Naked axons and symmetrical synapses in coelenterates

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With 6 plates (figs. 1 to 6)

Summary

Examination of sections of the marginal ganglion of the jellyfish *Cyanea* and the hydromedusan *Phialidium* by the electron microscope, in a region where nervous tissue is readily identified on account of its abundance, reveals the following features.

Nerve-cell bodies and axons are crowded together without special glial cells. The axons form a layer between the cell-bodies and the mesogloea and the spaces between them are continuous with other intercellular spaces and with the mesogloea.

Features typical of nerve-cells in other animals are mitochondria, Golgi region (= γ-cytomembranes), neurotubules (= canaliculi) about 16 mμ wide, and several types of vesicle ranging in size from 50 to 200 mμ, including synaptic vesicles of 50 to 100 mμ.

Features not typical of nerve-cells are the modified (possibly sensory) cilia on the dendrites of bipolar cells and the absence of clumps of Nissl substance and neurofilaments.

Synapses between axons (or with a perikaryon) have a synaptic cleft of 18 to 22 mμ and a crowded row of synaptic vesicles within the neurones on each side of the synapse.

Introduction

NERVE-FIBRES in Hydromedusae (Hertwig and Hertwig, 1878), in Scyphozoa (Schäfer, 1878), and in anemones (Hertwig and Hertwig, 1879) have long been known from fixed and stained preparations by the use of the light microscope. The distribution and functional morphology of coelenterate nerve-fibres have been more recently studied in considerable detail by selective staining methods for neurones, for example in anemones (Pantin, 1952; Batham, Pantin, and Robson, 1960) and in a jellyfish ganglion (Horridge, 1956). There may be one fairly homogeneous nerve-net, as in hydrozoan polyps, or a single regionally diversified net as in most parts of either of the body-layers of the best-known sea-anemones. In contrast, there may be two, possibly more, overlying and functionally distinct nets, as in parts of the subumbrellar epithelium of a jellyfish, or again there may be concentrated strands of nerve-fibres as in the two ring-nerves of Hydromedusae and in the neighbourhood of the marginal ganglia of jellyfish. Within the strands and ganglia of the two latter groups there is evidence that the neurones are functionally and anatomically of several different types, with at least a small proportion of one-way synapses. Physiologically distinct pathways of transmission have been demonstrated in the ring-nerves of Hydromedusae, where pathways which co-ordinate the tentacle movements round the bell are separate from those which initiate and co-ordinate the swimming rhythm. Similarly, the jellyfish ganglion contains

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physiologically distinct pathways. Two of these can be demonstrated when incoming excitation from the diffuse nerve-net of the bell and tentacles combines with intrinsic sensory excitation of the ganglion itself and initiates, or changes the rate of, the outgoing rhythmical sequence of impulses in the predominantly motor-nerve net which co-ordinates the swimming movements of the bell. Synapses within one nerve-net lie between equivalent fibres which, using a phrase of Romanes (1877, p. 749), 'are capable of vicarious action'. This is the best functional definition of a nerve-net as distinct from a plexus. The synapses, therefore, can be expected to transmit in either direction. However, in the special examples of synapses between fibres where different nerve-nets meet within the ganglion some one-way synapses must be inferred. From old figures by the Hertwig brothers on a variety of species of Hydromedusae, or from more recently acquired knowledge of the special tracts of motor-fibres running from the larval jellyfish ganglion (Horridge, 1956), it appears that a nerve-strand in these animals can be considered as a laterally compressed net with ganglion cells and synapses along its length rather than as a bundle of long axons alone. Neurones come together to form a net either by contact of their processes, as in the mesenteries of sea anemones (Pantin, 1952), or by fusion of processes of neurones to form a syncytium, as in one of the nets of *Porpita* (Mackie, 1960). The nerve-cells may be multipolar, as in hydrozoan polyps and parts of anemones, or they may be long and bipolar as in the rapidly conducting specialized motor-nets of anemone column and jellyfish bell. Most of the sensory cells, especially where they are crowded together as in the jellyfish ganglion, are bipolar cells which lie vertically in the epithelium and have one axon at their base. The axons run in the deeper layers of the epithelium of the ganglion; in the normal epithelial tissue of a coelenterate they make their way between the epithelial cells peripherally to the layer of muscle-fibres.

The fine structure of the nerve-fibres of coelenterates is of particular interest because these are the simplest animals where undoubted nerves are recognizable. Histologically the nerve-cells are distinguishable by their typical form, with axons and arborizations; and by their ability to take up specific stains, such as methylene blue, or be impregnated with silver. Physiologically they have the excitatory properties of nerves (Pantin, 1935) and when active produce an all-or-none action potential which may be recorded in favourable circumstances (Horridge, 1954). In fact they have all the attributes now considered to be characteristic of nerve-cells throughout most of the invertebrate phyla. The neurones are not, however, so clearly divided into categories as are those of higher animals. There is no distinction between peripheral ganglion cells of the nerve-net, inter-neurones and motor-neurones, but several distinct classes of sensory cells are distinguishable, including some within the marginal ganglia of jellyfish. Light-microscope studies have revealed no structures comparable to nerve-sheaths or glial cells nor any cellular nuclei which might be glial. The present examination by the electron microscope shows that in finer details the nerve-fibres in coelenterates also closely resemble the non-myelinated fine nerve-fibres of higher animals except for one feature;

no non-neural elements are specialized as sheath cells, the membranous investment adjacent to the axon membrane being formed by the plasma membranes of any epithelial cells or neighbouring neurones alongside which the axon happens to pass.

Methods

Nervous tissues of coelenterates are convenient for electron microscopy because the cells which concern us are located superficially in the ectoderm. Small pieces of the marginal ganglia of the jellyfish *Cyanea capillata* (North Sea blue variety, specimens about 15 cm in diameter), and of the hydromedusan *Phialidium hemisphericum* (about 1 cm in diameter) were fixed for ½ h in 0·1% osmic acid in sea-water, veronal buffered to pH 7·2, at 0° C. They were dehydrated in graded alcohols and embedded in Araldite. Thin sections cut with a Porter–Blum microtome, stained with lead acetate, were examined in an RCA EMU–3F electron microscope. Regions of the ganglion were identified by observation through the resin and comparison with light microscope sections, together with the construction of an electron-micrograph montage to show considerable areas of the ganglion.

Results

The epithelium of the ganglion, as seen in a montage of adjacent low-power views, is 20 to 100 μ thick, containing several layers of ectodermal oval nuclei each occupying most of the width of a tall columnar cell. The cell membranes are readily distinguishable, with irregular intercellular spaces about 50 mμ wide showing occasional dilations up to 150 mμ and with a few points of apparent contact (fig. 1, A). The nuclei, 4 to 5 μ long and 2 to 4 μ wide, also each have a double membrane which contains irregular long cavities up to 50 mμ wide and which meets at intervals where it is interrupted by pores. The only obvious intranuclear structure is the nucleolus. From their columnar arrangement and position along the sides of the stalk of the ganglion, most of the cells which can be distinguished by their thin layer of perinuclear cytoplasm are evidently the sensory nerve-cells long known from light-microscope studies to be crowded together in this area (Schäfer, 1878). This meagre cytoplasm contains several types of small inclusions, described below. In the outer layer of the ectoderm, comprising about a third of the thickness of this layer, the sensory cell membranes are predominantly oriented at right angles to the surface, and in some sections many of the cells have a peripheral process or dendrite which is frequently crowded with mitochondria. The dendrite extends towards the external surface and bears a cilium. In contrast, the deepest layer of the ectoderm, the inner third, contains tube-like structures which are oriented mainly parallel to the outer surface and to the thin layer of underlying mesogloea. These tubes, from their position, size, structure, and connexions with the sensory cells, we take to be nerve-fibres. Their thickness ranges from 150 to 1,000 mμ; in places they lie so closely stacked side by side that a horizontally oriented layer 5 μ in depth, adjacent to the mesogloea, is formed.

The membranes which define these tubes are apparently similar to those which surround the ectodermal sensory cells, ordinary ectodermal cells, and endodermal cells.

The cytoplasmic inclusions of the perikaryon of the ectodermal sensory cells closely resemble the inclusions of the underlying tubes. This fact contributes to the evidence that the tubes are nerve-fibres and that at least in parts of a ganglion they are mainly the axons of the sensory cells. Certain inclusions of the sensory cells are readily identifiable as mitochondria, Golgi complex (considered equivalent to γ-cytomembranes and to dictyosomes, see below), two types of vesicles, and several obscure bodies of unknown relationship. An endoplasmic reticulum is not obvious in the cytoplasm around the neuronal nucleus although strands, dense accumulations, and tightly spiralled whirls of appearance similar to endoplasmic reticulum are apparent here and there in the sections. The cytoplasm of the tubes in *Cyanea* but not in *Phialidium* is, however, readily distinguished by the presence of strands 10 to 20 mμ wide, described below. From their appearance and their position these strands will hereafter be called neurotubules. They closely resemble neurotubules(=canaliculi) which have been distinguished from axoplasmic filaments in some vertebrate neurones where the two types of cytoplasmic strand occur together (Rosenbluth and Palay, 1961). Neurotubules occur in axons from a wide range of animals, e.g. leeches (Couteaux, 1956), insects (Trujillo-Cenóz, 1959), and polychaetes (own unpublished observations).

Mitochondria of the sensory cells and of the nerve-fibres are usually slightly elongated, 400 to 1,000 mμ long and 200 to 400 mμ broad. They are most abundant in the peripheral processes of the sensory cells near the surface. They occur at irregular intervals in the nerve-fibres, even in the thinnest where they cause a bulge in the walls. The cisternae are notably irregular, especially in Hydromedusae, when compared with mitochondria of more familiar preparations from higher animals.

A Golgi complex can be found in many of the cells and probably occurs in all. In both ectodermal and endodermal cells at least one of these structures lies on the side of the nucleus which is directed away from the mesogloea. Similar structures are found in many other animals, appearing as a compact group of membranes of agranular endoplasmic reticulum associated with vesicles. In their studies of the lipidal inclusions which stain with neutral red in the

FIG. 1 (plate). A, *Cyanea*. The outer region of the ciliated sensory epithelium of the ganglion showing the main inclusions of a sensory neurone. Small vesicles are scattered through the cytoplasm. A structure (*x*) which we interpret as being the axon of another neurone can be seen in the cytoplasm.

B, *Cyanea*. Longitudinal section through several axons and through an axon hillock to show the smaller inclusions in the cytoplasm of these structures. Two axons run up and down to the left of centre; an axon hillock of another neurone lies to their right, and adjacent to another axon. The axons have neurotubules sparsely scattered in the axoplasm.

a, type-A vesicle; *bl*, large type-B vesicles; *bs*, small type-B vesicles; *cm*, cell membrane; *g*, Golgi complex (γ-cytomembrane or agranular reticulum); *l*, electron-dense bodies, probably lipid; *m*, mitochondria, with irregular cristae; *n*, nucleus; *r*, root of the cilium; *t*, neurotubules; *x*, axon.

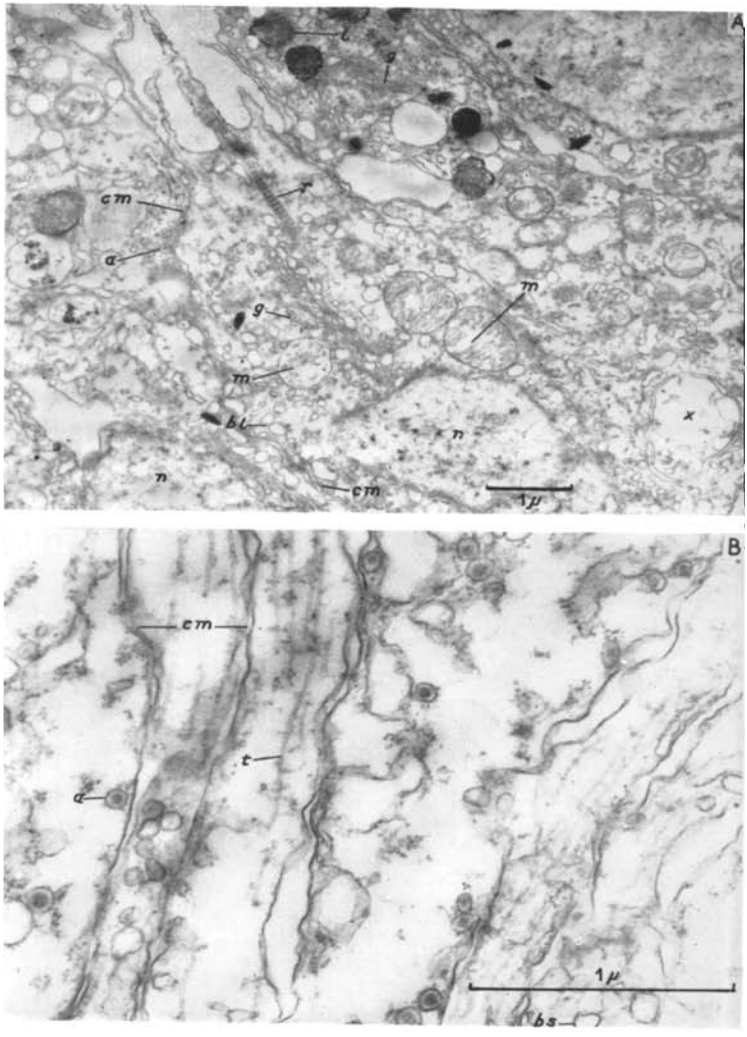

FIG. 1

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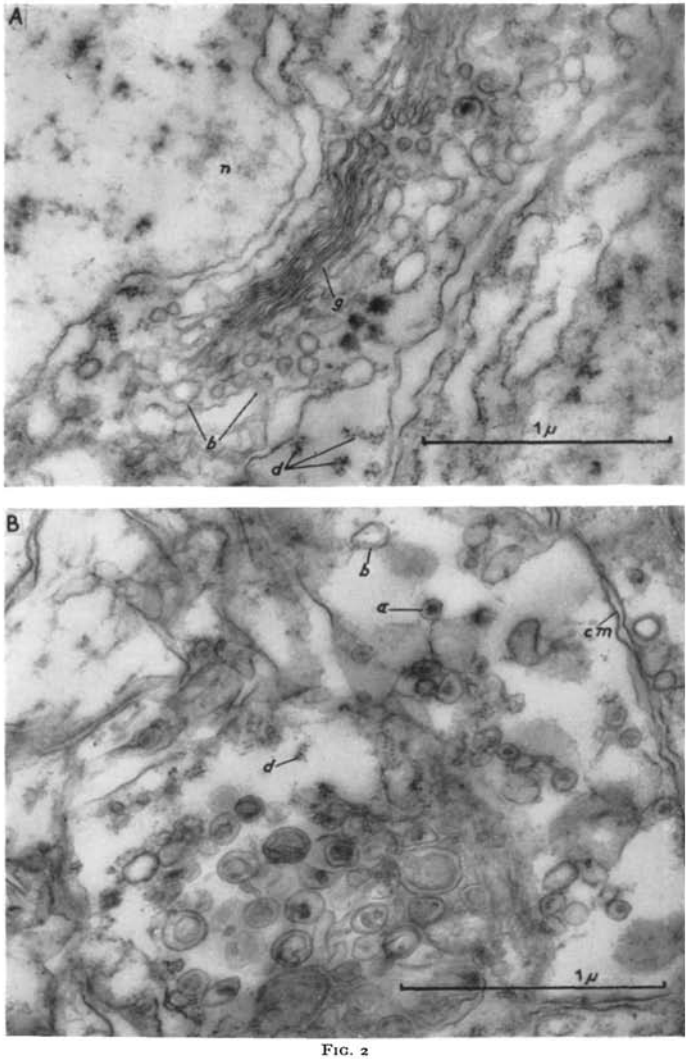

FIG. 2

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living neurones of the snail *Helix* and the prawn *Leander*, workers of the Oxford school have come to the conclusion that certain rounded lipidal bodies in the cytoplasm are surrounded by membranes which appear in flattened layers when fixed with osmic acid. When stained by classical silver methods and examined with the light microscope such groups of γ-cytomembranes may form the crescentic or rod-shaped dictyosomes which appear numerous in vertebrate neurones examined by such methods. As seen under the electron microscope the same structures in a variety of animals are revealed as groups of parallel membranes associated with vesicles (Lacy, 1957; Malhotra and Meek, 1960). In the jellyfish neurones the Golgi complex is an exactly comparable structure, fig. 2, A, consisting of 8 to 20 pairs of parallel membranes 20 to 30 mμ apart. The two members of each pair of membranes join at their edges and form inflated loops. In many of the nerve-cells, the nearby regions of the cytoplasm are crowded with hollow vesicles which appear to have been formed and released from these membrane loops. Possibly they are sections of tubular extensions of the folded membranes. Evidently the membranes of the Golgi complex are not remnants which once surrounded lipidal droplets since the membranes are not broken at their ends. In coelenterate neurones as in other examples of the Golgi complex, e.g. in plant cells (Mollenhauer and others, 1961), there is sufficient resemblance between the membranes of the swollen vesicular ends and those of the cytoplasmic and synaptic vesicles (which are typical of many phyla) for one to have confidence that these structures are preserved faithfully by the osmic fixative.

Vesicles are abundant but not crowded within the perikaryon of the sensory neurones and their axons. Similar vesicles are also found in other cells which cannot for certain be identified as nervous, so that vesicles are not necessarily diagnostic for neurones. The vesicles are varied in form; some, called type A, are distinguished from the others by a small spot, occupying about half of the cross-sectional area of the central region. This type is usually more round than oval and is of rather uniform size, 70 to 100 mμ in diameter. Other vesicles, type B, of a wide range of sizes, and more irregular and sometimes oval in shape, have no electron-dense contents. There is some evidence, drawn from their concentration at synapses, that the smaller type-B vesicles are in reality distinct from the larger ones, which are not so concentrated. This is illustrated in fig. 4, A where the large type-B vesicles are clearly different, both in size and distribution, from the small type-B vesicles which may be found mixed with type-A vesicles close to the synaptic cleft. Both small and large type-B vesicles are found associated with the Golgi complex of cytomembranes (fig. 2, A).

The larger, irregular type-B vesicles commonly measure 150 to 200 mμ in

FIG. 2 (plate). A, *Cyanea*. Golgi region of membranes with looped ends and vesicles, lying adjacent to the nucleus in a sensory cell-body.

B, *Cyanea*. Illustration of the diversity of vesicles within the cytoplasm of one cell.

a, type-A vesicles; *b*, type-B vesicles; *cm*, cell membrane; *d*, granular dots; *g*, Golgi complex; *n*, nucleus.

diameter; the smaller ones at synapses are relatively uniform, 70 to 100 mμ in diameter: vesicles over the whole size-range can be found scattered in the cytoplasm. Some cells and axons contain all types of vesicle, others have mainly one type. Some sections, especially some found by Chapman in the ganglia of the jellyfish *Aurelia*, show apparently hollow vesicles with the membrane on one side as if in various stages of invagination (Horridge, Chapman, and Mackay, 1962). Others (fig. 2, B) from *Cyanea* show intermediate forms between an internal membrane of horse-shoe shape and a solid electron-dense central spot. Taken together, the evidence strongly suggests that the spot in type-A vesicles is formed by invagination of the wall of the simple type-B vesicles. The distribution of the different types of vesicle in the axons is also compatible with this suggestion; type A are more abundant in the axons and type B in the cell-body, especially where they cluster round the Golgi complex.

Neurotubules are distinguishing features of the axons because they are consistently found in the latter in *Cyanea* and their presence contributes to the identification of small lengths of axons which are separated in the sections. These strands resemble the neurotubules (but not the axoplasmic filaments) already described in other nerve-cells, e.g. in the 8th nerve of the Goldfish (Rosenbluth and Palay, 1961). They are 10 to 20 mμ in breadth and very uniform in thickness, electron density and appearance (figs. 1, B; 3, C). Lengths up to 5μ can be seen in the larger fibres. They are less electron dense than the membranes of the axons, perikarya, or of the vesicles or nuclei which these contain, and of different appearance from the γ-cytomembranes. They are not found within the cell-body. A narrow axon of 150 to 400 mμ commonly contains 2 to 6 neurotubules but an axon of 1 μ may contain up to 20, regularly separated by spaces of 40 to 80 mμ. In the spaces between these filaments there are frequently rows of vesicles which appear to have been lined up by the mechanical effect of the neurotubules, to which they appear not to adhere. The neurotubules have so far not been encountered in the hydromedusan studied (*Phialidium*) although the tissues had similar treatment. Possibly associated with this lack of supporting strands, axons in *Phialidium* have much more wavy outlines than those in the jellyfish which were fixed and dehydrated at the same time.

The relationships between the axons are difficult to elucidate because they are seen only in section. In places they are crowded together (fig. 1, B), with irregular spaces between them. Such regions are adjacent to the mesogloea and the spaces between the axons are continuous with the mesogloeal space, just as are the narrow spaces between the ordinary epithelial cells. Evidently, therefore, the axons form a layer beneath the epithelium and between the epithelial cells, and they occupy spaces which are extracellular. In the

FIG. 3 (plate). *Cyanea*. A, synapse on the side of a cell-body.
B, symmetrical synapse in which most of the vesicles are of type A.
C, two nerve-fibres, one containing a concentration of vesicles; this photograph illustrates the difficulty in identifying a synapse when vesicles are present on only one side.
a, type-A vesicles; *b*, type-B vesicles; *cm*, cell membrane; *s*, synaptic cleft; *t*, neurotubules.

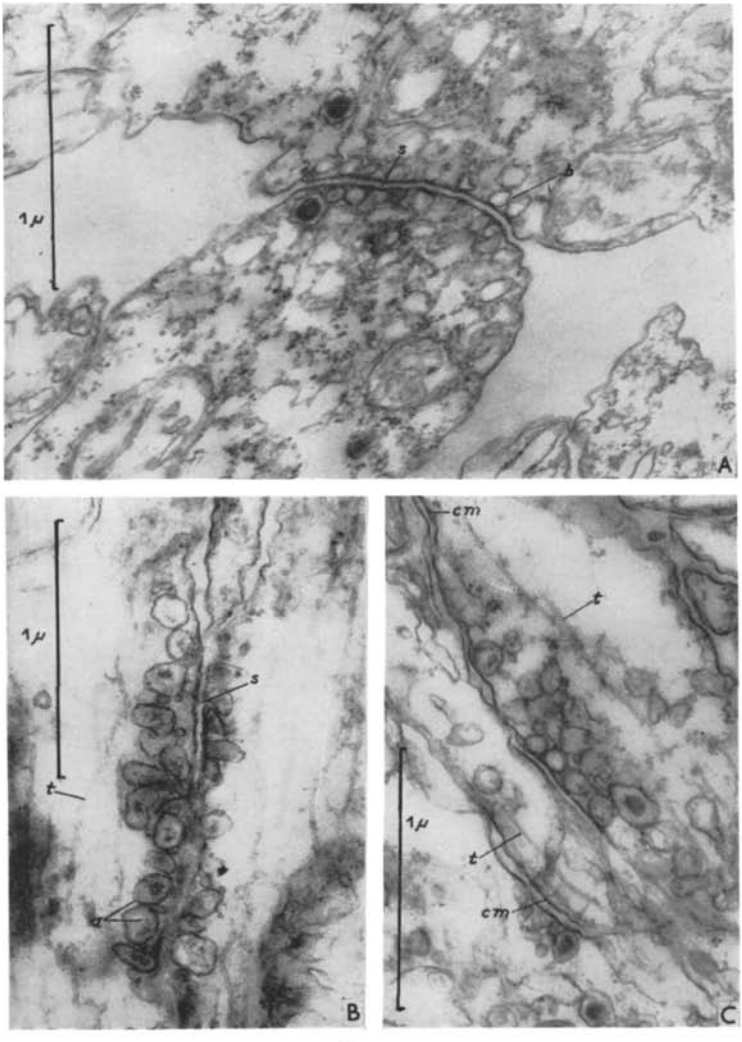

FIG. 3

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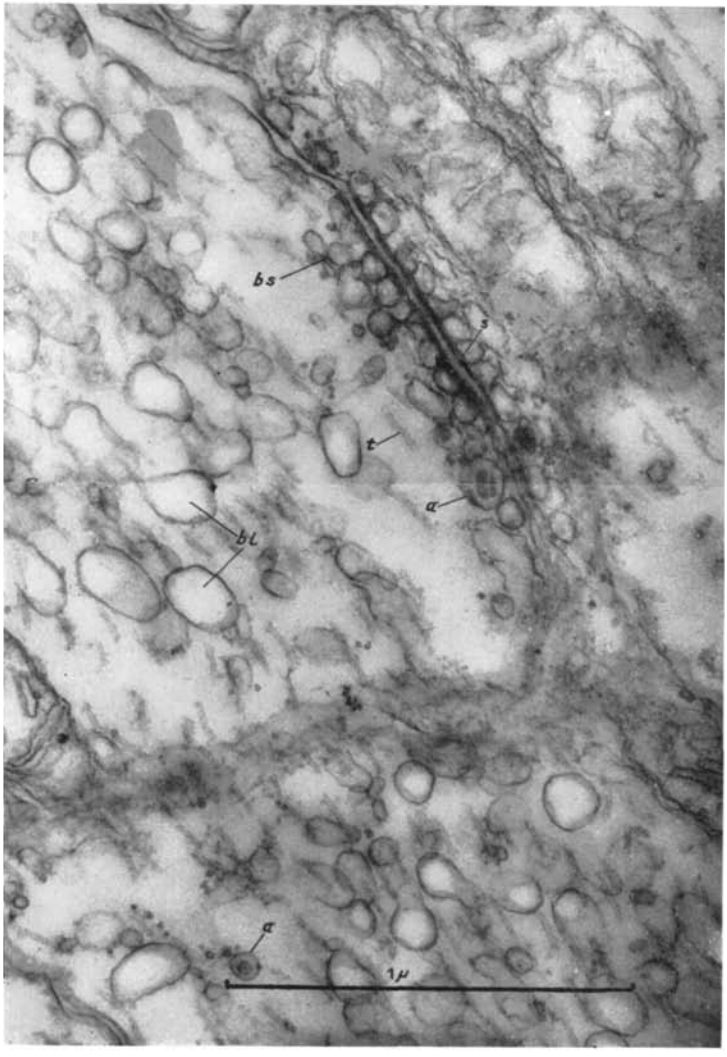

FIG. 4

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scyphozoan ganglion no axons have been found running from the epithelium through the mesogloea, although in Hydromedusae this has been seen in light-microscope preparations (Hertwig and Hertwig, 1878). In some regions the axons send off small side branches. Although the axons do not have any sheath or membrane in addition to their own membrane, they may push their way through other cells. In longitudinal section of an axon, a branch can sometimes be seen as a finger-like protrusion, which occupies a corresponding deep indentation or tunnel in the cytoplasm of another cell. Transverse sections of these tunnels are not easily identified but can be found by looking for double membranes surrounding cavities of the appropriate size (figs. 1, A; 5, A). The inner membrane belongs to the axon, the outer to the epithelial or sensory cell; the space between is extracellular. The axons lie mainly between epithelial cells but even so they are completely surrounded by their plasma membranes. Evidently, therefore, while the axons have no proper sheath they may be wrapped round by a single membrane of another cell which is itself specialized in other ways and which is not primarily a neuroglial cell. The relationships between crowded nerve-fibres and cells of the ganglion can be tentatively considered to conform to the situation in the animal as a whole since sheath-cell nuclei have never been found in peripheral nerve-nets. We then have the unique situation that there is no special class of sheath-cells which spread along the axons. Therefore neither transmission of nerve-impulses nor nutrition of nerve-cells depends upon glial tissue.

Evidently the situation is not so simple in some Hydromedusae. In *Amphinema*, Chapman (private communication) finds two or three layers of membranes surrounding an occasional axon of the ring-nerves. In *Phialidium* we find structures which may be axons surrounded by or containing two or three lamellae, but these are concentrically, not spirally arranged (fig. 6, C). In *Phialidium*, cells which lie within the nerve-ring have large irregular-shaped nuclei and cytoplasm which ramifies among surrounding nerve-fibres. The fibres themselves are sometimes grouped in bundles surrounded by the single invaginated membrane of a neighbouring cell (fig. 6, A, B). We have no reason to suppose that the large cells would fit a definition of glial cells; they do not appear to creep along the nerve-fibres in longitudinal section. They may be nerve-cell bodies which occur along the ring-nerves of Hydromedusae (Hertwig and Hertwig, 1878). The cytoplasmic contents of these cells are similar to those of the nerve-cells; vesicles in them resemble those in neighbouring axons (fig. 6, A, B). Definitive nerves appear for the first time in the animal kingdom as the marginal ring-nerves in Hydromedusae. Perhaps it will be possible in other members of this group to trace the earliest stages of the formation of the simplest sheaths and bundles.

FIG. 4 (plate). *Cyanea*. Synapse between two axons which are identified by their neurotubules. Three different types of vesicles are distinguishable in the large axon which occupies the centre of the figure.

a, type-A vesicles; *bl*, large type-B vesicles; *bs*, small type-B vesicles; *s*, synaptic cleft; *t*, neurotubules.

Synapses between axons have frequently been encountered in *Cyanea*; they are characterized by the following combination of features. The apposed membranes of the two axons or cell-body display an increased electron density, are alined parallel to one another, separated by a synaptic cleft of 18 to 22 mμ, and vesicles are lined up close to the membrane on each side of the synapse. The synaptic vesicles are predominantly but not invariably of type A with an electron-dense central spot (figs. 3, A, B; 4): they are mainly 50 to 100 mμ across, and are smaller than type-B vesicles of the axoplasm (fig. 4). No other structures are associated with the synapse. No asymmetrical synapses have so far been identified, perhaps because they would be more difficult to characterize (fig. 3, C). The material within the synaptic cleft is slightly more dense than the background. The dimensions imply that each synaptic membrane must have 500 to 2,000 vesicles. Synapses have not so far been discerned in *Phialidium*.

Discussion

In several respects these findings touch upon previous work and are relevant to the comparative study of nerve-cells.

First, the neurones in certain coelenterates have all the features of neurones of higher animals except that sheath-cells are absent. In fact, they behave physiologically like typical neurones, and therefore sheath-cells are not always essential for the electricity activity or the nutrition of nerve-cells. The coelenterates are thus the first phylum to be found without a definite glial tissue which creeps along the nerve-cells and their processes. Such axons, however, are not any less naked than are many of the thinner fibres in higher animals. Primary olfactory axons of vertebrates are crowded in groups which run within a fold in the membrane of a glial cell, but in any one cross-section many of the axons touch only neighbouring axons. These axons are still surrounded by spaces which are morphologically extracellular (Gasser, 1958). In *Cyanea* the axons in the ganglion have other axons, nerve-cell bodies or ordinary epithelial cells as neighbours. Some of them run, perhaps by chance, through an infolding in an epithelial cell. Elsewhere, in the muscle-sheet of the subumbrellar surface, and in sea anemones, the axons of the nerve-net run between the epithelial cells, which thus take the place of glial cells. Epithelial cells in a variety of tissues of other animals act in a similar way as supports for naked sensory terminations, e.g. trichogen cells in insect sensilla (Slifer, 1961) or as supporting cells for vertebrate olfactory mucosa (de Lorenzo, 1957).

The absence of glial cells suggests that this special class of cell is also not essential for transmission of excitation in these forms. On account of their

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FIG. 5 (plate). A, *Phialidium*. Large and small nerve-fibres containing granular substance and small vesicles. The nature of the fuzzy patch marked *y* is not known, but it appears to be different from the muscle-fibrils. No neurotubules have been found in *Phialidium* axons.

B, *Phialidium*. Two nerve-fibres, n_1 and n_2, running between mesogloea and a myoepithelial cell, one, n_1, containing a large mitochondrion.

cm, cell membrane; *f*, muscle-fibrils; *m*, mitochondrion; n_1 and n_2, nerve-fibres; *x*, axon in section; *y*, patch of unknown nature.

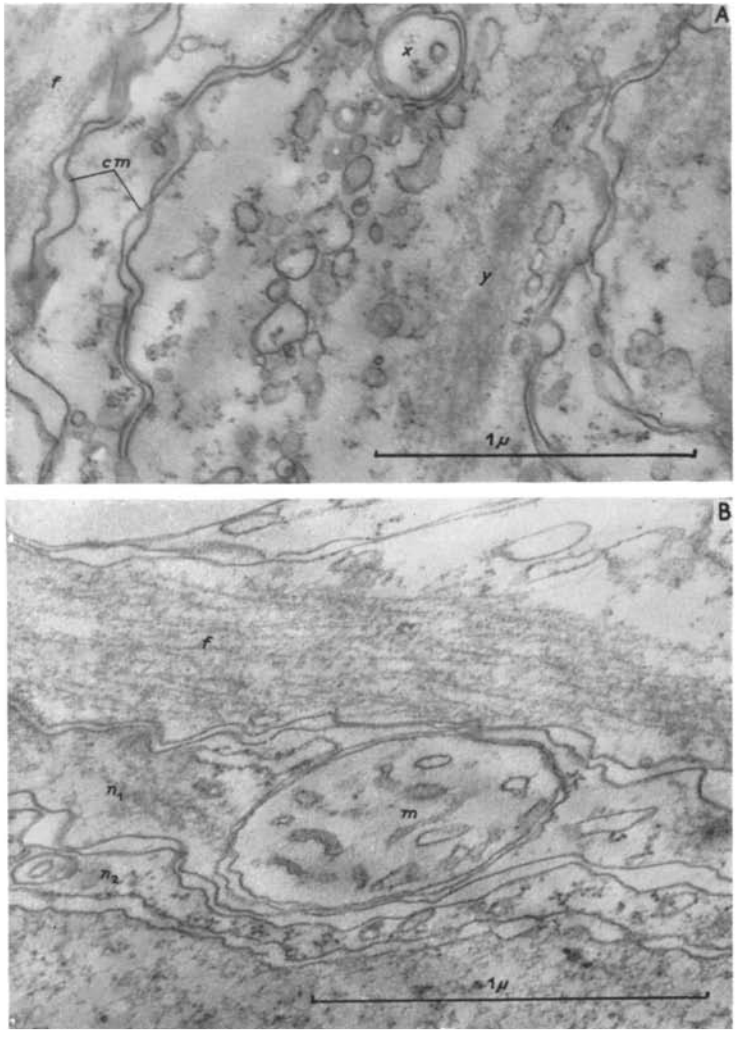

FIG. 5

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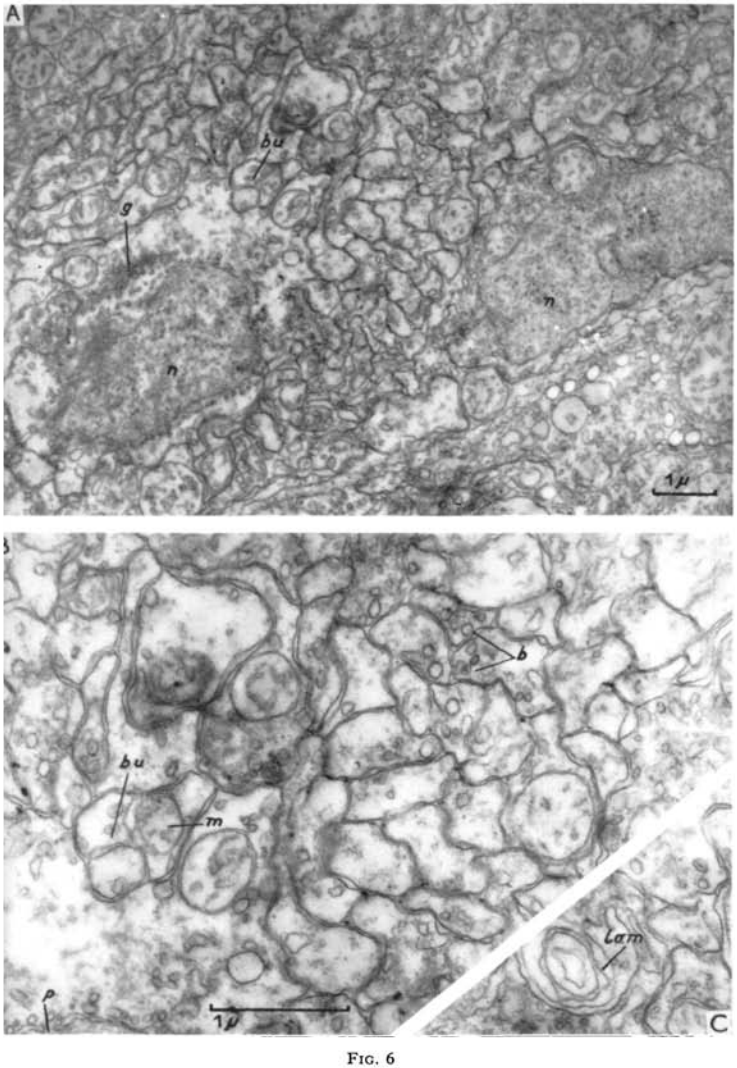

FIG. 6

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small size, little is known of the properties of the membranes of coelenterate neurones. They can be excited by electrical and other typical stimuli but have a long refractory period, up to 70 msec. Available detailed knowledge of the effects of increase and reduction of ionic concentrations on transmission and the initiation of spontaneous impulses in jellyfish nerves (Mayer, 1906; Bethe, 1908; Horridge, 1956) is compatible with the view that they operate by the ionic mechanism commonly accepted for all nerves which have been closely investigated. However, the nervous system in coelenterates differs from that in higher animals in that axons in a nerve-net can act vicariously for each other, i.e. within a net all are equivalent and there is no evidence to suggest that closely neighbouring neurones necessarily keep their excitation to themselves, except, perhaps, in the ring-nerves of Hydromedusae. It is in fact in Hydromedusae that folded lamellae and bundles of axons first appear. There may therefore be a functional consequence of the lack of glial cells in that 'accidental' lateral interaction of an ephaptic nature between crowded naked axons is not a disadvantage. In Hydromedusae, where there are distinct physiological pathways round the bell, there are at least two separated tracts, one associated with sensory excitation from the tentacles and the other having connexions with the subumbrellar motor system.

The lack of glial cells in jellyfish also suggests that the neurones can supply their own nutritive requirements. In some higher animals there is evidence that glial cells have this essential function, especially in ganglia, as in those of insects (Wigglesworth, 1959) or the lamprey (Schultz and others, 1956) which have no intramedullary blood-vessels. In coelenterates, however, passing of food between cells may be common, and the neurones may well participate, so that no new principles need be invoked.

The symmetrical synapses are presumably between axons which together constitute one nerve-net, or at least one system of fibres. They are likely to be mostly between axons of the sensory cells since these are the most abundant axons in the ganglion. The ganglion also contains some axons, but few cell-bodies, of the motor-net which co-ordinates the swimming movement. The sensory or diffuse net acts on the motor-net at junctions which can be shown by physiological experiments to transmit excitation in only one direction (Horridge, 1956). It is likely that such physiologically polarized synapses would be morphologically asymmetrical but that the symmetrical ones so far seen transmit in either direction. Neurones of the sensory net show no evidence from their fine structure of being pre- or post-synaptic at synapses. Although allowing a summation of excitation from different types of sensory cells, such a simple pattern of connexions implies a fundamental limitation in

FIG. 6 (plate). A, *Phialidium*, low-power view of a transverse section of the ring-nerve showing two large nuclei and between them many axons, some of which are arranged in groups as if in distinct bundles.

B, An enlarged view of the central part of A.

C, Part of the same material at the same scale, with a concentric arrangement of membranes.

b, type-B vesicles; *bu*, bundle of axons; *g*, Golgi complex; *lam*, lamellae with concentric arrangement; *p*, pore in nuclear membrane; *n*, nucleus.

the discrimination between sensory excitation of different kinds even in the ganglion.

Synapses so far described with vesicles on both sides have been shown to be electrically transmitting, i.e. the presynaptic action current is large enough to excite the postsynaptic fibre directly. Examples are the septate synapses in the earthworm and crayfish giant fibres, synapses between giant fibres and large motor axons in the crayfish (Hama, 1961); of these, each except the last mentioned transmit in either direction. Although a delayed chemical transmission cannot be ruled out, the electrical transmission throws into doubt the possible function of synaptic vesicles as agents of the most rapid transmission of excitation across these synapses, and suggests that the vesicles have an additional function, possibly a trophic one in maintaining the existence of the synapses.

The synaptic cleft in electrically transmitting axons is narrower than in normal axons, being about 10 mμ and down to 7 mμ wide in the earthworm septate synapses (Hama, 1961). Chemically transmitting synapses usually have a cleft of about 30 mμ (Bullock and Horridge, 1962): the 20 mμ cleft in *Cyanea* falls clearly into neither group. Although no examples of chemically transmitting synapses are already known with vesicles on both sides, the present data on the *Cyanea* synapses allows no conclusions as to the nature of the transmission. At present there is no direct evidence of the nature of the transmission in small axon-axon synapses in any of the lower animals.

The variety and size of vesicles within the axons are similar to some of the inclusions found in neurosecretory cells and the association of the coelenterate vesicles with the γ-cytomembranes is similar to that found by Bern and others (1961) in neurosecretory cells of a leech. The coelenterates are one of the few groups of animals where neurosecretion has so far not been reported histologically although physiological evidence comes from the old result that removal of the marginal ganglia reduces the rate of regeneration of other parts of the bell (Cary, 1917). In a cursory examination by the standard Gomori methods, particles which stain like neurosecretory products can be seen in the light microscope but they are too small for any valid inferences to be based upon them.

The diversity of nervous systems in coelenterates lies in the arrangement of the neurones in various patterns of nets and ganglia, while the cytological details of the cells themselves is rather constant, as has long been known from light-microscope studies. One can justifiably suppose, therefore, that the features outlined above are representative of the phylum. If this is so, the figures given here may be of value as an aid to the identification of nervous tissue under the electron microscope wherever it occurs in a less concentrated form in a peripheral nerve-net.

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