMONIA."

"THE PREVALENCE OF CoV-HKU1 IN HUMANS AS A CAUSE OF RESPIRATORY TRACT INFECTIONS REMAINS TO BE DETERMINED."

"FURTHER CLINICAL, SEROEPIDEMIOLOGICAL, AND PHYLOGENETIC STUDIES WOULD BE REQUIRED TO DETERMINE THE RELATIVE IMPORTANCE OF CoV-HKU1 compared to other respiratory tract viruses in causing upper and lower respiratory tract infections, its

seroprevalence, and the origin of the virus."

https://jvi.asm.org/content/79/2/884

In Summary:

-they start off by admitting that despite extensive laboratory investigations, the cause of respiratory disease for many patients remains unknown as no microbiological causes can be identified

-they report the discovery of HKU1 based on one 71 year old man with pneumonia

-isolation of the "virus" by culturing in various cell lines, mixed neuron-glia cultures, and through intracerebral inoculation of suckling mice were all UNSUCCESSFUL

-RNA was taken from NP swabs, urine, and feces

-RNA extracted from the unpurified nasopharyngeal aspirate was used as a template to create the genome and was converted to cDNA by a combined RANDOM-PRIMING and oligo(dT) priming strategy

-sequences were assembled and manually edited to produce the final "virus" genome

-they used "Coronavirus" specific PCR assays and compared their genome to "Coronavirus" genomes

-they used various prediction programs and if there was disagreement, they would use another to resolve it

-the 71 year old man, whom they "isolated" this new "Coronavirus" from, had a history of RESPIRATORY disease having had tuberculosis, being a chronic smoker, and having chronic obstructive airway disease, hyperlipidemia, and asymptomatic abdominal aortic aneurysm

-as stated before, no "virus" was cultured, no CPE was observed, quantitative RT-PCR results were negative, mice inoculated intracerebrally remained healthy, and his sputum was negative for bacterial and mycobacterial pathogens

-he recovered and was released 5 days later

-they then attempt to make excuses for why they were unable to isolate a "virus" from cell

cultures such as lack of susceptible cell line or the inherently low recovery rate of "Coronaviruses"

-they admit that "SARS-COV-1" can only be "isolated" from less than 20% of patients

-they then tested 400 swabs collected during the SARS epidemic and found one that matched their HKU1 sequence

-they concluded that despite the lack of "virus" from the 71 year old man and with the 1 positive result out of 400 from old SARS cases, this was proof enough that HKU1 was not an isolated incident

-they then state that the prevalence of HKU1 as a cause of respiratory disease was yet to be determined and that further clinical, seroepidemiological, and phylogenetic studies would be required to determine the importance of HKU1

No CPE observed, no "virus" was isolated from numerous cell and mixed neuron-glia cultures, quantitative RT-PCR results for "viral" replication were negative, the intracerebrally inoculated suckling mice remained healthy, no EM images were obtained, and the sample size was one 71 year old man with a history of respiratory disease....where is the proof of a new "Coronavirus" again?

https://docs.google.com/document/d/e/2PACX-1vQrxb_lyCbVvAviTJI_916jWtyJNFnvZimfKHOHT1x95JyiyI_tvNbYq_mundnzkulSuqb1ffFc1wD-/pub

ZAKI 2012 MERS CORONAVIRUS PAPER:

Remember the horrendous MERS "Coronavirus" outbreak of 2012? Yeah, me neither. Maybe it

was the fact that there were only 882 deaths associated with it globally? Or the fact that the "virus" didn't like to travel as 804 of those deaths were in Saudi Arabia? Or maybe it's because there has been no MERS "outbreak" since? Maybe the lack of evidence for the MERS "virus" is the best place to start. Highlights below:

ISOLATION OF A NOVEL CORONAVIRUS FROM A MAN WITH PNEUMONIA IN SAUDI ARABIA

"Case Report

A 60-YEAR-OLD SAUDI MAN was admitted to a private hospital in Jeddah, Saudi Arabia, on June 13, 2012, with a 7-day history of fever, cough, expectoration, and shortness of breath. He had no history of cardiopulmonary or renal disease, was receiving no long-term medications, and did not smoke. The physical examination revealed a body-mass index (the weight in kilograms divided by the square of the height in meters) of 35.1, a blood pressure of 140/80 mm Hg, a pulse of 117 beats per minute, a temperature of 38.3°C, and a respiratory rate of 20 breaths per minute."

"On day 1, treatment was started with oseltamivir, levofloxacin, piperacillin–tazobactam, and micafungin. On day 4, treatment with meropenem was started, SINCE KLEBSIELLA PNEUMONIAE THAT WAS SENSITIVE TO MEROPENEM WAS DETECTED ON BRONCHOSCOPY AND TRACHEAL LAVAGE PERFORMED ON DAY 2. STAPHYLOCOCCUS AUREUS, which was sensitive to a wide range of antimicrobials, WAS COLLECTED FROM A SPUTUM SAMPLE COLLECTED ON ADMISSION. ACINETOBACTER WAS DETECTED IN A TRACHEAL ASPIRATE SAMPLE COLLECTED ON THE DAY OF DEATH. No other pathogens were detected in respiratory specimens, and no bacterial growth was detected from blood samples."

"The patient tested negative for the human immunodeficiency virus; TESTING WAS NOT PERFORMED FOR PNEUMOCYSTIS PNEUMONIA."

"On day 11 after admission (June 24, 2012), the patient died of progressive respiratory and renal failure. A POSTMORTEM EXAMINATION WAS NOT PERFORMED."

"Methods

CLINICAL SPECIMENS AND VIRAL CULTURE

Blood samples were collected in vacutainers with and without EDTA. SPUTUM SAMPLES WERE

COLLECTED IN STERILE CUPS, AFTER WHICH VIRUS TRANSPORT MEDIUM WAS ADDED; samples were stirred and centrifuged at 2000 rpm for 10 minutes. Supernatant was transferred to a new sterile tube and USED TO INOCULATE VERO AND LLC-MK2 CELLS by adsorption for 1 hour at room temperature, AFTER WHICH 2% FETAL BOVINE SERUM IN MINIMAL ESSENTIAL MEDIUM EAGLE WAS ADDED. Flasks were incubated in a carbon dioxide incubator at 37°C and observed daily for 15 days for cytopathic changes WITH CHANGE OF MEDIUM EVERY 3 DAYS."

"The day 1 sputum sample tested negative by indirect immunofluorescence assays for influenza A and B viruses, parainfluenza viruses types 1 to 3, respiratory syncytial virus, and adenovirus. However, for a sputum sample obtained on admission, inoculation in LLC-MK2 and Vero cells RESULTED IN CYTOPATHIC CHANGES SUGGESTIVE OF VIRUS REPLICATION (Figure 2A). Cytopathic changes consisted of syncytium formation in LLC-MK2 cells at low pH and rounding and detachment of cells at neutral or alkaline pH in Vero and LLC-MK2 cells. On passage of the culture supernatant to fresh cells, the same cytopathic effects were observed within 5 days. Virus was not isolated from a blood sample collected on admission or from a tracheal aspirate sample collected 4 days after admission.

Indirect immunofluorescence assays for the detection of influenza A and B viruses, parainfluenza viruses types 1 to 3, respiratory syncytial virus, and adenovirus were performed with the infected cell cultures, but again with negative results. In contrast, when these slides were incubated with serum samples collected from the patient 10 and 11 days after admission, the samples reacted strongly when dilutions of 1:20 were tested on immunofluorescence assay specific for IgG antibodies. NO ATTEMPTS WERE MADE TO DETECT VIRUS-SPECIFIC IgM ANTIBODIES. In contrast, 2400 control serum samples collected from persons seeking medical attention at the Dr. Soliman Fakeeh Hospital in Jeddah from 2010 through 2012 remained negative in this assay. THESE DATA SUGGESTED THAT ANTIBODIES TO AN UNKNOWN VIRUS HAD DEVELOPED IN THE PATIENT, although such antibodies were not detectable in the general population over the previous 2 years.

Real-time PCR assays specific for adenovirus, enterovirus, human metapneumovirus, and human herpesvirus types 1 to 3 yielded negative results with the use of nucleic acids extracted from the inoculated cell-culture supernatants. Furthermore, family-wide PCR assays that can detect all known paramyxoviruses 6,7 also yielded negative results. However, FAMILY-WIDE PCR ASSAYS FOR THE DETECTION OF CORONAVIRUSES 3,8 YIELDED PCR FRAGMENTS OF THE EXPECTED SIZES."

"The PCR fragments of the pan-coronavirus PCR3 were sequenced. This sequence corresponded with a conserved region of open reading frame 1b of the replicase gene of a coronavirus. REFERENCE CORONAVIRUS GENOME SEQUENCES WERE DOWNLOADED FROM GenBank AND ALIGNED WITH THE AMPLIFIED FRAGMENT OF THE NEWLY DISCOVERED VIRUS, hereafter called HCoV-EMC (for Erasmus Medical Center). A maximum-likelihood tree was constructed to infer the phylogenetic relationships (Figure 2B)."

"Discussion

THE FIRST DECADE OF THE 21st CENTURY HAS WITNESSED AN INCREASE IN THE NUMBER OF CORONAVIRUSES THAT HAVE BEEN IDENTIFIED, along with a corresponding increase in the number of coronavirus genomes that have been sequenced. Such increases were due to the discovery of the SARS coronavirus, which resulted in a global outbreak of pneumonia in 2003 that affected persons in approximately 30 countries and resulted in about 800 deaths.12 BEFORE 2003, ONLY TWO HUMAN CORONAVIRUSES WERE KNOWN, HCoV-229E and HCoV-OC43, both discovered in the 1960s.13,14 After the emergence of the SARS-CoV in 2003, two additional human coronaviruses were discovered, HCoV-NL63 and HCoV-HKU1.15-17 HERE WE REPORT THE ISOLATION AND CHARACTERIZATION OF THE SIXTH CORONAVIRUS THAT APPARENTLY MAY INFECT HUMANS."

"As compared with other coronaviruses, HCoV-EMC WAS ISOLATED AND PROPAGATED RELATIVELY EASILY IN VERO AND LLC-MK2 CELLS. The only other human coronaviruses that replicate well in these monkey-cell lines are SARS-CoV and HCoV-NL63, which both use human angiotensin-converting enzyme 2 as their receptor. We hypothesize that one or more species of animals, possibly bats, were the reservoir host of this new coronavirus. Saudi Arabia harbors numerous bat species, including pipistrellus bats, which were found to carry BatCoV-HKU5 in Asia."

"Three months after the hospitalization of the patient in Jeddah, it was reported that a second patient with a history of travel to Saudi Arabia who had been transferred from a hospital in Qatar to a hospital in London was infected with the same virus.25 AT PRESENT, LINKS BETWEEN THE TWO INFECTED PATIENTS OR A POTENTIAL COMMON SOURCE OF INFECTION HAVE NOT BEEN IDENTIFIED. NO ADDITIONAL CASES HAVE BEEN IDENTIFIED, although several are still under investigation. Epidemiologic investigations, active case findings with the use of updated case definitions,25 and syndrome surveillance in combination with sensitive diagnostic tests will

be key to monitoring the present situation and — if necessary — to intervene in a potential outbreak. IT WILL BE EQUALLY IMPORTANT TO TEST WHETHER HCoV-EMC FULFILLS KOCH'S POSTULATES AS THE CAUSATIVE AGENT OF SEVERE RESPIRATORY DISEASE."

https://www.nejm.org/doi/full/10.1056/nejmoa1211721

In Summary:

-they never tested whether MERS fulfills Koch's Postulates in order to prove it is a causative agent of severe respiratory disease

END OF SUMMARY.

ACTUALLY THERE IS MORE

-MERS is based on the case of one 60 year old man

-Klebsiella Pneumoniae, Staphylococcus Aureus, and Acinetobacter were detected in the patient

-testing for Pneumocystis Pneumonia was not performed

-a postmortem examination was not performed

-sputum samples were placed into viral transport media and cultured in Vero and LLC-MK2 cells with FBS and MEME, which were replaced every 3 days

-they saw CPE SUGGESTIVE of "viral" replication

-No attempts were made to detect "virus-specific" IgM antibodies yet their data SUGGESTED that antibodies to an unknown "virus" were present

-PCR assays designed for "Coronavirus" shockingly (note sarcasm) yielded fragments of "Coronavirus" size

-"Coronavirus" reference genome sequences were downloaded and aligned with the "new" fragment

-they admit that the first decade of the 21st century has seen an increase in "Coronaviruses" discovered with there having been only 2 "Coronaviruses" from the 1960's to 2003

-they report the "isolation" and characterization of a "Coronavirus" that APPARENTLY MAY infect

humans

-they claim there was another patient infected with the "virus" but no link between the two patients nor a common infection source could be determined

-no other cases had been reported

So there you have it. MERS is based on one 60 year old patient. There were no EM images of a new "Coronavirus." They never fulfilled Koch's Postulates to prove it is a causative agent of severe respiratory disease.

End of story

https://docs.google.com/document/d/e/2PACX-1vSI1S72a1aUGQYZDgjzci-pfV8hChKZTxjn13cJJ-ZReWGJTtNZa2xgbsvpqz3qND4VnbIvXZgD6iV5/pub

ZHOU 2020 "SARS-COV-2" PAPER:

It is clear after having gone through the history of "Coronaviruses" from 1965 up to today, not a single one of these so-called "viruses" has ever been properly purified/isolated directly from a sick patient nor proven pathogenic by fulfilling Koch's Postulates. They always take the fluid from a sick patient and mix it with animal cells (usually from an African Green Monkey Kidney called Vero cells) along with a combination of antibiotics/antifungals, fetal bovine serum, "nutrients," and other chemicals. Even from this concoction, which can hardly be called an isolation of anything, they never purify any "virus" particles. Sometimes they take EM images directly from the cell culture supernatant which contains potentially billions of similar looking particles. They never prove pathogenicity in a natural way in animal models. This is as true today with "SARS-COV-2" as it was in 1965 with the forgotten B814. Highlights below from one of the first "SARS-COV-2" studies:

A PNEUMONIA OUTBREAK ASSOCIATED WITH A NEW CORONAVIRUS OF PROBABLE BAT ORIGIN

"Here we report the identification and characterization of a new coronavirus (2019-nCoV),

which caused an epidemic of acute respiratory syndrome in humans in Wuhan, China. The epidemic, which started on 12 December 2019, had caused 2,794 laboratory-confirmed infections including 80 deaths by 26 January 2020. Full-length genome sequences were obtained from five patients at an early stage of the outbreak. THE SEQUENCES ARE ALMOST IDENTICAL AND SHARE 79.6% SEQUENCES IDENTITY TO SARS-CoV. Furthermore, we show that 2019-nCoV is 96% IDENTICAL AT THE WHOLE-GENOME LEVEL TO A BAT CORONAVIRUS."

"THE DISEASE WAS DETERMINED TO BE CAUSED BY VIRUS-INDUCED PNEUMONIA BY CLINICIANS ACCORDING TO CLINICAL SYMPTOMS AND OTHER CRITERIA, including a rise in body temperature, decreases in the number of lymphocytes and white blood cells (although levels of the latter were sometimes normal), new pulmonary infiltrates on chest radiography AND NO OBVIOUS IMPROVEMENT AFTER TREATMENT WITH ANTIBIOTICS FOR THREE DAYS."

"Samples from seven patients with severe pneumonia (six of whom are sellers or delivery men from the seafood market), who were admitted to the intensive care unit of Wuhan Jin Yin-Tan Hospital at the beginning of the outbreak, were sent to the laboratory at the Wuhan Institute of Virology (WIV) for the diagnosis of the causative pathogen (Extended Data Table 1). As a laboratory investigating CoV, WE FIRST USED PAN-COV PCR PRIMERS TO TEST THESE SAMPLES 13, given that the outbreak occurred in winter and in a market—the same environment as SARS infections. WE FOUND FIVE SAMPLES TO BE PCR-POSITIVE FOR CoVs. ONE SAMPLE (WIV04), COLLECTED FROM THE BRONCHOALVEOLAR LAVAGE FLUID (BALF), WAS ANALYSED BY METAGENOMICS ANALYSIS USING NEXT-GENERATION SEQUENCING TO IDENTIFY POTENTIAL ASTROLOGICAL AGENTS. Of the 10,038,758 total reads—of which 1,582 total reads were retained after filtering of reads from the human genome—1,378 (87.1%) sequences matched the sequence of SARSr-CoV (Fig. 1a). By de novo assembly and targeted PCR, we obtained a 29,891-base-pair CoV genome that shared 79.6% sequence identity to SARS-CoV BJ01 (GenBank accession number AY278488.2). HIGH GENOME COVERAGE WAS CONTAINED BY REMAPPING THE TOTAL READS TO THIS GENOME (Extended Data Fig. 1)."

"WE THEN FOUND THAT A SHORT REGION OF RNA-DEPENDENT RNA POLYMERASE (RdRp) FROM A BAT CORONAVIRUS (BatCoV RaTG13)—which was previously detected in Rhinolophus affinis from Yunnan province—SHOWED HIGH SEQUENCE IDENTITY TO 2019-nCoV. We carried out full-length sequencing on this RNA sample (GISAID accession number EPI_ISL_402131). SIMPLOT ANALYSIS SHOWED THAT 2019-nCoV WAS HIGHLY SIMILAR THROUGHOUT THE GENOME TO RaTG13 (Fig. 1c), WITH AN OVERALL GENOME SEQUENCE IDENTITY OF 96.2%. Using the aligned genome sequences of 2019-nCoV, RaTG13, SARS-CoV and previously reported bat SARSr-CoVs,

no evidence for recombination events was detected in the genome of 2019-nCoV. Phylogenetic analysis of the full-length genome and the gene sequences of RdRp and spike (S) showed that—for all sequences—RaTG13 IS THE CLOSEST RELATIVE OF 2019-nCoV and they form a distinct lineage from other SARSr-CoVs (Fig. 1d and Extended Data Fig. 2). The receptor-binding spike protein encoded by the S gene was highly divergent from other CoVs (Extended Data Fig. 2), with less than 75% nucleotide sequence identity to all previously described SARSr-CoVs, EXCEPT FOR A 93.1% NUCLEOTIDE IDENTITY TO RaTG13 (Extended Data Table 3). The S genes of 2019-nCoV and RaTG13 are longer than other SARSr-CoVs."

"THE CLOSE PHYLOGENETIC RELATIONSHIP TO RaTG13 PROVIDES EVIDENCE THAT 2019-nCoV MAY HAVE ORIGINATED IN BATS."

"WE RAPIDLY DEVELOPED A QPCR-BASED DETECTION METHOD on the basis of the sequence of the receptor-binding domain of the S gene, which was the most variable region of the genome (Fig. 1c). Our data show that the primers could differentiate 2019-nCoV from all other human coronaviruses including bat SARSr-CoV WIV1, which shares 95% identity with SARS-CoV (Extended Data Fig. 4a, b). Of the samples obtained from the seven patients, WE FOUND THAT SIX BALF AND FIVE ORAL SWAB SAMPLES WERE POSITIVE FOR 2019-nCoV during the first sampling, as assessed by qPCR and conventional PCR."

"WE NEXT SUCCESSFULLY ISOLATED THE VIRUS (called 2019-nCoV BetaCoV/Wuhan/WIV04/2019) FROM BOTH VERO E6 AND Huh7 CELLS USING THE BALF SAMPLE OF PATIENT ICU-06. Clear cytopathogenic effects were observed in cells after incubation for three days (Extended Data Fig. 6a, b). The identity of the strain WIV04 was verified in Vero E6 cells by immunofluorescence microscopy using the cross-reactive viral N antibody (Extended Data Fig. 6c, d) and by metagenomics sequencing, most of the reads of which mapped to 2019-nCoV, and qPCR analysis showed that the viral load increased from day 1 to day 3 (Extended Data Fig. 6e, f). VIRAL PARTICLES IN ULTRATHIN SECTIONS OF INFECTED CELLS DISPLAYED A TYPICAL CORONAVIRUS MORPHOLOGY, as visualized by electron microscopy (Extended Data Fig. 6g)."

"The study provides a detailed report on 2019-nCoV, THE LIKELY AETIOLOGICAL AGENT RESPONSIBLE FOR THE ONGOING EPIDEMIC OF ACUTE RESPIRATORY SYNDROME in China and other countries. Virus-specific nucleotide-positive and viral-protein seroconversion was observed in all patients tested and provides evidence of an association between the disease and

the presence of this virus. However, there are still many urgent questions that remain to be answered. THE ASSOCIATION BETWEEN 2019-nCoV AND THE DISEASE HAS NOT BEEN VERIFIED BY ANIMAL EXPERIMENTS TO FULFIL THE KOCH'S POSTULATES TO ESTABLISH A CAUSATIVE RELATIONSHIP BETWEEN A MICROORGANISM AND A DISEASE. WE DO NOT YET KNOW THE TRANSMISSION ROUTINE OF THIS VIRUS AMONG HOSTS."

"Sample collection

Human samples, including oral swabs, anal swabs, blood and BALF samples were collected by Jinyintan hospital (Wuhan, China) with the consent of all patients and approved by the ethics committee of the designated hospital for emerging infectious diseases. Patients were sampled without gender or age preference unless indicated. FOR SWABS, 1.5 ml DMEM CONTAINING 2% FBS WAS ADDED TO EACH TUBE. The supernatant was collected after centrifugation at 2,500 rpm, vortexing for 60 s and a standing period of 15–30 min. The supernatant from swabs or BALF (no pre-treatment) was added to either lysis buffer for RNA extraction OR TO VIRAL TRANSPORT MEDIUM FOR ISOLATION OF THE VIRUS. THE VIRAL TRANSPORT MEDIUM WAS COMPOSED OF HANK'S BALANCED SALT SOLUTION (pH 7.4) CONTAINING BSA (1%), AMPHOTERICIN (15 μg ml−1), PENICILLIN G (100 units ml−1) AND streptomycin (50 μg ml−1). Serum was separated by centrifugation at 3,000g for 15 min within 24 h of collection, followed by inactivation at 56 °C for 1 h, and was then stored at 4 °C until use.

Virus isolation, cell infection, electron microscopy and neutralization assay

The following cell lines were used for virus isolation in this study: VERO E6 AND Huh7 CELLS, WHICH WERE CULTURED IN DMEM CONTAINING 10% FBS. All cell lines were tested and free of mycoplasma contamination, submitted for species identification and authenticated by morphological evaluation by microscopy. None of the cell lines was on the list of commonly misidentified cell lines (by ICLAC).

CULTURED CELL MONOLAYERS WERE MAINTAINED IN THEIR RESPECTIVE MEDIUM. The PCR-positive BALF sample from ICU-06 patient was spun at 8,000g for 15 min, filtered and DILUTED 1:2 WITH DMEM SUPPLEMENTED WITH 16 μg ml−1 TRYPSIN BEFORE IT WAS ADDED TO THE CELLS. After incubation at 37 °C for 1 h, THE INOCULUM WAS REMOVED AND REPLACED WITH FRESH CULTURE MEDIUM CONTAINING ANTIBIOTICS (see below) and 16 μg ml−1 trypsin. The cells were incubated at 37 °C and observed daily for cytopathogenic effects. The culture supernatant was examined for the presence of virus by qRT–PCR methods developed in this study, and cells were examined by immunofluorescence microscopy using the anti-SARSr-CoV

Rp3 N antibody that was generated in-house (1:1,000). PENICILLIN (100 units ml—1) AND STREPTOMYCIN (15 μg ml—1) WERE INCLUDED IN ALL TISSUE CULTURE MEDIA.

VERO E6 CELLS WERE INFECTED WITH THE NEW VIRUS at a multiplicity of infection (MOI) of 0.5 and collected 48 h after infection. CELLS WERE FIXED WITH 2.5% (w/v) GLUTARALDEHYDE AND 1% OSMIUM TETROXIDE, DEHYDRATED THROUGH A GRADED SERIES OF ETHANOL CONCENTRATIONS (from 30 to 100%) AND EMBEDDED WITH EPOXY RESIN. Ultrathin sections (80 nm) of embedded cells were prepared, deposited onto Formvar-coated copper grids (200 mesh), stained with uranyl acetate and lead citrate, and analysed using a 200-kV Tecnai G2 electron microscope."

"SAMPLES FROM PATIENT BALF OR FROM THE SUPERNATANT OF VIRUS CULTURES WERE USED FOR RNA EXTRACTION AND NEXT-GENERATION SEQUENCING (NGS) using BGI MGISEQ2000 and Illumina MiSeq 3000 sequencers."

https://www.nature.com/articles/s41586-020-2012-7#ref-CR13

In Summary:

-the discovery of this new "Coronavirus" started from the sequencing of a genome from the unpurified BALF of sick patients

-the sequences were only 79.8% similar to the original SARS

-they were, however, 96.2% similar to a bat "Coronavirus" named RaTG13

-the cases of disease were determined to be caused by a "virus" based on clinical symptoms and other measures as well as the lack of improvement after 3 days of antibiotic use

-they tested samples from seven patients with "Coronavirus" PCR primers based on the hunch that it may be SARS due to the location of the patients

-5 of the 7 tested positive by PCR for "Coronaviruses" and the BALF from one patient was sent for metagenomic sequencing as detailed here:

https://m.facebook.com/story.php?story_fbid=1015802029508576&id=502548575

https://m.facebook.com/story.php?story_fbid=10158048691828576&id=502548575

-they next quickly developed their own PCR test to detect "SARS-COV-2" in the BALF of 6 out of 7 patients and from the oral swabs of 5 out of 7 patients

-they finally decided to "isolate" the "virus" in Vero and HuH7 cell cultures AFTER they had determined their genome and made their PCR test

-they looked at unpurified cell culture supernatant in an Electron Microscope and saw "Coronavirus-like" particles (from which there are many similar looking particles within the sample) and decided that was their "virus"

-they concluded in their study that "SARS-COV-2" is the LIKELY aetiological agent causing disease

-they then admit that they did not fulfill Koch's Postulates to actually determine whether or not their new "virus" actually causes disease

-they admit animal studies to reproduce the same disease as seen in humans were still needed

-they admit the mode of transmission for the "virus" was unknown

As with MERS before it, this whole study and the hysteria surrounding "SARS-COV-2" can be completely thrown out due to the researchers admitting that they never fulfilled Koch's Postulates nor proved that their letters in a database actually exists nor causes disease. They mention EM images yet never supplied any in the study. They never mention any attempts at purification. They started with a genome before they ever attempted "isolating" a "virus." The genomes used as references to create "SARS-COV-2" came from unpurified and highly questionable sources. This paper is one big fraudulent mess.

If you can not see the lies of Virology by reading these papers, you aren't trying very hard as they are there, clear as day for all to see.

"CORONAVIRUS" OC43 GENOME:

In 1967, it was claimed a new "Coronavirus" labelled OC43 was discovered and "isolated." This was supposedly done through tissue cultures using tracheas from 5-9 month old aborted fetuses which were collected and stored in Hanks Salt Solution along with 10% FETAL BOVINE SERUM as well as penicillin and streptomycin. Fragments were partially covered in Leibovitz Medium, BOVINE ALBUMIN, glutamine, penicillin, and streptomycin. The culture goo was later "transferred" to suckling MICE brain cultures. This is all detailed here:

1967 Preliminary OC43 PAPER:

https://docs.google.com/document/d/e/2PACX-1vRoL-yEV689CBayF_6zu725uUjr0tS8vXS2WX-kKO8vdHPBsPWfrOKIMp3WiegnTrGLDxVBW5KVsAAS/pub

1967 OC43 PAPER:

https://docs.google.com/document/d/e/2PACX-1vQYafmjVpj2xXrP07mpMTsWDMrDmQjQM5U46GeckGMTnIqwYHyAlsHdCb_Jb9qRd_lsvDSgeaFGfhcX/pub

Upon sequencing the OC43 genome for the first time in 2005, the genomes for the BOVINE "Coronavirus" and the MHV MICE "virus" were used as references due to their genetic similarity. You don't think this could have had anything to do with the BOVINE ALBUMIN used to cover the tissues in or the fetal BOVINE serum used to store them in? Or maybe it could be due to passaging this mixture of human/bovine goo numerous times into mice brains? Well, according to this study, that had nothing to do with these genetic similarities. It was an unprovable leap from animal "virus" to man based on the hypothetical and assumption filled Molecular Clock. Highlights and summary below:

COMPLETE GENOMIC SEQUENCE OF HUMAN CORONAVIRUS OC43: MOLECULAR CLOCK ANALYSIS SUGGESTS A RELATIVELY RECENT ZOONOTIC CORONAVIRUS TRANSMISSION EVENTUALLY

"HCoV-OC43 and BCoV (ICTVdb code 03.019.0.01.002) SHOW REMARKABLE ANTIGENIC AND GENETIC SIMILARITIES (23, 29, 36, 44, 52, 63, 65). They both have hemagglutinating activity by attaching to the N-acetyl-9-O-acetylneuraminic acid moiety on red blood cells (33). BCoV causes severe diarrhea in newborn calves. The complete nucleotide sequences of different BCoV strains are known, but ONLY FRAGMENTS OF THE HCoV-OC43 GENOME HAD BEEN DETERMINED PREVIOUSLY."

"HCoV-OC43 WAS PROPAGATED IN A HUMAN RHABDOMYOSARCOMA (RD) CELL LINE, obtained from the European Collection of Cell Cultures (ECACC). The supernatant was harvested after 7 days of incubation at 33°C, and RNA was isolated by using the QIAamp viral RNA kit (QIAGEN,)."

"For BOTH RT-PCR AND SEQUENCING, oligonucleotide primers were designed in regions that were CONSERVED BETWEEN THE BCoV AND MHV GENOMES."

"To generate RT-PCR products containing the exact 3′-terminal sequence, we used oligonucleotide OC43R74 (5′-TTTTTTTTTTGTGATTCTTCCA-3′) BASED ON THE CONSERVED 3′-END SEQUENCE OF ALL KNOWN GROUP 2 CORONAVIRUSES."

"The SEQUENCE SIMILARITY among HCoV-OC43, BCoV, CRCoV, PHEV, ECoV, MHV, and SDAV was investigated by pairwise alignments of the corresponding ORFs and their proteins (Table (Table2).2). HCoV-OC43 SHOWED THE HIGHEST PERCENTAGE OF SIMILARITY TO BCoV in all ORFs except for the HCoV-OC43 E gene, which showed 99.6% identity on the nucleotide level and 98.8% identity on the protein level to the PHEV E gene. Maizel-Lenk dot matrix plots illustrate the similarity between HCoV-OC43 and BCoV (Fig. (Fig.33)."

"The prototype HCoV-OC43 strain (ATCC VR759) is a LABORATORY STRAIN that, since its isolation in 1967, has been PASSAGED 7 TIMES IN HUMAN EMBRYONIC TRACHEAL ORGAN CULTURE, followed by 15 PASSAGES IN SUCKLING MOUSE BRAIN CELLS and an UNKNOWN NUMBER OF PASSAGES IN HUMAN RECTAL TUMOR HRT-18 CELLS AND/OR VERO CELLS. DURING THE PASSAGE HISTORY, IT IS LIKELY THAT A NUMBER OF MUTATIONS HAVE ACCUMULATED. It would be interesting to analyze the complete nucleotide sequence of contemporary HCoV-OC43 strains that are FREE FROM IN VITRO EXPANSION MUTATIONS."

"We suggest that around 1890, BCoV might have jumped the species barrier and became able to infect humans, resulting in the emergence of a new type of human coronavirus (HCoV-OC43), a scenario similar to the origin of the SARS outbreak. INDISPUTABLE EVIDENCE FOR THE BOVINE-TO-HUMAN DIRECTION OF THE INTERSPECIES TRANSMISSION EVENT, instead of a human-to-bovine direction, IS NOT AVAILABLE. However, we consider the occurrence of a 290-nucleotide deletion (corresponding to the absence of BCoV ns4.9 and ns4.😎 in HCoV-OC43 relative to the BCoV genome to be a potential supporting argument, as this additional sequence fragment in BCoV is also present in MHV and SDAV. Consequently, we ASSUME that a deletion from BCoV to HCoV-OC43 RATHER THAN AN INSERTION IN THE OPPOSITE DIRECTION took place during evolution, and thus, we HYPOTHESIZE that the interspecies transmission event occurred from bovines to humans."

"Nevertheless, it is possible that two other group 2 coronaviruses, CRCoV and PHEV, might have played a role in the emergence of HCoV-OC43. CRCoV appears to be very closely related to BCoV and HCoV-OC43 (16), and for the HCoV-OC43 E gene, the highest percentage of similarity was found with the PHEV E gene, suggesting a possible recombination event. To elucidate the evolutionary relationship of HCoV-OC43 and BCoV with CRCoV and PHEV, complete genome sequence data of CRCoV and PHEV would be required. Molecular dating has frequently been used to investigate the origin of viral epidemics (31, 40, 48). The reliability of such an analysis is DEPENDENT ON THE VALIDITY OF THE MOLECULAR CLOCK HYPOTHESIS, which ASSUMES that the evolutionary rate is roughly constant in the lineages of a phylogenetic tree. ALTHOUGH THIS ASSUMPTION IS FREQUENTLY VIOLATED FOR VIRAL SEQUENCE DATA (28), a molecular clock test indicated that this HYPOTHESIS could not be rejected for the coronavirus data set investigated

here."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC544107/

In Summary:

-"HCoV-OC43 and BCoV are considered related due to antigenic and genetic similarities

-only fragments of the HCoV-OC43 genome had been determined previously

-HCoV-OC43 was cultured in a human rhabdomyosarcoma (RD) cell line

-unfortunately, no other cell culture information was provided but I found this upon doing a bit of digging:

1. Rhabdomyosarcoma (RMS) is the most common SOFT TISSUE SARCOMA of childhood and adolescence

2 The RD cell line used for the OC43 genome was DERIVED DIRECTLY FROM BIOPSY SPECIMENS of a 7-year-old female with a pelvic RMS PREVIOUSLY TREATED WITH CYCLOPHOSPHAMIDE AND RADIATION and found to have refractory disease

3. It is grown in EAGLE'S MEDIUM WITH 10% FETAL BOVINE SERUM

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3713458/

-for BOTH RT-PCR AND SEQUENCING, oligonucleotide primers were designed in regions that were CONSERVED BETWEEN THE BCoV (bovine coronavirus) AND MHV (murine coronavirus) GENOMES

-in other words, they used the genomes from cows and mice "coronaviruses" to create the genome for the human OC43 "coronavirus"

-to generate RT-PCR products containing the exact 3′-terminal sequence, they used oligonucleotide OC43R74 (5′-TTTTTTTTTTGTGATTCTTCCA-3′) BASED ON THE CONSERVED 3′-END

SEQUENCE OF ALL KNOWN GROUP 2 "CORONAVIRUSES"

-the SEQUENCE SIMILARITY among HCoV-OC43, BCoV, CRCoV, PHEV, ECoV, MHV, and SDAV was investigated by pairwise alignments of the corresponding ORFs and their proteins

-HCoV-OC43 showed the HIGHEST PERCENTAGE OF SIMILARITY to BCoV

-in other words, they used conserved regions from all Group 2 "Coronaviruses" to generate RT-PCR products and then somehow seem surprised that the genome created for OC43 has SIMILARITY to all of them and has the most SIMILARITY to BCoV which they used as a template for both RT-PCR and sequencing (!?)

-the prototype HCoV-OC43 strain (ATCC VR759) is a LABORATORY STRAIN that, since its "isolation" in 1967, has been PASSAGED 7 TIMES IN HUMAN EMBRYONIC TRACHEAL ORGAN CULTURE, followed by 15 PASSAGES IN SUCKLING MOUSE BRAIN CELLS and an UNKNOWN NUMBER OF PASSAGES IN HUMAN RECTAL TUMOR HRT-18 CELLS AND/OR VERO CELLS

-during the passage history, IT IS LIKELY THAT A NUMBER OF MUTATIONS HAVE ACCUMULATED

-they state that It would be interesting to analyze the complete nucleotide sequence of contemporary HCoV-OC43 strains that are FREE FROM IN VITRO EXPANSION MUTATIONS

-INDISPUTABLE EVIDENCE for the bovine-to-human direction of the interspecies transmission event, instead of a human-to-bovine direction, IS NOT AVAILABLE

-they ASSUME that a deletion from BCoV to HCoV-OC43 RATHER THAN AN INSERTION IN THE OPPOSITE DIRECTION took place during evolution, and thus, they HYPOTHESIZE that the interspecies transmission event occurred from bovines to humans

-they attempt to link the animal and human "Coronaviruses" through the molecular clock hypothesis which states that DNA and protein sequences evolve at a rate that is relatively constant over time and among different organisms

-however, they admit that the reliability of such an analysis is DEPENDENT ON THE VALIDITY of the molecular clock hypothesis, which ASSUMES that the evolutionary rate is roughly constant in the lineages of a phylogenetic tree

-although this ASSUMPTION IS FREQUENTLY VIOLATED for "viral" sequence data, a molecular clock test indicated that this hypothesis could not be rejected for the "coronavirus" data set investigated in this study

-in other words, their molecular clock test indicated that their molecular clock hypothesis could not be ruled out in regards to linking these "Coronaviruses" hence the subtitle of the study:

Molecular Clock Analysis SUGGESTS a Relatively Recent Zoonotic Coronavirus Transmission Event

To anyone looking at this logically, it is clear to see no "virus" was ever purified/isolated before the creation of this genome. Once again, look at what they admit to in the "isolation" of this "virus:"

"the prototype HCoV-OC43 strain (ATCC VR759) is a LABORATORY STRAIN that, since its "isolation" in 1967, has been PASSAGED 7 TIMES IN HUMAN EMBRYONIC TRACHEAL ORGAN CULTURE, followed by 15 PASSAGES IN SUCKLING MOUSE BRAIN CELLS and an UNKNOWN NUMBER OF PASSAGES IN HUMAN RECTAL TUMOR HRT-18 CELLS AND/OR VERO CELLS"

The "virus" itself is nothing more than human embryo tissue cultured goo mixed with bovine and mice DNA (and even African Green Monkeys judging by the use of Vero cells). The genome is a mixture of all of these sources stitched together off of cow/mice "virus" genome templates. They then claim a relation between all of these "viruses" and a zoonotic leap when the only "leaps" that occurred where in the cell culture dish and the numerous unfounded assumptions made from the results.

(Image is a stock image and did not come from any of these studies.)

https://docs.google.com/document/d/e/2PACX-1vRGHIDzDffFlLkiihzk2s3BIhDe1W48YCzqE3zeJD_cKrIyvvjwz_1BHY8HUPXXoPHkwmKzOj4tYMyl/pub

PARK 2020 "SARS-COV-2" PAPER:

“WE DID NOT OBTAIN AN ELECTRON MICROGRAPH SHOWING THE DEGREE OF PURIFICATION.”

Replying Author: Wan Beom Park

https://off-guardian.org/2020/06/27/covid19-pcr-tests-are-scientifically-meaningless

This paper is often cited by many as proof that "SARS-COV-2" exists and has been "isolated" from a sick patient. The problem is, they only read the title of the study. Had they read the study itself, it would be obvious to them that what the researchers call "isolation" of a "virus" is the exact opposite as they used the same cell culture soup full of foreign animal DNA, antibiotics/antifungals, "nutrients," chemicals, etc. that is used in the previous "Coronavirus" papers. Adding various ingredients together with the sample taken from a sick patient and incubating it for days until nonspecific cell damage called Cytopathic Effects (CPE) is observed is not isolation. It is nothing more than unpurified witches brew, as can be seen from the quote above by lead researcher Wan Beom Park admitting they did not purify anything. Highlights from his study below:

VIRUS ISOLATION FROM THE FIRST PATIENT WITH SARS-CoV-2 IN KOREA

"Here, we report the isolation of SARS-CoV-2 USING VERO CELLS from a patient entering Korea from Wuhan, China.

The patient with the FIRST LABORATORY-CONFIRMED SARS-CoV-2 INFECTION in Korea is published previously.6 Briefly, a 35-year-old woman developed fever, chill, and myalgia on January 18, 2020, and arrived at the Incheon airport from Wuhan on the next day. AFTER LABORATORY-CONFIRMED DIAGNOSIS OF SARS-CoV-2 INFECTION, she developed nasal congestion, cough, and sputum. Oxygen supplementation was started on day 4 of her illness, and her oxygen requirement increased to 6 L/min on day 7 of illness. Fever persisted for ten days and her maximum body temperature during her illness was 38.9°C on day 7 of illness.

THE PATIENT'S OROPHARYNGEAL SAMPLES WERE OBTAINED BY USING UTM™ kit CONTAINING 1 mL OF VIRAL TRANSPORT MEDIA (Copan Diagnostics Inc., Murrieta, CA, USA) on day 7 of her illness. WE INOCULATED MONOLAYERS OF VERO CELLS (ATCC ® CCL-81™) with the samples and cultured the cells at 37°C in a 5% carbon dioxide atmosphere. Until 5 days after inoculation, cytopathic effects were not distinct, which is compatible with the previous findings that no specific cytopathic effects were observed in the Vero E6 cells until 6 days after inoculation in the report about first isolation of SARS-CoV-2.3 FIVE DAYS AFTER INOCULATION, WE DID BLIND PASSAGE OF CULTURE SUPERNATANT INTO T-25 CULTURE FLASK (ThermoFisher Scientific Inc., Waltham, MA, USA) WITH MONOLAYERS OF VERO CELLS, and cytopathic effects consisting of rounding and detachment of cells were observed in the whole area of the T-25 flask 3 DAYS AFTER THE FIRST BLIND PASSAGE (Fig. 1A and B).

IN ORDER TO OBSERVE VIRUS PARTICLES, VERO CELL MONOLAYER SHOWING THE CYTOPATHIC EFFECTS WAS FIXED as previously described.7 It was cut on ultramicrotome (RMC MT-XL; RMC Boeckeler, Tucson, AZ, USA) at 65 nm. Ultrathin sections were stained with saturated 4% uranyl acetate and 1% lead citrate before examination with a transmission electron microscope (JEM-1400; JEOL USA Inc., Peabody, MA, USA) at 80 kV. Spherical particles with crown-like spikes ranging 66 to 81 nm in diameter were observed within the cytoplasmic vesicles and in the extracellular space adjacent to cell membrane (Fig. 1C and D).

FOR WHOLE GENOME SEQUENCING OF THE VIRUS ISOLATE (BetaCoV/Korea/SNU01/2020), CULTURE SUPERNATANT OF VERO CELLS INFECTED WAS USED FOR RNA EXTRACTION. RNA was extracted by using QIAamp viral RNA mini kit (QIAGEN, Valencia, CA, USA), according to the manufacturer's instructions. RNA libraries were prepared using TruSeq Stranded Total RNA Kit (catalog No. 20020596; Illumina, San Diego, CA, USA) according to the manufacturer protocol. Sequencing was performed on an Illumina Nextseq 500 platform, produced on average a total of 150 million reads, 150 bp per sample, as per the manufacturer's instructions in Macrogen Inc. (Seoul, Korea).8,9,10 FASTQ was used to trim the adapter and remove low quality bases and reads. QUALIFIED READS WERE MAPPED TO NC_045512, A SARS-CoV-2 GENOME REFERENCE using Burrows-Wheeler Aligner (v0.7.12-r1039), and a bam file was produced.11 In this bam file, the variation was confirmed by comparing with genome using SAMtools (v1.3.1).12 For genome-base phylogeny analysis, 37 strains including BetaCoV/Korea/KCDC03/2020 were used in combination with BetaCoV/Korea/SNU01/2020. The sequences used for analysis were downloaded from NCBI (http://www.ncbi.nlm.nih.gov/) and GISAID (http://www.gisaid.org). The 37 strain genomes were multiple-sequence aligned using MAFFT (v7.450), a sequence alignment tool, and were used to generate phylogenetic tree.13 Phylogenetic analysis of the aligned sequence was performed with 1,000 bootstrap replicates using MEGAX and a general time-

reversible model used as the nucleotide substitution model.14

NEXT-GENERATION SEQUENCING OF BetaCoV/Korea/SNU01/2020 (GenBank accession no. MT039890) REVEALED 9 MUTATIONS COMPARED TO THE NC_045512 REFERENCE GENOME ISOLATED FROM WUHAN (Table 1). Most of the mutations in our isolate consisted of 70% alternative genes and 30% reference genes (NC_045512). Five variants were found in ORF1ab, one variant in S gene, two variants in ORF3a, and one variant in E gene. Of the nine mutations, six also showed changes in amino acids. When comparing our isolate with the one isolated from the Korea Centers for Disease Control and Prevention (BetaCoV/Korea/KCDC03/2020), 12 VARIANTS INCLUDING THE ABOVE 9 MUTATIONS WERE FOUND. THESE MUTATIONS MAY OCCUR BY CELL CULTURE-ADAPTATION IN THAT OUR CULTURE ISOLATE WAS OBTAINED AFTER FIRST BLIND PASSAGE, or by micro-evolution of SARS-CoV-2 before acquisition in Wuhan. BECAUSE THOSE GENOME SEQUENCES ARE QUITE HOMOLOGOUS EACH OTHER, IT IS DIFFICULT TO VALIDATE THESE TWO HYPOTHESIS.``

"In summary, we ISOLATED SARS-CoV-2 USING VERO CELLS FROM THE FIRST LABORATORY-CONFIRMED SARS-COV-2-INFECTED PATIENT in Korea. Phylogenetic analyses of the whole genome sequences showed that it clustered with other SARS-CoV-2 reported from Wuhan, China."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7036342/

-In Summary:

-they "isolated" their "virus" using Vero cell (African Green Monkey kidneys) culture

-the case is considered the first laboratory-confirmed case in Korea

-they took an oropharyngeal (throat) sample and immediately placed it in Viral Transport Media (ingredients not listed)

-this sample was inoculated directly onto the Vero cells

-they did not observe any CPE after 5 days so they did a first blind passage into a new flask of Vero cells and 3 days later observed CPE

-the process of blind passaging cells can damage/alter the sample as detailed here:

https://m.facebook.com/story.php?story_fbid=10158084526738576&id=502548575

-in order to observe "virus," the Vero cells showing CPE (to which they admitted was not purified) were fixed for EM imaging in order to find the spherical crown-like particles they wanted to see

-for whole-genome sequencing, RNA was extracted from the same unpurified Vero cell sample

-"qualified" reads were then mapped to an already prepared "SARS-COV-2" reference genome

-there were 9 "mutations" found in the Korea "virus" that differed from the Wuhan reference "virus"

-when comparing to a genome from the Korean CDC, they found that their genome had 12 "mutations" including the 9 from the Wuhan reference

-they admit that the culturing process may have created these "mutations" through cell-culture adaptation as they obtained their genome after the first blind passage

The problem for papers like this, beyond the fact that they have completely inverted the meaning of the word ISOLATION, is that they do not perform the necessary controls to find out if the CPE they observe in the cell culture soup is created by a "virus" or from the cell-culturing process itself. The researchers here at least admit they are unable to determine if the "mutations" in their genome were created by the culturing process. If they took it a step further, they would realize that they can never prove that what they created through their chemistry experiments in a lab was ever in the original sample to begin with. This is why it is absolutely necessary to purify/isolate the particles believed to be "virus" DIRECTLY from the UNALTERED patient sample and not from experimental soup in a lab.

ZHU 2020 "SARS-COV-2" PAPER:

"[We show] an image of sedimented virus particles, NOT PURIFIED ONES."

-Replying Author: Wenjie Tan

https://off-guardian.org/2020/06/27/covid19-pcr-tests-are-scientifically-meaningless/

The Zhu study gets the infamous distinction of not only admitting to not purifying their "isolates" but also to not fulfilling Koch's Postulates.

They present similar evidence and findings as the earlier Zhou study yet neither of them were able to satisfy the proper scientific criteria needed to prove a new "virus" exists nor that it can also cause disease. Highlights below:

A NOVEL CORONAVIRUS FROM PATIENTS WITH PNEUMONIA IN CHINA, 2019

"VIRAL DIAGNOSTIC METHODS

FOUR LOWER RESPIRATORY TRACT SAMPLES, INCLUDING BRONCHOALVEOLAR-LAVAGE FLUID, WERE COLLECTED FROM PATIENTS WITH PNEUMONIA OF UNKNOWN CAUSE who were identified in Wuhan on December 21, 2019, or later and who had been present at the Huanan Seafood Market close to the time of their clinical presentation. SEVEN BRONCHOALVEOLAR-LAVAGE FLUID SPECIMENS WERE COLLECTED FROM PATIENTS IN BEIJING HOSPITALS WITH PNEUMONIA OF KNOWN CAUSE TO SERVE AS CONTROL SAMPLES."

ISOLATION OF VIRUS

"Bronchoalveolar-lavage fluid samples were collected in sterile cups TO WHICH VIRUS TRANSPORT MEDIUM WAS ADDED. Samples were then centrifuged to remove cellular debris. The supernatant was inoculated on human airway epithelial cells,13 WHICH HAD BEEN OBTAINED FROM AIRWAY SPECIMENS RESECTED FROM PATIENTS UNDERGOING SURGERY FOR

LUNG CANCER and were confirmed to be special-pathogen-free by NGS.14"

"Prior to infection, apical surfaces of the HUMAN AIRWAY EPITHELIAL CELLS WERE WASHED THREE TIMES WITH PHOSPHATE-BUFFERED SALINE; 150 µl OF SUPERNATANT FROM BRONCHOALVEOLAR-LAVAGE FLUID SAMPLES WAS INOCULATED ONTO THE APICAL SURFACE OF THE CELL CULTURES. After a 2-hour incubation at 37°C, UNBOUND VIRUS WAS REMOVED BY WASHING WITH 500 µl OF PHOSPHATE-BUFFERED SALINE FOR 10 MINUTES; human airway epithelial cells were maintained in an air–liquid interface incubated at 37°C with 5% carbon dioxide. EVERY 48 HOURS, 150 µl OF PHOSPHATE-BUFFERED SALINE WAS APPLIED TO THE APICAL SURFACES OF THE HUMAN AIRWAY EPITHELIAL CELLS, and after 10 minutes of incubation at 37°C the samples were harvested. Pseudostratified mucociliary epithelium cells were maintained in this environment; apical samples were passed in a 1:3 diluted vial stock to new cells. The cells were monitored daily with light microscopy, for cytopathic effects, and with RT-PCR, for the presence of viral nucleic acid in the supernatant. AFTER THREE PASSAGES, APICAL SAMPLES AND HUMAN AIRWAY EPITHELIAL CELLS WERE PREPARED FOR TRANSMISSION ELECTRON MICROSCOPY.

TRANSMISSION ELECTRON MICROSCOPY

Supernatant from human airway epithelial cell cultures that showed cytopathic effects was collected, INACTIVATED WITH 2% PARAFORMALDEHYDE FOR AT LEAST 2 HOURS, and ultracentrifuged to sediment virus particles. The enriched supernatant was negatively stained on film-coated grids for examination. Human airway epithelial cells showing cytopathic effects were collected AND FIXED WITH 2% PARAFORMALDEHYDE–2.5% GLUTARALDEHYDE AND WERE THEN FIXED WITH 1% OSMIUM TETROXIDE DEHYDRATED WITH GRADE ETHANOL EMBEDDED WITH PON812 RESIN. Sections (80 nm) were cut from resin block and stained with uranyl acetate and lead citrate, separately. The negative stained grids and ultrathin sections were observed under transmission electron microscopy."

"VIRAL GENOME SEQUENCING

RNA EXTRACTED FROM BRONCHOALVEOLAR-LAVAGE FLUID AND CULTURE SUPERNATANTS WAS USED AS A TEMPLATE TO CLONE AND SEQUENCE THE GENOME."

"DETECTION AND ISOLATION OF A NOVEL CORONAVIRUS

Three bronchoalveolar-lavage samples were collected from Wuhan Jinyintan Hospital on December 30, 2019. No specific pathogens (including HCoV-229E, HCoV-NL63, HCoV-OC43, and HCoV-HKU1) were detected in clinical specimens from these patients by the RespiFinderSmart22kit. RNA extracted from bronchoalveolar-lavage fluid from the patients was used as a template to clone and sequence a genome using a combination of Illumina sequencing and nanopore sequencing. More than 20,000 viral reads from individual specimens were obtained, and most contigs matched to the genome from lineage B of the genus betacoronavirus — SHOWING MORE THAN 85% IDENTITY WITH A BAT SARS-like CoV (bat-SL-CoVZC45, MG772933.1) GENOME PUBLISHED PREVIOUSLY. Positive results were also obtained with use of a real-time RT-PCR assay for RNA targeting to a consensus RdRp region of pan β-CoV (ALTHOUGH THE CYCLE THRESHOLD VALUE WAS HIGHER THAN 34 for detected samples). VIRUS ISOLATION FROM THE CLINICAL SPECIMENS WAS PERFORMED WITH HUMAN AIRWAY EPITHELIAL CELLS AND VERO E6 AND Huh-7 CELL LINES. The isolated virus was named 2019-nCoV."

"Electron micrographs of negative-stained 2019-nCoV particles were generally spherical WITH SOME PLEOMORPHISM (Figure 3). Diameter varied from about 60 to 140 nm. Virus particles had quite distinctive spikes, about 9 to 12 nm, and gave virions the appearance of a solar corona. Extracellular free virus particles and inclusion bodies filled with virus particles in membrane-bound vesicles in cytoplasm were found in the human airway epithelial ultrathin sections. THIS OBSERVED MORPHOLOGY IS CONSISTENT WITH THE CORONAVIRIDAE FAMILY."

"Discussion

We report a novel CoV (2019-nCoV) that was identified in hospitalized patients in Wuhan, China, in December 2019 and January 2020. Evidence for the presence of this virus includes IDENTIFICATION IN BRONCHOALVEOLAR-LAVAGE FLUID IN THREE PATIENTS BY WHOLE-GENOME SEQUENCING, DIRECT PCR, AND CULTURE. THE ILLNESS LIKELY TO HAVE BEEN CAUSED BY THIS CoV was named “novel coronavirus-infected pneumonia” (NCIP)."

"Molecular techniques have been used successfully to identify infectious agents for many years. Unbiased, high-throughput sequencing is a powerful tool for the discovery of pathogens.14,16 Next-generation sequencing and bioinformatics are changing the way we can respond to infectious disease outbreaks, improving our understanding of disease occurrence and transmission, accelerating the identification of pathogens, and promoting data sharing. WE DESCRIBE IN THIS REPORT THE USE OF MOLECULAR TECHNIQUES AND UNBIASED DNA

SEQUENCING TO DISCOVER A NOVEL BETACORONAVIRUS THAT IS LIKELY TO HAVE BEEN THE CAUSE OF SEVERE PNEUMONIA IN THREE PATIENTS IN WUHAN, CHINA.

Although establishing human airway epithelial cell cultures is labor intensive, they appear to be a valuable research tool for analysis of human respiratory pathogens.13 Our study showed that INITIAL PROPAGATION OF HUMAN RESPIRATORY SECRETIONS ONTO HUMAN AIRWAY EPITHELIAL CELL CULTURES, FOLLOWED BY TRANSMISSION ELECTRON MICROSCOPY AND WHOLE GENOME SEQUENCING OF CULTURE SUPERNATANT, was successfully used for visualization and detection of new human coronavirus THAT CAN POSSIBLY ELUDE IDENTIFICATION BY TRADITIONAL APPROACHES."

"ALTHOUGH OUR STUDY DOES NOT FULFILL KOCH'S POSTULATES, OUR ANALYSES PROVIDE EVIDENCE IMPLICATING 2019-nCoV IN THE WUHAN OUTBREAK. ADDITIONAL EVIDENCE TO CONFIRM THE ETIOLOGIC SIGNIFICANCE OF 2019-nCoV in the Wuhan outbreak include IDENTIFICATION OF A 2019-nCoV ANTIGEN in the lung tissue of patients by immunohistochemical analysis, DETECTION OF IgM AND IgG ANTIVIRAL ANTIBODIES IN THE SERUM SAMPLES FROM A PATIENT AT TWO TIME POINTS TO DEMONSTRATE SEROCONVERSION, AND ANIMAL (monkey) EXPERIMENTS TO PROVIDE EVIDENCE OF PATHOGENICITY. Of critical importance are epidemiologic investigations to CHARACTERIZE TRANSMISSION MODES, REPRODUCTION INTERVALS, AND CLINICAL SPECTRUM resulting from infection to inform and refine strategies that can prevent, control, and stop the spread of 2019-nCoV."

https://www.nejm.org/doi/full/10.1056/nejmoa2001017

In Summary:

-they collected samples, including BALF fluid, from 4 patients with pneumonia for whom they could not determine a cause

-they used samples from 7 patients with pneumonia of known causes as "controls"

-the BALF from the 7 patients was added to Viral Transport Medium

-they used human airway epithelial cells from lung cancer patients to culture their "virus" which were regularly washed with and stored in phosphate-buffered saline which can be toxic to cells:

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-the unpurified cell culture supernatant was used for the EM images in the study

-on top of the numerous toxic chemicals used during the culturing/washing process, paraformaldehyde was added to the supernatant for 2 hours to prepare it for TEM imaging

-more paraformaldehyde as well as glutaraldehyde were added and then the sample was fixed with osmium tetroxide dehydrated with grade ethanol and embedded in resin

-sections were cut from the resin and stained with uranyl acetate and lead citrate

-these processes not only kill and alter the cells, they can create artifacts in the TEM images as well:

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-RNA extracted from the unpurified BALF and culture supernatant were used as a template to clone and generate the genome

-their genome shared more than 85% identity match to the bat "Coronavirus" RaTG13

-PCR Ct Values were higher than 34 for the detected samples (which, according to Fauci, would be nothing but dead nucleotides)

-"virus" isolation was carried out in human airway epithelial cells, Vero cells, and HuH7 cells

-they observed "Coronavirus-like' particles with some pleomorphism (variability of size, shape, and staining of cells) in their unpurified cell culture sample

-their evidence consists of whole-genome sequencing from unpurified BALF of 3 patients, direct PCR, and cell culture

-the illness LIKELY TO HAVE BEEN CAUSED by their new "virus" was named 2019-nCoV

-they talk up their indirect molecular techniques as being suitable to identify a novel "virus" which is LIKELY TO BE THE CAUSE of an unknown pneumonia

-they state they were able to capture and identify this unknown "virus" through TEM images, cell cultures, and WGS sequencing from unpurified cell culture supernatant as it may have eluded identification by TRADITIONAL approaches

-they then admit that they did not satisfy Koch's Postulates, the very criteria needed to be met in order to prove a new pathogenic "virus" exists

-more evidence is needed to confirm its etiological significance such as: identifying an antigen, detection of IgG and IgM antibodies at two time intervals to show seroconversion, and animal experiments to prove pathogenicity

-further studies are also needed to characterize transmission modes, reproduction intervals, and determine clinical spectrum

Once you break down the "evidence" (or lack thereof), it is clear to see that the world was locked down, quarantined, masked, social distanced, and vaccinated based on nothing at all. Neither the Zhu nor the Zhou studies fulfilled Koch's postulates. Both left it up to future studies from different teams of researchers using different patients with different samples and different methods to prove their hypotheses for them. At the very least, Zhu provided a pretty TEM image of some particles he picked to REPRESENT his "virus" from potentially billions of similar particles in the unpurified cell culture supernatant. But TEM images of particles that may or may not belong to the A's, C's, G's, and T's in a computer database that may or may not represent something in reality is not evidence of a new "virus." It is absolute fraud to present it as such.

For more on TEM images and why purification/isolation of the particles is absolutely essential, read these related posts: THE ELECTRON MICROGRAPH CHAPTER OR for digial jump

https://www.facebook.com/502548575/posts/10158107068098576/

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CDC 2020 "SARS-COV-2" PAPER:

On March 7, 2020, the CDC released their study on the "isolation" and characterization of "SARS-COV-2" from the first US patient identified by PCR testing on January 22nd, 2020. As with every single paper before it, the CDC relies on unpurified cell cultures to claim "isolation" of a new "Coronavirus." They utilize the same unproven molecular tricks to create a genetic blueprint in a computer database for something they have never seen in reality. There are some interesting wrinkles I will point out with this particular study that put it squarely into unreliable territory along with all the others as well. Highlights below:

ISOLATION AND CHARACTERIZATION OF SARS-CoV-2 FROM THE FIRST US COVID-19 PATIENT

"RESULTS and DISCUSSION

A patient was identified with confirmed COVID-19 in Washington State on January 22, 2020 with cycle threshold (Cts) of 18–20 (nasopharyngeal(NP)) and 21–22 (oropharyngeal (OP)) (1). THE POSITIVE CLINICAL SPECIMENS WERE ALIQUOTED AND REFROZEN INOCULATION INTO CELL CULTURE on January 22, 2020. We first observed cytopathic effect (CPE) 2 days post inoculation and harvested viral lysate on day 3 post inoculation (Figure 1B and and1C).1C). Fifty µl of P1 VIRAL LYSATES WERE USED FOR NUCLEIC ACID EXTRACTION TO CONFIRM THE PRESENCE OF SARS-CoV-2 USING THE CDC MOLECULAR DIAGNOSTIC ASSAY (1). The Cts of three different nucleic acid extractions ranged from 16.0–17.1 for N1, 15.9–17.1 for N2 and 16.2–17.3 for N3, confirming isolation of SARS-CoV-2. A Ct of less than 40 is considered positive. The extracts were also tested for the presence of 33 additional different respiratory pathogens with the fast track 33 assay. No other pathogens were detected. Identity was additionally supported by thin section electron microscopy (Figure 1D). WE OBSERVED A MORPHOLOGY AND MORPHOGENESIS CHARACTERISTIC OF CORONAVIRUSES.

ISOLATES FROM THE FIRST PASSAGE OF AN OP AND AN NP SPECIMEN WERE USED FOR WHOLE GENOME SEQUENCING. The genomes from the NP specimen (Genbank accession MT020880) and OP specimen (Genbank accession MT020881) matched each other 100%. The isolates also matched the corresponding clinical specimen 100% (Genbank accession MN985325).

AFTER THE SECOND PASSAGE, OP AND NP SPECIMENS WERE NOT CULTURED SEPARATELY. VIRUS ISOLATE WAS PASSAGED TWO MORE TIMES IN Vero CCL-81 CELLS, and titrated by TCID50. The titers of the third and fourth passages were 8.65 × 106 and 7.65 × 106 TCID50 per mL, respectively."

"WE SUBSEQUENTLY GENERATED A FOURTH PASSAGE STOCK OF SARS-CoV-2 ON VeroE6 CELLS, ANOTHER FETAL RHESUS MONKEY KIDNEY CELL LINE. Viral RNA from SARS-CoV-2 passage four stock was sequenced and confirmed to have no nucleotide mutations compared with the original reference sequence (Genbank accession MN985325). Both SARS-CoV and MERS-CoV had been found to grow well on VeroE6 and Vero CCL81 respectively (12, 13). To establish a plaque assay and determine the preferred Vero cell type for quantification, we titered our passage four stock on VeroE6 and VeroCCL81. Following infection with a dilution series, we found that SARS-CoV-2 replicated in both Vero cell types; however, the viral titers were slightly higher in VeroE6 cells than Vero CCL81 (Figure 2A). In addition, plaques were more distinct and visible on Vero E6 (Figure 2B). As early as 2 days post inoculations, VeroE6 cells produced distinct plaques visible with neutral red staining. In contrast, Vero CCL81 produced less clear plaques and was most easily quantitated with neutral red 3 days post inoculation. On the individual plaque monolayers, SARS-CoV-2 infection of Vero E6 cells produced a cytopathic effect with areas of cell clearance (Figure 2C). In contrast, Vero CCL81 had areas of dead cells that had fused to form plaques, but the cells did not clear. Together, THE RESULTS SUGGEST THAT VeroE6 MAY BE THE BEST CHOICE FOR AMPLIFICATION AND QUANTIFICATION, but both Vero cell types support amplification and replication of SARS-CoV-2."

"As research is initiated to study and respond to SARS-CoV-2, information about cell lines and types susceptible to infection is needed. THEREFORE, WE EXAMINED THE CAPACITY OF SARS-CoV-2 TO INFECT AND REPLICATE IN SEVERAL COMMON PRIMATE AND HUMAN CELL LINES, including human adenocarcinoma cells (A549), human liver cells (HUH7.0), and human embryonic kidney cells (HEK-293T), in addition to Vero E6 and Vero CCL81. We also examined an available big brown bat kidney cell line (EFK3B) for SARS-CoV-2 replication capacity. Each cell line was inoculated with at high MOI and examined 24 hours post infection (Figure 3A). NO CYTOPATHIC EFFECT WAS OBSERVED IN ANY OF THE CELL LINES EXCEPT IN VERO CELLS which

grew to >107 PFU at 24 hours post infection. In contrast, both HUH7.0 and 293T cells showed only modest viral replication and A549 cells were incompatible with SARS-CoV-2 infection. These results are consistent with previous susceptibility findings for SARS-CoV and suggest OTHER COMMON CULTURE SYSTEMS INCLUDING MDCK, HeLa, HEP-2, MRC-5 cells, and embryonated eggs ARE UNLIKELY TO SUPPORT SARS-CoV-2 REPLICATION (14–16). In addition, SARS-CoV-2 FAILED TO REPLICATE IN THE BAT EFK3B CELLS which are susceptible to MERS-CoV. Together, the results indicate that SARS-CoV-2 maintain a similar profile to SARS-CoV in terms of susceptible cell lines."

"THE SARS-CoV-2 FOURTH PASSAGE VIRUS HAS BEEN SEQUENCED and maintains a nucleotide sequence identical to that of the original US clinical strain. These deposits make it available to the domestic and international public health, academic, and pharmaceutical sectors for basic research, diagnostic development, antiviral testing, and vaccine development."

"Specimen collection

Virus isolation from patient samples was deemed to be non-human subjects research by CDC National Center for Immunizations and Respiratory Diseases (research determination 0900f3eb81ab4b6e) Clinical specimens from the first identified US case of COVID-19 acquired during travel to china, were collected as described (1). NASOPHARYNGEAL (NP) AND OROPHARYNGEAL (OP) SWABS in 2 to 3 mL VIRAL TRANSPORT MEDIA were collected on day 3 post-symptom onset for molecular diagnosis and frozen. Confirmed PCR- positive specimens were aliquoted and refrozen until virus isolation was initiated.

Cell culture, limiting dilution, and isolation

VERO CCL-81 CELLS WERE USED FOR ISOLATION AND INITIAL PASSAGE. Vero E6, Vero CCL-81, HUH 7.0, 293T, A549, and EFKB3 cells WERE CULTURED IN DULBECCO'S MINIMAL ESSENTIAL MEDIUM (DMEM) SUPPLEMENTED WITH HEAT INACTIVATED FETAL BOVINE SERUM (5 or 10%) AND ANTIBIOTIC/ANTIMYOTIC (GIBCO). Both NP and OP swabs were used for virus isolation. For the isolation, limiting dilution, and passage 1 of the virus, 50 µl serum free DMEM was pipetted into columns 2–12 of a 96-well tissue culture plate. One-hundred µl clinical specimens were pipetted into column 1, and then serially diluted 2-fold across the plate. VERO CELLS WERE TRYPSINIZED AND RESUSPENDED IN DMEM + 10% FBS + 2X PENICILLIN-STREPTOMYCIN + 2X ANTIBIOTIC — ANTIMYCOTIC + 2 X AMPHOTERICIN B at 2.5 × 105 cells / ml. ONE HUNDRED µl OF CELL SUSPENSION WERE ADDED DIRECTLY TO THE CLINICAL SPECIMEN DILUTIONS AND MIXED GENTLY BY PIPETTING. The inoculated cultures were grown in a humidified 37°C

incubator with 5% CO_2 and observed for cytopathic effect (CPE) daily. Standard plaque assays were used for SARS-CoV-2 based on both SARS-CoV and MERS-CoV protocols (19, 20).

When CPE was observed, the cell monolayers were scrapped with the back of a pipette tip. FIFTY µl OF THE VIRAL LYSATE WERE USED FOR TOTAL NUCLEIC ACID EXTRACTION FOR CONFIRMATORY TESTING AND SEQUENCING. Fifty µl of virus lysate was used to inoculate a well of a 90% confluent 24-well plate."

"Inclusivity / Exclusivity testing

From the wells in which CPE were observed, CONFIRMATORY TESTING WAS PERFORMED USING CDC's rRT-PCR ASSAY and full genome sequencing (1) The CDC molecular diagnostic assay targets three portions of the N gene, and all three must be positive to be considered positive (https://www.cdc.gov/.../rt-pcr-detection-instructions.html) and (https://www.cdc.gov/.../lab/rt-pcr-panel-primer-probes.html). To confirm that no other respiratory viruses were present, Fast Track respiratory pathogen 33 testing was performed (FTD diagnostics)."

"Whole genome sequencing.

THIRTY-SEVEN PAIRS OF NESTED PCR ASSAYS SPANNING THE GENOME WERE DESIGNED BASED ON THE REFERENCE SEQUENCE, Genbank Accession No. NC045512. NUCLEIC ACID WAS EXTRACTED FROM ISOLATES AND AMPLIFIED BY THE 37 INDIVIDUAL NESTED PCR ASSAYS. Positive PCR amplicons were used individually for subsequent Sanger sequencing and also pooled for library preparation using a ligation sequencing kit (Oxford Nanopore Technologies), subsequently for Oxford Nanopore MinION sequencing. CONSENSUS NANOPORE SEQUENCES were generated using minimap 2.17 and samtools 1.9. CONSENSUS SEQUENCES BY SANGER SEQUENCES were generated from both directions using Sequencher 5.4.6, and WERE FURTHER CONFIRMED BY CONSENSUS SEQUENCES GENERATED FROM NANOPORE SEQUENCING."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7239045

In Summary:

-on January 22nd, 2020, a patient was confirmed positive by the CDC PCR test

- nasopharyngeal (NP) and oropharyngeal (OP) samples were inoculated onto cell cultures and frozen

-viral lysates from cell culture were used for nucleic acid extraction using the CDC PCR test to confirm "SARS-COV-2"

Quick sidenote: the CDC's PCR test was initially recalled due to producing too many false-positives. The new version also produced many false-positives and in one study both the negative controls and water tested positive for "SARS-COV-2:" Obviously, any data coming from their PCR tests should be discounted:

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-they observed "Coronavirus-like" morphology/morphogenesis in their unpurified EM images

-unpurified "isolates" from the first passage cell culture of both NP and OP swabs were used for whole-genome sequencing

-after second passage, both NP and OP samples were cultured together in Vero cells

-they generated "viral" stocks from fourth passaged VeroE6 (fetal rhesus monkey kidney) cells

-VeroE6 cells were determined to produce the "virus" the easiest

-they decided to test whether "SARS-COV-2" would replicate in other primate and HUMAN cell lines

-no cytopathic effect (CPE) occurred in any of the other cell lines used, only Vero cells

-they determined that the "virus" COULD NOT infect/replicate in HUMAN nor many other common cell lines including MDCK, HeLa, HEP-2, MRC-5 cells, and embryonated eggs nor in Bat EFK3B cells

-the NP and OP swabs were immediately placed in viral transport media

-VeroE6 cells were added to DMEM media along with fetal bovine serum and several

antibiotics/antimycotics

-VeroE6 cells were trypsinized and suspended in added DMEM, FBS, 2x penicillin-streptomycin, 2x amphotericin B

-the VeroE6 concoction was added to the NP/OP samples and mixed together

-this unpurified creation was used for total nucleic acid extraction, confirmatory testing, and sequencing

-for Whole-Genome Sequencing, ONLY 37 base pairs were used yet the "SARS-COV-2" genome consists of 30,000 base pairs:

"THIRTY THOUSAND BASE PAIRS MAKE UP THE (relatively tiny) SARS-CoV-2 GENOME. A singular genome holds limited information."

https://www.genengnews.com/news/mining-the-sars-cov-2-genome-for-answers/

Dr. Tom Cowan did a brilliant breakdown of why this is an issue:

"… we find that rather than having isolated the virus and sequencing the genome from end to end, THEY FOUND 37 BASE PAIRS FROM UNPURIFIED SAMPLES USING PCR PROBES. This means they actually looked at 37 out of the approximately 30,000 of the base pairs that are claimed to be the genome of the intact virus. THEY THEN TOOK THESE 37 SEGMENTS AND PUT THEM INTO A COMPUTER PROGRAM, WHICH FILLED IN THE REST OF THE BASE PAIRS."

"To me, this computer-generation step constitutes scientific fraud. Here is an equivalency: A group of researchers claim to have found a unicorn because they found a piece of a hoof, a hair from a tail, and a snippet of a horn."

"They then add that information into a computer and program it to re-create the unicorn, and they then claim this computer re-creation is the real unicorn. Of course, they had never actually seen a unicorn so could not possibly have examined its genetic makeup to compare their samples with the actual unicorn's hair, hooves and horn."

https://luis46pr.wordpress.com/2020/11/02/study-cdc-scientists-make-2-covid-admissions-that-destroy-official-narrative

In this CDC study, we have unpurified cell cultures, faulty PCR tests/data, evidence "SARS-COV-2" can not infect human cells but only Vero cells, and a genome made up almost entirely by consensus computer-algorithms.

In short, more "scientific" FRAUD.

DROSTEN 2020 "SARS-COV-2" PCR PAPER:

In Silico: in or on a computer : done or produced by using computer software or simulation

I won't go into too much detail regarding the Drosten PCR test as it was covered beautifully by people much better suited than I (linked below), but I would be remiss if I did not mention it at all. Drosten was instrumental in the fraud that was "SARS-COV-1" and naturally, his experience in regards to manipulating the masses with pseudoscience was put to good use with "SARS-COV-2." He has so much experience with these "viruses," he was able to create a "diagnostic" PCR test in silico in the absence of any "SARS-COV-2 isolates" based primarily off of social media reports. That takes talent folks. Highlights below:

DETECTION OF 2019 NOVEL CORONAVIRUS (2019-nCoV) BY REAL-TIME RT-PCR

"Background

The ongoing outbreak of the recently emerged novel coronavirus (2019-nCoV) poses a challenge for public health laboratories AS VIRUS ISOLATES ARE UNAVAILABLE while there is growing evidence that the outbreak is more widespread than initially thought, and international spread

through travellers does already occur.

AIM

We aimed to develop and deploy robust diagnostic methodology for use in public health laboratory settings WITHOUT HAVING VIRUS MATERIAL AVAILABLE.

METHODS

Here we present a validated diagnostic workflow for 2019-nCoV, ITS DESIGN RELYING ON CLOSE GENETIC RELATEDNESS OF 2019-nCoV WITH SARS CORONAVIRUS, MAKING USE OF SYNTHETIC NUCLEIC ACID TECHNOLOGY.

RESULTS

The workflow reliably detects 2019-nCoV, and further discriminates 2019-nCoV from SARS-CoV. Through coordination between academic and public laboratories, we confirmed assay exclusivity BASED ON 297 ORIGINAL CLINICAL SPECIMENS CONTAINING A FULL SPECTRUM OF HUMAN RESPIRATORY VIRUSES. Control material is made available through European Virus Archive – Global (EVAg), a European Union infrastructure project."

"A novel coronavirus currently termed 2019-nCoV was officially announced as the causative agent by Chinese authorities on 7 January. A viral genome sequence was released for immediate public health support via the community online resource virological.org on 10 January (Wuhan-Hu-1, GenBank accession number MN908947 [2]), followed by four other genomes deposited on 12 January in the viral sequence database curated by the Global Initiative on Sharing All Influenza Data (GISAID). THE GENOME SEQUENCES SUGGEST PRESENCE OF A VIRUS closely related to the members of a viral species termed severe acute respiratory syndrome (SARS)-related CoV, a species defined by the agent of the 2002/03 outbreak of SARS in humans [3,4]. The species also comprises a large number of viruses mostly detected in rhinolophid bats in Asia and Europe."

"Among the foremost priorities to facilitate public health interventions is reliable laboratory diagnosis. In acute respiratory infection, RT-PCR is routinely used to detect causative viruses from respiratory secretions. We have previously demonstrated the feasibility of introducing robust detection technology based on real-time RT-PCR in public health laboratories during international health emergencies by coordination between public and academic laboratories [6-12]. IN ALL OF THESE SITUATIONS, VIRUS ISOLATES WERE AVAILABLE AS THE PRIMARY

SUBSTRATE FOR ESTABLISHING AND CONTROLLING ASSAYS AND ASSAY PERFORMANCE.

IN THE PRESENT CASE OF 2019-nCoV, VIRUS ISOLATES OR SAMPLES FROM INFECTED PATIENTS HAVE SO FAR NOT BECOME AVAILABLE TO THE INTERNATIONAL PUBLIC HEALTH COMMUNITY. We report here on the establishment and validation of a diagnostic workflow for 2019-nCoV screening and specific confirmation, DESIGNED IN ABSENCE OF AVAILABLE VIRUS ISOLATES OR ORIGINAL PATIENT SPECIMENS. Design and validation were ENABLED BY THE CLOSE GENETIC RELATEDNESS TO THE 2003 SARS-CoV, AND AIDED BY THE USE OF SYNTHETIC NUCLEIC ACID TECHNOLOGY."

"Clinical samples and coronavirus cell culture supernatants for initial assay evaluation

CELL CULTURE SUPERNATANTS CONTAINING TYPED CORONAVIRUSES AND OTHER RESPIRATORY VIRUSES WERE PROVIDED BY CHARITÉ AND UNIVERSITY OF HONG KONG RESEARCH LABORATORIES. Respiratory samples were obtained during 2019 from patients hospitalised at Charité medical centre and tested by the NxTAG respiratory pathogen panel (Luminex, S´Hertogenbosch, The Netherlands) or in cases of MERS-CoV by the MERS-CoV upE assay as published before [10]. Additional samples were selected from biobanks at the Rijksinstituut voor Volksgezondheid en Milieu (RIVM), Bilthoven, at Erasmus University Medical Center, Rotterdam, at Public Health England (PHE), London, and at the University of Hong Kong. SAMPLES FROM ALL COLLECTIONS COMPRISED SPUTUM AS WELL AS NOSE AND THROAT SWABS WITH OR WITHOUT VIRAL TRANSPORT MEDIUM.

FAECAL SAMPLES CONTAINING BAT-DERIVED SARS-related CoV SAMPLES (identified by GenBank accession numbers) WERE TESTED: KC633203, Betacoronavirus BtCoV/Rhi_eur/BB98–98/BGR/2008; KC633204, Betacoronavirus BtCoV/Rhi_eur/BB98–92/BGR/2008; KC633201, Betacoronavirus BtCoV/Rhi_bla/BB98–22/BGR/2008; GU190221 Betacoronavirus Bat coronavirus BR98–19/BGR/2008; GU190222 Betacoronavirus Bat coronavirus BM98–01/BGR/2008; GU190223, Betacoronavirus Bat coronavirus BM98–13/BGR/2008.

ALL SYNTHETIC RNA USED IN THIS STUDY was photometrically quantified."

"Real-time reverse-transcription PCR

A 25 µL reaction contained 5 µL of RNA, 12.5 µL of 2 × reaction buffer provided with the Superscript III one step RT-PCR system with Platinum Taq Polymerase (Invitrogen, Darmstadt, Germany; containing 0.4 mM of each deoxyribose triphosphates (dNTP) and 3.2 mM magnesium sulphate), 1 µL of reverse transcriptase/Taq mixture from the kit, 0.4 µL of a 50 mM magnesium sulphate solution (Invitrogen), and 1 µg of NONACETYLATED BOVINE SERUM ALBUMIN (Roche). Primer and probe sequences, as well as optimised concentrations are shown in Table 1. ALL OLIGONUCLEOTIDES WERE SYNTHESIZED and provided by Tib-Molbiol (Berlin, Germany). Thermal cycling was performed at 55 °C for 10 min for reverse transcription, followed by 95 °C for 3 min and THEN 45 CYCLES of 95 °C for 15 s, 58 °C for 30 s."

"The INTENDED CROSS-REACTIVITY OF ALL ASSAYS WITH VIRAL RNA OF SARS-CoV allows us to use the assays WITHOUT HAVING TO RELY ON EXTERNAL SOURCES OF SPECIFIC 2019-nCoV RNA."

"RESULTS

BEFORE PUBLIC RELEASE OF VIRUS SEQUENCES FROM CASES OF 2019-nCoV, WE RELIED ON SOCIAL MEDIA REPORTS ANNOUNCING DETECTION OF A SARS-LIKE VIRUS. WE THUS ASSUMED THAT A SARS-RELATED CoV IS INVOLVED IN THE OUTBREAK. We downloaded all complete and partial (if > 400 nt) SARS-related virus sequences available in GenBank by 1 January 2020. The list (n = 729 entries) was manually checked and artificial sequences (laboratory-derived, synthetic, etc), as well as sequence duplicates were removed, resulting in a final list of 375 sequences. THESE SEQUENCES WERE ALIGNED AND THE ALIGNMENT WAS USED FOR ASSAY DESIGN (Supplementary Figure S1). UPON RELEASE OF THE FIRST 2019-nCoV SEQUENCE at virological.org, THREE ASSAYS WERE SELECTED BASED ON HOW WELL THEY MATCHED TO THE 2019-nCoV GENOME (Figure 1). The alignment was complemented by additional sequences released independently on GISAID (https://www.gisaid.org), CONFIRMING THE GOOD MATCHING OF SELECTED PRIMERS to all sequences. Alignments of primer binding domains with 2019-nCoV, SARS-CoV as well as selected bat-associated SARS-related CoV are shown in Figure 2."

"Assay sensitivity based on SARS coronavirus virions

To obtain a preliminary assessment of analytical sensitivity, we USED PURIFIED CELL CULTURE SUPERNATANT CONTAINING SARS-CoV STRAIN FRANKFURT-1 VIRIONS GROWN ON VERO CELLS.

The supernatant was ultrafiltered and thereby concentrated from a ca 20-fold volume of cell culture supernatant. THE CONCENTRATION STEP SIMULTANEOUSLY REDUCES THE RELATIVE CONCENTRATION OF BACKGROUND NUCLEIC ACIDS SUCH AS NOT VIRION-PACKAGED VIRAL RNA. The virion preparation was quantified by real-time RT-PCR using a specific in vitro-transcribed RNA quantification standard as described in Drosten et al. [8]."

"Discrimination of 2019 novel coronavirus from SARS coronavirus by RdRp assay

FOLLOWING THE RATIONALE THAT SARS-CoV RNA CAN BE USED AS A POSITIVE CONTROL FOR THE ENTIRE LABORATORY PROCEDURE, THUS OBVIATING THE NEED TO HANDLE 2019-nCoV RNA, we formulated the RdRp assay so that it contains two probes: a broad-range probe reacting with SARS-CoV and 2019-nCoV and an additional probe that reacts only with 2019-nCoV. By limiting dilution experiments, we confirmed that both probes, whether used individually or in combination, provided the same LOD for each target virus. The specific probe RdRP_SARSr-P2 detected only the 2019-nCoV RNA transcript but not the SARS-CoV RNA.:

"TO SHOW THAT THE ASSAYS CAN DETECT OTHER BAT-ASSOCIATED SARS-related VIRUSES, WE USED THE E GENE ASSAY TO TEST SIX BAT-DERIVED FAECAL SAMPLES available from Drexler et al. [13] und Muth et al. [14]. These virus-positive samples stemmed from European rhinolophid bats. DETECTION OF THESE PHYLOGENETIC OUTLIERS within the SARS-related CoV clade SUGGESTS THAT ALL ASIAN VIRUSES ARE LIKELY TO BE DETECTED. This would, theoretically, ensure broad sensitivity even in case of multiple independent acquisitions of variant viruses from an animal reservoir."

"Cross-reactivity with other coronaviruses

Cell culture supernatants containing all endemic human coronaviruses (HCoV)-229E, -NL63, -OC43 and -HKU1 as well as MERS-CoV were tested in duplicate in all three assays (Table 2). FOR THE NON-CULTIVABLE HCoV-HKU1, supernatant from human airway culture was used. Viral RNA concentration in all samples was determined by specific real-time RT-PCRs and in vitro-transcribed RNA standards designed for absolute quantification of viral load. Additional undiluted (but not quantified) cell culture supernatants were tested as summarised in Table 2. THESE WERE ADDITIONALLY MIXED INTO NEGATIVE HUMAN SPUTUM SAMPLES. None of the tested viruses or virus preparations showed reactivity with any assay."

"TECHNICAL QUALIFICATION DATA BASED ON CELL CULTURE MATERIALS AND SYNTHETIC CONSTRUCTS, as well as results from exclusivity testing on 75 clinical samples, were included in the first version of the diagnostic protocol provided to the WHO on 13 January 2020."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6988269/...

In Summary:

-no "viral isolates" were available at the time Drosten created his PCR test

-the aim of his study was to develop a test without needing "virus" material

-the workflow was based on the "close genetic-relatedness" between "SARS-COV-1" and "SARS-COV-2" (only 79% which is really not close at all) with the use of SYNTHETIC nucleic acid technology

-they based assay exclusivity off of 297 clinical samples containing a range of respiratory "viruses" but none with "SARS-COV-2"

-the genome sequence supplied by the Chinese SUGGESTED a "virus" related to "SARS-COV-1"

-in their previous test developments, "viral isolates" were available as the primary substrate for establishing and controlling assays and assay performance

-since no "virus isolates" were available this time around, they decided that the presumed relation to "SARS-COV-1" was enough to develop a reliable test with the use of synthetic nucleic acid technology

-cell culture supernatant for different "viruses" were provided by the Charite and various other sources

-samples were from sputum as well as nose and throat swabs with or without viral transport media

-they also used fecal samples supposedly containing Bat-related "Coronaviruses"

-synthetic RNA was used in the study

-during RT-PCR, various chemicals were used along with nonacetylated bovine serum albumin

-all oligonucleotides were synthesized

-the RT-PCR tests were run to 45 Cycles (remember, according to Fauci, anything above 35 is just dead nucleotides)

-the intended cross-reactivity of all assays to "SARS-COV-1" somehow meant they did not need any "SARS-COV-2" isolates

-they relied on social media reports and assumed from those that a "Coronavirus" related to "SARS-COV-1" was the culprit

-numerous different "SARS-COV-1" genomes (375) were aligned and used to design the assays for "SARS-COV-2"

-3 assays were chosen based on how well they matched up with the "SARS-COV-2" genome

-they used "purified SARS-COV-1" grown on Vero Cells to test analytical sensitivity

-they ultrafiltered the cell culture supernatant to REDUCE (not eliminate) the background nucleic acids such as not virion-packaged viral RNA

-they "rationalized" (cough assumed cough) that "SARS-COV-1" could act as a positive control for "SARS-COV-2" in all tests

-they determined that the E Gene assay can detect ALL ASIAN "VIRUSES

-they also admit HCoV-HKU1 is non-cultivable

-for some reason, they mixed "viral" culture supernatant with "non-viral" human sputum samples to test vs just using the unaltered human samples

-the originally submitted technological qualifications were based on cell culture materials and synthetic constructs

-the study itself was peer-reviewed and accepted in less than 24 hours

For an in-depth breakdown of this the Drosten PCR fraud, read the Corman-Drosten Review Report:

https://m.facebook.com/story.php?story_fbid=10157875675388576&id=502548575

And just in case you thought the CDC was above creating a PCR test without any "virus" available, know you would be wrong.

This is from the FDA emergency use authorization of the CDC's PCR test used in the USA:

"SINCE NO QUANTIFIED VIRUS ISOLATES OF THE 2019-nCoV ARE CURRENTLY AVAILABLE, assays designed for detection of the 2019-nCoV RNA were tested with characterized stocks of in vitro transcribed full length RNA (N gene; GenBank accession: MN908947.2) of known titer (RNA copies/µL) spiked into a diluent consisting of a SUSPENSION OF HUMAN A549 CELLS AND VIRAL TRANSPORT MEDIUM (VTM) TO MIMIC CLINICAL SPECIMEN."

https://www.fda.gov/media/134922/download

So it appears there is no need to have "viruses" available to create and validate tests anymore. Drosten and others can get on Twitter, read some social media reports, and then come up with a test for a "novel virus" on their computers without ever needing the actual "virus" present. They can just use the old not-as-closely-related-as-they-would-like-you-to-believe "viruses" as a stand-in. Pretty neat trick with how everything, from the "novel virus" itself to the test to detect it, are all computer-based. Those Virologists sure dodged a bullet with their molecular tricks and consensus computer algorithms as there is no need for the gold standard of a purified/isolated "virus." Social media driven computer-based synthetic creations are all the rage these days.

For more information on Christian Drosten's history with fraud:

https://m.facebook.com/story.php?story_fbid=10157751025113576&id=502548575

https://docs.google.com/document/d/e/2PACX-1vTmbyriIRnfUglRxLyutnN5iHKHp8_6HAehnWUyVq1f6O9I96G05LppwJtEqiG1Sm5SfSFd8hiJGFVa/pub

"SARS-COV-2" variants and mutations

With all the talk now with "SARS-COV-2" variants and mutations, especially with the recent discovery of 7 new variants in the US alone as well as 13 variants that were just discovered to have been in Wuhan in December 2019, it's a good time to look at the current state of these variants, mutations, and lineages.

Currently, there are hundreds of thousands of "variants" of "SARS-COV-2" that have been "discovered" since December 2019, with the amount growing rapidly:

"There are ALREADY TENS OF THOUSANDS OF SUPPOSED NEW STRAINS, “FOUND” since last winter all over the world. In fact, the GISAID virus data bank has NOW MORE THAN 452,000 DIFFERENT GENETIC SEQUENCES THAT CLAIM TO REPRESENT A VARIANT OF SARS-Cov2."

https://off-guardian.org/.../phantom-virus-in-search-of.../

These "variants" are all assigned to certain lineages. According to a recent study, in just the UK alone, there are over 1000 "SARS-COV-2" lineages:

"Rapid fluctuations in virus importation rates RESULTED IN >1000 LINEAGES"

"We identified a total of 1179 [95% highest posterior density (HPD), 1143 to 1286] UK TRANSMISSION LINEAGES."

https://science.sciencemag.org/content/371/6530/708

So we have hundreds of thousands of genome sequences, hundreds of thousands of "variants," and thousands of lineages all for one "virus."

Here is a sample of the numerous lineages that these "variants" of ONE "VIRUS" belong to:

https://cov-lineages.org/lineages/lineage_B.1.1.html

How these different genomes are classified is through a process call PHYLOGENETIC ANALYSIS:

What is phylogenetic analysis?

"In phylogenetic analysis, branching diagrams are made to represent the evolutionary history or relationship between different species, organisms, or characteristics of an organism (genes, proteins, organs, etc.) that are developed from a common ancestor."

https://www.news-medical.net/.../What-is-Phylogenetic...

"VIRUSES MAY EVOLVE AT HIGH, UNEVEN, AND FLUCTUATING RATES AMONG GENOMES SITES. The accumulated changes, through either mutation or recombination with other species, are first fixed in the genome of successful individuals that give rise to genetic lineages. THE RELATIONSHIP BETWEEN BIOLOGICAL LINEAGES RELATED BY COMMON DESCENT IS CALLED 'PHYLOGENY'. For inferring phylogeny, THE DIFFERENCES BETWEEN ALIGNED SEQUENCES OF GENOMES AND PROTEINS ARE QUANTIFIED AND DEPICTED IN THE FORM OF A TREE, in which contemporary species and their intermediate and common ancestors occupy, respectively, the terminal nodes, internal nodes, and the root. The tree is characterized by a topology, length of branches, shape, and the root position. A COMPLEX MATHEMATICAL APPARATUS HAS BEEN DEVELOPED FOR PHYLOGENY INFERENCE that can evaluate inter-species differences, facilitate tree building and comparison of trees, and assess the fit between data and tree through, typically, computationally intensive calculations. A RECONSTRUCTED TREE IS AN APPROXIMATION OF THE TRUE PHYLOGENY THAT PRACTICALLY REMAINS UNKNOWN."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7157450/

There are many issues with Phylogenetic Analysis. This is from a recent study in Italy:

"Our study has some limitations. The analysis of phylogenetic structures during such an early phase of the pandemic SHOULD BE INTERPRETED CAREFULLY, as the number of mutations that define phylogenetic lineages is small and MAY BE SIMILAR TO THE RATE OF POTENTIAL ERRORS INTRODUCED DURING REVERSE TRANSCRIPTION, PCR AMPLIFICATION, OR SEQUENCING 27"

https://www.nature.com/articles/s41467-020-20688-x

It would seem that they admit that these variations could all be errors that were introduced during the sequencing process. This is backed up by the source they cite:

"Importantly, it is not only sequences that inform phylogenies; multiple factors contribute to the outputs including MODEL ASSUMPTIONS, SAMPLING DENSITY, THE TIMING OF SAMPLE

COLLECTION, THE PORTION OF THE VIRAL GENOME SEQUENCED, QUALITY OF SEQUENCING DATA AND THE MUTATION RATE OF THE VIRUS ITSELF. Although it is important to extract as much information as possible from sequence data as outbreaks unfold, it is imperative to bear in mind that the historical relationships of strains (PHYLOGENIES) ARE HYPOTHESES THAT CAN BE CHALLENGED AS MORE DATA BECOMES AVAILABLE."

"The often-forgotten point is that phylogenies can rule out transmission, but if infections are caused by the same strains or identical viruses IT DOES NOT DEFINITIVELY PROVE TRANSMISSION."

"Even as the outbreak unfolds and more genomes are obtained, THEY ONLY REPRESENT A SNAPSHOT OF THE UNDERLYING GENETIC DIVERSITY. If phylogenies alone are considered, WE CANNOT CONCLUSIVELY ASSERT THE GEOGRAPHICAL ORIGINS OF THE VIRUS — or the extent of community transmission — as we are unable to distinguish between local transmission events and MULTIPLE INTRODUCTIONS OF GENETICALLY SIMILAR VIRUSES from geographically distinct sources if one aspect has not been sampled. In this way, UNEVEN SAMPLING CAN ALSO LEAD TO MISLEADING CONCLUSIONS on the geographical source, the number of introductions and the size and duration of local transmission chains11.

"The METHODS FOR VALID PHYLOGENETIC INFERENCE REQUIRE MULTIPLE ASSUMPTIONS WHICH ARE UNLIKELY TO BE MET during emerging outbreaks."

"Moreover, SOME MUTATIONS IN THE VIRAL GENOME SEQUENCE CAN BE DUE TO THE ERROR RATE OF THE SEQUENCING TECHNOLOGY, RECURRENT SEQUENCING ISSUES, HYPERMUTABILITY OR CONTAMINATION — issues which warrant caution with interpretations and especially with those concerning selection and recombination."

"PHYLOGENIES REPRESENT HYPOTHESES THAT ENCOMPASS DIFFERENT SOURCES OF ERROR AND THIS UNCERTAINTY NEEDS TO BE VISUALIZED AND COMMUNICATED FAR MORE TRANSPARENTLY."

https://www.nature.com/articles/s41564-020-0738-5

The problems with Phylogenetic Analysis include but are not limited to:

-based on hypotheses, inferences, and complex mathematical processes

-trees are approximations of true phylogeny that remains unknown

-can be influenced by errors introduced during reverse transcriptase, PCR amplification, and

sequencing

-multiple factors contribute such as sampling density, model assumptions, sample timing, quality of the sequence, portion of sequenced genome

-hypotheses are continually challenged by new data

-can not definitively prove transmission

-only a snapshot of genetic diversity

-cannot assert geographical locations of "viruses" if there are multiple similar "viruses"

-uneven sampling leads to misleading conclusions

-requires multiple assumptions that are unlikely to be met

-mutations could be due to contamination as well as sequencing technology limitations and errors

-the hypotheses encompass different sources of errors

It's clear to see that PHYLOGENETIC ANALYSIS is nothing but hypotheses, assumptions, and inferences that is prone to be influenced by the errors from the available technology.

"Variants" and "mutations" are nothing more than the inability to sequence the same genome every time. Instead of realizing this error, a whole "science" was built around justifying these errors by classifying them to fictional lineages. They can come up with and pull out new strings of letters in a database on a whim and claim them to be more contagious, more dominant, and more deadly at any time.

It keeps the FEAR CAMPAIGN rolling.

Related posts on the "Variants:"

https://m.facebook.com/story.php?story_fbid=10158050796268576&id=502548575

https://m.facebook.com/story.php?story_fbid=10158049233488576&id=502548575

https://docs.google.com/document/d/e/2PACX-1vSwugZCGN1YXfSp9uA6107GK7QEB4qRUWI1Dd4HK1ZeW0GfbKfxxzdXfmo4D8eRXIWTIVa3HGORqL1m/pub

Purification of a "virus" is impossible

It is becoming increasingly clear that purification of a "virus" is impossible. There are too many contaminants, variables, unknowns, and nanoparticles of similar shape/size to be able to say with certainty that the particles assumed to be a "virus" in a cell culture are the same ones imaged by TEM or for which the genome sequence is said to be based upon.

"EACH VIRUS POSES AN INDIVIDUAL PURIFICATION PROBLEM that is related to the properties of the virus, the nature of the host, and the CULTURE CONDITIONS. Consequently, IT IS NOT POSSIBLE TO OUTLINE A PURIFICATION PROCEDURE THAT WILL WORK WITH EQUAL EFFECTIVENESS FOR ALL VIRUSES."

"In these terms, PURITY MEANS THE DEGREE OF FREEDOM OF VIRAL PARTICLES FROM NONVIRAL COMPONENTS, or, conversely, the extent to which viral particles show gross physicochemical homogeneity. NO SINGLE TEST IS SUFFICIENT TO ESTABLISH THIS TYPE OF PURITY, but a consistent answer from each of several tests establishes the degree of homogeneity of the preparation in question and hence the reliance to be placed on analytical data and other results obtained with such a preparation."

"THE LOWER LIMIT OF CONTAMINANT DETECTABLE by either sedimentation analysis or electrophoresis IS VARIABLE, and is dependent upon the nature of the material and the circumstances of the test. As usually applied in testing virus preparations, THESE METHODS CANNOT BE EXPECTED TO DETECT LESS THAN A FEW PERCENT OF CONTAMINANT (Sharp 1953). For many purposes, it is satisfactory to measure purity to this degree, but as the tools for

chemical and biological analyses become sharper and sharper, it will be increasingly necessary to remember the limitations of sedimentation and electrophoresis measurements."

"The electron microscope can be used to examine directly the physical homogeneity of a virus preparation. Under favorable conditions it is possible to detect an impurity present in a concentration of as little as 1 percent of the virus (Williams 1954). IT IS OBVIOUS, OF COURSE, THAT IMPURITIES WILL ESCAPE DETECTION IF THEY HAVE THE SAME SIZE AND SHAPE AS THE VIRUS PARTICLES, OR IF THEY ARE BELOW THE SIZE RESOLVED BY THE MICROSCOPE. Also, particles present in small number but large in mass ARE EASILY OVERLOOKED, owing to sampling difficulties (Lauffer 1951)."

"In summary, NO SINGLE CRITERION OF PURITY IS SUFFICIENT TO ESTABLISH THE HOMOGENEITY OF A PREPARATION OF VIRUS. This must be done by applying critically as many tests as possible (see Knight 1974)."

https://link.springer.com/content/pdf/10.1007/978-3-642-85899-4_2.pdf

https://docs.google.com/document/d/e/2PACX-1vTjyUqE8tA2J7hdxTrmdgsa5o8EOR2DjAI08i52V6X_gAyHA6vMNas3dRwgm10FmYM12JKWeTrmyXUs/pub

Two undeniable facts about "SARS-COV-2" (and any other "virus" for that matter):

1. "SARS-COV-2" has NEVER been properly purified/isolated directly from an unaltered sample taken from a sick patient, EM photographed, characterized, sequenced, and proven pathogenic by infecting an animal in a natural way.

2. Without purification/isolation, the PCR, antigen, and antibody tests are all MEANINGLESS. This is why they come with disclaimers such as this:

"DETECTION OF VIRAL RNA MAY NOT INDICATE THE PRESENCE OF INFECTIOUS VIRUS OR THAT INFLUENZA OR SARS-CoV-2 VIRUSES ARE THE CAUSATIVE AGENT FOR CLINICAL SYMPTOMS."

Due to these two undeniable facts, there is no basis and no credible evidence for lockdowns, quarantines, social-distancing, masks, vaccines, etc.

In this day and age, IGNORANCE is a choice. If you are just going along with the program without looking at the evidence, without doing your own research, and without using critical thinking and logic, then you are just as much a part of the problem as those who have lied and taken our freedoms to get us into this mess.

If this upsets or offends anyone, I'm sorry. My hope is that it compels you to try and prove me wrong. I hope you decide to no longer sit on the sidelines and look at the "science" which has been presented to us as "evidence." I hope you look into:

-"virus" purification/isolation

-the faults of different tests

-the manipulated statistics

-the genomes/variants and how they are created

-the lack of credible science and reproducibility

My hope is that through this process, you will come to realize what myself and many others have come to realize: there is no "virus," there is no pandemic, there is no need for masks/vaccines, and there is no need for FEAR.

The worst thing one can do at this time is be indifferent to all of this and just assume it will end. There is far too much at stake for our us and for our future generations.

Don't sit helplessly on the sidelines anymore.

Be a part of the solution.

https://docs.google.com/document/d/e/2PACX-1vTSc_Ciw5cB2quctJcKN4YRnk1oV74t-5nqTNBXHvRu5pVxHHtHpxngE0nWIbnCG6_ddCyEnTvOdePn/pub

TRANSMISSION

When was the last time you were feeling perfectly healthy, went to the Dr. for a check-up, and they diagnosed you with a "virus" based on no symptoms at all?

As Florence Nightingale put it: “There are no specific diseases, there are specific disease conditions”.

John Snow, considered to be the father of epidemiology. ...Used scientific methods to identify the environment in which cholera was spreading. By disrupting this environment, he ended the epidemic.

Current Sars CoV2 public health protection protocols (social distancing, lock-downs, masking, etc) are rationalized on the scientifically unsupported theory of asymptomatic transmission. This hypothesis arose from majority of individuals testing positives for Sars CoV2 (Covid 19 infection) with PCR testing do not present symptomatically.

However, as documented above, asymptomatic presentation would be the expected result with PCR test over amplifying sample material through high CT rates resulting in up to 90% false positives per studies reported in NYT; as individual are not ill or contagious.

UP to 86% of Individuals with Positive PCR test have No Core Symptoms of Covid 19 Infection

https://www.cnbc.com/2020/10/08/more-than-80percent-of-people-with-coronavirus-had-no-symptoms-uk-study.html

Additionally, advocates of the asymptomatic spread theory utilize research that is not designed to study for method of transmission of Sars CoV2 but rather evaluates viral load detected on RT PCR tests to hypothesize asymptomatic transmission as a main driver of virus spread. As in this CNN example using a study published in JAMA Medicine:

https://www.cnn.com/2020/08/07/health/covid-asymptomatic-transmission-study-wellness/index.html

The study authors admit that their research is not designed to determine method of transmission of virus and states:

"Although the high viral load we observed in asymptomatic patients raises a distinct possibility of a risk for transmission, our study was not designed to determine this," the researchers wrote.

"It is important to note that detection of viral RNA does not equate infectious virus being present and transmissible," the researchers wrote. "

This is a STUNNING statement, as it admits that a positive test result is not reliable proof that the infection originated from Sars CoV2.

This was further documented in the Corman Drosten report above which no longer links to full text article, please refer to archived link above for sourcing:

The design errors described here are so severe that it is highly unlikely that specific amplification of SARS-CoV-2 genetic material will occur using the protocol of the Corman-Drosten paper

According to this statement from 22 top scientific experts in relevant research fields, the WHO/FDA/CDC and all government health organizations have been recommending & implementing the use of a test that is highly unlikely to detect Sars Cov2 virus due to severe problems with testing design. Almost the entire basis of the Sars CoV2 Covid-19 pandemic has been rationalized on the results of these tests. This renders all data results from PCR testing severely corrupted.

CDC Admitted in 2011 PCR Inappropriate for Screening Asymptomatic Individuals for Infection:

CDC warned in 2011 that PCR testing should not be used in diagnosis of petussis infections due to significant potential for false positive results. Nothing inherently has changed about PCR testing to explain for the CDC's change from it former recommendations:

Testing Patients with Signs and Symptoms of Pertussis Early signs and symptoms of pertussis are often non-specific, making it difficult to determine clinically who has pertussis in the earliest stages (http://www.cdc.gov/pertussis/clinical/features.html). However, only patients with signs and symptoms consistent with pertussis should be tested by PCR to confirm the diagnosis. Testing asymptomatic persons should be avoided as it increases the likelihood of obtaining falsely-positive results. Asymptomatic close contacts of confirmed cases should not be tested and testing of contacts should not be used for post-exposure prophylaxis decisions.

https://www.cdc.gov/pertussis/clinical/downloads/diagnosis-pcr-bestpractices.pdf

https://www.unite4truth.com/post/part2-petition-asymptomatic-presentation-positive-covid-19-test-result-due-to-flawed-tests-methods

NO DIRECT TRANSMISSION STUDIES had ever been done:

From a CDC study published July 2020 entitled:

Absence of Apparent Transmission of Sars-Cov-2 from Two Stylists After Exposure at a Hair Salon with a Universal Face Covering Policy

"ALTHOUGH NO STUDIES HAVE EXAMINED SARS-CoV-2 TRANSMISSION DIRECTLY, data from previous epidemics (6,7) support the use of universal face coverings as a policy to reduce the spread of SARS-CoV-2"

https://www.cdc.gov/mmwr/volumes/69/wr/mm6928e2.htm

Keep in mind that this is a good 6-7 months after the original "SARS-COV-2" studies came out and that NO DIRECT TRANSMISSION STUDIES had ever been done. Beyond the lack of a purified/isolated "virus," it is clear that evidence of transmission is a very important piece of the "virus" puzzle that is clearly missing from the beginning.

---MY GOAL IS TO BRING YOU CLOSER TO UNDERSTANDING SPREAD / NOT JUST ASYMPTOMATIC IS THE BIGGER LIE WE HAVE BEEN SOLD ---

"New evidence has emerged from China indicating that the LARGE MAJORITY OF CORONAVIRUS INFECTIONS DO NOT RESULT IN SYMPTOMS.

Chinese authorities began publishing daily figures on 1 April on the number of new coronavirus cases that are asymptomatic, with the first day's figures suggesting that AROUND FOUR IN FIVE CORONAVIRUS INFECTIONS CAUSED NO ILLNESS." (1)

"VAST MAJORITY INFECTED WITH CORONAVIRUS ARE ASYMPTOMATIC when tested, study finds UCL scientist FIND 86 PERCENT DID NOT HAVE A COUGH, TEMPERATURE OR LOSS OF TASTE OR SMELL - 77 PERCENT of those tested SHOWED NO SYMPTOMS" (2)

"UP TO 95 PERCENT OF THE CORONAVIRUS CASES IN KARACHI HAVE BEEN ASYMPTOMATIC, researchers said Tuesday, shedding possible light on why Pakistan has been able to weather the pandemic."(3)

"PEOPLE WHO WERE ASYMPTOMATIC ACCOUNTED FOR 86% OF THE PEOPLE WHO TESTED POSITIVE FOR COVID-19 in a UK sample population during lockdown, a study showed on Thursday" (4)

"For COVID-19, DATA TO DATE SUGGEST THAT 80% OF INFECTIONS ARE MILD OR ASYMPTOMATIC, 15% are severe infection, requiring oxygen and 5% are critical infections, requiring ventilation. These fractions of severe and critical infection would be higher than what is observed for influenza infection." (5)

UP TO 80% OF COVID-19 INFECTIONS ARE ASYMPTOMATIC, a New Case Report Says (6)

"In our area, which includes upper Manhattan and the Bronx, about 15 percent of patients who came to us for delivery tested positive for the coronavirus, BUT AROUND 88 PERCENT OF THESE WOMEN HAD NO SYMPTOMS OF INFECTION." (7)

And before anyone suggests these "asymptomatic" cases will go on to develop symptoms later, guess again:

"COVID-19 PATIENTS WITH NO SYMPTOMS LIKELY TO STAY THAT WAY, Japan researchers say

WHAT ARE THE ODDS COVID-19 PATIENTS WITH NO SYMPTOMS COULD DEVELOP THEM LATER ON?

QUITE SLIM, according to a recent study led by a group of researchers in Aichi Prefecture, who have discovered that asymptomatic coronavirus patients tend to recover within nine days of having their infections confirmed via polymerase chain reaction (PCR) tests. (8)

(1) https://www.bmj.com/content/369/bmj.m1375

(2) https://www.telegraph.co.uk/news/2020/10/08/majority-people-test-positive-coronavirus-asymptomatic-tested/

(3) https://time.com/5842669/coronavirus-asymptomatic-transmission/

(4) https://www.reuters.com/article/us-health-coronavirus-britain-asymptomat-idUSKBN26T2O7

(5) https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200306-sitrep-46-covid-19.pdf

(6) https://time.com/5842669/coronavirus-asymptomatic-transmission/

(7) https://www.washingtonpost.com/outlook/2020/04/20/we-tested-all-our-patients-covid-19-found-lots-asymptomatic-cases/

(8) https://www.japantimes.co.jp/news/2020/06/15/national/science-health/asymptomatic-covid-19-patients

https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200306-sitrep-46-covid-19.pdf

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THE INFECTIOUS MYTH BUSTED: PART 2

Some interesting highlights/conclusions about the flu and the inability throughout the decades to show evidence of human-to-human transmission of the so-called "influenza virus:"

"An eighth conundrum – one not addressed by Hope-Simpson – IS THE SURPRISING PERCENTAGE OF SERONEGATIVE VOLUNTEERS WHO EITHER ESCAPE INFECTION OR DEVELOP ONLY MINOR ILLNESSES AFTER BEING EXPERIMENTALLY INOCULATED WITH A NOVEL INFLUENZA VIRUS. The percentage of subjects sickened by iatrogenic aerosol inoculation of influenza virus is less than 50% [3], although such experiments depend on the dose of virus used. Only three of eight subjects without pre-existing antibodies developed illness after aerosol inhalation of A2/Bethesda/10/63 [4]. Intranasal administration of various wild viruses to sero-negative

volunteers only resulted in constitutional symptoms 60% of the time; inoculation with Fort Dix Swine virus (H1N1) – a virus thought to be similar to the 1918 virus – in six sero-negative volunteers FAILED TO PRODUCE ANY SERIOUS ILLNESS, with one volunteer suffering moderate illness, three mild, one very mild, and one no illness at all [5]. Similar studies by Beare et al on other H1N1 viruses found 46 of 55 directly inoculated volunteers failed to develop constitutional symptoms [6]. IF INFLUENZA IS HIGHLY INFECTIOUS, WHY DOESN'T DIRECT INOCULATION OF A NOVEL VIRUS CAUSE UNIVERSAL ILLNESS IN SERONEGATIVE VOLUNTEERS?"

"After confronting influenza's conundrums, Hope-Simpson CONCLUDED THAT THE EPIDEMIOLOGY OF INFLUENZA WAS NOT CONSISTENT WITH A HIGHLY INFECTIOUS DISEASE SUSTAINED BY AN ENDLESS CHAIN OF SICK-TO-WELL TRANSMISSIONS [2]. Two of the three most recent reviews about the epidemiology of influenza state IT IS "GENERALLY ACCEPTED" that influenza is highly infectious and repeatedly transmitted from the sick to the well, BUT NONE GIVE REFERENCES DOCUMENTING SUCH TRANSMISSION [11-13]. Gregg, in an earlier review, also reiterated this "generally accepted" theory but warned:

"SOME FUNDAMENTAL ASPECTS OF THE EPIDEMIOLOGY OF INFLUENZA REMAIN OBSCURE AND CONTROVERSIAL. Such broad questions as what specific forces direct the appearance and disappearance of epidemics still challenge virologists and epidemiologists alike. Moreover, at the most basic community, school, or family levels of observation, even the simple dynamics of virus introduction, appearance, dissemination, and particularly transmission vary from epidemic to epidemic, locale to locale, SEEMINGLY UNMINDFUL OF TRADITIONAL INFECTIOUS DISEASE BEHAVIORAL PATTERNS." [14] (p. 46)

Questioning a generally accepted assumption means asking anew, "WHAT DOES THE EVIDENCE ACTUALLY SHOW?"

"In 2003, Bridges et al REVIEWED INFLUENZA TRANSMISSION AND FOUND "NO HUMAN EXPERIMENTAL STUDIES PUBLISHED IN THE ENGLISH-LANGUAGE LITERATURE DELINEATING PERSON-TO-PERSON TRANSMISSION OF INFLUENZA."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2279112/

Let's look at all the ways that they say you CAN NOT get "SARS-COV-2:"

DIRECT CONTACT AND FOMITES:

"There is currently no conclusive evidence for fomite or direct contact transmission of SARS-CoV-2 in humans."

DOMESTIC PETS AND FARM ANIMALS:

"There are no confirmed cases of transmission from domestic pets to humans."

VERTICAL TRANSMISSION:

"In addition, breast milk can harbor viral RNA, although no confirmed transmissions to infants from breast milk have been reported (90–92). Taken together, these studies suggest that vertical transmission of SARS-CoV-2 rarely occurs."

FECAL–ORAL (or FECAL AEROSOL) TRANSMISSION:

"No evidence currently supports fecal–oral transmission in humans, and intragastric inoculation of SARS-CoV-2 in macaques did not result in infection."

SEXUAL TRANSMISSION:

"No current evidence supports sexual transmission of SARS-CoV-2."

BLOODBORNE TRANSMISSION:

"The proportion of persons with viral RNA detectable in blood is currently unknown."

"To date, no replication-competent virus has been isolated from blood samples, and there are no documented cases of bloodborne transmission."

It appears that there is no evidence "SARS-COV-2" can be transmitted from:

*direct contact/fomites

*domestic animals

*mother-to-baby

*fecal to oral

*sexual activity

*bloodborne transmission.

What about RESPIRATORY TRANSMISSION?

"The accumulated evidence SUGGESTS that most transmission is respiratory, with virus suspended either on droplets or, less commonly, on aerosols."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7505025/

The evidence SUGGESTS.

There is no direct evidence of human-to-human transmission of "SARS-COV-2" from respiratory transmission. Everything that has come out is indirect evidence.

DIRECT EVIDENCE is defined as evidence THAT DIRECTLY PROVES A KEY FACT AT ISSUE.

INDIRECT EVIDENCE, also referred to as circumstantial evidence, is evidence that relies on an INFERENCE to connect it to a conclusion.

There is NO DIRECT EVIDENCE of human-to-human transmission of "SARS-COV-2." It is all INDIRECT EVIDENCE from which assumptions, hypotheses, and theories are drawn from and sold as conclusive.

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"Since then I have seen with my own eyes and smelled with my own nose smallpox growing up in first specimens, either in closed rooms or in overcrowded wards, where it could not by any possibility have been 'caught', but must have begun. I have seen diseases begin, grow up, and pass into one another. Now, dogs do not pass into cats."

- Florence Nightingale

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FACT VS FICTION IN TRANSMISSION

Many Departments of Health are putting forward high numbers of confirmed cases. Some of these so-called confirmed cases are not even from PCR test that have been said to be inaccurate at the least. The inventor of the test Kerri Mullins denounced PCR as a tool for identifying virus entirely.

Speaking solely on the other, calling confirmed cases with out even using a questionable diagnostic tool, which seems a bit strange to begin with, how are we assuming more cases without clinical data?

A research paper

published on November 20th highlights a case study of almost 10 million people in China. What the study found was there were 300 cases of Coronavirus in the population being carried without any symptoms at all. So the scientists then tracked the asymptomatic carriers. The contact tracing of 1,174 "close contacts" with the asymptomatic carriers showed ZERO

transmission. Not a few, not a couple, but zero -none- not a single transmission of Coronavirus from a person without symptoms....

....So why is the entire world engaged in COVID-19 mitigation processes to block the transmission of a virus that has never, not even once, been identified as occurring?

https://www.nature.com/articles/s41467-020-19802-w

"Among 1,001 child contacts of these six cases there were no confirmed cases of COVID-19. In the school setting, among 924 child contacts and 101 adult contacts identified, there were no confirmed cases of COVID-19."

"In summary, examination of all Irish pediatric cases of COVID-19 attending school during the pre-symptomatic and symptomatic periods of infection (n = 3) identified no cases of onward transmission to other children or adults within the school and a variety of other settings."

No evidence of secondary transmission of COVID-19 from children attending school in Ireland, 2020

https://www.eurosurveillance.org/.../1560-7917.ES.2020.25...

Out of 110 cases, 27 created secondary exposures - of which 23 were in closed environment.

Conversely 71 cases were in closed environment and did not generate a secondary exposure.

As is, the data presented is statistically insignificant... it does not prove that closed environments increase the risk of COVID Exposure.

https://www.medrxiv.org/con.../10.1101/2020.02.28.20029272v2

No evidence of secondary transmission of COVID-19 from children attending school in Ireland, 2020

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7268273/

No more lockdowns: World Health Organization warns that the price is too high

By TimesLIVE -August 3, 2020

The World Health Organization has urged countries not to reimpose national lockdowns in an attempt to stem the spread of Covid-19 due to social and economic repercussions.

https://www.therep.co.za/.../no-more-lockdowns-world.../

Public Health England Admits NO PROOF COVID-19 Is Contagious

- At the start of this 'pandemic' I read about four separate experiments/ investigations carried out by the US Military during the Spanish Flu, all of which demonstrated that the Spanish Flu could not be passed from very sick to healthy persons, even by getting the healthy to drink the warm sputum of the sick.

I wondered why no similar investigation was being conducted into the transmission of COVID19. Such experiments would not have to be so 'gross'.

Furthermore. It's not like this is an issue of no importance.

I sent FOI requests to the Department of Health and Social Care who, in a first reply (to the question of isolation of the virus), admitted that they held "no information relating to the isolation of Sars-Cov-2", a pretty astonishing statement the elevates Sars-Cov-2 to the same mythical status as that of the unicorn, an extraordinary thing that no one has ever seen.

The main difference between a unicorn and Sars-Cov-2 is that no one has yet invented a "scientific" test of supposed constituent parts that "proves" the existence of a unicorn, which is a great shame as it would be quite something to observe how many people would be convinced if a 'unicorn test' ever returned a 'positive'.

The DHSC also held no information about transmissibility/ contagion but suggested I sent my request to Public Health England.

PHE has replied (at last).

Here are the relevant lines:

Please could you forward any information you have relating to experimental evidence demonstrating that COVID-19 is person-to-person transmissible.

PHE can confirm it does not hold information in the way specified by your request.

What this means is that no specific investigation has been carried out into the most central assumption (and that's all it is) that has driven the global "response" to this supposed pandemic!

It would quite obviously be a straightforward issue to prove or disprove contagion (i.e. contagion-via-transmitted-droplet) experimentally. There is NO EXCUSE for not investigating this directly. Science could easily resolve contradicting beliefs about this, one way or the other.

In my opinion, it already has done. That's why the failure to investigate is, in itself, evidence of bad faith and the enforcement of a diabolical lie.

Scientific papers that demonstrate the uselessness of lockdowns and mask-wearing in protecting people against COVID 'infection' offer further indirect evidence that flu-like illnesses are NOT person-to-person transmissible. This is simply not how such illnesses work. Other factors, external and internal, define who becomes ill and when.

Here was my second FOI request:

If no such experimental evidence exists for COVID-19 please could you forward any available evidence collected, targeting this particular issue over the past 150 years, that demonstrates person-to-person transmissibility for any other influenza type illness?"

Reply:

PHE can confirm it does hold this information. However, the information is exempt under section 21 of the FOI Act because it is reasonably accessible by other means, and the terms of the exemption mean that we do not have to consider whether or not it would be in the public interest for you to have the information. However, for your convenience we have included a link to the report 'Impact of mass gatherings on Influenza.'

The first part of the response indicates, in my opinion, that PHE are admitting that they hold or are aware of the scientific evidence collected during the Spanish Flu (that used to be online in 'The US Surgeon General's Report 1919 [which disappeared from the document last October]).

By referring to not having to consider "whether or not it is in the public interest" that they release this information they are covertly admitting that they know the investigation demonstrated non-contagion and that it might be "in the public interest" that we be told this.

In fact, under our new global 'Communitarian' system (yes, we're already in it folks) what is defined as "the public interest" is decided by rulers …. as anyone with a brain should realise by now.

Truth, or even what we understand as the public interest (i.e. the common good) has nothing to do with anything any more … as American voters recently found out the hard way.

The linked report, in my opinion, has little to do with my FOI request. The weak 'conclusion' of "The impact of mass gatherings on Influenza" suggests correlation without demonstrating proof of anything at all. The probabilities suggested in the Conclusion are, yet again, based on assumptions that the author does not even care to define.

Correlation between future infection and mass gatherings without investigation of other factors inherent to mass gatherings (e.g. everyone being in approximately the same place and therefore subject to multiple identical environmental influences at the same time) surely means nothing scientifically. The report admits there is no proof of causation but suggests it is "prudent" to discourage them.

Why, one wonders, does it not suggest it would be prudent to investigate the scientific community's own primary assumption, that these illnesses are in any way contagious at all?

See this link to the full document.

Here is its 'conclusion'.

In conclusion there is limited data indicating that mass gatherings are associated with influenza transmission and this theme is continued with the inclusion of new evidence for the update.

Certain unique events such as the Hajj, specialised settings including civilian and military ships- a new theme for this update, indoor venues and crowded outdoor venues provide the primary evidence base to suggest mass gatherings can be associated with Influenza outbreaks.

Some evidence suggests that restricting mass gatherings together with other social distancing measures may help to reduce transmission. However, the evidence is still not strong enough to warrant advocating legislated restrictions.

Therefore, in a pandemic situation a cautious policy of voluntary avoidance of mass gatherings would is still the most prudent message. Operational considerations including practical implications of policy directed at restricting mass gathering events should be carefully considered.

After reading the entirety of the FOI response, here is my own conclusion:

PHE admits that government's assumption of human-to-human transmissibility of COVID-19 is based on … NO SCIENCE AT ALL!

Source and reference: KevBoyle.blogspot.com; Assets.publishing.service.gov.uk [pdf]

humansarefree.com

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Rosenau 1919 Spanish Flu experiments

Here is something you will never see in the news. During the 1918 Spanish Flu which is considered to be the most contagious "virus" of all, researchers for the Public Health Service and the U.S. Navy tried to determine what caused the flu and how infectious it was. The results of their experiments proved that the flu is not infectious at all:

"Perhaps the most interesting epidemiological studies conducted during the 1918–1919 pandemic were the human experiments conducted by the Public Health Service and the U.S. Navy under the supervision of Milton Rosenau on Gallops Island, the quarantine station in Boston Harbor, and on Angel Island, its counterpart in San Francisco. The experiment began with 100 VOLUNTEERS from the Navy WHO HAD NO HISTORY OF INFLUENZA. Rosenau was the first to report on the experiments conducted at Gallops Island in November and December 1918.69 His first volunteers received FIRST ONE STRAIN and THEN SEVERAL STRAINS of Pfeiffer's bacillus BY SPRAY AND SWABS INTO THEIR NOSES AND THROATS AND THEN INTO THEIR EYES. When that procedure FAILED TO PRODUCE DISEASE, OTHERS WERE INOCULATED WITH MIXTURES OF OTHER ORGANISMS ISOLATED FROM THE THROATS AND NOSES OF INFLUENZA PATIENTS. Next, SOME volunteers RECEIVED INJECTIONS OF BLOOD FROM INFLUENZA PATIENTS. Finally, 13 of

the volunteers were taken into an influenza ward and exposed to 10 influenza patients each. EACH VOLUNTEER WAS TO SHAKE HANDS WITH EACH PATIENT, to TALK WITH HIM AT CLOSE RANGE, AND TO PERMIT HIM TO COUGH DIRECTLY INTO HIS FACE. NONE OF THE VOLUNTEERS IN THESE EXPERIMENTS DEVELOPED INFLUENZA. Rosenau was clearly puzzled, and he cautioned against drawing conclusions from negative results. He ended his article in JAMA with a telling acknowledgement: “We entered the outbreak with a notion that we knew the cause of the disease, and were quite sure we knew how it was transmitted from person to person. PERHAPS, IF WE HAVE LEARNED ANYTHING, IT IS THAT WE ARE NOT QUITE SURE WHAT WE KNOW ABOUT THE DISEASE.”69 (p. 313)

The research conducted at Angel Island and that continued in early 1919 in Boston broadened this research by INOCULATING WITH the MATHERS STREPTOCOCCUS AND BY INCLUDING A SEARCH FOR FILTER-PASSING AGENTS, BUT IT PRODUCED SIMILAR NEGATIVE RESULTS.70–72 IT SEEMED THAT WHAT WAS ACKNOWLEDGED TO BE ONE OF THE MOST CONTAGIOUS OF COMMUNICABLE DISEASES COULD NOT BE TRANSFERRED UNDER EXPERIMENTAL CONDITIONS."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2862332/...

And then there's this:

Influenza Studies III. Attempts to Cultivate Filtrable Viruses from Cases of Influenza and Common Colds

Sara E Branham, Ivan C Hall

The Journal of Infectious Diseases, 143-149, 1921

"THESE EXPERIMENTS OFFER NO EVIDENCE IN SUPPORT OF THE THEORY THAT THE CAUSE OF EITHER COMMON COLDS OR INFLUENZA IS A FILTRABLE VIRUS. In attempting to cultivate filtrable viruses from the nasopharyngeal secretions in colds and influenza, NO BODIES WERE FOUND IN THE "CULTURES" WHICH COULD NOT BE FOUND ALSO IN THOSE FROM NORMAL PERSONS, IN CONTROLS IN ALL SIMPLE MEDIUMS EXAMINED, AND ON BLANK SLIDES. It is recognized that negative experiments, limited to the attempted cultivation of a filtrable virus, and including no attempts to reproduce the disease in animals, do not offer conclusive evidence that such a virus is not involved. No conclusions can be drawn concerning influenza, on account of the few cases examined, together with the fact that samples of such were not collected during the earliest stages of the disease. However, THE UNIFORMLY NEGATIVE RESULTS

OBTAINED WITH A LARGE AND REPRESENTATIVE NUMBER OF COLDS ARE NOT WITHOUT SIGNIFICANCE."

https://scholar.google.com/scholar...

Downloaded From: http://jama.jamanetwork.com/ by a Simon Fraser University User on 05/31/2015

Evidence continues to prove social distancing and herd immunity are unnecessary agendas based on the myth that "viruses" are contagious which has no basis in reality.

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"Researchers have yet to prove that a virus causes any of these conditions" - Dr. Tom Cowan

“if we see flies on a manure pile which do we think is more intelligent – to fight disease be swatting flies or to remove the pile of manure.” -Dr. J. Baldor, Surgeon, Florida

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Rosenau 1919 spanish flu experiments

[PDF] EXPERIMENTS TO DETERMINE MODE OF SPREAD OF INFLUENZA | Semantic Scholar

Https://www.semanticscholar.org/paper/EXPERIMENTS-TO-DETERMINE-MODE-OF-SPREAD-OF-Rosenau/40e4027b4d2f0b9b26a963f10022ea79ffed84e4?

This contains scientific references of many studies that were undertaken to try and prove that germs cause disease. All of the studies failed

Where is the evidence that viruses cause disease? I have been asking for almost 12 months now, and no one has been able to provide me with a single peer reviewed journal article showing an isolated virus causes disease. It should be so easy to look through the literature and find a study in a couple of minutes, yet no one seems to be able to do such a thing.

Scientists and doctors have already done countless experiments to try and prove germ theory over the course of 120+ years, and all have failed.

So I will ask again, can anyone provide me one such study, showing an isolated virus causes disease in humans? If so, I will gladly stand corrected and recount everything I have ever said on this matter.

There needs to be a truly scientific and intellectually honest conversation about this. This is the beauty of the scientific method, that we can ask questions, challenge our beliefs, put forward new ideas (that may or may not be correct) and learn new things.

Here are just some of the experiments that have been done on the common cold / flu. Many studies like this have been done in other diseases like measles and chicken pox as well, and they have not been able to prove viral causation or contagion.

Thanks NORTHERN TRACEY - DANIEL ROYTAS

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In 1758,

Francis Home attempted to inoculate 15 children with experimental measles by making a superficial incision in to an infected persons arm, right over a measles lesion. He then stuffed

cotton inside the wound and let it sit for three days. Home then made an incision in a healthy persons arm, and stuffed the cotton that was inside the sick persons arm inside the healthy persons wound. He reported that in many cases he observed that a “mild and modified form of measles” occurred. He also soaked cotton in the watery fluid coming from the eyes of infected people, made an incision in the arms of healthy people and applied the cotton over the wound. He found that within 6 days the person became unwell and within a couple of days the person was better(1).

Erasmus Darwin attempted to replicate these experiments, but found when he exposed healthy children to the blood and tears of measles patients, they did not become ill. Hoffman also questioned the results of Home, as he found that the blood of children infected with small pox was not infectious. C.J Themmen was also doubtful of Home’s results, as he found that natural measles breaks out on the 14th day after exposure to an infected person. Themmen tried to replicate Home’s work and undertook five experiments where he exposed incisions on the arms of healthy children with the blood, tears and perspiration of infected children. None of the children contracted measles. Themmen even questions whether Home even ever undertook such experiments(1).

Home’s notes were examined by several doctors and scientists years later. they concluded that none of the 15 children were ever actually infected with measles as a result of the inoculation(1).

In 1799, Dr. Green reported that he successfully infected three children by exposing them to the fluid of measles scabs, however there are no reliable records on this(1).

In 1801, Chapman repeatedly tried to infect healthy people with measles by exposing them to the blood, tears, nasal mucous, lung fluid and the discharge from measles scabs, however none of the participants became sick(1).

In 1809, Willan tried to infect three children by exposing them to the fluid of measles lesions from sick people. None of the children became sick(1).

In 1810, Waschel claimed to have experimentally infected an 18-year-old man with measles, however these claims were disputed by others at the time. The man became sick 22 days after inoculation and it is said the man actually contracted measles naturally and not from the inoculation(1).

In 1822, Dr. Frigori tried to infect 6 children with measles using the above-mentioned methods used by Home. Whilst the children developed mild non-specific symptoms, they did not develop measles. Not happy with his results, Frigori attempted to infect himself but without success(1).

In 1822, Dr. Negri tried to infect two children using Home's methods, however he had the same negative results as Dr. Frigori.

In 1822, Speranza attempted to infect 4 children using similar methods, but without success(1).

In 1834, Albers tried to infect four children with measles, however none fell ill(1).

Between 1845 – 1851 Mayr is said to have successfully infected 6 children with measles, however it seems to be a modified form of the disease (in other words, not measles)(1).

In 1890, Hugh Thompson tried to infect children with measles in two separate instances, however both attempts failed(1).

References: 1. Hektoen L. Experimental Measles. J Infect Dis. 1905;2(2):238-255. doi:10.1093/infdis/2.2.238

All the experiments trying to experimentally induce influenza, chickenpox and measles have all failed. Well, it should come as no surprise to you that scientists and doctors tried to do the same with Scarlett fever, and failed time and time again.

Source: HEKTOEN, L. (1923). THE HISTORY OF EXPERIMENTAL SCARLET FEVER IN MAN. JAMA:

The Journal of the American Medical Association, 80(2), 84.
doi:10.1001/jama.1923.02640290014005

https://jamanetwork.com/.../jama/article-abstract/232168...

In 1817, Themmen undertook five experiments where he exposed incisions on the arms of healthy children with the blood, tears and perspiration of infected children. None of the children contracted measles.

In 1799, Dr. Green reported that he successfully infected three children by exposing them to the fluid of measles scabs, however there are no reliable records on this(1).

In 1905, Ludvig Hektoen reports that he was able to successfully infect two healthy people with the blood of infected measles patients(1). It should be noted that the blood was mixed with other substances, such as ascites fluid before it was injected. This experiment is considered to be the best evidence that proves beyond any doubt that the measles virus causes disease(2).

There are few specific details about the signs and symptoms that these patients actually exhibited, so there is some doubt as to whether they really had measles(3).

In 1915, Charles Herman swabbed the nasal mucosa of 40 infants with cotton buds covered in the nasal secretions of infected measles patients. The majority of the infants had no reaction, 15 infants had a slight rise in body temperature and a “few” were said to develop some red spots on their skin. At 1 year of age, 4 of these infants had intimate contact with infected people. None of the infants became sick and this is said to be due to the infants having “immunity”(4).

In 1919, Sellards tried to inoculate 8 healthy men (with no previous exposure to measles) with the blood of measles patients, using the same methods as Hektoen. None of the men became sick(3,5). It is interesting reading the authors commentary, where he describes how he intensified his efforts to try and infect the patients, but was still unable to infect them.

A few weeks later, the volunteers were exposed to an infected measles case, yet none of them became sick.

Nasal secretions were then taken from measles patients and syringed up in to the nasal passages of the healthy participants. None became sick(3,5).

Sellards also conducted another experiment to try and infect another 2 healthy human volunteers with measles by injecting them subcutaneously and intramuscularly with the blood of two infected patients. Neither man became sick(3,5).

In 1919, Alfred Hess makes a comment about Sellards results. He states "It is remarkable that Sellards was unable to produce this highly infectious disease by means of the blood or nasal secretions of infected individuals, not long ago I was confronted with a similar experience with chicken pox, thus we are confronted with two diseases, the two most infectious of the endemic diseases in this part of the world, which we are unable to transmit artificially from man to man" (6).

In 1924, Harry Bauguess wrote a paper and stated "A careful search of the literature does not reveal a case in which the blood from a patient having measles was injected into the blood stream of another person and produced measles". He reports two cases where he observed people contract measles from blood transfusions.

In one case a critically ill 9-month-old child received a blood transfusion from her mother, who was in perfect health. Approximately 13 days later, the child developed a rash and the diagnosis of measles was made. The child then developed bronchopneumonia and died about 10 days later. Bauguess explains that the reason why the child contracted measles, was because two days after the transfusion, the mother developed measles. It was concluded the child contracted measles from the mothers blood(6).

In a second case, Bauguess reports another critically ill child who was 3-months-old receiving a blood transfusion. The mother was in perfect health at the time of the transfusion. The child

seemed to be recovering, however about 10 days later the child began to become ill again and developed a rash, which was diagnosed as measles. Just like the previous case, two days after the transfusion, the mother developed measles. It was said that the child contracted measles from the mothers blood(6).

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7. BAUGUESS H. MEASLES TRANSMITTED BY BLOOD TRANSFUSION. Am J Dis Child. 1924;27(3):256. doi:10.1001/archpedi.1924.019200900610

In March of 1919 Rosenau & Keegan conducted 9 separate experiments in a group of 49 healthy men, to prove contagion. In all 9 experiments, 0/49 men became sick after being exposed to sick people or the bodily fluids of sick people.

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Rosenau 1919 spanish flu experiments

[PDF] EXPERIMENTS TO DETERMINE MODE OF SPREAD OF INFLUENZA | Semantic Scholar

Https://www.semanticscholar.org/paper/EXPERIMENTS-TO-DETERMINE-MODE-OF-SPREAD-OF-

Rosenau/40e4027b4d2f0b9b26a963f10022ea79ffed84e4?

In November 1919, 8 separate experiments were conducted by Rosenau et al. in a group of 62 men trying to prove that influenza is contagious and causes disease. In all 8 experiments, 0/62 men became sick.

Another set of 8 experiments were undertaken in December of 1919 by McCoy et al. in 50 men to try and prove contagion. Once again, all 8 experiments failed to prove people with influenza, or their bodily fluids cause illness. 0/50 men became sick.

In 1919, Wahl et al. conducted 3 separate experiments to infect 6 healthy men with influenza by exposing them to mucous secretions and lung tissue from sick people. 0/6 men contracted influenza in any of the three studies.

https://www.jstor.org/stable/30082102?seq=1...

In 1920, Schmidt et al conducted two controlled experiments, exposing healthy people to the bodily fluids of sick people. Of 196 people exposed to the mucous secretions of sick people, 21 (10.7%) developed colds and three developed grippe (1.5%). In the second group, of the 84 healthy people exposed to mucous secretions of sick people, five developed grippe (5.9%) and four colds (4.7%). Of forty-three controls who had been inoculated with sterile physiological salt solutions eight (18.6%) developed colds. A higher percentage of people got sick after being exposed to saline compared to those being exposed to the "virus".

https://pubmed.ncbi.nlm.nih.gov/19869857/

https://catalog.hathitrust.org/Record/102609951

In 1921, Williams et al. tried to experimentally infect 45 healthy men with the common cold and influenza, by exposing them to mucous secretions from sick people. 0/45 became ill.

https://pubmed.ncbi.nlm.nih.gov/19869857/

In 1924, Robertson & Groves exposed 100 healthy individuals to the bodily secretions from 16

different people suffering from influenza. The authors concluded that 0/100 became sick as a result of being exposed to the bodily secretions.

https://academic.oup.com/.../article.../34/4/400/832936...

In 1930, Dochez et al. attempted to infect a group of men experimentally with the common cold. The authors stated in their results, something that is nothing short of amazing.

"It was apparent very early that this individual was more or less unreliable and from the start it was possible to keep him in the dark regarding our procedure. He had inconspicuous symptoms after his test injection of sterile broth and no more striking results from the cold filtrate, until an assistant, on the second day after injection, inadvertently referred to this failure to contract a cold.

That evening and night the subject reported severe symptomatology, including sneezing, cough, sore throat and stuffiness in the nose. The next morning he was told that he had been misinformed in regard to the nature of the filtrate and his symptoms subsided within the hour. It is important to note that there was an entire absence of objective pathological changes".

https://pubmed.ncbi.nlm.nih.gov/19869798/

In 1937 Burnet & Lush conducted an experiment exposing 200 healthy people to bodily secretions from people infected with influenza. 0/200 became sick.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2065253/

In 1940, Burnet and Foley tried to experimentally infect 15 university students with influenza. The authors concluded their experiment was a failure.

https://onlinelibrary.wiley.com/doi/abs/10.5694/j.1326-5377.1940.tb79929.x

Part 3 - Animal contagion studies in measles. After decades of trying to prove the infectivity of measles in humans with many different experiments, scientists moved on to trying to infect monkeys. In 1911, Anderson and Goldberg tried to replicate the result of a previous study they had conducted years prior, where they report successfully infecting monkeys with measles. These results have been questioned by other authors(7).

In this experiment they conducted three separate experiments where they tried to experimentally infect monkeys with measles. In experiment one, 0/18 monkeys became ill after being injected with nasal secretions of measles patients. In experiment two, two monkeys had measles scabs aka “scales” from infected people syringed in to their nostrils. Neither monkey became sick. In experiment three, measles “scales” were injected in to two monkeys and neither became ill. In 1911, Nicolle and Conseil reported to have successfully transferred measles from humans to monkeys, despite the monkeys having no signs or symptoms of measles, apart from a slightly raised temperature(7).

In 1912, Tunnicliff injected a monkey with the blood of a measles patient. The monkey did not develop any symptoms(9).

In 1914, Jurgelunas tried to infect three monkeys with the blood and five monkeys with the nasal secretions of measles patients, however none of the monkeys became ill. He also put two monkeys in to a measles ward in a hospital and none of the monkeys became ill(7).

In 1918 and 1919, Sellards and Wentworth tried to experimentally infect five monkeys with the blood of measles patients. None of the monkeys became sick(7).

In 1919, Sellards injected the blood of two measles patients into two monkeys. Only one of the monkeys developed symptoms, however the symptoms were not suggestive of measles(3).

In another set of experiments by the same author (Sellards), swabs covered in the mucous of infected measles patients were rubbed over the conjunctiva, nasal and pharyngeal mucous membranes of two monkeys. Neither monkey became ill(3).

In a second experiment, two additional monkeys were also inoculated the same way and neither monkey became sick(3).

In 1920, Blake and Trask report that they were able to successfully infect monkeys with measles by exposing them to blood of infected people(7).

In 1921, Blake and Trask report that they successfully inoculated 8/10 monkeys with measles, after exposing them to the mucous secretions of infected people. Interestingly, other authors state that the rash observed in these monkeys didn't differ significantly from the maculopapular rashes that are usually observed in healthy monkeys(3).

It is therefore unlikely the monkeys had contracted measles.

In a review paper in 1924, Sellards states "There is certainly, at present, no exact proof of the susceptibility of monkeys to measles" and "Personally I am not willing to accept as established the various characteristics of the virus of measles as worked out this way. Thus the important conclusion that the virus is filterable rests primarily upon more or less vague results obtained in three monkeys. I prefer to consider the filterability of the virus as an entirely open question"(3).

In the years that follow, doctors and scientists try to infect rabbits and guinea pigs with measles but get results similar to those of the monkey trials. They then move in studies with rats and mice, again with mixed and unfavorable results.

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There are a number of things that are known to cause "flu-like symptoms", all of which occur when a person is detoxifying after exposure to poisonous or toxic substances. Well known causes of influenza like illness include; - Smelters chills / Monday morning fever (heavy metal toxicity / exposure) - Dippers flu (acute pesticide exposure) - Polymer fume fever (acute Teflon exposure) - Caffeine withdrawal - Organophosphate exposure - Phenol exposure - Smokers flu (nicotine withdrawal) - Alcohol detoxification - Chemotherapy (RADIATION POISON) - Anti-depressant medication discontinuation - Sick building syndrome So we know that flu-like symptoms are associated with the detoxification process. Some people suggest that the flu is simply a "spring clean" that our body goes through to detoxify itself. ....More than 150 years of germ theory, not a single piece of evidence, using an isolated virus has ever proven that germs do in fact cause disease. There was considerable research undertaken by people like Antione Bechamp, Claude Bernard, Gunther Enderlein and Gaston Naessans in support of the "terrain theory", however this research was never given the time or attention it deserved by the mainstream, as it challenged the "belief" that germs cause disease. When a claim is made that X causes Y, yet dozens of pieces of research, including controlled trials show X doesn't cause Y, then an alternative cause should be investigated, rather than continuing to say X causes Y because of dogma or long held belief. This literature review from 1924 provides many hypotheses about what may cause influenza. It really is a fascinating read. The reason I highlight this piece of work, is because it was published back when people still thought critically and the scientific method was alive and well. A Review of the Literature on Influenza and the Common Cold Front Cover - James Gayley Townsend

https://books.google.com.au/books/about/A_Review_of_the_Literature_on_Influenza.html?id=8FIkTBdeJOwC&redir_esc=y&fbclid=IwAR0iUML7PTxb4vrIakUQ3Cyz73a8tDYZDNCwNZdu_QHIYxtWRnh_swzvTw8

Swine Flu Expose

a book by Eleanora I. McBean, Ph.D., N.D.

I heard that This was in an army camp, so I wrote to the Government for verification. They sent me the report of U.S. Secretary of War, Henry L. Stimson. The report not only verified the report of the seven who dropped dead from the vaccines, but it stated that there had been 63 deaths and 28,585 cases of hepatitis as a direct result of the yellow fever vaccine during only 6 months of the war. That was only one of the 14 to 25 shots given the soldiers. We can imagine the damage that all these shots did to the men. (See the chapter on What Vaccinations Did to Our Soldiers.)

The first World War was of a short duration, so the vaccine makers were unable to use up all their vaccines. As they were (and still are) in business for profit, they decided to sell it to the rest of the population. So they drummed up the largest vaccination campaign in U.S. history. There were no epidemics to justify it so they used other tricks. Their propaganda claimed the soldiers were coming home from foreign countries with all kinds of diseases and that everyone must have all the shots on the market.

The people believed them because, first of all, they wanted to believe their doctors, and second, the returning soldiers certainly had been sick. They didn't know it was from doctor-made vaccine diseases, as the army doctors don't tell them things like that. Many of the returned soldiers were disabled for life by these drug-induced diseases. Many were insane from postvaccinal encephalitis, but the doctors called it even though many had never left American soil.

The disease brought on by the many poison vaccines baffled the doctors, as they never had a vaccination spree before which used so many different vaccines. The new disease they had created had symptoms of all the diseases they had injected into the man. There was the high fever, extreme weakness, abdominal rash and intestinal disturbance characteristic of typhoid. The diphtheria vaccine caused lung congestion, chills and fever, swollen, sore throat clogged with the false membrane, and the choking suffocation because of difficulty in breathing followed by gasping and death, after which the body turned black from stagnant blood that had been deprived of oxygen in the suffocation stages. In early days they called it The other vaccines cause their own reactions — paralysis, brain damage, lockjaw, etc.

When doctors had tried to suppress the symptoms of the typhoid with a stronger vaccine, it caused a worse form of typhoid which they named But when they concocted a stronger and more dangerous vaccine to suppress that one, they created an even worse disease which they didn't have a name for. What should they call it? They didn't want to tell the people what it really was — their own Frankenstein monster which they had created with their vaccines and suppressive medicines. They wanted to direct the blame away from themselves, so they called it It was certainly not of Spanish origin, and the Spanish people resented the implication that the world-wide scourge of that day should be blamed on them. But the name stuck and American medical doctors and vaccine makers were not suspected of the crime of this widespread devastation — the It is only in recent years that researchers have been digging up the facts and laying the blame where it belongs.

Some of the soldiers may have been in Spain before coming home, but their diseases originated in their own home-based U.S. Army Camps. Our medical men still use that same dodge. When their own vaccines (required for travel) cause vaccine diseases abroad they use this as grounds for a scare campaign to stampede people into the vaccination centers. Do you remember the Hong Kong Flu and the Asian Flu and the London Flu scares? These were all medically-made epidemics mixed with the usual common colds which people have every year.

The experiment began with 100 volunteers from the Navy who had no history of influenza. Rosenau was the first to report on the experiments conducted at Gallops Island in November and December 1918.69 His first volunteers received first one strain and then several strains of Pfeiffer's bacillus by spray and swab into their noses and throats and then into their eyes. When that procedure failed to produce disease, others were inoculated with mixtures of other organisms isolated from the throats and noses of influenza patients. Next, some volunteers received injections of blood from influenza patients. Finally, 13 of the volunteers were taken into an influenza ward and exposed to 10 influenza patients each. Each volunteer was to shake hands with each patient, to talk with him at close range, and to permit him to cough directly into his face. None of the volunteers in these experiments developed influenza. Rosenau was clearly puzzled, and he cautioned against drawing conclusions from negative results. He ended his article in JAMA with a telling acknowledgement: "We entered the outbreak with a notion that we knew the cause of the disease, and were quite sure we knew how it was transmitted from person to person. Perhaps, if we have learned anything, it is that we are not quite sure what we know about the disease."

The State of Science, Microbiology, and Vaccines Circa 1918 (nih.gov)

Https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2862332/?fbclid=IwAR0a1IpdU3tY1j_nMFCbVlH1NwE43FFuTGZi-sGJFyHB8AU0n8bvsCWjrbI

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It is more important to know what sort of person has a disease than to know what sort of disease a person has.

-Hippocrates

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COURT CASES OF SMALLPOX EPIDEMICS DECLARED WHEN THERE WAS NO SMALLPOX

(Report from THE ADVERTISER'S PROTECTIVE BUREAU of Kansas City)

"In the fall of 1921 the health of the city was unusually good, but dull for the doctors. So the Jackson Medical Society met and resolved to make an epidemic in the city.

According to the record:

"MOTION WAS MADE AND SECONDED THAT A RECOMMENDATION BE MADE BY THE COMMITTEE TO THE BOARD OF HEALTH THAT AN EPIDEMIC OF SMALLPOX BE DECLARED TO EXIST IN THE CITY AT THE PRESENT TIME.

"MOVED AND SECONDED THAT A DAY BE SET ASIDE TO BE TERMED ON WHICH PHYSICIANS WILL BE STATIONED AT ALL SCHOOLS, CLINICS, PUBLIC BUILDINGS AND HOSPITALS ... TO VACCINATE, FREE OF CHARGE. (No shots are free. The taxpayers are charged for them.)

"It is further recommended that wide publicity be given, stating that vaccination is a preventitive of smallpox and urging the absolute necessity of vaccination for every man, woman and child in the city.

Those who investigated this fake, doctor-made epidemic searched for cases of smallpox to justify this vaccination drive, but could not find one case in the city.

The scare-head vaccination propaganda showed a picture of a child covered with sores (probably from empetigo, psoriasis or congenital syphilis), and called it smallpox. People are easily frightened when public officials tell them a disease is contagious and is in their midst. The fear vanishes when people learn the truth. Smallpox is not contagious, and compulsory

vaccination is illegal. (See the chapter on smallpox for information on tests which proved smallpox and other diseases are not contagious, and can be avoided with the right knowledge and application of that knowledge, given in the big book, "VACCINATION CONDEMNED BY COMPETENT DOCTORS."

Most people do not question the decision of their doctors and public officials so they trustingly put their lives and the lives of their children into the hands of the unprincipled drug vendors. The results were disastrous for the people but good for the doctors. The hospitals were soon filled with vaccine poisoned people and the doctors had business all winter.

According to the record, the doctors made $500,000 from that Kansas City vaccination spree, not counting the millions of dollars from the hospital cases.

ANOTHER MEDICALLY-MADE EPIDEMIC IN PITTSBURGH IN 1924

The same procedure was carried out as for Kansas. The doctors called a meeting and moved and seconded that an epidemic be declared in the city. As usual, they spread their scare propaganda far-and-wide and herded the people into the vaccination centers.

When the vaccination campaign was at its height, the report showed the death-rate rose 22% in three months, from July 1 to Sept. 30. These deaths were all among the recently vaccinated. Were the doctors and pharmaceutical companies charged with murder and given the death sentence? No. They "got by with murder;" they usually do.

This doctor-made epidemic never would have been brought to public attention if it had not been for an active, and well informed group of non-medical, health-minded citizens known as THE PITTSBURGH HEALTH CLUB. They, and their legal aid investigated this manufactured epidemic and brought the instigators to trial.

According to the Health Club's attorney:

"THE DIRECT MONEY LOSS TO THE CITY OF PITTSBURGH IN THIS VACCINATION RAID in 1924 was $3,096,616 of which the doctors took $2,000,000. .

"This does not represent the indirect losses such as deaths, permanent injury, business loss from work, etc."

Every city and town today should have an active, capable, and well prepared NON-MEDICAL health group such as those in Pittsburgh and Kansas City to warn the people against the many questionable practices of the medical doctors, because they continue to stage deadly vaccination campaigns, both large and small in all cities across the country every year, but most

of them are not investigated or questioned.

We are in the middle of one of these medically-made epidemics right now.............

http://whale.to/vaccine/sf1.html

Dr. Stefan Lanka: The history of infection theory

(English transcript) By: Abrupt Earth Changes *see also AEC's new essay "Covid-19 is the new Mediaeval Leprosy: a Historical Comparison of Isolation, Religious Fervor and Medical Tyranny" AEC brought to light this brilliant transcription of Stefan Lanka's interview The title of Lanka's original video is "Grippe pandemie und Tamiflu" copy here. DoCuments: klein-klein-media.de Translation from German and original subtitled video: Sacha Dobler, AbruptEarthChanges.com "My name is Stefan Lanka, I am a biologist and virologist. I discovered the first virus, which was in the ocean. That's how I became involved in this matter. First, I recognized that this virus doesn't cause any harm. Secondly, the Austrian professor Fritz Pol alerted me to the fact, that something was wrong with the entire AIDS affair and the virus might not even exist at all. I checked this and realized, that was indeed the case. I thought this couldn't be and I remained silent for half a year, for I assumed, I misunderstand something. I couldn't imagine that the entire world would go along with this." 2:00 Then I started researching and became involved in the infection theory. I realized that everything was wrong, it initially started with an error, that then turned into fraud, political fraud already under Otto von Bismarck, this can easily be proven and reconstructed. This fraudulent concept was abandoned after World War II, then reestablished by the Americans in order to provoke fear and to conduct population control. Copy of the translated video: 2:45 Further, I recognized, as I will demonstrate here, how the infection theory gave rise to the gene theory and the so-called molecular technology and gene manipulation and -technology. Today's model of the infection theory is used in the form of vaccines, of fear from material contagion, in the form of pandemics, just as in the current one, which is predicted to erupt any moment, or it will erupt in the second phase or even later and that it will then be encountered with the drug called Tamiflu. Incidentally, Tamiflu stands for toxic amiflue, there was an apparent glitch in the naming process of this chemo-therapeutic drug. 03:32 I'm going to tell the story, how everything developed, in order for you to comprehend, how an error turned into a fraud, a fraud turned into a crime, and how through the industrialization of this crime, the madness developed, a kind of madness that endangers all of us, the entire human race. 04:03 We begin at a point in history of ancient Greece there this concept infection developed. It is the basis of today's system of medicine that is ruled by systematic fighting and poisoning. It is also why we are supposed to swallow Tamiflu even preemptively and, after the pandemic is declared, it should be take in large quantities. 04:55

Before we delve into ancient Greece: Goethe tried to warn of the 2006 influence pandemic, and also of AIDS and of the swallowing of Tamiflu. He did this in Faust I, not in the first edition, that went through censorship, not in the second, but in the third or fourth edition he included this essential passage, in which he describes how even back then a single doctor killed thousands of people: First he describes in alchemist's language, how the Tamiflu and chemotherapy of his time was produced: organic mercury compounds, mixed with sirup, Latwergen stands for sirup mixtures, and these will be the hellish Latwergen in alchemistic language and how they were produced. Here are the important lines: (Here was the Tamiflu, the patients died (will die?) And no one asked: who recovered? No one asked: is the theory correct? Does the virus exist at all?) And we'll treat the black plague also, a political disease just as AIDS, influenza, SARS, BSE, just as small pox, the collective term of leprosy, from which black plague was derived, and also the collective term of polio. Goethe. He has not been taken seriously 'till today. Whenever there is a tsunami or an earthquake, I think to myself: Goethe is speaking again. For he warned and this warning was dismissed. 06:49 How did it get to this, what is the mistake, the overall false assumption? The general false assumption, on which the entire western academic medicine is based on, is this: In the frameworks of the doctrine of juices, it was believed that disease was brought about by an unbalance of juices or fluids in the body. We have many different fluids in the body, about 270 different types, in the joints, in the eye, sweat glands, digestive glands, fluids of the inner ear, brain fluids,spinal fluids and so on. It was assumed that a non-equilibrium of juices would lead to the development of disease-causing toxins. And it was believed from experience, that the administration of small amounts of poisons would cause in the body the reaction of production of an antidote, anti-poison. This idea derived from the experience with the cell toxin alcohol. Consumed in small quantities it can be fun, it can also diminish anxieties and the like, but if a young person who never had been in contact with alcohol, drinks half a bottle of liquor, when he is paralyzed enough to not be able to spit, then his stomach must be pumped empty, otherwise he dies of alcohol poisoning. Jelzin and others, they make world politics after two liters. 08:12 This observation was the basis premise : to ingest a poison little by little. You can try it yourself, quit drinking alcohol for half a year, and then drink two glasses of wine, you'll almost fall over. But not because the body made the anti-poison, but because the body is trained, it has prepared the enzymes to quickly process and neutralize and excrete the alcohol. This doctrine of juices in this form is the explicit basis of the entire western academic medicine, including the false believe in an immune system. Why? They believed, that a disease-causing poison could develop, and if one preemptively took a poison, then the body would make an anti-poison, so one would obtain immunity. And whenever the disease-causing poison arrives, then I'm already invulnerable. That's why Rasputin and Napoleon (this can be measured in the hair) frequently took different kinds of toxins in small quantities in order to be immune against a poison attack. But then in the 19th century, when it was possible to detect small amounts of toxins, it was found that in no illness, toxins can be detected. In non of them, until today. 9:56 So the theory proved to be wrong. But the entire thinking was based on it, that means to give mercury compounds preemptively, as in Goethe's times, so whenever the

disease-causing toxin comes about in form of an epidemic, the person is already invulnerable. This perception of epidemics already developed in the history of ancient Greece. It was claimed that the illness demon would possess and defile a person. From this, the Greek word miasma is derived. Defilement, one is tarnished by the illness demon and one can transmit the disease to others. The disease demon infects me, reproduces within me, and can be passed on to others and disease-causing toxins would be produced. That's why anti-poisons are administered preemptively, just as Goethe describes it, and the survivors applauded. “And now from the withered old must hear How men praise shameless murderers.“ As It were, Goethe revived Paracelsus in the figure of Faust, but he is ashamed of having been celebrated by peasants before, as they call after him: “You saved our lives!“ but thousands perished. He was ashamed of this and sat down with his Atlantus Wagner on this certain rock near the village and meditates and recounts what he did, and what his father had done. 11.56 It had been observed that bacteria produce toxins. This was investigated, and they were all very certain, that bacteria could only produce toxins in the dead body. That is because bacteria run their metabolism in an aerobic environment and produce substances in the intestines during digestion such as necessary vitamins. But when these bacteria are deprived of oxygen, which is the case in a dead body after a couple of days, then a few of them can survive, they change their metabolism. Just as the yeast changes its metabolism under exclusion of oxygen to produce the toxic alcohol, in this way these bacteria produce their toxins, but only under complete exclusion of oxygen. 13:03 This was well known, bacteria cannot be the cause of disease. Professor Henle further solidified this knowledge, he phrased his postulates and said: If you claim that bacteria can be transmitted and then produce their poisons, then you need to identify that bacterium in every case of a disease, which you claim was caused by this bacterium. And that was not the case. Bacteria are only insufficiently identifiable in a test-tube, it can be done only with a few of them. Of all bacteria, which we know based on their performance, only about 2 % are cultivatable and multipliable. And what is defined as a bacterium in the laboratory is not the same as the original bacterium outside. Why? 14:11 Because the idea of bacteria in the lab representing one single type, is a laboratory artifact. For, bacteria exchange information among one another continuously and they change their form and function. This was recently confirmed in a large study: bacteria, as individual as they are in terms of their biochemistry, they are very similar in terms of their nucleic acid. They adapt. If we extract a bacterium and cultivate it in isolation, it looses its properties after some time and it can't survive. Thus, I must produce a large quantity of them, freeze them and then I always work with those. But this already constitutes a massive intervention into nature, and doesn't represent the reality of bacteria exchanging their information amongst one another, and thus the definition of types that was imposed on them, was not scientifically justifiable. That was the first problem. For instance, they didn't manage to find the famous tuberculosis bacterium, the cultivation of which was successfully done by Robert Koch: It could only be found in about half the cases. That remains the same to the present day. 15:46 The second Henle Postulate states: this isolated pathogen must be observed, in case of a bacterium it must be multiplied, and it must be observed whether or not

it can do what is assigned to it. In all these experiments they found, the bacteria couldn't produce toxins in the living organism, only after a few days in the dead body, after an animal or human died. That was also determined, throughout the entire scientific community without exception. 16:23 Henle formulated the third Postulate, which states: Then, the pathogen that was isolated and multiplied, must be injected into an organism and the same disease must develop. And this has never happened, never ever. 17:00 But how did it happen, that suddenly Robert Koch was celebrated as the discoverer of the transmissibility of diseases? That is the question. 17:03 The question is easily answered. Robert Koch deserved reputation for having managed to make photography adaptable to visual microscopy and to make photographs of bacteria. Photography itself had been rediscovered in Europa in 1885. This brought him much reputation deservedly. Photography was considered to be sacred, no one could imagine that a negative could be retouched, that double exposures could be used, that it could be manipulated. It was deemed as inherently scientific and objective. They simply made claims along with photography, and this acted in a very hypnotic way, much like television today, so people just accepted these claims. 18:00 He simply photographed bacteria that can be found everywhere. From this, two different concepts derived. Of course, these bacteria don't cause disease, but the Third Postulate (which states bacteria much cause the same disease) was violated by Koch, he introduced the scientific fraud, that plays the central role until today, in cases like AIDS, vaccination, influenza pandemic, and so on including Tamiflu. 18:41 He said, the inoculation of the test animal with this bacteria culture leads to the development of a SIMILAR illness. Not the same, but a SIMILAR illness. And this is one of the general acts of fraud of the entire infection theory: development of a SIMILAR illness. Read for yourself, that's homework number one, don't just believe me, go the library, read what Robert Koch did. Anthrax, just one example: he kills mice with corps toxins. This corps poison you can make at home: leave a potato salad standing outside in the summer for a weak, spoiled egg meal, bacteria spores are floating in the air everywhere, they settle, grow, reproduce, they consume the oxygen. They transfer into the anaerobic state, mostly in the centre of the potato salad or the egg meal or in a dead body. And then, toxins are produced. The toxins themselves they can kill, if this is fed to a person little by little, and the foulness is covered up with strong spices or taste enhancers. In his way, a person can be chronically poisoned, or be caused to suffer severe diarrhea and cramps. 20:10 Koch produces these toxins in a meat broth, as you can replicate at home, he injects them into the vein of a mouse, the mouse dies, the milt is swollen, he extracts the milt of the mouse, and transplants it under the skin of a frog. The frog convulses and dies and this is called skin anthrax. Robert Koch, scientific fraud. 20:42 Now you can imagine which animal experiments were made to claim lung anthrax. The milt of the killed mouse was implanted into the lungs of the frog. That is what is done until the present day, that is what is done in the influenza pandemic: Animals are being killed with incisions of the trachea, liquids are inserted continuously, the animals die, and then it is claimed, it was the virus. You can study this on our influenza virus information flyer, which is attached to this file. On this you will find the literature on how they operate concerning influenza. No control group animals, if they were to inject

ordinary liquids into the animals, the exact same were to happen. 21:32 The second thing that was derived from Germany and Robert Koch, was this: Robert Koch relied on new colorants to be ably to dye bacteria. And naturally, he received these dyes from the colorant industry. Then, all other medical researcher took the same colorants, took healthy tissue, they acidified the tissue and discovered they had the same coloring reaction and the exact same bacteria can be seen and photographed, just as Robert Koch did. 22:12 But then they also discovered, these dyes killed bacteria by making holes in them, they inhibit the DNA of the bacteria, these can no longer reproduce, the bacteria die. From this, antibiotics were derived, from colorants. BASF, BAYER, IG Farben, Hoechst, Merk and so on. The pharma- industry was derived from colorant manufacturers based on the infection hypothesis. The American capital built up their parallel structures in Switzerland, in order to keep up with the revolutionary knowledge of the Germans, who were assumed to be capable of having something important just as the H. Bosch process, were nitrogen is extracted from air. By this, the Germans no longer relied on importing sodium nitrate from Chile for bombs and grenades, as the oceans were blocked. That's the historic background. 23:24 But why did the German government employ Robert Koch? He already had to flee from Berlin before because he had killed thousand with his magic drug tuberculin against tuberculosis. This drug's ingredients were kept secret against the law. He fled, Otto von Bismarck called him back, he desperately needed a pretext against the British who had seized the Suez canal illegally and thus had significant military and political advantages, for they didn't have to sail around Africa, but they came through the Suez canal from India with their troops and goods, such as spices, serving as anti-oxidants, and the like and they sailed through the calm Mediterran sea. The German tried to deprive the English form this advantage with the allegation they were bringing home anthrax, smallpox, the black pledge from India. Thus, quarantine was demanded, they weren't allowed to dock at any Mediterranean port and at Gibraltar they were shot at. Therefore, Robert Koch, who was on the run, was called back and was offered 100,000 Reichsmark in order to create the argument that the English would bring in black plague, small pox and anthrax, the latter we already discussed. We noted, that this colorant business lead to the emergence of antibiotics, later to the chemotherapy and the weaponized gasses, including the pharma- industry with its entire capital, with more revenue then all military budgets globally combined. 25:18 Robert Koch committed scientific fraud by not upholding the first postulate. He could cultivate some bacteria, which he didn't find in every case of a disease. This is still done in the same way today. He could never reproduce the disease as in the third postulate, and neither could he again isolate the same pathogen from these organisms. That is the date when the brutal animal experiments were introduced. 26:02 How did the idea of a virus come to life? Koch's French counterpart was Luis Pasteur, scientific fraudster employed by the French, as the French were at war with Germany in 1872. The dead were later declared as victims of a small pox epidemic. The Germans claimed it came from France, the French said it came from Germany. Pasteur, knew from Bechamp and other scientist, what bacteria could do and what they couldn't do, he first denied the new knowledge in order to play along with the church, he claimed he had proven the primordial creation, only to later take a reverse stand, once he was

employed by the state, he said it was all wrong, bacteria are in a continuum, there are spores, they cannot be created in the primordial soup. Pasteur sold these insights as his own, but he knew what bacteria can and what they cannot do, and has earned some merits for this. 27:16 But the same Pasteur, who knew, that bacteria cannot cause diseases, applied a trick. In order to maintain the model of the doctrine of juices and disease, which the entire western medicine is based on, a disease-causing toxin had to be postulated. Especially since this concept of pandemics had been used many times to suppress upheavals, to control starvation situations and so on. It all started, with the early Vatican creating fear of diseases, by claiming the disease is coming from the disease demon, just as in ancient Greece. Thus, in order to establish total control, the early Vatican claimed that illness was a punishment by God. 28:26 This concept was interrupted for a few years by the Stauffers, when emperor Otto, at the re-foundation of the Holy Roman Empire on Jan 1st, 1000 AD employed the French Humanist Gerbert de Aureac for the purpose of establishing a medical system, which was obviously not present before. For, the military faction, the western Roman wing, had separated from Rome, that is well known. They had only military knowledge, but no technical knowledge, they were separated from the universities and culture. The early gothic was able to build small windows only, no tall buildings, the building plans were useless, whenever the knowledge of craftsmen and engineers, which always must go together, didn't converge. This is visible in early gothic architecture. 29:33 So, they recruited Gerbert de Aureac, alias Pope Silvester. He brought in the Arabs in each garrison from which the monasteries were derived from, with the aim to obtain the ancient knowledge of the Chinese via the Arabs, who themselves had further developed medicine. For, in China the concept of contagion didn't exist, there is the concept of too much and too little energy, there is the influence, latin influenza, by the decrease and increase of light and warmth in spring and autumn, but the idea of contagion plays no role. Contagion is not part of arjuvedic medicine. The concept of contagion is typically war- oriented, European. 30:12 The idea of disease being something vile was already retracted from by Galenus, the great physician of Marcus Aurelius. He refrained from this concept and stated that they had recognized: it wasn't sin that makes people sick, but rather that diseas may cause sins. And today, if we think about the insights of German New Medicine by Dr. Hamer, this is becoming very up to date. We find psychoses, that can be visualized in CT scans, as a result of diverse constellations, that is, activities in the brain in different locations and sudden alterations from mania to depression and other properties. Gerbert de Aureac again pursued this (oriental) approach and the foundation of the empire was humane. However, this was quickly changed to the contrary, when Pope Silvester died a few years later. After this, health tribunals were installed throughout the entire Holy Roman Empire of German Nations. 31:26 The Vatican didn't manage to confiscate all the documents in all archives from this time, especially in the later protestant regions. From these city chronicles and book we learn that there were health tribunals in the entire Holy Roman Empire, headed by a priest, accompanied by community or city councilors, and they decided who was a sacred sick person, or who was an evil sick person, who was punished by god and therefore needed to be expelled. The German word for leprosy is Aussatz, which means to cast away. This leprosy/

expulsion- concept is identical in all regions in which the data was preserved throughout the Holy Roman Empire in the beginning of the 11th century. The definition included natural illnesses such as hair loss, acne zits, swellings and so on, but it also included trickier diagnoses such as the claim someone had a nightmare. He might have called out in his sleep or the like, and even more wicked (this could be called the first AIDS test in medieval times): goose bumps as a reaction to a draft. That was a criterium to be expelled, the person was tattooed (marked), received the last oiling, and was forced to leave all inhabited territories, and was forbidden to come near a settlement the by threat of death penalty. 30:58   That was the leprosy concept (i.e. Aussatz, expulsion) beginning with the 11th century. Then, after the onset of the Small Ice Age in 1308, when great pressure of migration from the north arrived, as the northern apple and wheat plantations became less productive due to severe cold, large tensions, hunger catastrophes and so on, arose in the new Holy Roman Empire of German Nations. Especially after the strong earthquake with epicenter in Friuli of 1348, which devastated many Mediterranean cIties. This was interpreted by the orthodox as a proof of the antichrist, as law and order collapsed because the central hub of global trade, Venice, as well as all trade lines and currencies, also collapsed. At this point, this exact concept with the exact same disease definition was adapted by the priests and city officials, to declare entire groups of the population as punished by god and they were claimed to have the black plague. Entire city quarters were quarantined, put under lock down, starved to death, slaughtered and poisoned, just as Goethe describes it. 34:34   They simply renamed Leprosy into black plague. Later, as the Vatican's power of definition was reduced by national revolutions, French Revolution, American Revolution, the same concept was renamed into small pox, but the same principle remained. Today, it is carried on in unacceptable disease definitions such as AIDS. At any rate, the public was terrified to no ends, whenever epidemics were declared, for this meant they could be put into quarantine, they could be killed, they could be forced to take medications, just as Goethe described. Meanwhile, thousands died because there was no food, there were social upheavals. And the survivors applauded. This medical system was always immediately repressive in times of crisis and in its history it always regarded disease as something vile, evil: the illness demon, that takes hold of someone and grows and rages like a cancer, and above all, can even be spread and transmitted to others like an evil spell, the disease demon. This fear was extremely ingrained in society, and the medical system, from which the pharma industry as the most powerful entity on this planet, arose, will not give up this power on their own account. For this, we need to become active citizens. For this, I will provide you with more information. The idea of a virus was realized, and from this idea the field of gene technology was derived. 36:32   To pick up with Pasteur again: Pasteur knew that bacteria could not cause diseases, period. Enough studies and experiments were conducted and published in Germany and elsewhere, among other by Max von Pettenkofer, who demonstrated what cholera was and how cholera was easily prevented. Pasteur worked on contract to find an argument to not let the English through the Mediterranean Sea, he came up with the idea to claim there was a new pathogen, and this one would make its disease-toxins also in the living human body and this he called: poison! Latin:

virus. 37:22 That was the idea. He said it is a thousand times smaller then bacteria, we use such dense filters where bacteria can't pass through. He presses the liquid, the poison from a dead animal, through the filter, he injects the liquid into the brain of a dog that was tied onto a pole vertically. He used a third of the volume of the dogs brain, the liquid comes out the over side, the dog convulses, barks, foams from the mouth and dies. That was called rabies, that's what Pasteur did. 38:20 Pasteur also claimed to have the antidote to his virus, to push the vaccine concept. This vaccination agenda was propagated primarily in France, for the Germans had their antibiotics and chemotherapy. Pasteur committed fraud in all his undertakings. But he was humane enough to document his deceits in diaries parallel to his primary lab books. He decreed that these records must never be publicized. His family naturally obtained great wealth. But the last male ancestor of Pasteur didn't obey to that decree and leaked the records to the Princeton university and in 1993 Professor Gerard Geisson published an analysis in the English language that revealed that Pasteur had committed massive fraud in all his studies. For instance, vaccinated animals, if they survived, had not been poisoned, the control group animals that died without vaccines were poisoned massively and so on. That was Pasteur. 39:30 Pasteur is the inventor of the idea of a smaller pathogen that cannot be seen in the optical microscope, but that always makes its poison, the disease- causing poison. This supported the standard model of illness which was use for centuries, a model that is based on the premise of war, not on the premise of symbiosis, as is the real workings of Nature. In order to solidify this model and to have political leverage against England, Pasteur postulates the idea of a virus. But Pasteur didn't anticipate that there would be a microscope in the future, an electron microscope, which has a much higher magnification as the optical microscope, that would allow to see small structures not visible before. And with this electron microscope, available to science after WW II, it was possible to visualize structures one thousands of the size of a bacterium. They observed spores, that were still capable of staying alive. It was recognized that bacteria generate spores, when they die slowly. If they die rapidly, when they are for example heated or dried out, then they produce even smaller particles that can't live by themselves, but they consist of proteins and bear a nucleic acid in the center, and they will provide other bacteria, the ones that survived, with nutrients, so the latter can overcome the crisis situation. 41:22 This was observed in the cases of bacteria, in other very simple organisms, in fungi, in amoeba. In my own research, I first found it in a very simple algae from the ocean. But it was never observed in a human or animal or plant. You can verify this with little effort. beginning of part2 42:40 As a first step, you can check the virus question by asking: How are viruses detected nowadays? If a virologist claims he isolated an influenza virus, then he refers to the chicken egg and the chicken embryo, as we can see in the media for the planning for the current H1N1 influenza pandemic. They work with chicken eggs, they kill chicken embryos, that's the modern form of animal tests. This method goes back to Robert Koch. If the embryo dies, they say it was a virus and that they had isolated the virus. They took something from a diseased animal or a human, they inject it into the egg and then, depending on how the embryo dies, on what location it is becoming spotted first, they will claim it was this type of virus or that one. That is proclaimed to be isolation, when chicken

embryos are killed. 42:44 Needless to say, there is no control group: if you inject a sterile solution of the same amount, the chicken embryo dies as well. You can also verify it by taking a look at the photos that claim to depict viruses and you will find they are identical with images from completely normal cells, that is with electron micrographs from normal cells. Here, we see a section from the centre of a cell, which is very productive, the so-called Golgi apparatus, that produces various substances, and these are separated in small vesicles, they are all of different sizes, but they have no nucleic acid within them. 43:32 A larger fat particle is called a small pox virus, here we see the bacteria within the cells, the mitochondria, which process the oxygen, here we see two small ones. These particles are mitochondria, a muscle cell has 1000, a liver cell has 2000 mitochondria, and these were extracted from the cell in a thin cross section, embedded in artificial resin and cut in a very thin slice with a diamond blade. When they are protruded with an electron beam, we see the cap of such a mitochondrion. And such particles are then sold as virus without ever having isolated them, without having shown them in an isolated condition, in order to demonstrate that these look identical to what is shown in the photo of the cell. 44:32 Here, for instance, the photo that circulated as the photo of HIV, published by Montagnier. Here, we see how particles are excreted or absorbed from the cell into or out of the cell cavity. In biology, we call this endocytosis or exocytosis. Whenever something goes into or out of the cell liquid. This has nothing to do with particles that have a stable structure, that carry nucleic acid within them, that can leave the organism, such as the viruses or bacteria, that are called phages, or with the things I discovered in the ocean, they are harmless. These particles here are normal components of cells. In the case of the funny photos that are presented as influenza virus: these are simply mixtures of fats and proteins. If these are shaken in an ultrasound bath and then visualized (they decompose quickly) they don't have an nucleic acid and they are of various sizes. That's how you can tell fraud. 45:45 Further, they don't even claim that they observed this within the cells or that the particles were isolated. What is striking in the current swine flue pandemic: they are trying to present more pictures of particles that are more or less equally shaped. You can reseach this by demanding a scientific publication, in which it is proven and documented that the virus exists, it was seen in the organism, it was isolated from the organism, purified from all foreign components. Just as on world savings day [or when you fill out your Tax form], your Euros must be isolated, buttons and chewing-gums are not excepted. Then the isolated particles must be analyzed biochemically. In the first step, a scientific publication can be recognized in the title of the scientific journal and of course the two dates, first the date of submission of the paper to the editor and second the date when it was checked and accepted by three work group, that were not previously know to the authors… I have not been able to determine if or when I will have the time to translate the second part of the presentation. If it will be completed, I will link to it on this article. via: Sacha Dobler of Abrupt Earth Changes https://joedubs.com/the-history-of-infectious-disease-by-stefan-lanka/?fbclid=IwAR2ftgw9p_dqBujSHy3Cyrh5F1wFeAZAqYy_HzDCHXPnOnyKzKH9HinSaYk

There are no experiments where an isolated varicella zoster viruses has been shown to cause disease, so it is difficult to say that it is indeed the cause. I don't claim to know what causes these diseases, it seems that no one really does, however there are some hypotheses that have been put forward by others. There is the work of Nobel prize winner and French virologist Luc Montagnier. He conducted experiments where he was able to transfer the DNA of bacteria and "viruses" from one test tube to another test tube using electromagnetic frequencies. https://pubmed.ncbi.nlm.nih.gov/26098521/ There is also the work by Rupert Sheldrake on the phenomena known as morphic resonance. Sheldrake says, morphic resonance is "the idea of mysterious telepathy-type interconnections between organisms and of collective memories within species" and accounts for phantom limbs, how dogs know when their owners are coming home, and how people know when someone is staring at them.

https://www.scientificamerican.com/article/ruperts-resonance/#:~:text=Morphic%20resonance%2C%20Sheldrake%20says%2C%20is,someone%20is%20staring%20at%20them

We also know the human body is abe to communicate silently via the observations of women synching their menstrual cycles when living together. It is understood there is a "non-contagious" mechanism that causes this for example.

https://docs.google.com/document/d/e/2PACX-1vQmOweCdbDZWcxAyS6c4_4Xx4rJ5MMiouf8SB3MDWBftdNABpk72BWSWh7h8dGa5sn2MOF7xbHYGFop/pub

https://nateserg808.wixsite.com/my-site/post/5-staple-items-for-autumn

LACK OF KNOWLEDGE ON

"VIRAL" MODES OF TRANSMISSION:

Much has been made about the lack of evidence for human-to-human transmission of "viruses," especially in regards to the failed transmission experiments during the 1918 Spanish Flu. There were also many failed transmission experiments for Chickenpox, Scarlet Fever, Measles, etc. So it should come as no surprise that given the failure to actually transmit "viruses" from human to human, very little is known about how these "viruses" actually spread. The transmission studies are actually a hodgepodge of conflicting indirect experiments that lead to contradictory results, hence the non-conclusive terms/phrases such as "the evidence suggests," "it is believed/thought," "most probably caused by," etc. Two different studies highlight the lack of knowledge and contradictory information regarding inter-human modes of transmission:

From 2021:

TRANSMISSIBILITY AND TRANSMISSION OF RESPIRATORY VIRUSES

"WE KNOW LITTLE ABOUT THE RELATIVE CONTRIBUTION OF EACH MODEL TO THE TRANSMISSION OF A PARTICULAR VIRUS IN DIFFERENT SETTINGS, AND HOW ITS VARIATION AFFECTS TRANSMISSIBILITY AND TRANSMISSION DYNAMICS. Discussion on the particle size threshold between droplets and aerosols and the importance of aerosol transmission for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and influenza virus is ongoing."

"RESPIRATORY VIRUS INFECTIONS OFTEN CANNOT BE DIFFERENTIATED CLINICALLY. Respiratory viruses belong to diverse virus families that differ in viral and genomic structures, populations susceptible to infection, disease severity, seasonality of circulation, transmissibility and modes of transmission."

"Alternatively, volunteer transmission studies, where transmission is observed in susceptible volunteers who are exposed to other volunteers who are either experimentally or naturally infected8, may be used to provide important information on the effectiveness of interventions and the importance of pre symptomatic or asymptomatic transmission in a controlled setting25. HOWEVER, THESE STUDIES CAN BE CHALLENGING AND EXPENSIVE TO CONDUCT, AND MAY BE CRITICIZED

AS TOO ARTIFICIAL."

"MATHEMATICAL OR STATISTICAL MODELS ARE OFTEN USED TO ESTIMATE TRANSMISSIBILITY OF A RESPIRATORY VIRUS IN THE POPULATION, especially during pandemics to assess the extent of transmission. With use of data from surveillance, observational and interventional epidemiological studies, or simulation from modelling studies, TRANSMISSIBILITY IS USUALLY ASSESSED BY THE ESTIMATION OF THE BASIC REPRODUCTION NUMBER (R0) OR SECONDARY ATTACK RATE (SAR)."

"Respiratory viruses are transmitted between individuals when the virus is released from the respiratory tract of an infected person and is transferred through the environment, leading to infection of the respiratory tract of an exposed and susceptible person. There are a number of different routes (or modes) through which transmission could occur, the chance of which is modified by viral, host and environmental factors. ALTHOUGH THERE IS EVIDENCE IN SUPPORT OF INDIVIDUAL MODES OF TRANSMISSION, THE RELATIVE CONTRIBUTION OF DIFFERENT MODES TO A SUCCESSFUL TRANSMISSION EVENT, AND THE RELATIVE EFFECT OF EACH FACTOR ON EACH MODE OR MULTIPLE MODES SIMULTANEOUSLY, IS OFTEN UNKNOWN."

"TRADITIONALLY, IT IS BELIEVED that respiratory viruses are transmitted directly via physical contact between an infected individual (infector) and a susceptible individual (infectee), indirectly via contact with contaminated surfaces or objects (fomites) or directly through the air from one respiratory tract to another via large respiratory droplets or via fine respiratory aerosols."

"Various approaches, including environmental sampling, experimental animal and volunteer transmission studies, and epidemiological observations (mostly from outbreak investigations), have been used to provide evidence in support of each individual mode of transmission for different respiratory viruses, ALTHOUGH FOR EACH, SOME MAY CRITICIZE THEIR RELEVANCE 6,7. Furthermore, although attempts have been made to classify each mode as ‘obligate’, ‘preferential’ or ‘opportunistic’15,50, LIMITED RESEARCH WAS DONE TO QUANTIFY THE RELATIVE IMPORTANCE OF EACH MODEL TO TRANSMISSION9."

https://www.nature.com/articles/s41579-021-00535-6#ref-CR8

In Summary (Part 1):

-they admit WE KNOW LITTLE about the relative contribution of each mode to the transmission of a particular "virus" in different settings, and how its variation affects transmissibility and transmission dynamics

-respiratory "virus" infections often CANNOT BE DIFFERENTIATED CLINICALLY

-human transmission studies are said to be CHALLENGING (?) and expensive to conduct, and may be CRITICIZED AS TOO ARTIFICIAL (?)

-mathematical or statistical models are often used to ESTIMATE transmissibility of a respiratory "virus" in the population

-transmissibility is usually assessed by the ESTIMATION of the basic reproduction number (R0) or secondary attack rate (SAR)

-although there is evidence in support of individual modes of transmission, the relative contribution of different modes to a successful transmission event, and the relative effect of each factor on each mode or multiple modes simultaneously, IS OFTEN UNKNOWN

-traditionally, IT IS BELIEVED that respiratory "viruses" are transmitted directly via physical contact between an infected individual (infector) and a susceptible individual (infectee), indirectly via contact with contaminated surfaces or objects (fomites) or directly through the air from one respiratory tract to another via large respiratory droplets or via fine respiratory aerosols (in other words: "it is believed" = THEY DON'T KNOW)

-VARIOUS APPROACHES, including environmental sampling, experimental animal and volunteer transmission studies, and epidemiological observations (mostly from outbreak investigations), have been used to provide evidence in support of each individual mode of transmission for different respiratory "viruses," ALTHOUGH FOR EACH, SOME MAY CRITICIZE THEIR RELEVANCE

-although attempts have been made to classify each mode as ‘obligate’, ‘preferential’ or ‘opportunistic’ LIMITED RESEARCH WAS DONE TO QUANTIFY THE RELATIVE

IMPORTANCE OF EACH MODE TO TRANSMISSION

From 2018:

TRANSMISSION ROUTES OF RESPIRATORY VIRUSES AMONG HUMANS

"Most studies on inter-human transmission routes are INCONCLUSIVE.

The relative importance of respiratory virus transmission routes is NOT KNOWN."

"Many outbreaks have been investigated retrospectively to study the possible routes of inter-human virus transmission. THE RESULTS OF THESE STUDIES ARE OFTEN INCONCLUSIVE and at the same time DATA FROM CONTROLLED EXPERIMENTS IS SPARSE. Therefore, FUNDAMENTAL KNOWLEDGE ON TRANSMISSION ROUTES that could be used to improve intervention strategies IS STILL MISSING."

"Transmission via each of these three routes is complex and depends on many variables such as environmental factors (e.g. humidity and temperature), crowding of people, but also on host factors such as receptor distribution throughout the respiratory tract. THE FACT THAT ALL THESE VARIABLES AFFECT THE DIFFERENT TRANSMISSION ROUTES OF THE DIFFERENT RESPIRATORY VIRUSES IN A DISSIMILAR WAY, MAKES IT VERY DIFFICULT TO INVESTIGATE THEM EXPERIMENTALLY."

"OUR OBSERVATIONS UNDERSCORE THE URGENT NEED FOR NEW KNOWLEDGE ON RESPIRATORY VIRUS TRANSMISSION ROUTES and the implementation of this knowledge in infection control guidelines to advance intervention strategies for currently circulating and newly emerging viruses and to improve public health."

"Measles virus (MV)

Measles is one of the most contagious viral diseases in humans that has been associated with aerosol transmission for a long time [12, 13, 14••, 15, 16, 17, 18••]. However, it should be noted that MV also replicates systemically, and that there is a role for dead cell debris-associated virus spread via fomites. In the late 1970s and early

1980s, data from RETROSPECTIVE OBSERVATIONAL STUDIES obtained during outbreaks in pediatric practices, a school, and a sporting event SUGGESTED TRANSMISSION THROUGH AEROSOLS [14••, 15, 16, 17, 18••]. Indeed, THOSE STUDIES SHOWED THAT MOST SECONDARY CASES NEVER CAME IN DIRECT CONTACT WITH THE INDEX PATIENT AND SOME WERE NEVER EVEN SIMULTANEOUSLY PRESENT IN THE SAME AREA AS THE INDEX CASE [14••, 18••] Examination of airflow in the pediatricians' offices showed that aerosols were not only dispersed over the entire examination room but also accumulated in the hallway and other areas [14••, 18••]. Furthermore, based on the investigation of air circulation in a sport stadium, in which a MV outbreak occurred, AUTHORS SUGGESTED that MV had been dispersed through the ventilation system [16]. THUS IT WAS CONCLUDED THAT MV CAN BE TRANSMITTED VIA AEROSOLS. Although coughing is a common symptom associated with measles disease, index patients were described to cough frequently and vigorously in the outbreak reports of pediatric practices. REMINGTON et al. CALCULATED THE INFECTIOUS DOSE OF MV PRODUCED BY THE INDEX CASE THROUGH COUGHING, USING A MATHEMATICAL MODEL BASED ON AIRBORNE TRANSMISSION. They found that the index case produced a very high infectious dose compared to cases from other outbreaks and mentioned a phenomenon called superspreading [18••]."

"Parainfluenza (PIV) and human metapneumovirus (HMPV)

THERE IS A SUBSTANTIAL LACK OF (EXPERIMENTAL) EVIDENCE ON THE TRANSMISSION ROUTES OF PIV (types 1–4) AND HMPV. For both viruses, contact and droplet transmission are commonly accepted transmission routes [23, 24, 25]. However, only virus stability on various surfaces has been investigated so far and it has been shown that PIV and HMPV are stable on non-absorptive surfaces and can barely be recovered from absorptive surfaces [26, 27, 28, 29, 30]."

"Respiratory syncytial virus (RSV)

TRANSMISSION OF RSV AMONG HUMANS IS THOUGHT TO OCCUR VIA DROPLETS AND FOMITES [1, 7]. In the 1980s three potential transmission routes of RSV were studied in humans by dividing infected infants and healthy volunteers into three groups, representing: Firstly, all transmission routes, secondly, transmission via fomites and finally, airborne transmission by allowing the volunteers to have either, firstly, direct contact with infants (cuddlers), secondly, touching potential fomites (touchers) or finally, sitting next to the infant (sitters). Volunteers in the group of the cuddlers and touchers but not the sitters became infected, SUGGESTING THAT DIRECT CONTACT AND DROPLET TRANSMISSION WERE THE PROBABLE ROUTES FOR EFFICIENT INFECTION OF THE VOLUNTEERS AND THAT TRANSMISSION VIA AEROSOLS WAS LESS LIKELY [31]. Another study on the

transmission via fomites showed that RSV could be recovered from countertops for several hours, but only for several minutes from absorptive surfaces such as paper tissue and skin [32••]. Later on, in the late 1990s, Aintablian et al. detected RSV RNA in the air up to 7 m away from a patient's head [33]. In spite of that, SINCE VIRUS INFECTIVITY COULD NOT BE DEMONSTRATED, POTENTIAL AIRBORNE TRANSMISSION OF RSV HAS BEEN CONSIDERED NEGLIGIBLE AND TRANSMISSION OF RSV WAS THOUGHT TO OCCUR MAINLY THROUGH CONTACT AND DROPLET TRANSMISSION. However, in a recent study authors were able to collect aerosols that contained viable virus from the air around RSV infected children [34••]. ALTHOUGH THE DETECTION OF VIABLE VIRUS IN THE AIR IS BY ITSELF NOT ENOUGH TO CONFIRM AEROSOL TRANSMISSION, the general PRESUMPTION that RSV exclusively transmits via droplets should be reconsidered and explored further."

"Rhinovirus

EXTENSIVE HUMAN RHINOVIRUS TRANSMISSION EXPERIMENTS HAVE NOT LED TO A WIDELY-ACCEPTED VIEW ON THE TRANSMISSION ROUTE [35, 36, 37, 38••, 39••, 40]. Inhalation of aerosols (0.2–3 μm) resulted in efficient rhinovirus infection [41], but LITTLE TO NO INFECTIOUS RHINOVIRUS COULD BE DEMONSTRATED IN SNEEZES AND COUGHS as detected by virus titration."

"Influenza A virus

Due to the severity of the yearly influenza epidemics and the potential of zoonotic influenza A viruses to cause severe outbreaks, there have been many studies on influenza A virus transmission among humans. Different kinds of studies, such as air sampling and intervention studies, as well as human challenge studies have been conducted. In addition, transmission events have been described extensively after outbreaks in aircrafts, households and hospital settings. HOWEVER, UNTIL TODAY, RESULTS ON THE RELATIVE IMPORTANCE OF DROPLET AND AEROSOL TRANSMISSION OF INFLUENZA VIRUSES STAY INCONCLUSIVE AND HENCE, THERE ARE MANY REVIEWS INTENSIVELY DISCUSSING THIS ISSUE.

"The presence of virus in aerosols COULD INDICATE POTENTIAL AIRBORNE TRANSMISSION, although many studies only quantified the amount of viral RNA [55, 57•, 61]. A few studies quantified viable virus, ALTHOUGH THIS WAS ONLY RECOVERED FROM A MINORITY OF SAMPLES."

"Coronavirus

In humans, alpha (229E and NL63) and beta coronaviruses (OC43, HKU1, SARS and MERS) ARE ASSOCIATED with respiratory disease [62, 63]. Alpha coronaviruses have a high attack rate early in life and spread rapidly during outbreaks, indicating efficient human to human transmission [63]. Furthermore, samples obtained from staff and patients of a neonatal and pediatric intensive care unit showed a high incidence of human coronaviruses HCoV-229E and HCoV-OC43, SUGGESTING staff-to-patient and patient-to-staff transmission [64]. Unfortunately, THERE IS VERY LITTLE DATA TO CORROBORATE ON THE HCoV-229E, HCoV-NL63 AND HCoV-OC43 TRANSMISSION ROUTES."

"The SARS outbreak was primarily linked to healthcare settings, with ≥49% of the cases linked to hospitals [71], MOST PROBABLY CAUSED BY AEROSOL-GENERATING PROCEDURES ON SEVERELY ILL PATIENTS [72, 73]. Aerosol-generating procedures like intubation, the use of continuous positive-pressure ventilation and drug delivery via nebulizers ARE LIKELY TO PRODUCE 'fine infectious droplets', which travel further than droplets from coughs [74]. Additionally, superspreading events contributed to the dispersion of the SARS outbreak [73, 75, 76, 77], particularly in the Hotel Metropole and the Prince of Wales Hospital in Hong Kong [76]. Moreover, a link with transmission to healthcare workers was observed when they were in close proximity (<1 m) to an index patient, SUGGESTING DIRECT CONTACT OR DROPLET TRANSMISSION [73, 78•, 79•]. Air samples and swabs from frequently touched surfaces in a room occupied by a SARS patient tested positive by PCR, ALTHOUGH NO VIRUS COULD BE CULTURED FROM THESE SAMPLES [80]. In the Amoy gardens outbreak fecal droplet transmission was SUGGESTED [81, 82]."

"To date, THERE IS LITTLE DATA ON THE HUMAN-TO-HUMAN MERS-CoV TRANSMISSION ROUTE [83]."

"**Adenovirus**

This is illustrated by, for example, outbreaks among military recruits for which AIRBORNE SPREAD WAS SUGGESTED [92, 94, 99]. It is difficult to eliminate adenovirus from skin, fomites and environmental surfaces [100]. An outbreak in a mental care facility WAS PROBABLY ENHANCED by spending the day mainly in a crowded room while sharing cigarettes and soda cans, SUGGESTING INDIRECT FOMITE SPREAD [101]."

"During a military training period, increased numbers of adenovirus infections occurred over time, which correlated with an increased detection of PCR-positive air filters. Additionally, a correlation between disease and the extent of ventilation was observed, with more ventilation resulting in fewer disease cases [103•]. In a more recent study in military recruits, positive viral DNA samples were mainly obtained from pillows, lockers and rifles, although adenovirus DNA was also detected in air samples. NO CONSISTENT CORRELATION BETWEEN INCREASED POSITIVE ENVIRONMENTAL SAMPLES AND DISEASE WAS OBSERVED [104]."

"Studies on the transmission routes of respiratory viruses have been performed since the beginning of the 20th century [105]. DESPITE THIS, THE RELATIVE IMPORTANCE OF TRANSMISSION ROUTES OF RESPIRATORY VIRUSES IS STILL UNCLEAR"

"Inter-human transmission has been studied under many different (experimental) conditions. A summary of the advantages and disadvantages of the different study designs (Table 3) HIGHLIGHTS THE DIFFICULTY OF HUMAN TRANSMISSION EXPERIMENTS. As a consequence, CONTRASTING RESULTS HAVE BEEN OBTAINED FOR MANY VIRUSES. This is also reflected in Table 2, summarizing the experimental data on inter-human transmission. Besides the difficulty of performing studies under well-controlled conditions, another key issue is that often (attenuated) laboratory strains are studied in healthy adults, WHICH DOES NOT REFLECT THE NATURAL CIRCUMSTANCES and target group and HENCE INFLUENCE THE OUTCOME OF THE STUDIES."

"Unfortunately, TERMS AND DEFINITIONS OF RESPIRATORY TRANSMISSION ROUTES AND ISOLATION GUIDELINES ARE NOT ALWAYS USED IN A UNIFORM WAY, LEAVING ROOM FOR PERSONAL INTERPRETATION. But more importantly, information on the transmission route does not always reflect the isolation guidelines (e.g. for PIV and rhinovirus, Figure 1). As a proxy for transmission route, virus stability is often referred to in the guidelines, however, this can only imply a role for indirect contact transmission but is by no means conclusive on the transmission route. In hospital settings, prevention of contact transmission is generally implemented in standard infection prevention precautions such as strict hand hygiene and cough etiquette. IT IS IMPORTANT TO NOTE DIFFERENCES IN ISOLATION GUIDELINES BETWEEN DIFFERENT ORGANIZATIONS AND THE LACK OF CORRELATION TO SCIENTIFIC DATA. The variation in described transmission routes and associated isolation guidelines among the different organizations UNDERSCORES THE LACK OF CONVINCING DATA."

"Well-designed human infection studies could be employed to investigate the role of transmission routes of respiratory viruses among humans [112••]. However, SINCE HUMAN TRANSMISSION EXPERIMENTS ARE VERY CHALLENGING, ANIMAL TRANSMISSION MODELS CAN PROVIDE AN ATTRACTIVE ALTERNATIVE AND SHOULD BE EXPLORED AND DEVELOPED FOR ALL RESPIRATORY VIRUSES. In such experiments, the influence of environmental factors on transmission routes can also be investigated [113]. HOWEVER, BEFORE EXTRAPOLATING EXPERIMENTALLY GENERATED DATA TO HUMANS, IT IS IMPORTANT TO UNDERSTAND THE LIMITATIONS OF THESE MODELS, AND APPRECIATE THE HETEROGENEITY OF EXPERIMENTAL SETUPS EMPLOYED IN LABORATORIES [114]. Furthermore, quantitative data such as viral load in the air can be obtained by air sampling methods in various environments, such as hospital settings. Air sampling of viruses is an increasingly used technology in animal and human experiments. HOWEVER, WHEREAS MOST STUDIES RELY ON THE DETECTION OF VIRAL GENOME COPIES, viability assays such as plaque assays or virus titration SHOULD BE INCLUDED TO GAIN INFORMATION ON VIRUS INFECTIVITY.

Ultimately, THE KNOWLEDGE GAP ON INTER-HUMAN TRANSMISSION SHOULD BE FILLED BY DEVELOPING AND PERFORMING STATE-OF-THE ART EXPERIMENTS IN A NATURAL SETTING. Combined with animal transmission models and air sampling in different (health care and experimental) settings, THESE DATA SHOULD RESULT IN A THOROUGH SCIENTIFIC UNDERSTANDING OF THE INTER-HUMAN TRANSMISSION ROUTES OF RESPIRATORY VIRUSES.``

https://www.sciencedirect.com/science/article/pii/S1879625717301773

In Summary (Part 2):

-most studies on inter-human transmission routes are INCONCLUSIVE

-the relative importance of respiratory "virus" transmission routes is NOT KNOWN

-the results of these studies are often INCONCLUSIVE and at the same time data from controlled experiments is SPARSE

-fundamental knowledge on transmission routes that could be used to improve intervention strategies IS STILL MISSING

-the fact that all these variables affect the different transmission routes of the different respiratory "viruses" in a dissimilar way, MAKES IT VERY DIFFICULT TO INVESTIGATE THEM EXPERIMENTALLY

-their observations underscore the URGENT NEED FOR NEW KNOWLEDGE on respiratory "virus" transmission routes

-for Measles (MV), data from RETROSPECTIVE OBSERVATIONAL STUDIES from the 1970's and 80's obtained during outbreaks in pediatric practices, a school, and a sporting event SUGGESTED transmission through aerosols

-those studies showed that MOST SECONDARY CASES NEVER CAME IN DIRECT CONTACT WITH THE INDEX PATIENT and some were NEVER EVEN SIMULTANEOUSLY PRESENT IN THE SAME AREA as the index case

-based on the investigation of air circulation in a sport stadium, in which a MV outbreak occurred, AUTHORS SUGGESTED that MV had been dispersed through the ventilation system

-thus it was concluded that MV can be transmitted via aerosols

-Remington et al. calculated the infectious dose of MV produced by the index case through coughing, USING A MATHEMATICAL MODEL based on airborne transmission

-for Parainfluenza (PIV) and human metapneumovirus (HMPV), there is a SUBSTANTIAL LACK OF (EXPERIMENTAL) EVIDENCE on the transmission routes

-for Respiratory syncytial "virus" (RSV), transmission among humans is THOUGHT TO OCCUR via droplets and fomites

-since "virus" infectivity COULD NOT BE DEMONSTRATED, potential airborne transmission of RSV has been considered negligible and transmission of RSV was THOUGHT TO OCCUR mainly through contact and droplet transmission

-the detection of "viable virus" in the air is by itself NOT ENOUGH TO CONFIRM aerosol transmission

-extensive human rhinovirus transmission experiments HAVE NOT LED to a widely-accepted view on the transmission route

-LITTLE TO NO INFECTIOUS RHINOVIRUS could be demonstrated in sneezes and coughs

-until today, results on the relative importance of droplet and aerosol transmission of influenza "viruses" STAY INCONCLUSIVE and hence, there are many reviews intensively discussing this issue

-the presence of "virus" in aerosols COULD INDICATE POTENTIAL AIRBORNE TRANSMISSION, although many studies only quantified the amount of "viral" RNA

-a few studies quantified "viable virus," ALTHOUGH THIS WAS ONLY RECOVERED FROM A MINORITY OF SAMPLES

-in humans, alpha (229E and NL63) and beta coronaviruses (OC43, HKU1, SARS and MERS) are ASSOCIATED with respiratory disease

-there is VERY LITTLE DATA to corroborate on the HCoV-229E, HCoV-NL63 and HCoV-OC43 transmission routes

-"SARS" was MOST PROBABLY CAUSED by aerosol-generating procedures on severely ill patients

-aerosol-generating procedures like intubation, the use of continuous positive-pressure ventilation and drug delivery via nebulizers ARE LIKELY TO PRODUCE ‘FINE INFECTIOUS DROPLETS’

-a link with transmission to healthcare workers was observed when they were in close proximity (<1 m) to an index patient, SUGGESTING direct contact or droplet transmission

-air samples and swabs from frequently touched surfaces in a room occupied by a "SARS" patient tested positive by PCR, ALTHOUGH NO "VIRUS" COULD BE CULTURED FROM THESE SAMPLES (I guess detecting "virus" with PCR doesn't mean "virus" is present... 🤔)

-to date, THERE IS LITTLE DATA ON THE HUMAN-TO-HUMAN MERS-CoV TRANSMISSION ROUTE

-for "adenovirus," airborne spread WAS SUGGESTED among military recruits

-an outbreak in a mental care facility WAS PROBABLY ENHANCED by spending the day mainly in a crowded room while sharing cigarettes and soda cans, SUGGESTING INDIRECT FOMITE SPREAD

-in a more recent study in military recruits, positive "viral" DNA samples were mainly obtained from pillows, lockers and rifles, although "adenovirus" DNA was also detected in air samples yet NO CONSISTENT CORRELATION BETWEEN INCREASED POSITIVE ENVIRONMENTAL SAMPLES AND DISEASE WAS OBSERVED (another strike against PCR results = "virus" 🤔)

-the relative importance of transmission routes of respiratory "viruses" is still UNCLEAR

-a summary of the advantages and disadvantages of the different study designs HIGHLIGHTS THE DIFFICULTY OF HUMAN TRANSMISSION EXPERIMENTS

-as a consequence, CONTRASTING RESULTS HAVE BEEN OBTAINED for many "viruses"

-besides the DIFFICULTY OF PERFORMING STUDIES UNDER WELL-CONTROLLED CONDITIONS, another key issue is that often (attenuated) laboratory strains are studied in healthy adults, WHICH DOES NOT REFLECT THE NATURAL CIRCUMSTANCES and target group and HENCE INFLUENCE THE OUTCOME OF THE STUDIES

-unfortunately, terms and definitions of respiratory transmission routes and isolation guidelines are NOT ALWAYS USED IN A UNIFORM WAY, leaving room for PERSONAL INTERPRETATION

-it is important to note differences in isolation guidelines between different organizations and the LACK OF CORRELATION TO SCIENTIFIC DATA

-the VARIATION IN DESCRIBED TRANSMISSION ROUTES and associated isolation guidelines among the different organizations UNDERSCORES THE LACK OF CONVINCING DATA

-SINCE HUMAN TRANSMISSION EXPERIMENTS ARE VERY CHALLENGING, animal transmission models can provide an attractive alternative and should be explored and developed for all respiratory "viruses"

-however, before extrapolating experimentally generated data to humans, IT IS IMPORTANT TO UNDERSTAND THE LIMITATIONS OF THESE MODELS, and appreciate the heterogeneity of experimental setups employed in laboratories

-however, whereas MOST STUDIES RELY ON THE DETECTION OF "VIRAL" GENOME COPIES, viability assays such as plaque assays or "virus" titration SHOULD BE INCLUDED to gain information on "virus" infectivity

-the KNOWLEDGEABLE GAP on inter-human transmission should be filled by developing and performing state-of-the art experiments IN A NATURAL SETTING

-these data SHOULD RESULT in a thorough scientific understanding of the inter-human transmission routes of respiratory "viruses"

In other words, after over a hundred years and countless studies, we still do not have a thorough understanding of the inter-human transmission routes of respiratory "viruses." Now why would that be...?

https://docs.google.com/document/d/e/2PACX-1vSmTmG9VehA_XMea2ctjZW3f_yw4ZIL74pWpznt7eV-qpGyXmOHMlMMEBQITeCBcgSeXa5GAzalbwo_/pub

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The test procedure to detect the alleged new Corona virus was developed by Prof. Christian Drosten even before the nucleic acid of the alleged new Corona virus was “decoded.” The Chinese virologists who had mentally constructed the nucleic acid of the alleged new virus using alignment, claimed that it has not been proven that this virus has the potential to produce diseases. They assumed that the new virus was very similar to harmless and difficult-to-transmit viruses in animals. The “positive” results of Prof. Drosten’s PCR test were used to justify the claim that the new virus was “definitely” detected and that there was easy human-to-human transmission. These rash actions of Prof. Drosten had the effect of escalating a local SARS hysteria in Wuhan (triggered by an ophthalmologist) to a global Corona crisis.

S-LANKA - VIRUS MISCONCEPTION PIII

RECOMENDED READING FROM HERE:

The Virus Misconception Part II - The beginning and the end of the corona crisis
https://wissenschafftplus.de/uploads/article/wissenschafftplus-the-virus-misconception-part-2.pdf

The Virus Misconception part III - Corona simple and understandable

https://wissenschafftplus.de/uploads/article/wissenschafftplus-the-virus-misconception-part-3.pdf

The Virus Misconception part 1 - Measles as an example
https://wissenschafftplus.de/uploads/article/wissenschafftplus-the-virus-misconception-part-1.pdf

Virologists by Dr. Stefan Lanka (translated from this original article)
https://wissenschafftplus.de/uploads/article/wissenschafftplus-virologen.pdf

PAPER ON THE TRIAL WITH STEFFAN LANKA GOGO-VIRUS

https://truthseeker.se/wp-content/uploads/2021/01/go-VIRUS-go-by-Dr-Stefan-Lanka.pdf

https://nateserg808.wixsite.com/my-site/post/trials-and-revelations

THIS LINK IS TO THE PAPERS FROM THE INVESITIGATION DURRING THE TRIAL

Https://wplus-verlag.ch/de_DE/p/buy/wissenschafftplus-ausgabe-4-2017?fbclid=IwAR32IhaUzZVvkCNENA_uNKPZ7N6IPTWOFnC14n1IASEQdeIyCU2ECZEgkp8

More papers from Biologist STEFFAN LANKA

https://wissenschafftplus.de/cms/de/wichtige-texte

Interview with Dean Braus

https://odysee.com/@DeansDanes:1/cpe-english:f

ANTIBODY 1

https://nateserg808.wixsite.com/my-site/post/anti-bodies

ANDYBODY 2

https://nateserg808.wixsite.com/my-site/post/antibody-2

Antibody studies are non-reproducible:

https://docs.google.com/document/d/e/2PACX-1vQKy0dFmzzadiL9EkG-mLfQE8UWds8sKfo5ykgxlov_OcNkc7H-9DAv35oc7ny9bAMH9RJfToAf5hBC/pub

ANTIBODY STUDIES

https://docs.google.com/document/d/e/2PACX-1vS5jTh_Jo-hTt7mbMhQRF57rClqbiKBTWGuLKRBhzPt2QD77SYpMIGzTLi9fMcJKw7Kxg5IrssdGcwD/pub

Antibody studies are non-reproducible:

https://docs.google.com/document/d/e/2PACX-1vQKy0dFmzzadiL9EkG-mLfQE8UWds8sKfo5ykgxlov_OcNkc7H-9DAv35oc7ny9bAMH9RJfToAf5hBC/pub

WHERE MOST OF THIS CAME FROM ---->

THE MIKE STONE DOCUMENTS

https://docs.google.com/document/d/e/2PACX-1vQtVav4dmSOSafCDWBdelyXQRx8Y_ACCSz3rqtYw2b5Cs9aEWSxFc70I3b5JmWHEUS8cUrJZxFiXO1x/pub?

fbclid=IwAR0AbeKL2e_iMNPhLXwd7Rc2oPknq3IvthSlsWao0n8nSYJHv9ca1k5okBo

OTHER VERY IMPORTANT READING MATERIAL

-VIRUS MANIA

-WHAT REALLY MAKES YOU ILL?

-INVISABLE RAINBOW

-CONTAGION MYTH

-BECAHMP OR PASTURE

-CELL BIOLOGY IN DIRE STRAITS

-