Moffett, A. A. (1932). ' J. Genet.,' vol. **25**, p. 315.
Nawaschin, S. G. (1912). ' Bull. Acad. Sci. St.-Pétersb.,' vol. **6**, p. 373.
—— (1927). ' Ber. deuts. bot. Ges.,' vol. **45**, p. 415.
Newton, W. C. F., and Darlington, C. D. (1929). ' J. Genet.,' vol. **21**, p. 1.
—— (1930). ' J. Genet.,' vol. **22**, p. 1.
Richardson, M. M. (1936). ' J. Genet.,' vol. **32**, p. 411.
Schrader, F. (1932). ' Z. wiss. Zool.,' vol. **142**, p. 520.
Schrader, S. H. (1931). ' Z. Zellforsch.,' vol. **13**, p. 743.
Smith, F. H. (1935). ' Cytologia,' vol. **6**, p. 165.
Trankowsky, D. A. (1930). ' Z. Zellforsch.,' vol. **10**, p. 736.
Turrill, W. B. (1932). ' R.H.S. Lily Year Book, 1932,' p. 78.
Upcott, M. B. (1936). ' Cytologia,' vol. **7**, p. 118.

611 . 018 . 83 : 595 . 3
611 . 018 . 83 : 594 . 5

The Structure of Nerve Fibres in Cephalopods and Crustacea

By J. Z. Young, Department of Zoology and Comparative Anatomy and Magdalen College, Oxford

(*Communicated by Sir Henry Dale, F.R.S.—Received* 20 *May*, 1936)

[Plates 14, 15]

Introduction and Methods

The nerves of Crustacea have been used for many years for physiological and biochemical investigations, but the value of the results obtained has been seriously reduced by the absence of reliable data as to their histological structure. The present paper attempts to describe the axons and their sheaths not only in a Crustacean, *Maia*, but also in Cephalopods, which may prove to be very suitable material for the investigation of the properties of non-medullated nerve, and in which the giant nerve fibres provide exceptional opportunities for study of the axon.

Most of the observations were made with *Maia* and *Sepia*, which were obtained from the laboratories of the Marine Biological Association at Plymouth, and my thanks are due to Dr. E. J. Allen and his staff for their assistance. The histological work was done in the Department of Zoology and Comparative Anatomy at Oxford, and I am most grateful to Professor E. S. Goodrich for his advice during the course of the work.

I have also to thank Professor A. V. Hill for suggestions as to the types of histological data required.

The nerves of *Sepia* which are most convenient for study are those running to the mantle, shown diagrammatically in fig. 1. To prepare them the animal is killed by decapitation and laid on its back. The mantle is opened by a midventral cut and the fin nerve on each side is then seen running past the stellate ganglion, whence it can easily be dissected through the muscles on to the back. In this way a stretch of nerve more than 10 cm. in length can be obtained from a large *Sepia*.

In fig. 1 are shown on the right the connexions of the giant fibres, on the left those of the smaller neurons. The main nerve leaving the C.N.S. for the mantle is the mantle connective or pallial nerve (*m.c.*). This gives off first a medial branch to the retractor muscle of the head (*n. retr. cap.*), then the large fin nerve (*fin. n.*); finally it runs into the stellate ganglion (*st. gn.*), from which stellar nerves (*st. n.*) radiate to the mantle muscles (*mant. musc.*). Afferent fibres (*aff.*), having their cell bodies at the periphery, run both in the fin nerve and stellar nerves, from which they pass through into the pallial nerve, probably without synapse in the ganglion. The efferent path of all fibres to the mantle muscles contains a synapse in the stellate ganglion, but the fibres to the chromatophores (*cr.*) run through the ganglion without synapse. For further details *see* Sereni and Young (1932) and the literature there quoted.

For histological study all nerves were removed from the animals a few minutes after death, and were fixed stretched at approximately their natural length on pieces of card or tied to capillary tubing. The methods used for fixation and staining are described in the text. The best results were obtained by dissolving the fixing substances in sea water (*see* Young, 1935, *a*).

The Sheaths of the Nerve Fibres of Cephalopods

The sheaths surrounding the axons in the peripheral nerves of Cephalopods have already been described by Sereni and Young (1932) as composed of continuous sheets, containing nuclei, and apparently consisting of connective tissue. In that paper the axons were described as being sometimes much smaller than the connective tissue tubes in which they run. This appearance is now known to be an artefact. In a properly fixed Cephalopod nerve each axon, large or small, entirely fills its sheath, and that this is the normal condition in all states of extension of the nerve can be verified by observation of unfixed fibres examined in sea water. Under such conditions, no free space can be seen between the outer surface of the axon and the inner surface of the sheath. Nuclei of the

sheath, lying in its very innermost layers, may indent the surface of the axon, and at such points it can be seen most clearly, both in living nerves

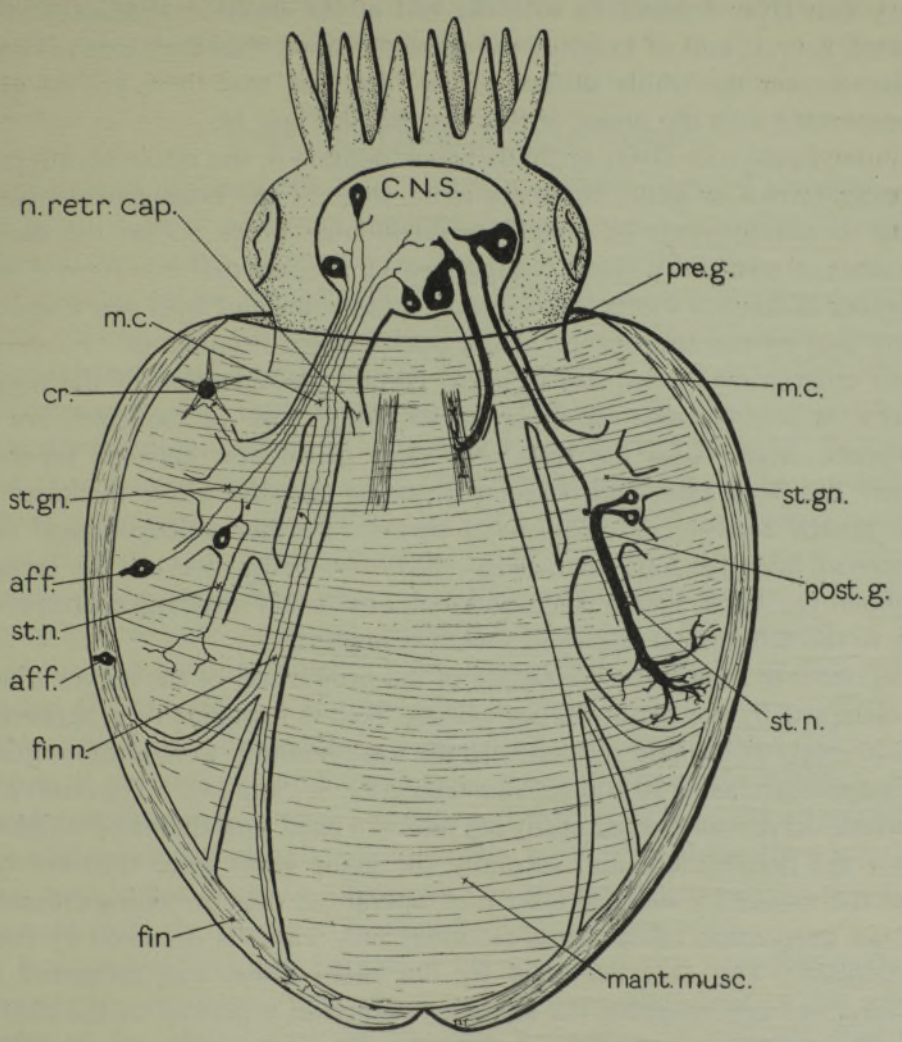


FIG. 1—Diagram of the innervation of the mantle in *Sepia officinalis*. The details of the connexions of the giant fibres are not known; *pre. g.*, represents the " pre-ganglionic " fibres, which arise in the palliovisceral ganglion; *post. g.*, the " post-ganglionic " fibres, which take origin from the fusion of the processes of several cells. For further explanation *see* text.

and suitably fixed preparations, that there is no trace of any intervening fatty sheath (figs. 5, 6, 7, Plate 14).* Indeed, these innermost sheath nuclei

* Since the MS. of this paper was written, it has been shown by the use of polarized light that a thin layer of oriented fatty molecules is present *outside* the layer whose nuclei indent the axon (Bear, Schmitt, and Young, *unpublished*).

sometimes lie so close to the axon as to appear to be part of the latter. Careful observation will show, however, that they are surrounded by a very thin layer continuous with the rest of the sheath. Examination of teased nerves, and of longitudinal sections shows that the sheath is continuous over the whole surface of the fibre and that there are no gaps comparable with the nodes of Ranvier (fig. 9, Plate 14).

After fixation in OsO_4 or fluids which contain it, the whole sheath may appear brown or grey, but there is no trace of the homogeneous black ring so characteristic of vertebrate medullated fibres. That the sheath consists of connective tissue is suggested by the fact that it is formed of a number of fibrous sheets staining with anilin blue and with van Gieson's stain and by the appearance of the nuclei which it contains. However, such criteria are inadequate to allow certain identification of the tissue, and it is possible that the inner nuclei, lying close to the axon, are of different origin from the rest. No clear distinction between separate layers has been seen, and it seems probable that the whole thickness of the sheath consists of collagenous sheets. Further embryological and chemical investigations are needed, however, before we can be certain whether the whole sheath is mesodermal, or whether anything corresponding to the ectodermal Schwann sheath is present.

In order to obtain some estimate of the proportion of the nerves which is occupied by the axons, stellar nerves, fixed in formaldehyde, sectioned transversely at 3 μ and stained with the azan method, were photographed at magnifications of 90 and 660 diameters (fig. 8, Plate 14). The outline of a whole nerve was then carefully cut out on a print and the piece weighed. First the perineurium and secondly the giant fibres were then cut out and the remainder weighed after each operation, thus providing estimates of the proportion of the cross-sectional area which is occupied by these structures. The area occupied by the small fibres was estimated by cutting out and weighing the axons contained in a portion of the photograph magnified to 660 diameters.

By this method the perineurium was found to comprise 8% of the cross-sectional area of a larger stellar nerve and 12% of that of a smaller one, while the giant axons made up 13% of the former and 12% of the latter nerve. The smaller axons occupied 74% of the area examined, which was chosen so as not to include any perineurium or giant fibres.

No doubt the density with which the axons are packed varies in different areas of any one nerve and between different nerves, but since the fin nerve is very similar to the small-fibre portion of the stellar nerves it is probable that about 65% of the cross-sectional area of the fin nerve is occupied by axoplasm, 10% by the perineurium, and the remaining 25% by other

tissues, including the sheaths of the axons, the grosser bundles of connective tissue, the blood vessels and spaces containing tissue lymph. In the stellar nerves, on account of the presence of the giant fibres, the proportion of axoplasm is somewhat greater.

Sizes and Frequencies of Fibres

The axons of *Sepia officinalis* vary from minute fibres of less than 1 μ diameter to giant axons as much as 200 μ across. In *Loligo* the giant axons in the stellar nerve may be nearly 1 mm. in diameter.

Estimates of the numbers of fibres of the various sizes in the nerves were obtained by photographing transverse sections of nerves, fixed in formaldehyde and stained by the azan technique, at a magnification of 200 diameters, and then measuring and counting the fibres on the prints. The diameters measured were those of the connective tissue tubes, not of the axons, since these latter are often much shrunken. The number of fibres in the parts of the pallial nerve is so great that a sampling method was adopted, four rectangles being taken at right angles, each along a radius of the nerve from near the centre to the perineurium. The fibres in each area were measured, counted, and grouped by intervals of 5 μ, and the four frequency distributions thus found compared by means of a χ^2 test (Fisher, 1930). The test showed that the fin nerve is sufficiently homogeneous for this method of sampling to be appropriate, but for the nerve to the stellate ganglion the differences between the different rectangles are greater than would be expected from errors of random sampling. The difference, however, lies mainly in the proportion of the fibres less than 5 μ, and is not likely to affect any conclusions which can be drawn at present from the shape of the size-frequency distribution.

Estimates of the total numbers of fibres in the nerves were then made by discovering the proportion which the areas counted bear to the whole areas of the nerves, and the standard deviations of the means of the numbers in the four samples of each nerve calculated, in order to give some measure of the accuracy of the estimates. Two fin nerves were treated in this way and the numbers of fibres agreed within the limits of accuracy of the method.

In the nerve to the stellate ganglion there is a bundle of giant axons segregated by themselves in a corner of the nerve. Since this bundle contains only very few smaller fibres it was treated separately from the rest of the nerve, its fibres being measured and counted individually. For comparison this giant fibre bundle was counted in two separate nerves.

TABLE I—COMPOSITION OF NERVES OF *Sepia officinalis*

Nerve	Total fibres		< 5 μ	10 μ	15 μ	20 μ	25 μ	30 μ	35 μ	40 μ	> 45 μ
N. pinnae (*Sepia* DI)	7409 ± 855	%	42·3	23·0	14·8	9·5	7·0	2·2	1·2		
n. pinnae (*Sepia* GI)	6080 ± 456	%	38·7	27·8	20·4	10·5	2·6				
n. to *st.* ganglion (excl. giant fibres)	8500 ± 556	No.	5645	1547	629	280	212	68	51	59	9
Giant fibres	48	No.					9	9	3	2	25
Whole nerve (*Sepia* DI) ..		%	66·0	18·1	7·4	3·3	2·6	0·9	0·6	0·7	0·4
Giant fibres (*Sepia* GI) ..	47	No.					11	9	6	7	14
Stellar nerve	338	No.	162	145	28	1			1		1
		%	47·9	42·9	8·3	0·3			0·3		0·3

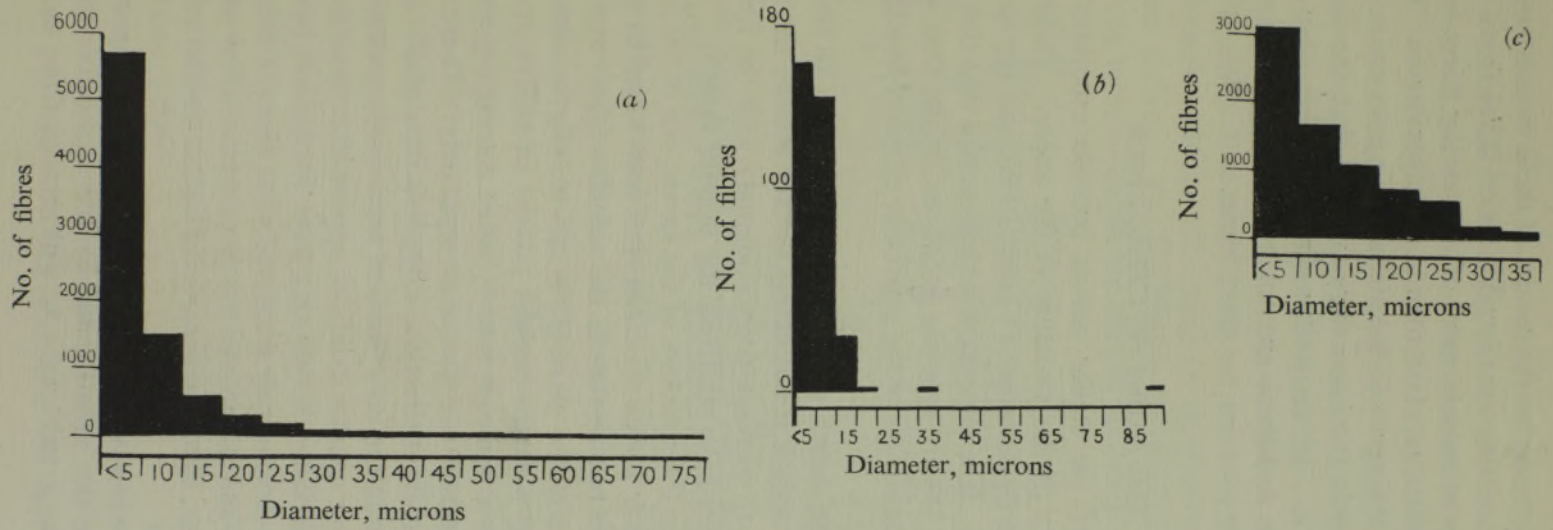


FIG. 2—Sizes and frequencies of fibres in some nerves of *Sepia officinalis*. Measurements made on material fixed in formaldehyde and embedded in paraffin, in life all the fibres would be somewhat larger. (*a*) Nerve to stellate ganglion; (*b*) stellar nerve; (*c*) fin nerve.

Table I and fig. 2 summarize the results of these measurements. In all the nerves there is a continuous distribution of fibres from the smallest to 20 μ or 30 μ, proportionately more of the smaller fibres being present in the nerve to the stellate ganglion than in the other nerves. The smallest of the giant fibres in the nerve to the stellate ganglion are no larger than the largest fibres outside the giant fibre area, so that the distribution is continuous up to 75 μ. In the stellar nerves, on the other hand, there are fewer giant fibres, and these are much larger than any others in the nerve. The fin nerve contains no giant fibres.

The Axons of Cephalopods

The giant fibres of the stellar nerves provide especially favourable material for the study of the structure of the axons. Their connexions have already been briefly described (Young, 1936), and further details will be published shortly. It is important here to notice that they are not the axons of single cells, but are syncytia produced, in the case of the largest fibres of *Loligo*, by the fusion of the processes of many hundreds of small cells.

The structure of the sheath appears to be similar in all fibres, whether large or small. The sheath is absolutely thicker around the larger fibres, but does not appear to increase proportionately with the fibre diameter, so that the larger fibres have relatively the thinner sheaths.

In order to examine the structure of the axons, one of the stellar nerves is taken from a *Sepia* or *Loligo* which has just been killed by decapitation, and is teased out with fine needles in the animal's blood or a drop of sea water. One or more of the giant fibres can usually be separated out in this way and studied with the highest powers of the microscope. The axon then appears as a homogeneous cylinder, having no central core or definite neurofibrils which can be traced separately. There is, however, a very delicate longitudinal striation, extending throughout the thickness of the fibre. The striae are very fine and exceedingly numerous. Since, as will be shown below, coarser striations appear after damage to an axon, it is difficult to be certain whether this faint fibrillation be not also an artefact. Dr. B. Sen has recently examined giant fibres of *Sepia* with dark ground illumination, and he informs me that no fibrillation was to be seen under these conditions, the axon containing only a number of granules in Brownian movement.

When a giant fibre is severed, the substance of the axon may flow out from the cut end of the tube formed by the connective tissue sheath, fig. 10, Plate 14 (Young, 1935, *b*). As will be seen from fig. 3, the flow

begins soon after the cut has been made, and the axoplasm pours out into the surrounding medium while the sheath slowly collapses. While this outflow is going on, movements can be seen in the axoplasm at a distance of several millimetres from the cut surface. The flow is seldom uniform across the whole cross-section of the fibre, being sometimes faster at the edges, sometimes at the centre, depending apparently on the shape of the opening. When the flow is fastest at the edges, it sometimes results in the leaving of a central core of altered axoplasm, which only partly fills the connective tissue tube (*see* p. 329).

Very noticeable changes take place in the finer structure of the axon close to a cut surface. The fibrillation becomes much more distinct in the axon which remains inside the sheath, so that it is sometimes possible

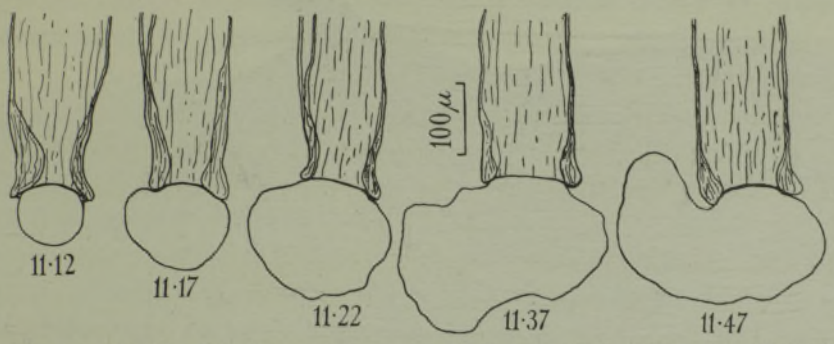


FIG. 3—Camera lucida drawings of the cut end of a giant fibre of *Sepia officinalis*, the cut having been made at 11·05 a.m. The outflow is often faster than in this case

to recognize distinct strands or fibres in such a region. As the stream emerges at the cut surface, this longitudinal fibrillation disappears. Coarser granules appear in the exudate, these being apparently artefacts, not present in the intact axon. The substance which emerges does not mix very readily with sea water, though it will do so if disturbed, but, on the other hand, it does not become separated from the water by the formation of any visible surface membrane. Brownian movement, which is not visible by transmitted light in the normal axon, appears in the exudate, especially at its outer edges.

All of these phenomena are seen equally well if the sheath of the axon is punctured by means of a needle at some point along its length. As shown in fig. 4, there is then a lateral outflow of axoplasm and the fibrillation becomes much more distinct in the affected region, with lines which follow the lines of flow.

These changes in the appearance of the axoplasm close to an injured point are a warning against placing too much reliance on observations of the visible or physical structure of axons which have been injured, or

punctured with microdissection needles. A fibre removed from the body to sea water maintains for some time a constant appearance, except, of course, close to its cut ends: but the operation of removal is necessarily a drastic one for the fibre, and we cannot even be sure that the appearance seen under these conditions is that of the fibre in the living body, though all the indications point to this being so.

Any injury to the sheath rapidly produces the fibrillation and granulation reported above, and the axoplasm has then certainly changed its properties. The fact that the axoplasm flows out from the cut end of an

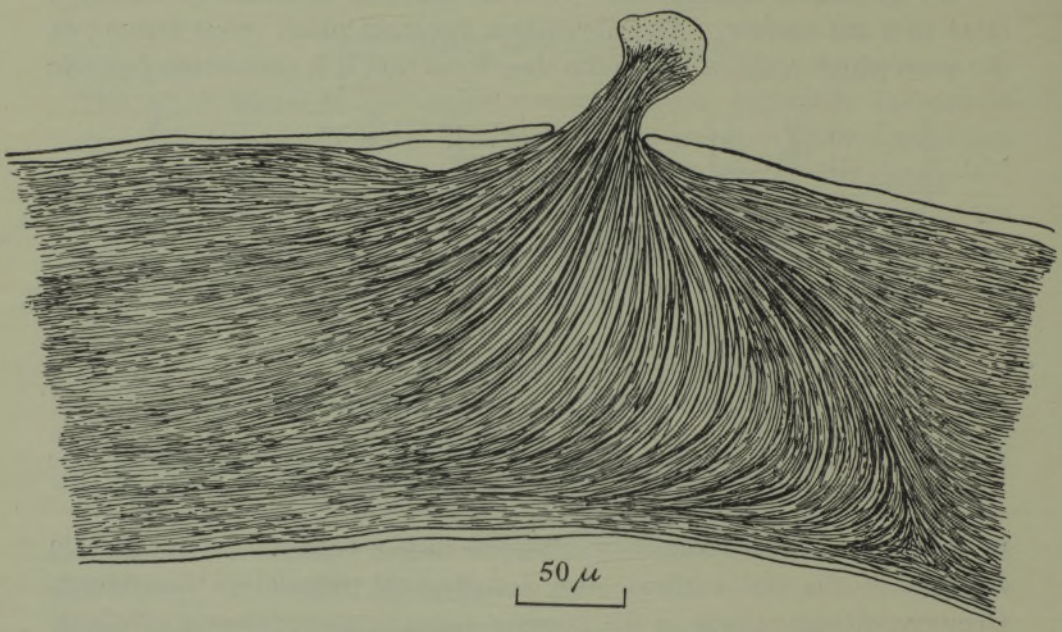


FIG. 4—Giant axon of *Sepia officinalis* which has been punctured laterally by a fine needle. The main lines of fibrillation were drawn with a camera lucida, but the details are diagrammatic. Note that the fibrils are more definite close to the injured point.

axon does not, therefore, make it certain that it is fluid in the living state, though this is suggested by the fact that the flowing movements can be observed in the axoplasm at some distance from the cut. These observations indicate, then, that the giant axons consist of a rather viscous fluid, whose substance shows some longitudinal organization but no definite continuous neurofibrils, though these latter readily appear under abnormal conditions.

Although these giant fibres are syncytia, originating by the fusion of the processes of many cells, yet no trace of their composite nature can be

detected either by study of the living, intact axons, or of those from which the axoplasm is flowing out, or in fixed preparations examined in longitudinal or transverse section. It is concluded, therefore, that the fusion of the separate axons is complete, the result being a giant axon, which functions as a single unit. Further details of this remarkable condition will be published shortly.

It is to be noticed that neither by observation of the intact giant fibres, nor by watching the process of outflow from them, does there appear any evidence of the existence of a surface membrane optically separable from the contents of the fibre. No doubt the molecules at the outside of the fibre take on special orientations to form a limiting membrane whose progressive depolarization probably constitutes the nerve impulse, but there is no reason to suppose that such a surface region of orientated molecules would be optically detectable.

Axons in Fixed Preparations

The study of fixed preparation of Cephalopod axons confirms the above interpretation based on the study of the living nerves. When well fixed by solutions having the appropriate saline constitution the axon completely fills its sheath, as in the living state, and the axoplasm appears to be homogeneous, except for a faint, wavy, longitudinal striation. No definite neurofibrils traceable for long distances are visible.

The faint longitudinal striation appeared after all the histological methods used, including fixation in Flemming's fluid, with or without acetic acid, formaldehyde, Bouin's fluid, mercuric chloride, with and without acetic acid, and various modifications of the methods of Cajal and Bielschowsky. The appearance varied somewhat according to the fixation, the striation being, for instance, somewhat coarser after fixation in formaldehyde (fig. 11, Plate 14) than after Flemming's fluid, which gave an appearance closely comparable with that of the living fibres.

None of the techniques employed produced in the axons any appearance of grosser neurofibrils traceable for long distances in the axon. In some of the preparations, however, there appeared central cores with a different staining reaction, and a more fibrillar appearance than the rest of the axon. Such cores were never seen in living intact nerves, and they appear to be artefacts, due to the currents and eddies which are set up in the axoplasm by the outflow from the cut end of the fibre. They occur close to the cut end of a nerve, and often take on remarkably complex forms (fig. 12, Plate 15). Sometimes these more darkly staining cores may stand out very distinctly, as, for instance, in the case shown in fig. 13, Plate 15,

where the core is markedly basophil and appears sharply demarcated from the rest of the axoplasm.

Smaller Axons of Cephalopods

The smaller nerve fibres in the mantle connective, fin nerve and stellar nerves, are essentially similar to the giant fibres. When examined in sea water they are seen to consist of an axon entirely filling its connective tissue sheath. The axon has no visible surface membrane and shows a faint longitudinal fibrillation. Comparable appearances are seen in fixed preparations (figs. 5, 6, 7, Plate 14).

When these smaller fibres are severed they do not immediately flow out from the cut ends of their sheaths, presumably because of the surface tension, which would tend to restrain the outflow of axoplasm from a narrow tube, while allowing it from a wider one. The initial stages of regeneration of these fibres have been studied by making preparations of the stumps a few hours after they had been cut, and it appears that the first stages of the process consist of an outflow of axoplasm, essentially similar to the faster outflow from a giant fibre.

Sheaths of Nerve Fibres in Crustacea

In *Palaemon* and related genera, and in Mysids, the nerve fibres are surrounded by a fatty sheath which was first discovered by Retzius (1888 and 1890) and later investigated by Göthlin (1913) and Nageotte (1922). These sheaths are said to differ from those of Vertebrates in that (1) the sheath is only thinned and not completely interrupted at the nodes of Ranvier; (2) there are no incisures of Schmidt-Lantermann; and (3) the "myelin" is separated from the axon by a nucleated inner sheath.

The nerves in the walking legs of Lobsters and Crabs, however, are commonly supposed to be "non-medullated" (*see* Mangold, 1905, and Retzius, 1890, for early literature), but Lullies (1933) claims to have demonstrated myelin sheaths. Göthlin (1913) showed that in *Homarus* and *Astacus* the sheaths of the leg nerves are not negatively birefringent, as are the medullary sheaths in prawns and Vertebrates, but positively so, as is the axon of medullated fibres. He made the interesting observation that, after dehydration with glycerine, these nerves became negatively birefringent. His suggestion, that this was due to some orientation in the fatty substances contained in the nerves, has been confirmed by Schmitt, Bear, and Clark (1935). They also showed that, contrary to the

opinion of Boehm (1933) and Schmitt and Wade (1935), much connective tissue is present, as was shown independently by Young (1935, *c*).

In order properly to evaluate the results of investigations of the action potentials, heat production, metabolic exchanges, excitation characteristics, X-ray diffraction patterns and other features of these nerves, further information is needed about the visible structure of the sheaths, the possible presence of any especially fatty layers and the proportion of the nerves which is occupied by the axons.

To obtain information on these points, nerves were removed from the walking legs of *Maia*. The legs were cut open with bone cutters and the nerves carefully dissected out and fixed, stretched to their natural length, on pieces of card. It is very difficult to obtain good fixation, especially of the smaller axons. Rapidly acting fixatives, such as Bouin's fluid or mercuric chloride, produce very serious distortion. Flemming's fluid, made up in sea water and containing very little or no acetic acid, gave better results, but the best were obtained with 1% chromic acid, dissolved in sea water.

The leg nerve is made up of a number of separate bundles, each surrounded by a thin perineurium of collagen fibres. This perineurium is continuous with an endoneurium forming an irregular meshwork of very fine fibrils extending throughout the nerve, nuclei being scattered at intervals. Around the nerve fibres the collagen seems to be thickened, so that each axon runs in a connective tissue tube, very much as in Cephalopods (figs. 14, 15, Plate 15). There are irregular spaces throughout the nerve which stain faintly with red by the azan technique, and are presumably occupied by tissue fluids.

The axons of the leg nerves vary in diameter from less than 1 μ to 20 μ, but, whereas the sheaths of the smaller fibres are very thin, those of the larger are excessively thick, so that, with its sheath, a fibre whose axon is 15 μ in diameter may be as much as 80 μ across. Thus the larger fibres have relatively thicker sheaths than the smaller, this being exactly the opposite of the condition in Cephalopods, and perhaps indicating that, in *Maia*, the sheath plays some definite part in the activity of the axon.

It seems probable that these larger axons run to the muscles, including, perhaps, both excitatory and inhibitory fibres (*see* Biedermann, 1887, Hardy, 1894, Mangold, 1905, Barnes, 1931, Pantin, 1934, and others). There are relatively few of them in the nerve and they usually run in separate bundles, accompanied by few or no smaller fibres. Their numbers and sizes vary at different levels along the leg. Towards the base a few very large fibres, of the type shown in fig. 17, Plate 15, can be seen running quite separate from all other bundles.

The sheaths of these very large fibres have been investigated with especial care, since it was in them that Lullies (1933) claimed to have found " eine besonders deutliche Markscheide ". Examination of preparations fixed and stained in a variety of ways has shown that the sheaths consist of concentric sheets of what is, apparently, collagenous connective tissue, continuous with the rest of the endoneurium (figs. 15–18, Plate 15). This tissue stains distinctly with anilin blue, and red with van Gieson's stain, though in neither case is the stain very dark, the fibres being very fine. Nuclei are scattered throughout the thickness of the sheath, and some of them lie in its very innermost layers, often pressed close against the axon. Between the collagenous lamellae are narrow channels, filled with a finely granular substance which stains red with the azan technique, and is presumably tissue lymph.

After fixation with osmium tetroxide or Flemming's fluid, no dense black ring comparable with the medullary sheath of a vertebrate axon can be seen; the sheaths may be somewhat blackened, but not more darkly than the connective tissue in other organs. The collagenous sheets darken more than the lymph between them. After fixation with Flemming's fluid the concentric lamellae are still stainable with anilin blue, a reaction which distinguishes them sharply from vertebrate medullary sheaths.

I have not been able to make any clear distinctions between inner and outer layers of the sheath, such as were found by de Renyi (1929) in the sheaths of the giant fibres of *Homarus*. The innermost layer sometimes has a smoother appearance than the rest, from which it may also separate somewhat after fixation. It is also often rather more darkly stained with osmium tetroxide (fig. 15, Plate 15).* Its nuclei, however, are similar to those elsewhere in the sheath. Although further embryological and chemical investigations are highly desirable, we have sufficient data to conclude that *both the large and small axons of the leg nerves of* Maia *are enclosed in continuous sheaths, formed of sheets of a collagen-like substance, interspersed with nuclei, containing some fat, and uninterrupted by any breaks comparable with the nodes of Ranvier*. Between the fibrous sheets are spaces filled with lymph, so that, except for the very thin innermost layer, the axon is probably in continuity with the interstitial fluids of the nerve, which in turn is only separated from the outside by a very thin perineurium.

* Drs. F. O. Schmitt and R. S. Bear, in a paper of which they kindly showed me the MS., have demonstrated by the use of polarized light that there is a layer of oriented fatty molecules in this region.

Proportion of the Nerve Occupied by Axons

Accurate estimates of the proportion of the nerves occupied by the axons are very difficult to obtain, because of the difficulty of fixing the smaller axons. Exact measurements would, in any case, be of little value, since the different bundles vary considerably in their make-up. The following estimates were obtained by cutting out and weighing appropriate portions of photographs of transverse sections of nerves fixed in 1% chromic acid dissolved in sea water, embedded in paraffin, sectioned and stained by the azan technique.

In a bundle consisting of small fibres only (less than 5 μ), the axons comprised 65%, the connective tissue and interfibrillary spaces 35% of the cross-sectional area. The bundles of larger fibres contain a much smaller proportion of the axoplasm. Thus a bundle of 7 axons of 7–15 μ in diameter contained only 25% of axon in cross-sectional area. In the very large axons at the base of the limb the proportion is even lower. In the case of a fibre which, with its sheath, was 70 μ in diameter, weighing the photograph showed that only 10% of the cross-sectional area was occupied by axoplasm.

Since these bundles of large fibres occupy only a small portion of the whole nerve, we may estimate that in a preparation of the middle portion of a leg nerve of *Maia*, such as is commonly used for physiological investigation, between 60 and 70% of the cross-sectional area is occupied by axoplasm.

In order to make quantitative comparison between the heat production or metabolic exchanges of *Maia* nerves and those of other animals, we need to know not only what proportion of the nerve is made up of axons, but also whether any nerve cell bodies are present in the trunks. Two nerves were therefore removed from the first walking leg along their whole lengths, folded carefully into bundles 1–2 cm. long, and sectioned serially. Careful search through these preparations failed to show any nerve cell bodies. Such work is very tedious and it is possible that isolated cells were missed, but it may safely be concluded that in these highly developed Arthropods, as in Vertebrates, the great majority of cell bodies are collected into definite ganglia, few or none being scattered along the peripheral nerves.*

* This investigation does not exclude the possibility that there is a network of neurons at the *periphery*, as suggested by Tonner (1933), though I have no reason to believe that such neurons exist.

Discussion

This histological study, although it does not substantiate the claim of Lullies that there are true medullated fibres in the nerve trunks of *Maia*, nevertheless confirms the most important aspect of his work in showing that fibres of different sizes are present (*see also* Barnes, 1931). Even though none of these have true myelin sheaths, yet one would expect, on the analogy of vertebrate nerves, that they would differ in excitation characteristics and conduction velocity. This was shown by Lullies to be so, and he has therefore indicated that the relation between fibre size, excitability, and conduction velocity is in a general way similar in vertebrates and in *Maia*, although the positive identification of his groups of fibres with the B2, B3, and C groups of vertebrates is hardly justified.

The conception of the nerve fibres of these invertebrates, as consisting of a more fluid axoplasm contained within a nucleated sheath, is a partial return to the views held during the last century, before the fibrillar theories of axon structure become dominant. The description of " Nervenröhren ", in such works as those of Retzius (1890), more accurately represent the structure of the living fibres than do the accounts of most later workers. Jenkins and Carlson (1904) have shown experimentally that the physiological length of nerves can be altered by stretching and relaxation, as would be expected on this view of their structure.

It was widely known during the last century that axons may be of semi-fluid consistency. Thus Huxley (1880) speaks of the contents of the nerve fibres of *Astacus* as " a fluid of gelatinous consistency ", and Hardy (1894) saw granules moving about within large axons of the same animal. More recently Bozler (1927) has shown that the " neuroplasma " which surrounds the neurofibrils visible in the living nerve cells of the medusa *Rhizostoma* is " sehr dünnflüssig ". de Renyi (1929) has shown the same for the axoplasm of the giant fibres of *Homarus*. In the frog, however, he believed the axon of medullated nerve fibres to be rather rigid gel.

The disagreements of histologists about the structure of nerve fibres have centred mainly around the question of whether the axons are further internally differentiated into neurofibrils. The literature of this controversy has been reviewed by Parker (1929) and Peterfi (1929). Except in *Rhizostoma* and *Homarus*, definite optically isolable fibrils are not visible in the living state, but numerous workers have reported them as faintly indicated (" swach angedeudet ", Ettisch and Jochims, 1926). They become clearer after injury to the fibre, and after appropriate fixation and staining.

The present observations have failed to reveal any definite continuous fibrils within the axon, either in living or fixed preparations, but there is nevertheless a faint visible striation in the axoplasm.

The widespread occurrence of this elusive longitudinal visible striation, together with such facts as the birefringence of the axon (Schmidt, 1934) and the thermal shortening of nerve (Schmitt and Wade, 1935), point very strongly to the conclusion that there is a pattern of micelles oriented along the axon. Change in the state of such a system might be expected readily to produce grosser fibrils by coagulation of the parallel micelles, especially if the change involves dehydration, as do so many histological techniques.

Boehm (1933) and Schmitt, Bear, and Clark (1935) have tried to elucidate this micellar pattern by X-ray diffraction studies. They have provided much interesting information about the structure of the medullary sheath, but have not so far revealed any diffraction pattern which is positively due to the axon. Since they have not been able to examine any preparations consisting exclusively of axoplasm, it is impossible yet to be certain whether there are systems of orientated molecules in the axon capable of giving X-ray diffraction patterns. It is hoped that the giant fibres of Cephalopods, which can be readily isolated and have only thin sheaths, may provide suitable material for investigation of this problem.

Summary

In Cephalopods every axon of the peripheral nerves is covered by a continuous sheath formed of nucleated sheets of a tissue resembling collagenous connective tissue.

The substance of each axon completely fills its sheath; it contains no definite neurofibrils traceable as separate entities, but there is a very faint longitudinal striation visible both in living and fixed fibres. More distinct fibrillae appear if the axon be damaged in any way.

After section of a giant nerve fibre, the axoplasm flows out from the connective tissue tube. The substance of the axon is therefore a rather viscous fluid, the faint visible striation suggesting the presence of longitudinally orientated micelles which, by coagulation, produce the more definite fibrils seen in damaged axons.

65–70% of the cross-sectional area of Cephalopod nerves is occupied by axoplasm, the remainder being connective tissue sheaths, blood vessels, and tissue lymph spaces.

Estimates of the numbers and diameters of the axons in the fin nerve, nerve to the stellate ganglion, and stellar nerves are given.

The leg nerves of *Maia* consist of separate bundles each bounded by a thin perineurium. Each axon is surrounded by a connective tissue sheath which is nucleated, continuous over the whole surface of the axon and containing some fat, although not histologically comparable with the medullary sheath of vertebrates.

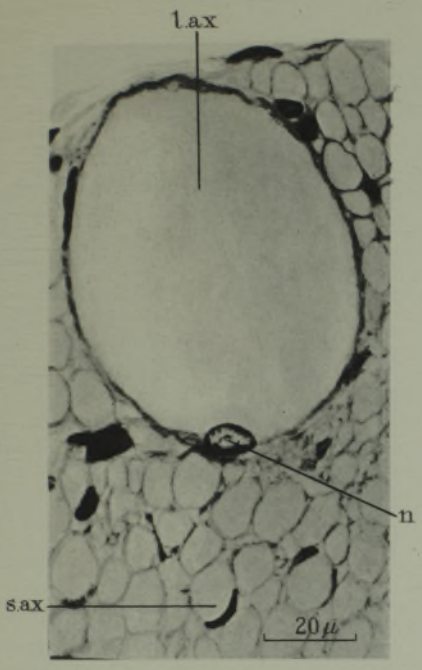
The sheaths around the larger axons of *Maia* are very thick, consisting of many concentric layers, interspersed with tissue lymph spaces.

60–70% of the cross-sectional area of the leg nerves of *Maia* is occupied by axoplasm. In the case of the largest fibres, running in separate bundles, as little as 10% of the cross-sectional area is axoplasm, the remainder being sheath.

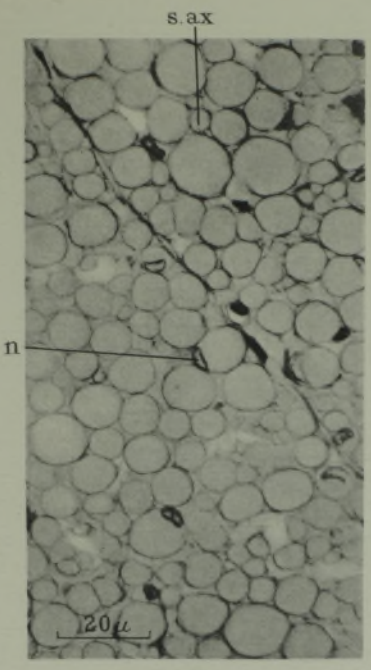
No nerve cell bodies were found anywhere along the length of leg nerves of *Maia* (ischus to dactylus), by the examination of serial sections.

References

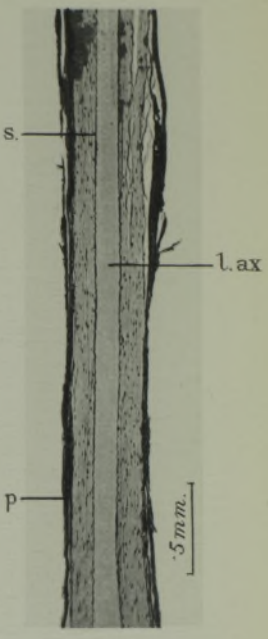
Barnes, T. C. (1931). ' J. Physiol.,' vol. **71**, p. xii.
Biedermann, W. (1887). ' S.B. Akad. Wiss. Wien.,' vol. **95**, p. 7.
Boehm, G. (1933). ' Kolloid Z.,' vol. **62**, p. 22.
Bozler, E. (1927). ' Z. vergl. Physiol.,' vol. **6**, p. 255.
Ettisch, G., and Jochims, J. (1926). ' Pflügers Arch.,' vol. **215**, p. 519.
Fisher, R. A. (1930). " Statistical Methods for Research Workers," 3rd Ed., London.
Göthlin, G. F. (1913). ' K. svenska. VetenskAkad. Handl.,' vol. **51**, p. 1.
Hardy, W. B. (1894). ' Phil. Trans.,' B, vol. 185, p. 83.
Huxley, T. H. (1880). " The Crayfish," London.
Jenkins, O. P., and Carlson, A. J. (1904). ' J. Comp. Neurol.,' vol. **14**, p. 85.
Lullies, H. (1933). ' Pflügers Arch.,' vol. **233**, p. 584.
Mangold, E. (1905). ' Z. allg. Physiol.,' vol. **5**, p. 136.
Nageotte, J. (1922). " L'organisation de la matière dans ses rapports avec la vie," Paris.
Pantin, C. F. A. (1934). ' J. exp. Biol.,' vol. **11**, p. 11.
Parker, G. H. (1929). ' Quart. Rev. Biol.,' vol. **4**, p. 155.
Peterfi, T. (1929). Bethe's ' Handb. norm. pathol. Physiol.,' vol. **9**, p. 79.
de Renyi, G. S. (1929). ' J. comp. Neurol.,' vol. **47**, p. 405, and vol. **48**, p. 441.
Retzius, G. (1888). ' Biol. föreningens. forhandl.,' vol. **1**, p. 58.
—— (1890). ' Biol. Unt. Neue Folge.,' vol. **1**, p. 1.
Sereni, E., and Young, J. Z. (1932). ' Pubb. Staz. zool. Napoli,' vol. **12**, p. 173.
Schmidt, W. J. (1934). ' Handb. biol. Arbeitsmeth.,' vol. **5**, teil 10, heft 3.
Schmitt, F. O., and Wade, L. J. (1935). ' Amer. J. Physiol.,' vol. **3**, pp. 159, 169.
Schmitt, F. O., Bear, R. S., and Clark, G. L. (1935). ' Radiology,' vol. **25**, p. 131.
Tonner, F. (1933). ' Z. vergl. Physiol.,' vol. **19**, p. 762.
Young, J. Z. (1935, *a*). ' Nature,' vol. **135**, p. 823.
—— (1935, *b*). ' J. Physiol.,' vol. **83**, 27 P.
—— (1935, *c*). ' J. Physiol.,' vol. **85**, 16 P.
—— (1936). ' Quart. J. micr. Sci.,' vol. **78**, p. 367.


l.ax
n
s.ax
20μ

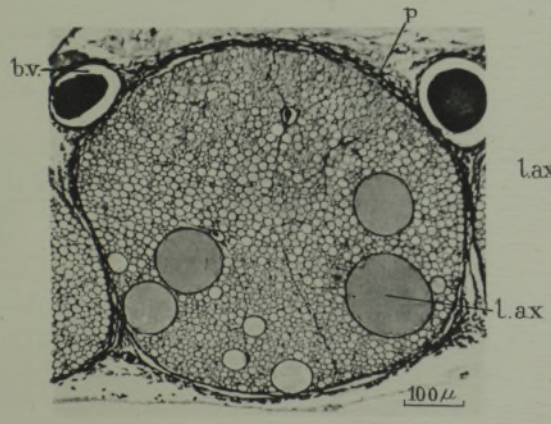
5


s.ax
n
20μ

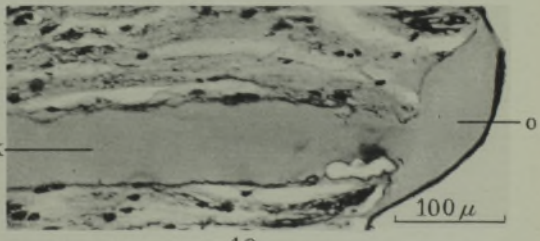
6


s.
l.ax
p
·5 m.m.

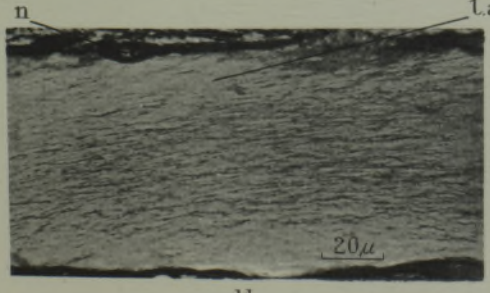
9


p
b.v.
l.ax
100μ

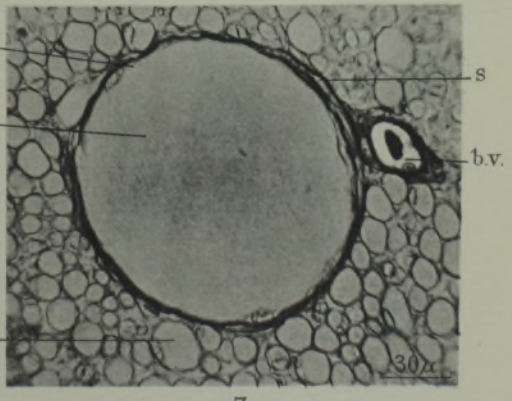
8


o
l.ax
100μ

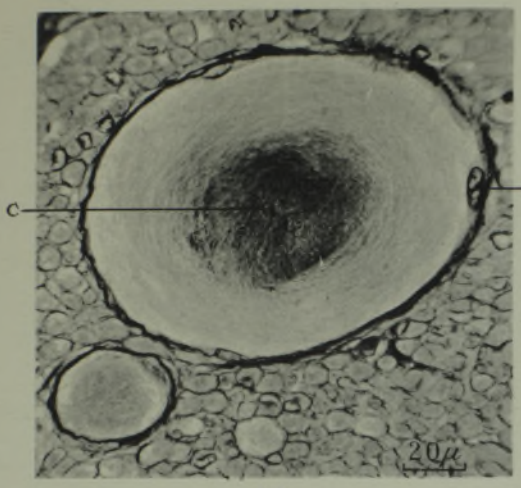
10


n
l.ax
20μ

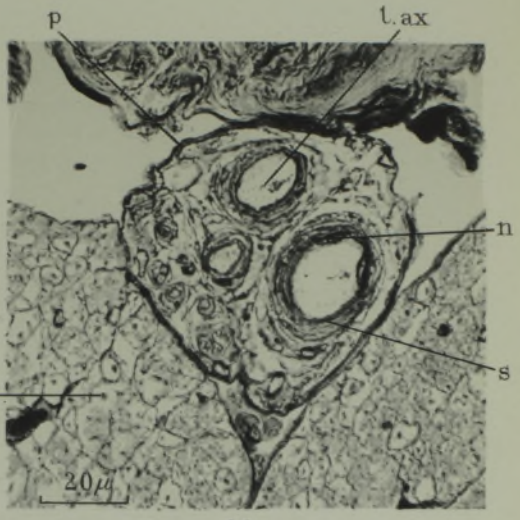
11


n
s
l.ax
b.v.
s.ax
30μ

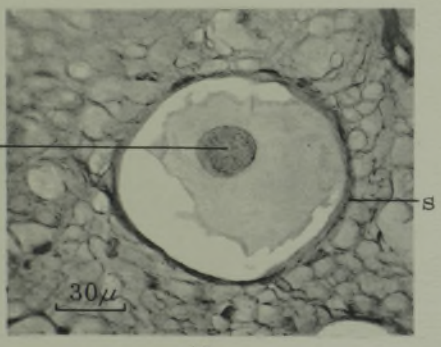
7



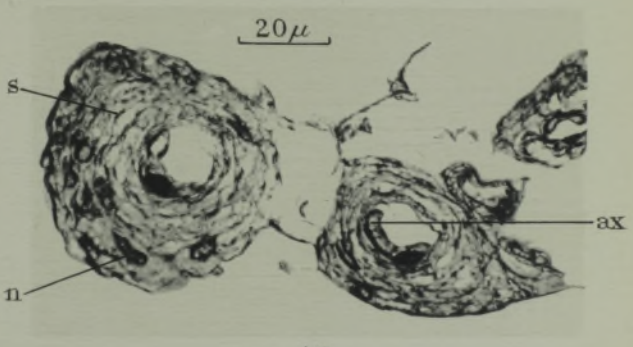
12



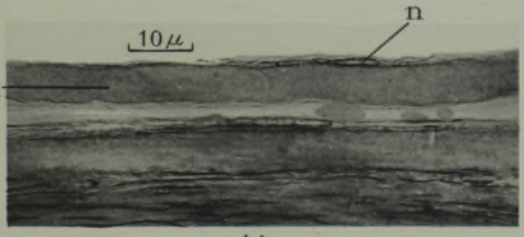
16



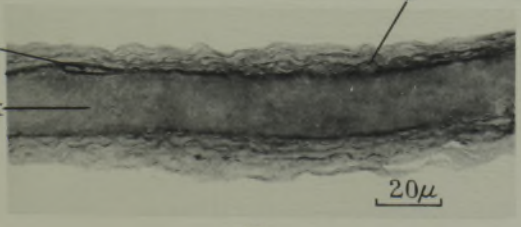
13



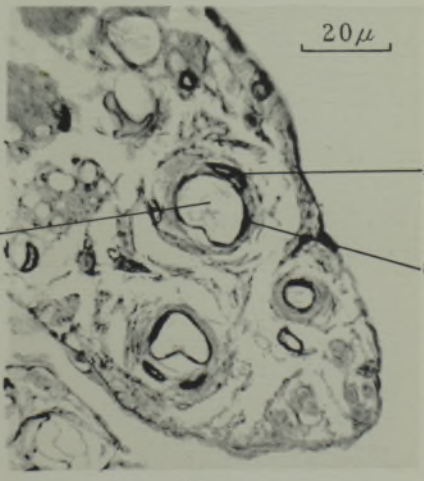
17



14



15



18

Explanation of Plates

Figs. 5 to 13 are of *Sepia officinalis*, figs. 14 to 18 of *Maia squinado*.

Lettering—*ax.*, shrunken axon; *b.v.*, blood vessel; *c.*, darkly staining core of axon; *l. ax.*, large axon; *n.*, nucleus of sheath; *o.*, substance which has flowed out from cut end of axon; *p.*, perineurium; *s.*, sheath; *s. ax.*, small axon.

Plate 14

Fig. 5—Portion of T.S. stellar nerve, showing large and small fibres. Fixed mercuric chloride and acetic acid; stained iron haematoxylin.

Fig. 6—Portion of T.S. stellar nerve, showing the structure of the smaller fibres. Fixed saturated picric acid in sea water, stain azan.

Fig. 7—T.S. giant fibre from stellar nerve. Fixation picroformol in sea water, stain azan.

Fig. 8—T.S. whole stellar nerve. Fixation and staining as fig. 6.

Fig. 9—L.S. whole stellar nerve, showing a giant fibre. Fixation Bouin's fluid, stain azan.

Fig. 10—L.S. end of a stellar nerve, cut 15 minutes before fixation. Some of the axoplasm of the giant fibre has flowed out. Fixation potassium bichromate in sea water, stain iron haematoxylin.

Fig. 11—L.S. giant fibre of *Sepia* in the stellate ganglion. Fixation formaldehyde in sea water, stain Cajal's method.

Plate 15

Fig. 12—T.S. giant fibre in stellar nerve to show central core formed as an artefact. Note radial pattern of the fibrillation, as if the axoplasm had been swirled around. Fixation picro-formol in sea water, stain azan.

Fig. 13—T.S. stellar nerve to show appearance of basophil core as an artefact at centre of a giant fibre. Fixation formaldehyde in sea water, stain iron haematoxylin.

Fig. 14—Small fibres from leg nerve. Fixed in 2% osmium tetroxide and teased in glycerine.

Fig. 15—Large fibre from leg nerve, preparation as fig. 14.

Fig. 16—T.S. portion of leg nerve showing large and small fibres. Fixation 1% chromic acid in sea water, stain azan.

Fig. 17—T.S. very large nerve fibres from base of leg nerve. Fixation 0·5% osmium tetroxide in sea water, no further staining.

Fig. 18—T.S. portion of leg nerve to show fibres of medium size. Fixation 1% chromic acid, stain iron haematoxylin.