A Dimethyltryptamine-Forming Enzyme in Human Blood

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An enzyme capable of forming the hallucinogen dimethyltryptamine was found in human red blood cells, plasma, and platelets. The enzyme activity in red blood cells and plasma was not significantly different in psychiatric patients from that in normal subjects. The enzyme activity in platelets was higher in psychotic subjects than in nonpsychotics and was apparently related to the presence of a dialyzable inhibitor in the normal subjects.

FOR 20 YEARS there has been speculation about substances within the blood of certain groups of psychotic patients that are capable of producing psychotomimetic methylated amines (1, 2). The only agent known that is both psychotomimetic in man and possibly made in man is dimethyltryptamine (DMT) (3). In 1961, an enzyme in the lung of rabbits that N-dimethylated tryptamine to form DMT was described (4). Subsequently, Mandell and Morgan (5) described a similar enzyme in chick, sheep, and human brain. Saavedra and Axelrod also found an enzyme in human and rat brain that N-methylated tryptamine and, in addition, demonstrated that the rat brain was capable of forming DMT in vivo (6).

Because an abnormality in this enzyme could be related to psychosis, it seemed desirable to look for this enzyme in an easily accessible tissue and to examine whether there were differences in the activity of this enzyme in psychotic patients. This paper describes the presence of an N-methyltransferase that is capable of forming DMT in human blood, especially platelets. In order to demonstrate the problems in the search for and utilization of the tryptamine-methylating enzyme in man, the methods and results are presented in the chronological order in which they were done.

SUBJECTS

The subjects studied in this series of experiments were drawn from the following groups:

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Normal subjects: selected from a group of normal volunteers and laboratory personnel at NIMH.

Alcoholic patients: chronic alcoholics who were free of physical illness, drugs, and alcohol during the preceding six months while hospitalized either voluntarily or by court order at the Washington, D.C., Rehabilitation Center for Alcoholics.

Psychotic depressives: a total of 13 patients with psychotic affective disorders who were studied at least two weeks after all drugs had been discontinued. The patients were hospitalized on NIMH research wards at either the Clinical Center in Bethesda, Md., or at Saint Elizabeths Hospital, Washington, D.C.

Chronic schizophrenics: a total of 30 male chronic schizophrenic patients who were housed on a special NIMH research ward at Saint Elizabeths Hospital. Eleven were free from all drugs for at least 30 days; 19 were taking phenothiazines equivalent to less than 400 mg. of chlorpromazine per day.

Acute schizophrenics: a total of ten patients who were psychotic at the time of blood drawing, were demonstrably ill for less than six months, and were free of drugs for at least two weeks. They were studied on a research ward at the Clinical Center or at the emergency room of Saint Elizabeths Hospital.

All patients were diagnosed according to the criteria of APA's *Diagnostic and Statistical Manual of Mental Disorders*, second edition (7).

TRYPTAMINE-METHYLATING ENZYME IN RED BLOOD CELLS (SERIES 1)

Method

Because several enzymes of importance to brain function have recently been described in the red blood cells, this was the first blood component we examined (see appendix 1).

Results

All samples in this and the following series were run without the experimenter's knowing whether they came from patients or controls. There was no difference in enzyme activity in red blood cells among 20 normal adults, 22 chronic schizophrenics, ten acute schizophrenics (drug-free for at least 14 days), and five patients with psychotic affective disorders (see table 1). There was no difference in enzyme activity for the two groups of schizophrenic patients.

TABLE 1
Comparison of Methylating Enzyme Activity in Red Blood Cells of Normal and Patient Groups

Group	Number of Subjects	Counts per Minute/5 μl. of Whole Blood Mean ± S.D.
Normal subjects	20	620 ± 43
Chronic schizophrenics		
Total	22	561 ± 33
Drug-free	11	600 ± 25
Acute schizophrenics	10	680 ± 40
Psychotic depressives	5	611 ± 39

ENZYMATIC METHYLATION OF N-MONOMETHYLTRYPTAMINE IN PLATELETS (SERIES 2, EXPERIMENT 1)

Method

Platelets were separated from other blood components by differential centrifugation (see appendix 2). Figure 1 demonstrates the enzyme activity in platelets for different protein concentrations, using monomethyltryptamine and ^{14}C S-adenosyl-methionine (SAM) as substrates. The radioactive extractable product formation was linear for high protein concentrations, but at low protein concentrations the product increased inversely with the amount of protein. This suggested the presence of a dilutable inhibitor, which had to be taken into consideration, since the platelet-protein concentrations used in the assay varied from subject to subject.

Identification of the enzymatic product was attempted after drying the samples both under nitrogen and in the vacuum desiccator. At least five different solvent systems were used. Figure 2 shows results typical of these systems. Only about 25 percent of the total radioactive product could be identified as authentic DMT, with a high percentage (50 percent) of the unknown material migrating with the front. Because of the failure to fully identify the product of the enzyme, the enzyme activity was expressed in total counts per minute (cpm.) per milligram of protein.

The protein determinations were done by the method of Lowry and associates (8). Five assays from the same person, with blood drawn over a five-month period, produced a maximum range of 22 percent around the mean.

Results

The results are summarized in table 2 and figure 3. Among the normal subjects, men and women had the same enzyme activity in platelets. There was no significant correlation with age, which ranged from 19 to 60 years. The mean value for the alcoholic patients was not statistically different from that of the normal group.

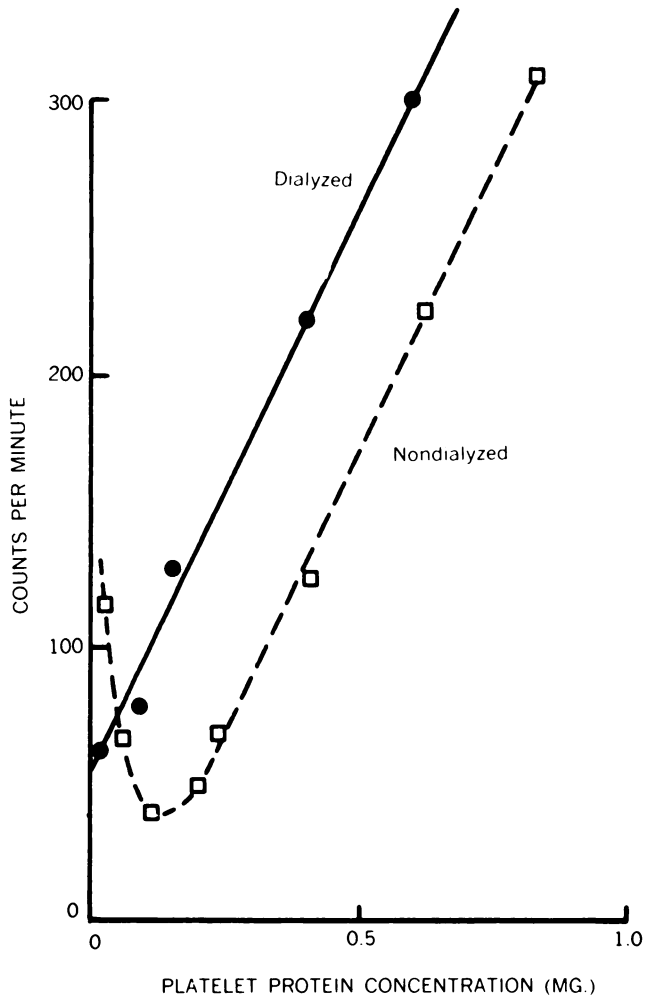
Using a two-tailed t test, it was found that the mean enzyme activity for the psychotic depressives was significantly elevated ($p < .01$) above that of the normal group. The mean activity of the drug-free chronic schizophrenics was also significantly higher ($p < .01$) than that of normal controls, while the group of chronic schizophrenics taking phenothiazines had a mean that was not statistically different from that of the "nonmedicated" group. The combined mean cpm. for the 30 schizophrenic patients was significantly higher ($p < .001$) than that of the normal group. The mean platelet enzyme activity of the group of acute schizophrenics was significantly higher ($p < .01$) than that of the normal group.

N-METHYLATION OF TRYPTAMINE IN DIALYZED PLATELETS (SERIES 2, EXPERIMENT 2)

Method

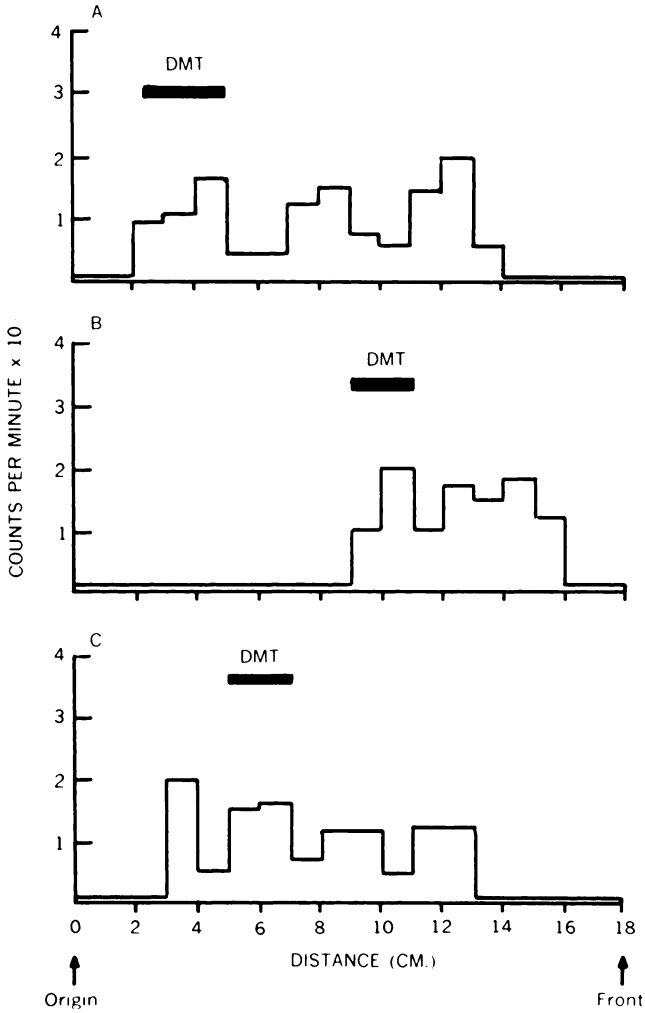
Concomitantly with the above studies, we found that

FIGURE 1
*Protein Concentrations Versus Product Formation for Dialyzed and Nondialyzed Platelets**



*Dialysis increases enzyme activity and also prevents the J-shaped curve that occurred in the nondialyzed sample; the curve is probably due to the presence of a dilutable inhibitor.

FIGURE 2
Identification of the Product in Nondialyzed Platelets in Three Solvent Systems Using N-monomethyltryptamine as the Substrate*



*A = toluene : acetic acid : ethyl acetate : water (80:40:20:5); B = isopropanol : ammonium hydroxide (ten percent) : water (200:10:20); C = N-butanol : acetic acid : water (12:3:5). Using these and other solvent systems, only about 25 percent of the total radioactive product was isographic with authentic DMT (indicated by the black bar).

the activity of methylating enzymes in platelets and human brain could be greatly increased by dialysis. In view of this finding, it appeared possible that the above difference in platelet enzyme activity could be due to the difference in a dialyzable inhibitory factor rather than to enzyme activity per se. Platelets were obtained and assayed in the same manner as that in experiment 1. In addition, sonicated platelet material in 0.5 ml. of 0.01 M TRIS buffer (pH 8.6) was dialyzed against a similar buffer for 18 hours with three changes of buffer. As can be seen in figure 1, this had two effects: it increased the enzyme activity per milligram of platelet protein and prevented the dilution effect seen at low protein concentrations in nondialyzed platelets (in practice, assays with

TABLE 2
Comparison of Methylating Enzyme Activity in Nondialyzed Platelets of Normal and Patient Groups

Group	Number of Subjects	Counts per Minute per mg. of Protein Mean ± S.D.
Normal subjects		
Men	32	125 ± 8
Women	17	126 ± 6
Combined	49	126 ± 7
Alcoholics	10	104 ± 13
Psychotic depressives	13	190 ± 23*
Chronic schizophrenics		
Drug-free	11	217 ± 22*
Taking phenothiazines	19	182 ± 19*
Combined	30	199 ± 15**
Acute schizophrenics	5	239 ± 42*

*$p < .01$ (two-tailed t test), compared with normal subjects.
**$p < .001$ (two-tailed t test), compared with normal subjects.

protein concentrations of less than 0.2 mg. occasionally demonstrated this effect and were discarded prior to the breaking of the patient identification code).

The radioactive product was identified using three solvent systems on thin-layer chromatography (figure 4). The chromatographic spot isographic with authentic DMT represented 70 to 80 percent of the total radioactivity. The isographic spot was scraped from the chromatographic plate and eluted with 0.5 ml. of methanol and co-crystallized with cold DMT for four recrystallizations (9). There was constant specific activity through the four recrystallizations. The Michaelis constants (K_ms) for tryptamine, N-methyltryptamine, and SAM did not change appreciably with dialysis.

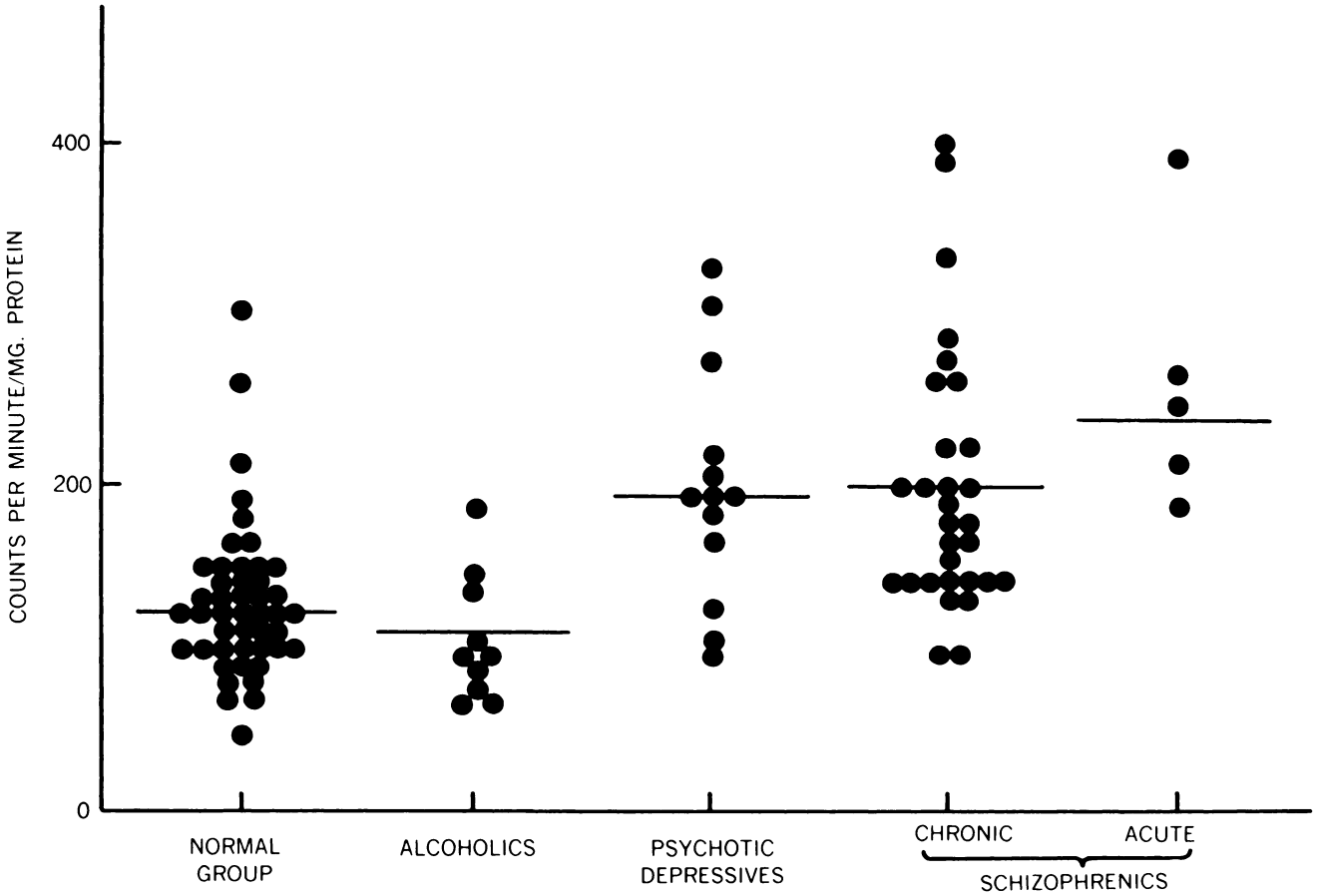
Results

The results are summarized in table 3 and figure 5. Among normal subjects, the N-methyltransferase activity in dialyzed platelets was 50 to 100 percent higher than in nondialyzed platelets ($p < .001$). There was no statistical difference in activity between men and women and there was no significant correlation of enzyme activity with age. Five samples taken from the same subject over a two-month period produced a maximum range of 52 percent around the mean.

The mean value for 11 chronic alcoholics was higher ($p < .02$) than the nondialyzed values from the same subjects but was not statistically different from the mean for dialyzed samples from normal subjects. The same was true for nine psychotic depressives, whose mean activity was significantly higher than their nondialyzed values but not different from the mean for dialyzed samples from normal subjects.

The mean level of activity for eight drug-free chronic schizophrenics was not significantly different from that for nine chronic schizophrenics receiving phenothiazines. However, their combined mean activity was statistically higher ($p < .01$) than that in the nondialyzed

FIGURE 3
*Enzyme Activity in Nondialyzed Platelets**



*Using a two-tailed t test for comparison with the normal group, there were significant differences for the psychotic depressives ($p < .01$), chronic schizophrenics ($p < .001$), and acute schizophrenics ($p < .01$).

samples from chronic schizophrenic patients. It was barely higher ($p < .05$) than the values found in dialyzed samples from normal subjects. If the highest single value from the schizophrenic patients was dropped, the difference was not statistically significant. Also, the nonparametric Mann-Whitney U test did not demonstrate this difference (10). Ten samples from acute schizophrenics had a mean value that was not different from that of the nondialyzed samples from acute schizophrenics or from that of dialyzed samples from the normal subjects.

N-METHYLATION ACTIVITY IN PLASMA (SERIES 3)

Method

Platelet-free plasma was obtained from the supernate of the platelet preparation. The assay was run identically to that in series 2, experiment 1, except that 200 μl. of plasma instead of the platelet preparation was added to the incubation mixture to make up a volume of 450 μl. On thin-layer chromatography, the product was found to be isographic with authentic DMT.

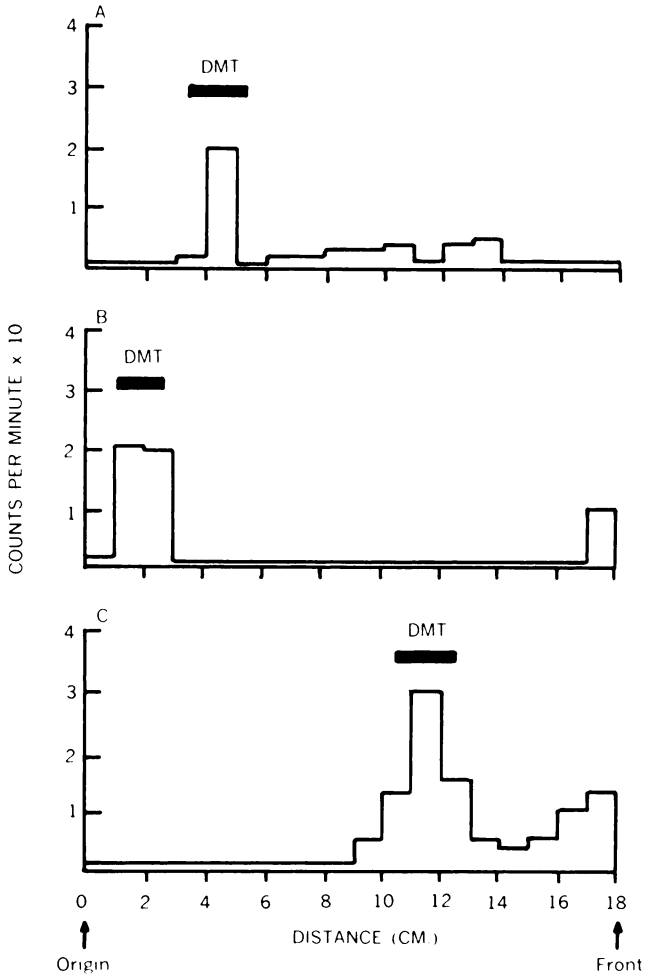
Results

As shown in table 4, the activity for ten acute male schizophrenics (drug-free for 14 or more days) was not significantly different from the extractable product of activity for ten age-matched (age = 20 ± 4 years) normal male subjects.

DISCUSSION

There is enzyme activity in human red blood cells, plasma, and platelets that is capable of converting tryptamine to monomethyltryptamine and monomethyltryptamine to DMT. This activity is present in a number of mammalian species, including the primitive opossum. The level of activity, however, for all three blood components is very low (less than one percent of the total radioactivity was recovered). Because of the

FIGURE 4
Identification of the Product in Dialyzed Platelets in Three Solvent Systems Using N-monomethyltryptamine as the Substrate*



*A = N-butanol : acetic acid : water (12:3:5); B = toluene : acetic acid : ethyl acetate : water (80:40:20:5); C = isopropanol : ammonium hydroxide (ten percent) : water (200:10:20). Using these solvent systems, 60 to 80 percent of the total radioactive product was isographic with authentic DMT (indicated by the black bar).

platelets' relatively higher activity (on a milligram of protein basis) and physiologic substrate affinities (K_ms) they seemed to provide the best approach to the development of a sensitive assay. The product of the nondialyzed platelets was only partially identified on the thin-layer chromatography. The reasons for this are not clear. One possibility is that DMT is rapidly broken down by a substance present in the nondialyzed platelets but not in the dialyzed samples. Conceivably, this substance could account for the differences between the dialyzed and nondialyzed platelet values.

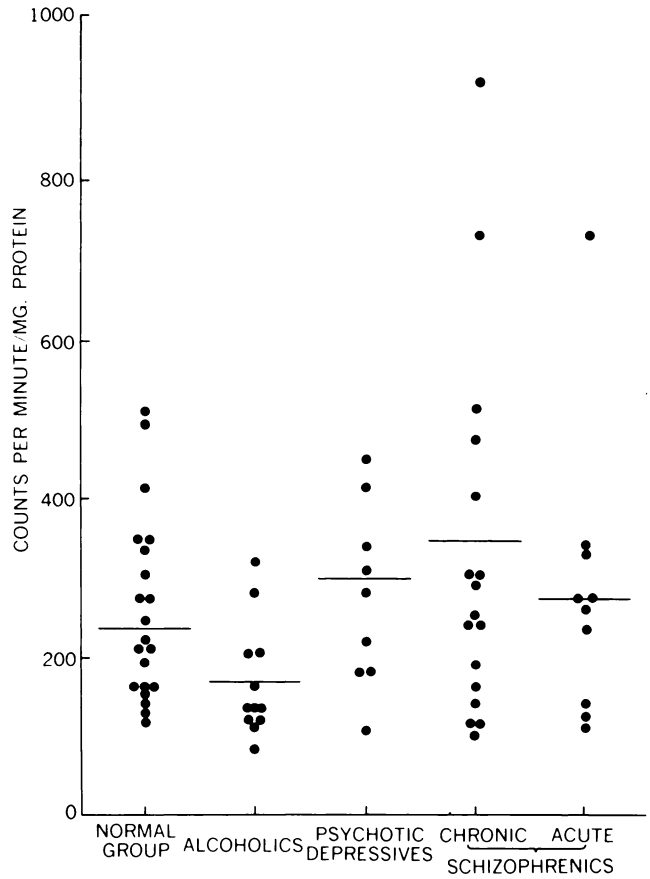
Assays of enzyme activity of both red blood cells and plasma showed no difference between psychotic patients and normal subjects. Thus, while allowance must be made for possibly different patient populations and meth-

TABLE 3
Comparison of Methylating Enzyme Activity in Dialyzed Platelets of Normal and Patient Groups

Group	Number of Subjects	Counts per Minute per mg. of Protein Mean ± S.D.
Normal subjects		
Men	15	220 ± 27
Women	6	282 ± 33
Combined	21	238 ± 22
Alcoholics	11	171 ± 22
Psychotic depressives	9	300 ± 42
Chronic schizophrenics		
Drug-free	8	327 ± 86
Taking phenothiazines	9	364 ± 64
Combined	17	347 ± 51*
Acute schizophrenics	10	276 ± 57

*$p < .05$ (two-tailed t test), compared with normal subjects.

FIGURE 5
*Enzyme Activity in Dialyzed Platelets**



*The chronic schizophrenic group had a significantly higher ($p < .05$) value than the normal subjects; this difference disappeared when the highest patient value was omitted or the nonparametric Mann-Whitney U test was used.

TABLE 4
Comparison of Methylating Enzyme Activity in Plasma of Normal Subjects and Acute Schizophrenics

Group	Number of Subjects	Counts per Minute per 200 μl. of Plasma Mean ± S.D.
Normal subjects	10	75 ± 17
Acute schizophrenics	10	63 ± 26

odology, the radioassay described here was unable to confirm the report of Narasimhachari and associates (11) of increased N-methyltransferase activity in the sera of schizophrenic patients.

The results of the clinical assay with the platelets must be given careful consideration. The samples of nondialyzed platelets from the acute schizophrenics, chronic schizophrenics, and psychotic depressives had higher levels of activity than normal subjects and chronic alcoholics. The chronic alcoholics were included to test whether hospitalization itself was responsible for the differences. It apparently was not. One factor that was not controlled for was the possible influence of stress, which is probably greater in the psychotic groups than in the normal subjects or alcoholics. The fact that the enzyme activity was elevated in both depressed and schizophrenic patients increases the likelihood that the abnormality is nonspecific.

It was of particular interest that the phenothiazines (equivalent to 400 mg. of chlorpromazine or less) had a relatively small effect on the enzyme activity. If anything, it tended to decrease the activity and probably cannot be used to explain the differences between the chronic schizophrenic groups for the nondialyzed platelets.

The difference in enzyme activity between psychotic and nonpsychotic subjects was greatly diminished by dialysis. It therefore appears that the difference in enzyme activity between psychotic and normal subjects is due to the presence of a dialyzable inhibitor (or a substance capable of metabolizing DMT) in normal subjects and not due to a quantitative difference in the enzyme. The identity of this inhibitor is not known.

From the red blood cell, plasma, and platelet data, it appears that there is no evidence for an enzymatic difference between psychotics and nonpsychotics for the enzyme capable of forming DMT. The question of whether there is an inhibitor (or substance capable of metabolizing DMT) in the platelets has not been resolved. If there is such a substance, it is decreased in depressed as well as schizophrenic patients and is not specific to one diagnostic group. Such a substance could be related to the recent finding of low platelet monoamine oxidase in some depressed patients and schizophrenics (12, 13).

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APPENDIX 1

Details of Methods in Series 1

For each patient, 30 ml. of blood was collected in plastic syringes and transferred into tubes containing 0.01 mg. of sodium heparin USP (Hynson, Westcott, and Dunning). The red blood cells were separated by centrifuging at 125G for ten minutes at 4C, the supernate was discarded, and the red blood cells were washed twice in saline solution. The red blood cells were then centrifuged at 12,000G for ten minutes at 4C and lysed by freeze-thawing twice.

The methylating activity was examined by incubation of 50 μl. of a mixture of one part lysed and packed red blood cells (equivalent to 5 μl. of whole blood) to four parts of 0.01 M sodium phosphate buffer (pH 7.9), 5 mμmoles of S-adenosyl-1-[methyl-^{14}C] methionine (SAM) with a specific activity of 54.6 millicuries/mmole (New England Nuclear), and 200 μg. of tryptamine (Aldrich). This mixture was made up to 260 μl. with the sodium phosphate buffer. The blank consisted of the same substances with tryptamine omitted. The reaction was stopped after four hours of incubation at 37C with 0.6 ml. of 0.5 M sodium borate buffer (pH 10). The radioactive product was extracted into 6 ml. of 97 percent toluene and three percent isoamyl alcohol; this solvent mixture extracts any methylated tryptamines.

The organic phase was separated by centrifugation at 5,000G for five minutes; 5 ml. of the organic phase was transferred to a counting vial and dried in a ventilated oven at 80C for eight hours to remove the volatile ^{14}C methanol produced by a methanol-forming enzyme. Using this procedure, about 6 μμmoles of radioactive product was formed. When the incubation mixture was preheated for ten minutes at 80C, no radioactive product was formed.

Other conditions tried but not found to alter activity were the addition of dithiothreitol (Cleland's reagent) in five concentrations ranging from 7.4 x 10^{-3}M to 4.6 x 10^{-5}M, pargyline (2.6 x 10^{-3}M), disodium ethylenediamine tetra acetate, calcium chloride, ferric sulfate, manganese sulfate, cupric sulfate, magnesium sulfate in four concentrations ranging from 3.7 x 10^{-3}M to 3.7 x 10^{-6}M, and dialysis. TRIS and phosphate buffers of 0.2 and 0.01 M with pHs from 7.0 to 8.6 were tested. The optimal pH was found to be 7.9 in a 0.01 M phosphate buffer.

Using serotonin creatinine sulfate as a substrate, the experimental to blank (no substrate) ratio could not be increased above 3:2 and was felt to be unreliable. The ratio of radioactive product extracted in the presence and absence of tryptamine was at least 4:1. The higher ratio for tryptamine than for serotonin indicated that tryptamine provided a more sensitive assay.

The Michaelis constant (K_m) of the crude enzyme preparation was about 10^{-2}M for tryptamine and 9 x 10^{-3}M for N-monomethyltryptamine. The K_m of SAM for both substrates was about 2.6 x 10^{-5}M. Since the K_m value indicates the enzyme's affinity for its substrate, the high K_ms for tryptamine and monomethyltryptamine indicate that an extremely high concentration of these substrates would be necessary to produce any significant product. Very high concentrations of tryptamine (58 mM) inhibited the reaction. Five samples taken from the same person over a two-month period were within 20 percent of the mean.

The identification of the ^{14}C-methylated tryptamine was done by thin-layer chromatography on Eastman chromatogram sheets of silica gel, 100 microns in thickness. All sheets were activated by heating for 30 minutes at 90C immediately before using. Seventy-five percent of the radioactive product was isographic to authentic N-monomethyltryptamine and 25 percent was isographic to authentic DMT.

The relative enzyme activity of four species, as compared with man (100 percent), was: Virginia opossum = 77 percent, domestic cat = 61 percent, guinea pig = 42 percent, and white laboratory rat = ten percent. The relative activity per milligram of protein for platelets, red blood cells, and plasma was 100 percent:22 percent:six percent.

APPENDIX 2

Details of Methods in Series 2

To separate platelets from other blood components, 16 ml. of whole blood was collected in 2 ml. of water containing 16 mg. of citric acid, 49 mg. of sodium citrate, and 41 mg. of dextrose (ACD solution A) in a glass Vacutainer tube (Becton-Dickinson). We were able to store the blood in ice for at least five hours without any change in the enzyme activity. The blood was centrifuged in a refrigerated instrument at 170G for ten minutes. The platelet-rich plasma supernatant was removed, provided that no visible red color from the red blood cells was present. This procedure was repeated at 380G and 680G.

The platelet-rich plasma was centrifuged at 3,020G for ten minutes and the platelet-poor plasma was poured off. The platelet pellet was washed with 2 ml. of saline solution. We were able to store the pellet for as long as 30 days at -20C without measurable loss of activity.

For the enzyme assay, 0.5 ml. of 0.01 M TRIS buffer (pH 8.6) was added to the pellet and the sample was sonicated on a Branson S-125 instrument at 5 amps (DC) for 15 seconds. Microscopic examination (x 1000) of oil immersion indicated complete lysis of the platelets. The incubation mixture used contained 0.2 to 0.5 mg. of platelet protein, 25 mμmoles of ^{14}C SAM with a specific activity of 54 millicuries/mmole (New England Nuclear), and 200 μg. of N-monomethyltryptamine (Aldrich) diluted to 300 μl. with 0.01 M TRIS buffer (pH 8.6). The incubation mixture without the N-monomethyltryptamine served as a blank.

The samples were incubated for 90 minutes at 37C and the reaction was terminated by adding 1.2 ml. of 0.5 M sodium borate buffer (pH 10). The product was extracted from the aqueous phase into 3 ml. of 95 percent heptane and five percent isoamyl alcohol. This solvent gives the lowest blank value and extracts the product almost quantitatively. The organic phase was washed with 1.2 ml. of borate buffer. To reduce the blank, 2 ml. of the organic phase was evaporated under 15 cm. of mercury in a desiccator heated to 60C for 30 minutes. This eliminated the volatile ^{14}C methanol produced by the methanol-forming enzyme. The residue was taken up in 2 ml. of ethanol and 10 ml. of phosphor, and the radioactivity was measured in a Beckman Scintillation Counter with an internal standard to correct for quenching. This procedure produced a more sensitive assay by reducing the blank.

The extractable radioactive product formation was linear with an incubation time of up to three hours. The optimal pH for N-monomethyltryptamine was 8.6 in a TRIS buffer (0.01 M) and 7.9 in a phosphate buffer (0.01M). Though the phosphate buffer (pH 7.9) produced nine percent higher total extractable counts, the experimental to blank ratio was 7:1 for the TRIS buffer versus 6:1 for the phosphate buffer. The K_m for the unpurified enzyme was about 1.6 x 10^{-4}M for N-monomethyltryptamine and 1.7 x 10^{-5}M for SAM. The Km for tryptamine was about 9 x 10^{-4}M.