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ULTRASTRUCTURE OF THE NERVE PLEXUSES OF THE MAMMALIAN INTESTINE: THE ENTERIC GLIAL CELLS

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Abstract—The ultrastructure of the glial cells in the enteric plexuses of the rat, guinea-pig, rabbit, cat and sheep has been investigated by freeze–fracture and by thin-section electron microscopy. In all the ganglia studied, glial cells outnumber neurons. They are readily identified by their shape, position and ultrastructure (particularly the abundant amount of gliofilaments) but could not be subdivided into separate types. They provide a partial sheath to the ganglion neurons (but large areas of neuronal membrane lie directly beneath the basal lamina and collagen fibrils) and have long laminar processes extending between nerve processes. Most nerve processes are in direct membrane-to-membrane contact with each other; the glial cells only separate groups of them and rarely form a sheath around an individual neurite.

The gliofilaments are anchored to conspicuous dense bodies beneath the cell membrane at the surface of ganglia. The possible significance of these systems of gliofilaments (and the high number of intermediate junctions) is discussed in the light of the severe mechanical stresses imposed on the ganglia by the contractile activity of the gut wall.

Numerous specialized contacts, of unknown significance, are found between vesicle-containing nerve varicosities and glial cell bodies or glial processes. In freeze–fracture preparations (cat and guinea-pig), a specific pattern of intramembrane particles allows the cell membrane of the enteric glial cells to be readily identified.

THE ENTERIC nerve plexuses are attracting growing interest and, in the past few years alone, a wealth of data on their electrophysiological, pharmacological and histochemical properties has been published. The fine structure of the plexuses, however, has not attracted much interest, or perhaps just enough to show their extraordinary complexity without throwing much light on it (reviewed by GERSHON, DREYFUS & ROTHMAN, 1979; GABELLA, 1979; FURNESS & COSTA, 1980). Much of the work has understandably been concentrated on the ultrastructure of the nerve endings, of which a large variety are found, whereas only little attention has been paid to other aspects of the ganglion organization. One such aspect is the morphology of the glial cells of the intramural plexuses, briefly described in previous investigations on the guinea-pig (GABELLA, 1972; COOK & BURNSTOCK, 1976). Although they are referred to as Schwann cells by COOK & BURNSTOCK (1976), it had already been noted (GABELLA, 1972) that they have some characteristic structural features which are not found in other glial cells of the peripheral nervous system. This paper analyses in more detail the fine structure of the glial cells of the enteric plexuses in several mammalian species. The data obtained by conventional thin-section electron microscopy are supplemented with freeze–fracture observations.

EXPERIMENTAL PROCEDURES

Rats (five Wistar rats, body weight about 200 g), guinea-pigs (over twenty Grayston guinea-pigs, body weight 400–600 g), rabbits (two rabbits weighing about 2.0 kg), cats (three cats weighing about 2.5 kg) and sheep (three pregnant ewes) were used. Under deep anaesthesia, portions of the small and large intestine were excised and put in oxygenated Krebs' solution at room temperature. After 5–20 min the lumen was cannulated and gently distended with Krebs' solution, after putting cotton thread ligatures at both ends of the gut segment. This was then immersed in fixative, made of 5% glutaraldehyde in 100 mM Na cacodylate (often with 2–5 mM $CaCl_2$ added) at pH 7.4, at room temperature. After about 1 h the intestine was sliced into short rings which were immersed in fresh fixative for 2–18 h. The specimens were then washed in buffer, post-fixed in 2% osmium tetroxide in the same cacodylate buffer, washed again in buffer and block-stained for 30 min in a saturated aqueous solution of uranyl acetate. Following dehydration in ethanol and epoxypropane the specimens were infiltrated in Araldite. Thin sections were cut with glass knives, collected on coated copper grids and stained with uranyl acetate and lead citrate.

For freeze–fracture the specimens (from the small intestine of guinea-pig and cat) were fixed as described above, then washed in buffer and infiltrated with 25% glycerol. Under a dissecting microscope the muscle coat was separated from the rest of the intestinal wall and cut into small blocks about 1 × 1.5 mm. These were mounted flat on support discs and frozen in liquid Freon 22 cooled to −150°C with liquid nitrogen. Freeze–fracture was carried out in a Balzer 300 apparatus. The blocks were fractured at −105°C and immediately shadowed with platinum carbon at 45° followed by carbon at 90°. The tissue was removed with sodium hypochlorite, and after cleaning in double distilled water the replicas were collected on 200-mesh uncoated grids. Replicas and thin sections were

examined in Philips 300 and 400 electron microscopes equipped with goniometer stage and rotating specimen holder.

RESULTS

Identification

Glial cells of the intramural ganglia are structurally similar in all the species studied and are readily identified on the basis of their shape, position and ultrastructural features (Figs 1, 3). By comparison with the ganglion neurons, glial cells are smaller in size and lack the large expanses of cytoplasm which are common in neurons. The glial nuclei are elongated, about 2–3 μm in width, with deep crenations. The electron-dense chromatin material is abundant, usually incrusted on the inner aspect of the nuclear envelope. Neuronal nuclei are, by contrast, larger, tending to be spherical or ovoid and with low electron-density (vacuolar aspect) (Fig. 3). The glial cytoplasm contains scattered sacs of smooth and rough endoplasmic reticulum, mitochondria, ribosomes and numerous microtubules (Fig. 4). The most abundant component in the cytoplasm of the glial cell body and the glial processes is filaments (gliofilaments) of about 10 nm dia.; they are gathered in bundles running in various directions within the cell body and mainly along the long axis in glial processes.

Number

In the ganglia of the species studied glial cells are more numerous than ganglion neurons (Fig. 1). The preponderance is particularly obvious in the myenteric ganglia of the guinea-pig ileum, where glial cells outnumber neurons by about 2 to 1; it is, however, somewhat less marked in the ganglia of the submucosal plexus of the guinea-pig and the myenteric ganglia of the other species. In the connecting meshes of the plexuses, apart from the rare occurrence of neuronal perikarya, all the cells are glial cells (Fig. 2).

Shape and position

The shape of glial cells in the intestinal plexuses tends to be flattened and to be moulded over that of the adjacent neuronal structures. Glial cells spread over the surface of neuronal perikarya (Fig. 4) and in the spaces between neuronal processes (which tend to be round in profile). No differences were found between the species studied.

Unlike the satellite cells of sympathetic ganglia, the glial cells of the enteric ganglia do not form a complete 'capsule' around a neuron. Indeed, a characteristic feature of the myenteric plexus is that large areas of the surface of perikarya and large dendrites are not covered by a cellular sheath at all, but are directly coated by the basal lamina surrounding the whole ganglion and by collagen fibrils. A felt of material about 50 nm thick, partly amorphous, partly microfibrillar, lies in the cytoplasm beneath these 'naked' areas of the neuronal membrane. In addition, glial cells do not cover the neuronal membrane at numerous sites where nerve processes lie directly over the perikaryal surface (a small number of these processes are varicosities, rich in vesicles and forming synaptic junctions). Other areas in which a glial covering is also missing are those in which two neuronal perikarya are in direct membrane-to-membrane contact; such areas are found only occasionally in ganglia of adult animals, whereas they are common during development.

Some glial cells have long and elaborate processes which extend tens of microns away from the cell body and stand mainly in relation with nerve processes. They find their way in the angular spaces between neuronal processes, and surround completely some individual processes or small bundles of them. The filling of spaces by the glial processes is almost complete, so that a narrow gap of about 20 nm between adjacent membranes is virtually all the extracellular space that can be found within the ganglia. Only some neuronal processes are fully wrapped by a glial sheath, and neuronal processes lying in direct membrane-to-membrane contact with each other are very common. Many, if not all, glial cells also have processes reaching the surface of the ganglion, where they form an expansion covered by the basal lamina (Fig. 6). It is common to find glial cells which have laminar processes reaching both the surfaces of the ganglion (one facing the circular, the other the longitudinal musculature).

In the connecting strands of the plexus neurons are very rare. Virtually all nuclei belong to glial cells, which have a characteristic arrangement. Their small cell bodies are situated in the central part of the strand and send laminar expansions, radially arranged, between the neuronal processes (axons). The expansions reach the surface of the strand where they spread and interdigitate with other expansions. Only few neuronal processes are directly exposed to the surface of the strand. Many axons are tightly packed together without intervening glial processes. The numerical ratio between axons and glial cells varies with the size of the connecting strand. In the guinea-pig myenteric plexus, up to 600 axons have been counted in strands which displayed a single, centrally-placed glial cell with radially symmetric processes. Strands made of a greater number of axons contain two glial cells at any level of section.

Cell junctions

Structural specializations of the glial cell membrane are involved in a variety of cell junctions. There are symmetrical membrane densities, i.e., intermediate junctions, between two glial cells or between a glial cell and a neuron (or between two neuronal structures) (Fig. 3). These junctions mostly measure about 0.1 μm in length, but some can be as long as 0.4 μm. They are not identified in freeze-fracture preparations, an observation suggesting that they are not of the desmosome type (in which case a typical

FIG. 1. Tangential section of the muscle coat of the guinea-pig ileum, with an *en face* view of the myenteric plexus. Stained with toluidine blue. In the ganglion one can count 31 neurons and 47 glial cells (of which mainly the nucleus is visible). To the left is the longitudinal musculature, and to the right (top and bottom) the circular musculature. × 675

FIG. 2. Transverse section of the muscle coat of the guinea-pig ileum. Between the circular muscle (top) and the longitudinal muscle (bottom) is a connecting strand of the myenteric plexus; in this, centrally placed, is the nucleus of a glial cell. × 675. Marker: 50 μm

FIG. 3. Electron micrograph of a myenteric ganglion of the rabbit ileum. A glial cell, showing part of its nucleus (top left) lies adjacent to a neuron also showing part of its nucleus (top right). Around the two cells there are numerous neurites and glial processes. Gliofilaments are conspicuous in the glial cell, ribosomes in the neuron. Both cells contain rough endoplasmic reticulum and numerous microtubules. An intermediate junction is present in the area of contact between the two cells (arrow). Two nerve varicosities form specialized contacts with glial processes. × 42,500. Marker: 1 μm

FIG. 4. Myenteric plexus of the guinea-pig ileum. Parts of two ganglion neurons with well developed endoplasmic reticulum and ribosomes; both cells show subsurface cisternae. There is a glial cell body with part of the nucleus (*n*) and several glial processes: one of these (*g*) is spread over the surface of the neuron at the bottom left. A vesicle-containing nerve varicosity forms a synapse on the neuron at the top, while two other varicosities form specialized contacts with the glia (arrows). × 35,000. Marker: 1 μm

FIG. 5. A very oblique section through a glial process (left), the surface of the myenteric ganglion, the interstitial space and an adjacent muscle cell of the circular layer (right). Gliofilaments are well in evidence in the glial process and some of them (arrow) are seen to penetrate into the electron-dense material beneath the cell membrane. The smooth muscle cell mainly display thin (actin) filaments. × 48,000. Marker: 0.5 μm

FIG. 6. Two glial processes reach the surface of the myenteric ganglion and lie beneath the basal lamina and the collagen fibrils of the interstitial space. The latter also contains some small processes from interstitial cells. The glial processes, which are very rich in gliofilaments and (less) in microtubules, have conspicuous dense bodies projecting into the cytoplasm from the cell membrane. × 26,000. Marker: 1 μm

FIG. 7. One of the processes of Fig. 6 is shown at higher magnification in an adjacent section to illustrate details of the gliofilaments and the electron-dense material associated with the cell membrane. × 65,000. Marker: 0.5 μm

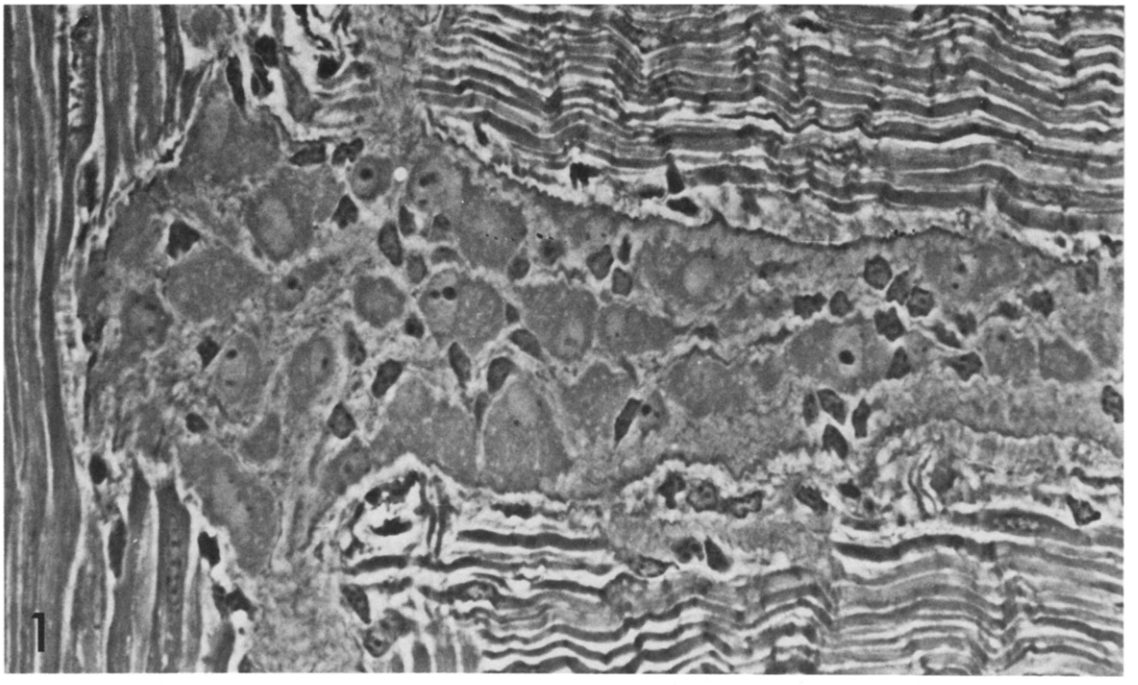
FIGS 8–10. Freeze-fracture preparations of the myenteric plexus of the small intestine of the guinea-pig (Figs 8, 9, 11) and cat (Fig. 10).

FIG. 8. A glial cell process whose cytoplasm is exposed by the fracture plane shows a characteristic pattern of intramembrane particles on the P-face of its cell membrane (*g*) (compare with the membrane of an adjacent neurite *n*). × 49,000. Marker: 0.5 μm

FIG. 9. The characteristic clustering of intramembrane particles into small groups on the P-face of the glial cell membrane. × 100,000. Marker: 0.25 μm

FIG. 10. A similar appearance is found in glial cells from the cat myenteric plexus. × 57,000. Marker: 0.5 μm

FIG. 11. Array of intramembrane particles characteristic of gap junctions in the cell membrane of a glial cell. × 77,000. Marker: 0.5 μm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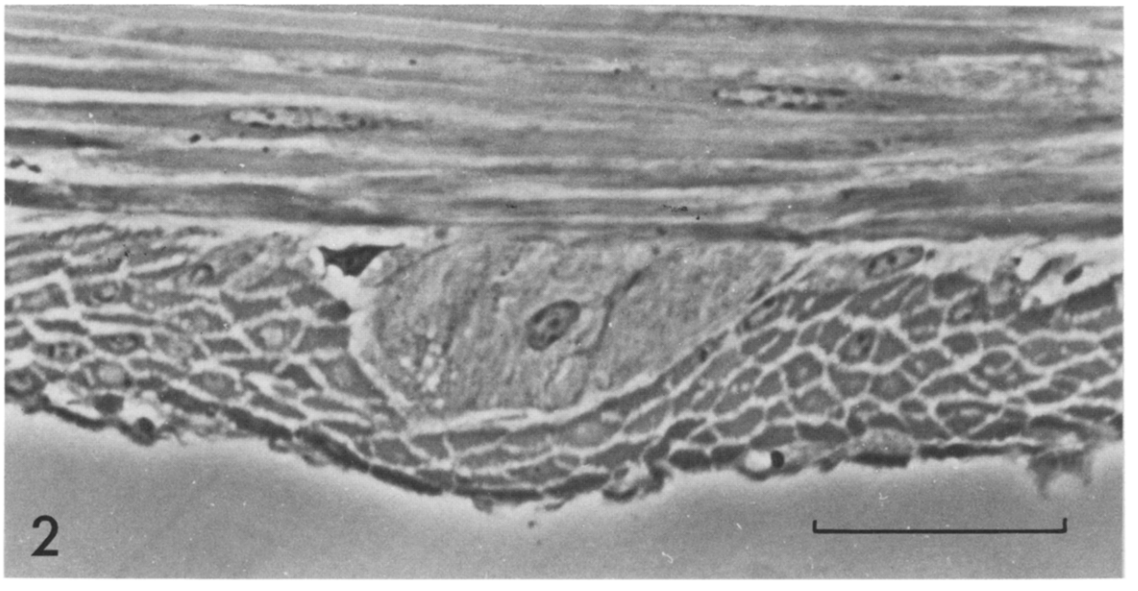
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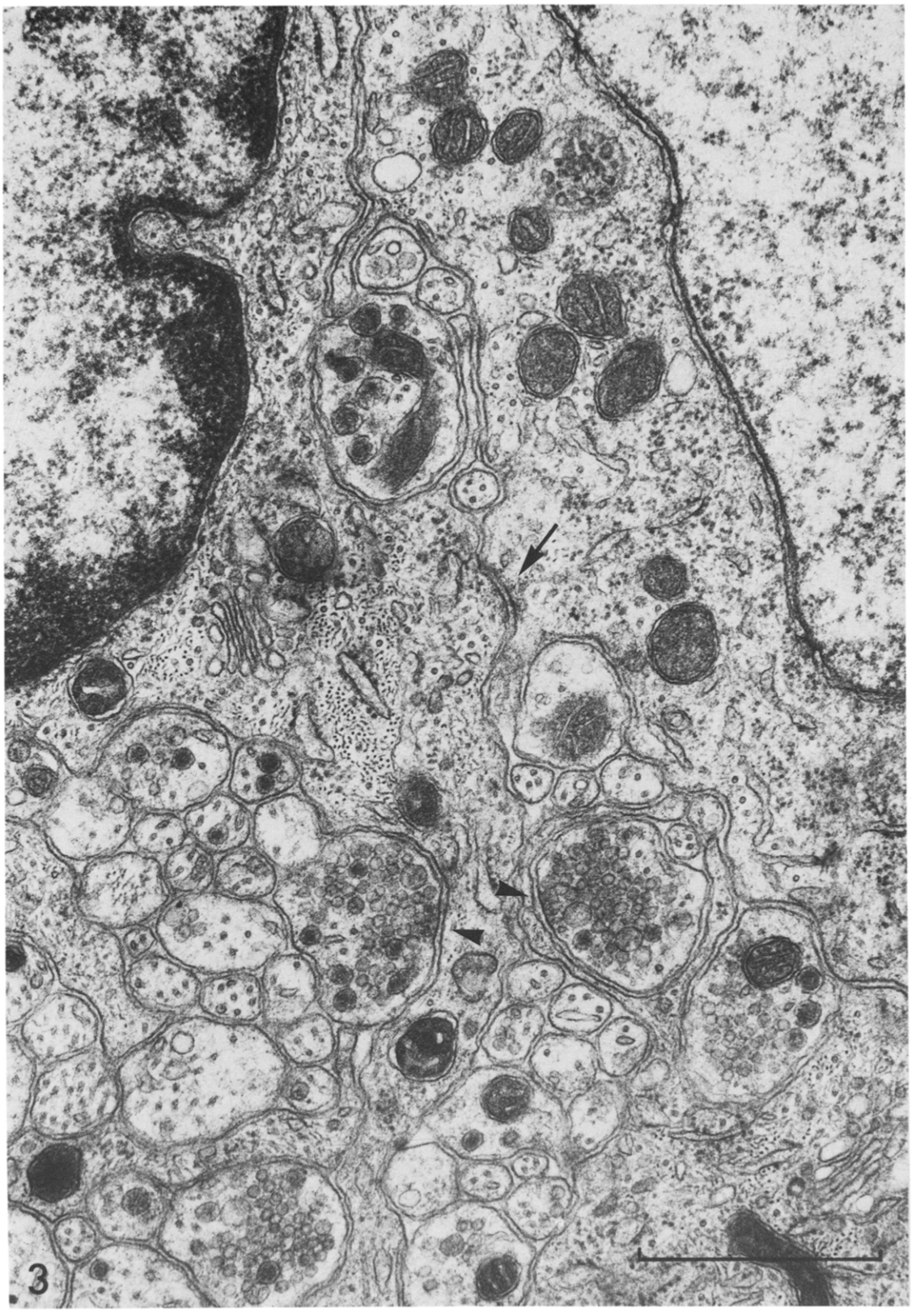
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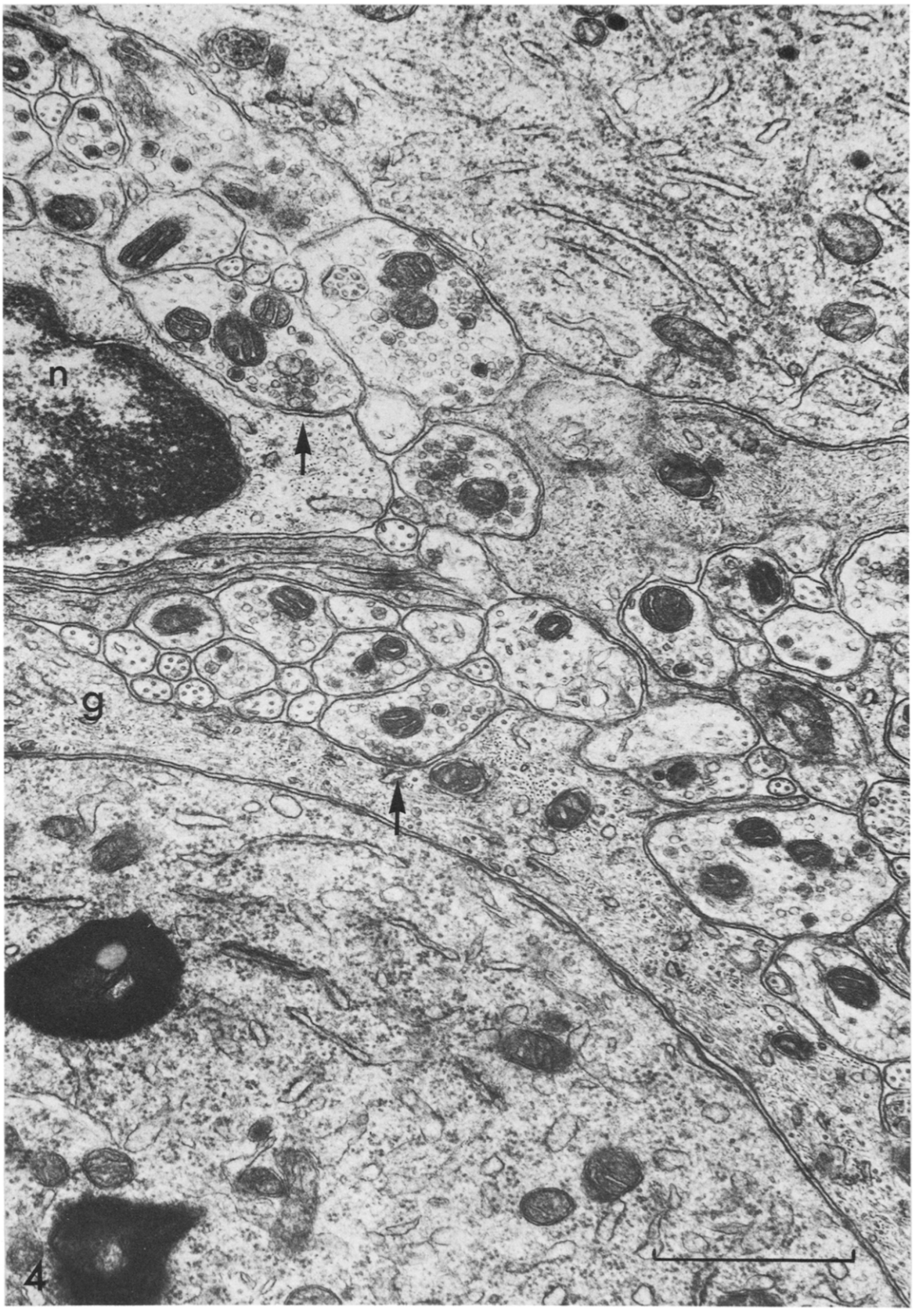
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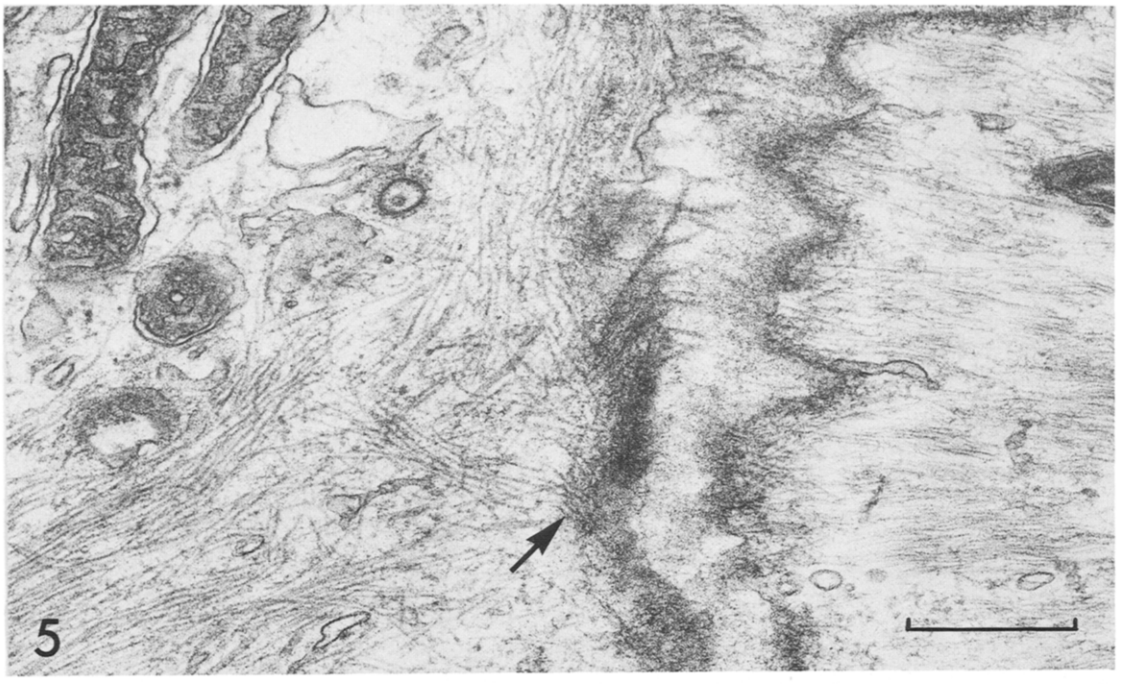
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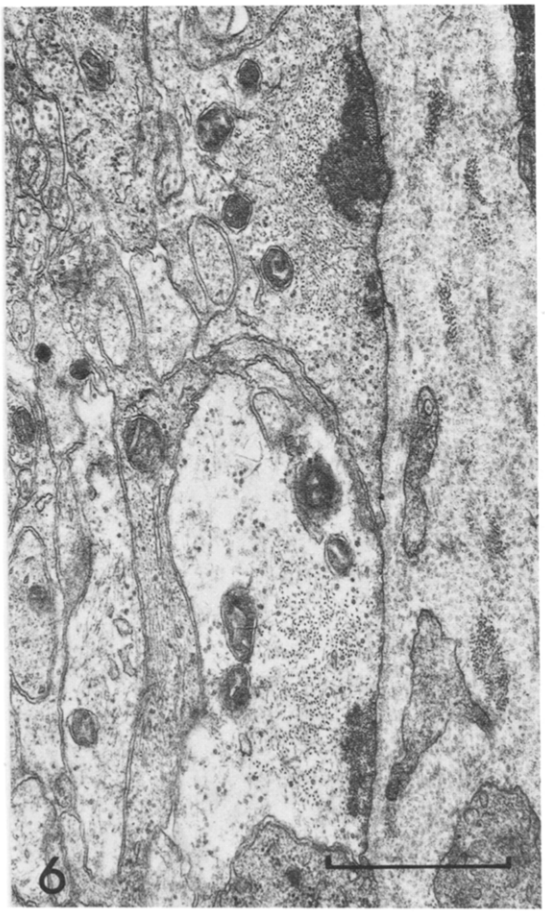
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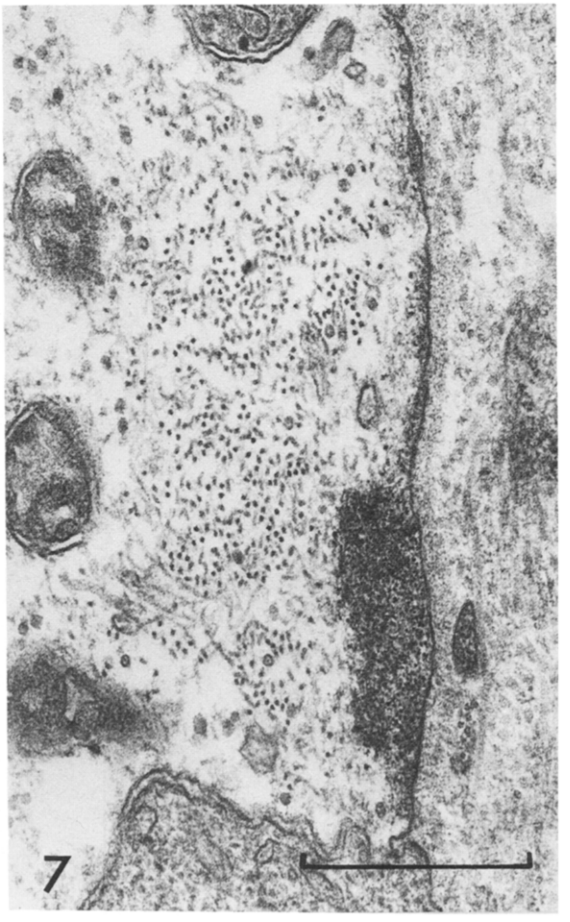
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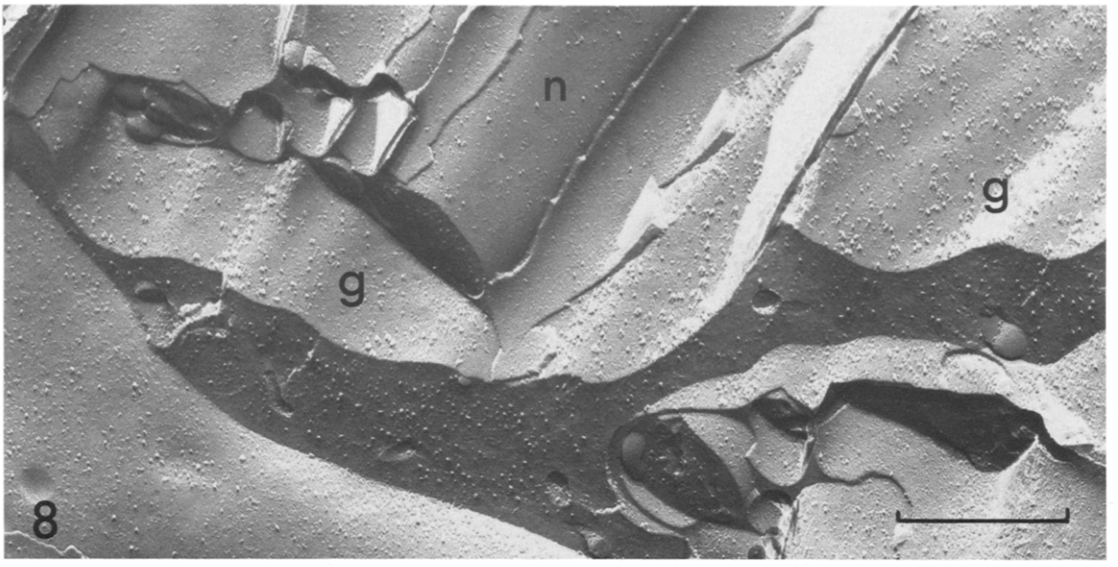
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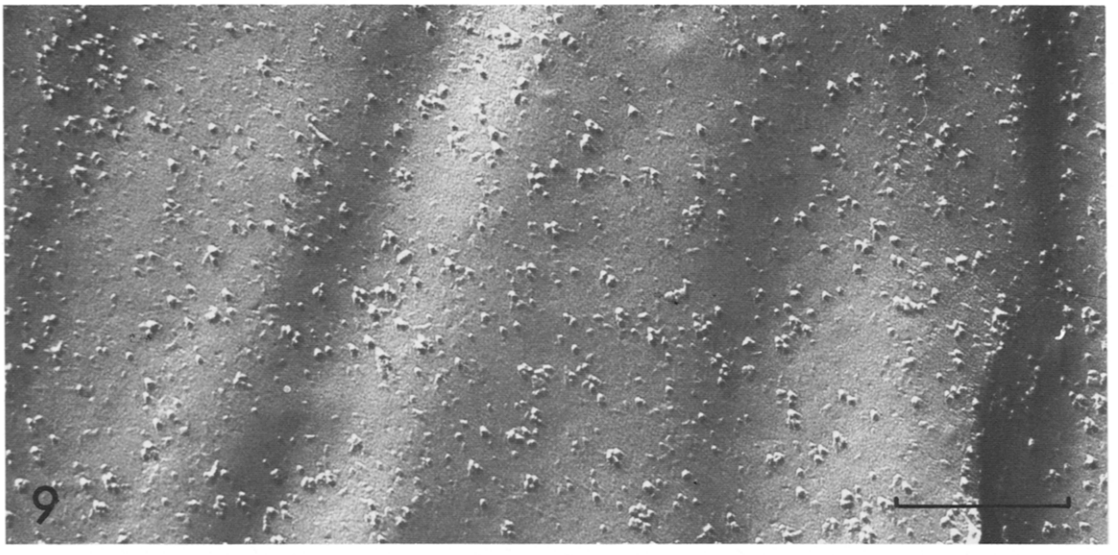
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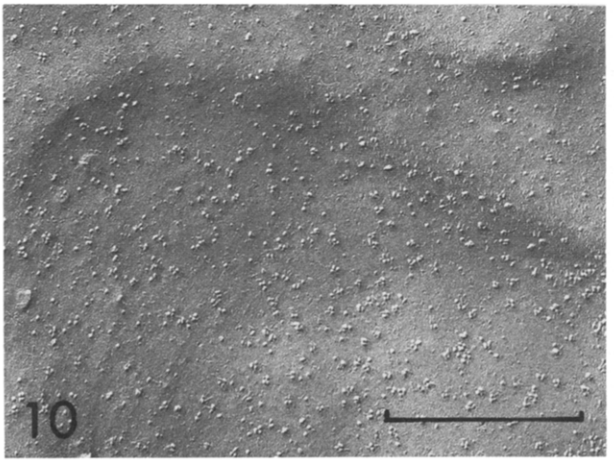
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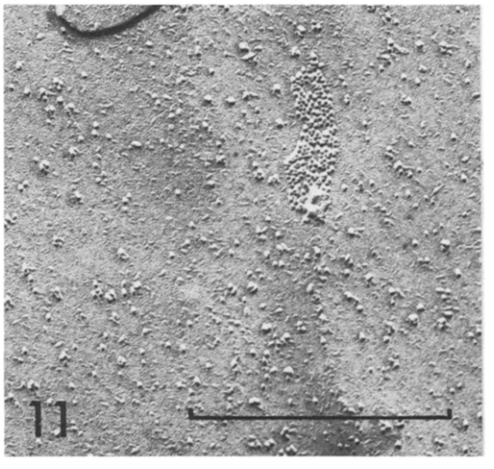
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pattern of large intramembrane particles would be expected). They are usually not associated with gliofilaments.

Gap junctions are very rare in the intestinal plexuses. The few which have been found (in the myenteric plexus of the small intestine of the cat and guinea-pig) are small, sometimes punctuate, and occur between two glial cells. In freeze–fracture, they show the well-known clustering of intramembrane particles on the P-face of the glial membrane (Fig. 10).

A very common type of junction occurs between vesicle-containing axons and glial cell bodies or glial processes. There neuro-glial junctions are consistently present in the myenteric and submucosal ganglia of all species studied. The intercellular cleft is of uniform width and often is slightly wider than in adjacent areas. The apposed glial membrane, however, shows no specializations. The nerve varicosities involved are usually small (about 0.5 μm in dia.) and contain mainly small agranular vesicles and a variable number of large granular vesicles (Figs 3, 4). Nerve varicosities with small granular vesicles, those with heterogenous granular vesicles or those with an almost pure population of small agranular vesicles (according to the classification discussed in GABELLA, 1972) have not been observed to form specialized contacts with glial cells. These contacts with glial cells are also absent in varicosities forming synapses on dendrites or perikarya, and in varicosities located at the ganglion surface.

Finally, the numerous glial filaments found in the glial processes fan out inside the glial expansions at the surface of the ganglion and penetrate into electron-dense material incrusting the cytoplasmic aspect of the cell membrane (Fig. 5). A basal lamina, microfibrils and collagen fibrils cover these areas of the cell membrane. In some processes, the electron-dense material forms very prominent inward projections from the cell membrane into which numerous gliofilaments penetrate (Fig. 7); this occurrence is far more common in the myenteric than in the submucous plexus.

Freeze–fracture studies

Due to the almost fortuitous position of the plane of fracture, there are considerable difficulties in studying the enteric plexuses by freeze–fracture (a full report of these studies will be published elsewhere). On occasions, a glial cell of a myenteric ganglion can be positively identified in a replica on the basis of shape and relation to neuronal processes (Fig. 8). These instances have permitted recognition of a characteristic pattern of intramembrane particles on the P-face of the glial cell membrane of the cat and the guinea-pig myenteric plexus. The intramembrane particles do not appear evenly distributed, since in addition to numerous particles occurring singly (not unlike what is found in the adjacent neuronal structures), many other particles are clustered in characteristic assemblies of 3 to 12 (mostly 3 to 5) particles (Figs 9, 10). These assemblies are distributed over the entire glial cell surface, and were never found in identified neuronal membranes.

DISCUSSION

The morphological observations reported allow some considerations on the significance of the glial cells in the intramural ganglia of the gut to be made. The glial cells outnumber the neurons but they are quite small in size and the chances that a microelectrode penetrates into them (and remains there) in random impalements of a ganglion must be rather small. Some glial cells are spread over the surface of neuronal perikarya but they do not form a complete sheath around them, since (a) there are many neural processes (synaptic and non-synaptic) in contact with the neuronal membrane, and (b) there are extensive areas of the perikarya and the large dendrites which are 'naked' and directly covered by the basal lamina of the ganglion. The glial cells, therefore, certainly do not form a barrier between neurons and connective tissue or interstitium. Nutrients and other blood-borne substances which diffuse through the capillary endothelium can directly reach the neuronal surface. However, blood vessels do not penetrate into the enteric ganglia, and GERSHON & BURSZTAJN (1978) have reported that there is a blood–myenteric plexus barrier similar to the blood–brain barrier (the main evidence being that the capillaries supplying the plexus are of the continuous type and have tight junctions). However, the lack of fenestrated capillaries may not be sufficient evidence for a barrier in the same sense that there is a barrier between blood and central nervous tissue. Moreover, interstitial cells are only loosely arranged around the ganglia (and can be absent altogether around some of the small ones), and part of the neuronal surface is freely exposed to the interstitial space. GERSHON & BURSTAJN'S (1978) suggestion is supported by their experiments with systemically-injected tracers, which do not penetrate into the myenteric ganglia. JACOBS (1977), on the other hand, has shown that systemically-injected horseradish peroxidase readily penetrates into the myenteric ganglia of the guinea-pig. The question of the existence of a barrier around the enteric ganglia has important consequences for the interpretation of the results of pharmacological experiments in the gut.

An important role for the glial cells of enteric ganglia is related to the fact that during the mechanical activity of the muscularis externa the ganglia (and the myenteric ganglia to a greater extent than the submucosal ganglia) are exposed to very intense mechanical stresses. There are changes in width and thickness of the ganglia as well as changes in the shape of the individual neurons. The glial cells (because of their shape and their richness in gliofilaments) probably play a major role in this structural re-arrangement of the ganglia, which must include allowing changes in shape and sliding of structures past each other

and at the same time holding the ganglion together. Mechanically, some glial cells seem to form, by means of their radial processes packed with gliofilaments, robust cross members spanning the full thickness of a ganglion from one surface to the other. The large number of intermediate contacts is also possibly related to this circumstance. Although not as numerous as in the enteric ganglia, intermediate contacts are also found in sympathetic ganglia (ELFVIN, 1963, 1971; TAMARIND & QUILLIAM, 1971; TAXI, GAUTRON & L'HERMITE, 1969; MATTHEWS & NELSON, 1975). ELFVIN (1971) has proposed that some of these junctions (in the cat inferior mesenteric ganglion) may be sites of chemical interaction, while MATTHEWS (1974) has pointed out that they may also have important mechanical functions in those ganglia that are exposed to the pulsations of major arteries such as carotid and aorta.

The gap junctions which have been seen between glial processes (none were found between neuronal processes) were so few in number with relation to the amount of material examined, that they must represent a very rare occurrence. The situation is not very different from that of the central nervous system of vertebrates, where gap junctions are not a common occurrence and are nearly always between glial cells (with some notable exceptions, such as the lamprey spinal cord [PFENNINGER & ROVAINEN, 1974] and the rabbit olfactory bulb [LANDIS, REESE & RAVIOLA, 1974]). Gap junctions between astrocytes have been described by PETERS (1962), BRIGHTMAN & REESE (1969) and PALAY & CHAN-PALAY (1974), and some between ependymal cells by BRIGHTMAN & PALAY (1963). Gap junctions (of the inverted type) are also found between glial cells in the nervous system of certain insects (LANE, SKAER & SWALES, 1977). Studies by intracellular recording have shown electrical coupling between glial cells in the leech (KUFFLER & POTTER, 1964) and in the amphibian central nervous system (KUFFLER & NICHOLLS, 1966).

The specialized contacts between vesicle-containing varicosities and glial cells, whose occurrence has been reported before (GABELLA, 1972), are a puzzling feature of the glial cells of the enteric plexuses. They are undoubtedly very common in all the ganglia studied, but their significance is completely obscure. They may be tentatively labelled synaptoid contacts or, better, neuro-glial junctions, although the absence of detectable structural specializations on the glial side makes it even uncertain whether these are junctions at all. The possibility that these contacts are sites of release of substances from nerve endings is suggested by the clustering of vesicles, but whether such substances act directly on the adjacent glial membrane or diffuse along the narrow intercellular space, acting in a more diffuse way, is unknown. It has been known for some time that 'synaptic-like contacts' between axons and glial cell processes exist in spinal cord explants *in vitro* (GRAINGER, JAMES & TRESMAN, 1968; JAMES & TRESMAN, 1969). HENRIKSON & VAUGHN (1974) have shown that 'axoglia synapse-like contacts' are a normal feature of the radial glial processes in the embryonic mouse spinal cord, but they disappear long before birth. Both groups of authors favour the view that such contacts represent developmental errors which are corrected when "appropriate' synaptic junctions are formed. The neuro-glial junctions of the enteric ganglia are probably not transient structures since they are also seen in senescent animals (guinea-pigs over 2 y old). The finding that the varicosities involved in a specialized contact with the glial are not seen to form a conventional axoneuronal synapse (although such occurrence cannot be fully ruled out without an extensive analysis of serial sections) may be interpreted as indicating that these varicosities have failed to establish a proper synaptic connection and 'fall back' on a less specific contact with a glial cell. Rare synaptic junctions between axons and ependymal cells have been found by EBNER & COLONNIER (1975) in the visual cortex of the turtle.

The finding of a characteristic pattern of intramembrane particles in the plasma membrane of the glial cells of the gut provides a useful marker to identify even small areas of glial cells in freeze–fracture preparations (although it cannot yet be stated for certain that it is present in *all* glial cells). The astrocytes of the central nervous system too display a special pattern of intramembrane particles, the 'membrane associated orthogonal particle complexes' (DERMIETZEL, 1974). Four particles of 5 nm dia. are assembled in a square sub-unit and several sub-units may aggregate into large rectangular groups (DERMIETZEL, 1974; LANDIS & REESE, 1974). This pattern is considered a specific marker of astrocytes (SANDRI, VAN BUREN & AKERT, 1977).

Conclusions

In conclusion, the morphological data available from the literature (GABELLA, 1972; COOK & BURNSTOCK, 1976) and those obtained in the present investigation on the mammalian enteric ganglia, clearly indicate that the glial cells found in these ganglia have a number of unique structural features. They represent a distinct group of glial cells, and, until more is known about the variety of glial cells of the central and peripheral nervous system, it may be appropriate to label them enteric glial cells. The term is as non-committal as our current ignorance on these cells requires, but at the same time recognizes a group of cells with unique structural features. These cells are clearly different in some essential aspects from the satellite cells of the autonomic ganglia (TAXI, 1965; MATTHEWS, 1974) and from the Schwann cells of peripheral nerves (autonomic and somatic) (reviewed in LANDON, 1976).

Broadening the comparison, it appears that cells which are more similar in appearance to the enteric glial cells are the astrocytes of the central nervous system. This similarity may be only a superficial one and it should not obscure the important differences

between the glial cells of the two tissues. It is, however, an interesting speculation which may help in the search for the role of these cells. When the notion of a similarity in the basic structural plan of the enteric ganglia and the central nervous system, including certain similarities between enteric glial cells and astrocytes, was formulated (GABELLA, 1972), it was regarded as indicating little more than a curiosity. Investigations carried out in the past few years, particularly on the presence of transmitters and neuroactive substances and on the multiplicity of neuronal types (reviewed in GERSHON *et al.*, 1979; GABELLA, 1979) have brought to light several other features shared by the brain and the enteric ganglia. As regards the glial cells, JESSEN & MIRSKY (1980) have recently shown by immunofluorescence histochemistry that a protein called glial fibrillary acidic protein is present in the glial cells of the rat myenteric plexus. The protein is associated with gliofilaments (intermediate or 10-nm filaments) (SCHACHNER, HEDLEY-WHYTE, HSU, SCHOONMAKER & BIGNAMI [1977]), and it had been up to now considered to be specific of the astrocytes of the central nervous system (BIGNAMI, ENG, DAHL & UYEDA, 1972). Oligodendrocytes, Schwann cells and glial cells of sympathetic ganglia give a negative reaction (RAFF, FIELDS, HAKOMORI, MIRSKY, PRUSS & WINTER, 1979).

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