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1752

Nervous degeneration and regeneration in Cephalopods

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1) Introduction

In two previous notes (SERENI, 1929; YOUNG, 1929) short accounts were given of physiological and histological changes observed after section of certain nerves of Cephalopods. These animals provide particularly suitable material for such experiments, and since the above notes were written the studies have been continued and extended, making it possible to compare the processes observed with those which are so well known in vertebrates as the result of a long series of studies culminating in those of CAJAL (1928) and CASTRO (1930). Such studies are of great interest to neurologists because of the light which they throw on the nature of nervous organisation in general and of their usefulness in the

analysis of particular mechanisms (fibre tracts, etc.). No research of this sort appears to have been carried out with any invertebrates, although it should prove particularly interesting in these animals since the workers who have been led to believe in neurofibrillar continuity between neurons have relied largely on observations on invertebrates (BETHE, APATHY, etc.).

The general scheme of the work is due to Professor SERENI, who at the date of his most unfortunate death had already almost finished the physiological experiments, while he had given the nerves to me (J. Z. Y.) for histological examination.

The present paper, in its physiological aspects, is an attempt to extract the more important results from his notes, very kindly placed at my disposal by Signora Dvora Sereni. Thanks to the great care and orderliness with which Professor Sereni kept his notes the task has not proved excessively difficult, and I hope that I have given a true representation of his views. If, however, there is any inaccuracy or incompleteness the responsibility must rest on me for false interpretation of the experiments which are so clearly set out in his notes.

The work was mostly done at the Stazione Zoologica di Napoli, but E. S. made a few experiments at Woods Hole, Mass., U. S. A. in 1929, and J. Z. Y. some at Plymouth, England in 1930. The histological examination of the pieces was made in the Department of Zoology and Comparative Anatomy, at Oxford.

Professor E. S. Goodrich, F. R. S. kindly read through the M. S. before publication.

2) Technique

The material used consisted of more than 200 animals belonging to the following species :

Eledone moschata Leach

Eledone cirrosa d' Orbigny

Octopus vulgaris Lam

Octopus macropus Risso

Sepia officinalis L

Loligo vulgaris Lam

Loligo pealei Lesueur.

The greater part of the work was done at Naples on *Eledone moschata* and *Octopus vulgaris* which can be kept for long periods in the laboratory. The Decapods survive only for a short time in captivity and are therefore less suitable than the Octopods for experiments of this sort.

The operations in question are themselves very slight and were performed either without anaesthesia or after immersion in solutions of ethy-

lurethane (2 %) or magnesium chloride (4 %) in sea water. The nerves were either cut with a fine pair of scissors or, in a few cases, pinched with forceps. In the case of the mantle connective regeneration is only possible if the nerve alone is cut, leaving the muscular bridge intact, since after severance of the latter the two stumps become widely separated.

Before removal from the body for fixation the nerves were tied to pieces of thin glass tubing, in order to keep them straight and normally stretched.

For fixation of the axons Bouin's, Zenker's, Helly's and corrosive sublimate fluids were all found to be useless, whereas strong Flemming's and Champy's solutions preserved the uninjured fibres very well and had the advantage of indicating the presence of fat in the nerve; the nuclei being afterwards stained with safranin and the fibres themselves with picronigrosin. Ciaccio's method was used for the recognition of lipins.

For the finer study of the nerve fibres a number of variations of the methods of CAJAL and BIELSCHOWSKY were used. The latter was not found suitable for general use since it invariably stained the connective tissue sheaths, leaving the contained nerve fibres unstained. Cajal's method was found to be better in this respect but also stained the young, unsheathed, regenerating fibres more readily than the adult fibres in their sheaths.

In general it was found necessary to use the solutions of silver nitrate very concentrated and for long periods.

The following two methods were found to be the most useful.

A. 1. Fixation for 24 hours in :	Pyridine	20 c. c.
	Chloral hydrate	2.5 gm.
	Alcohol (97 %)	40 c. c.
	Distd. water	40 c. c.

2. Thorough washing for 24 hours in several changes of distilled water.

3. 97 % alcohol for 24 hours.	
4. 5 to 10 % silver nitrate at 37°C. for about 14 days.	
5. Reduction in Pyrogallic acid (or Hydroquinone)	1 gm.
Neutral formol (40 %)	10 c. c.
Distd. water	90 c. c.

6. Short wash in distilled water, rapid dehydration in alcohol, inclusion in paraffin through benzol or methylbenzoate-celloidin.

7. Sections were cut 10-15 μ thick and either mounted without further staining or else counterstained with toluidin blue, which was found greatly to improve many preparations. Gold toning was not found to give any improvement.

B. This method is similar to the last except that the fixation is in
 Neutral formol (40%) 15 c. c.
 Sea water 85 c. c.

The pieces can be left in the fixative indefinitely. Before staining they are washed first for 24 hours in running tap water and then in distilled water and so on as from 2 above.

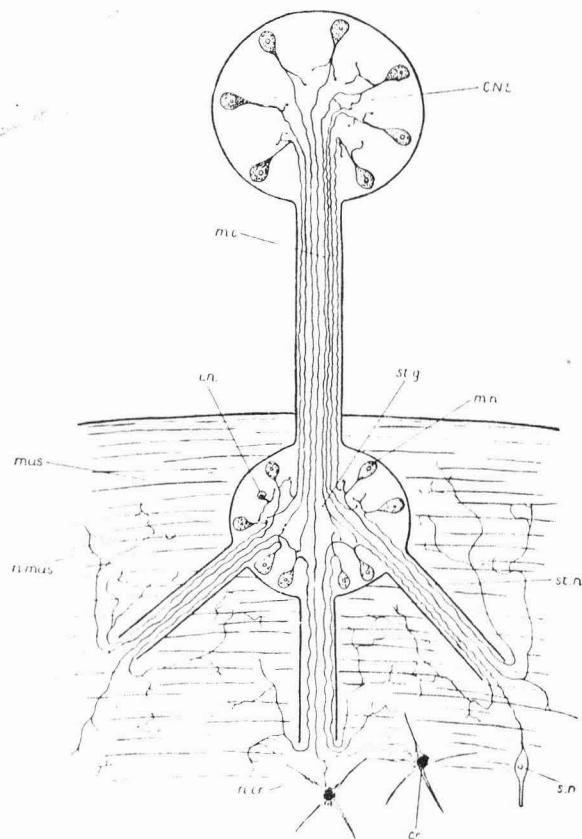


Fig. 1.—Diagram of the innervation of the mantle of an Octopod.
 cr., chromatophore; C.N.S., circum-oesophageal ganglia; i.n., intercalary neuron; mc., mantle connective; m.n., motor neuron; mus., mantle muscles; n.cr., nerves to chromatophores; n.mus., nerves to mantle muscles; s.n., sensory neuron; st.g., stellate ganglion; st.n., stellar nerve.

3) Anatomy and histology of the nerves of the mantle

I. — Anatomy

The nerves concerned in the innervation of the mantle of Cephalopods are the mantle connective, stellate ganglion and stellar nerves. The

first of these is a large nerve running backwards from the peri-oesophageal centres and crossing on a muscular bridge to the mantle. In Octopods all of the connective runs into the stellate ganglion, but in Decapods part of it, the nervus pinnae, containing motor fibres for the fin and for some of the chromatophores, does not run through the ganglion. From the stellate ganglion a number of stellar nerves run out radially to innervate the muscles of the mantle and the chromatophores. A diagram of these relations is seen in Figure 1.

II. — Structure of the stellate ganglion

The stellate ganglion consists, like most invertebrate ganglia, of layers of cells towards the outside whose processes run inwards towards the centre of the ganglion where they branch and anastomose with the incoming fibres of the mantle connective, forming a complex network, the neuropil (Fig. 2). The majority of the cells of the ganglion send out motor fibres in the stellar nerves to end in the muscles of the mantle. This is shown by the fact that after section of these nerves there was retrograde degeneration of the Nissl substance and Golgi element (YOUNG 1932) and disappearance of the nerve-endings in the muscles (p. 187). No changes were observed in the cells of the ganglion after section of the mantle connective, so that few, if any of them send fibres forwards to the central nervous system. After section of the stellar nerves, however, some of the smaller cells showed no retrograde changes and these are presumed to be intercalary neurons whose fibres do not extend outside the ganglion.

The cells themselves are all unipolar,¹⁾ but where they reach the

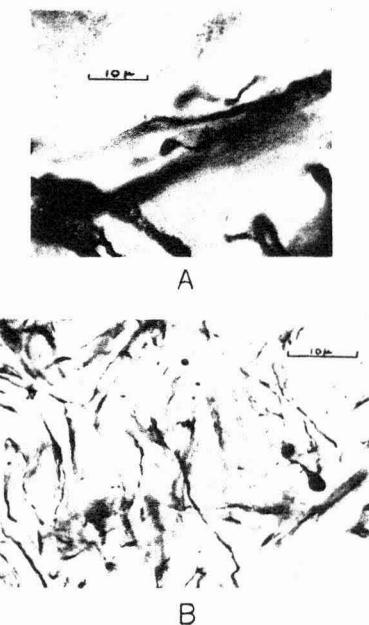


Fig. 2.—Terminal boutons in neuropil of stellate ganglion.
Octopus vulgaris. Cajal's stain. Photo. Zeiss apo. 90 1.3.

¹⁾ After section of the stellar nerves very close to the ganglion it was sometimes observed that the bodies of the cells put out short processes like dendrites, but these were presumably a response of the cell to the stimulus of cutting and not concerned with the conduction of impulses.

neuropil they divide into a great number of branches. The mass of fibres is so complex that it was never found possible to trace out the ramifications of single cells, though somewhere in this tangle they must make contact with the incoming fibres of the mantle connective and FRÖHLICH (1910) showed that there was a delay of about 0.01 second in conduction through the stellate ganglion, due presumably to the presence of a synapse. In fact the endings themselves can be seen in good CAJAL preparations in the form of terminal knobs scattered throughout the neuropil and especially in its outer layers (Figs. 2 and 3); very rarely, they were found actually among the cells themselves. They are rounded or pear-shaped in form and about 4.5μ long by 2μ broad. They are usually uniformly stained but occasionally there is a more lightly staining central region (Fig. 2A). It is difficult to observe their relations with the branches of the cells of the ganglion since the latter stain very poorly, but in some cases the boutons were clearly seen to be applied to large fibres in which the neurofibrils were faintly visible. In no case was there any evidence of continuity between the substance of the bouton and the fibrils of the larger fibre; indeed in many cases the bouton lies at right angles to the latter.

Besides the small boutons larger, terminal swellings were also observed (Fig. 4). No boutons could be seen attached to them and their nature remains uncertain, possibly they are collaterals of the motor nerve cells.

Fig. 3.—Terminal boutons in neuropil of stellate ganglion. *Octopus vulgaris*. CAJAL's stain. Photo. Zeiss apo. 90 1.3. *a.*, axon of cell of ganglion; *b.*, terminal bouton.

Endings of these two types were observed in all well stained preparations, even after the use of fixatives as various as formal and chloral hydrate and there can be no doubt that they give a fairly accurate picture of the synaptic junctions in the ganglion. Most of the smaller boutons are the terminal branches of fibres in the mantle connective, as is shown by the fact that they degenerate after section of this nerve (p. 195). The few boutons which remain intact after this operation are presumably collaterals of the cells of the ganglion.

Special interest attaches to the small boutons on account of their similarity to the structures observed by CAJAL and others in the central



nervous system of vertebrates, but never yet described in any invertebrate. In the vertebrates most of the boutons are in the form of loops whereas those observed during the present study mostly appeared solid. It is possible however that this uniform staining was due to the great concentrations of silver nitrate employed and it is significant that loop forms were occasionally observed (Fig. 3).

Considerable controversy has centred round the question as to whether these boutons are really terminal or whether, as maintained by MARUI (1919) and TIEGS (1931, etc.) there are further prolongations, penetrating into the substance of the cell to which they are attached. HOFF (1932) has recently studied the question in detail in cats, and concludes that they represent the final terminations and that there is no evidence of neurofibrillar continuity across the synapse. This is also the conclusion reached from the present study of the boutons of Cephalopods and the experiments described below bring further confirmation since, after section of the mantle connective, degeneration extends to the ends of the boutons but not into the cells of the stellate ganglion.

III.—Nerve-Endings in the Mantle Muscles

The exact nature of the connections of the fibres in the stellar nerves with the mantle muscles has never been made out. MIKHAILOFF (1921) maintained that there were motor cells present in the muscles, but gave figures only of what he took to be sensory cells. Impregnation with silver nitrate reveals a great number of fibres running among the muscles, and from the fact that these degenerate after section of the stellar nerves (p. 187) it is concluded that they are the terminal branches of the axons of the cells of the stellate ganglion, which thus innervate the muscles directly, without the intervention of any further nerve cells. It is extremely difficult to trace the fibres to their endings on the muscles. B. V. DER SPRENKEL (1929) has recently studied the motor innervation of the arms of *Sepia* and concluded that the fibres end either straight or as



Fig. 4.—Large terminal knobs in neuropil of stellate ganglion. *Octopus vulgaris*. CAJAL's stain. Photo. Zeiss apo. 90 1.3.

loops, penetrating the substance of the muscle cells and ending near the nucleus. The preparations of the mantle muscles studied during the present work seem to indicate that the finest branches run at right angles across the muscle fibres, giving off short lateral branches, or bearing swellings which are applied to the outside of the muscle fibres. There was no indication that the endings actually penetrate into the muscle cells, but it is impossible to be certain that the fibres have been impregnated up to their extreme ends. It is proposed to return to this important question in a later paper.

V. DER SPRENKEL also claims to have seen interstitial cells (lemnoblasts) at the points of bifurcation of the fibres, but in my preparations there appear only thickenings of the fibres at such points, without any trace of a nucleus.

In fact no nerve cells at all were seen in the circular muscles which make up the greater part of the mantle or in the strands of longitudinal fibres which run between them. On the other hand cells are certainly present in the thin coats of muscle cells which lie outside the main mass of muscles. Presumably these are the cells which were seen by MIKHAILOFF. Two of them are illustrated in Fig. 5. They are

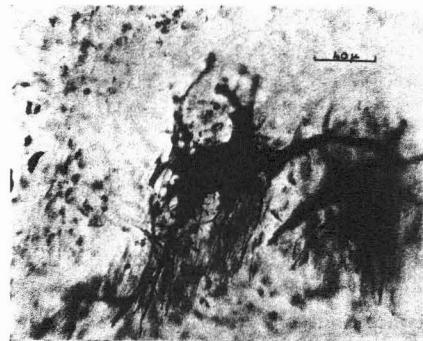


Fig. 5.—Nerve cells from the superficial layers of muscle on the inside of the mantle. *Octopus vulgaris*. Formol-Cajal stain. Photo. Watson 1/6 in.

large in area but much flattened in the plane of the sheets of muscle; they have very many branches, some ending near the cell, others running for long distances along the muscle fibres, against which some of them seem to end. For the present it remains uncertain whether these are sensory neurones as supposed by Mikhailoff or, and this seems more probable, peripheral motor cells. There is some physiological evidence that these muscles receive their motor innervation direct from the circum-oesophageal ganglia without synapse in the stellate ganglion and these cells may constitute a second link in the motor chain.

IV. — Histology of the nerve trunks

The nerve trunks of Cephalopods consist of numbers of nerve fibres, each running in a separate sheath composed of connective tissue (Figs. 6 and 7). It may be that occasionally more than one fibre runs within

a single sheath but I am of the opinion that all the appearances in Fig. 6 are due to turning of the fibres. When the nerves were fixed stretched out tight all the fibres were seen to run straight, but if the nerve was allowed to contract before fixation, then the axons were seen to take a spiral course and even to turn backwards so as to be cut through several times in a single transverse section. APATHY (1897) observed that the neuro-fibrils in the nerve trunks of Annelids run similar courses. This behaviour shows that the fibres are not attached to the walls of the tubes but are free to move inside them. This is of interest in view of the work of CARLSON (1911) who found that, in the slug *Arion*, stretching the nerves increased the conduction time, and interpreted this as being due to increase in length of the fibres. If in Cephalopods under normal conditions the nerves fold up when they shorten, then it would be expected that the conduction time would be the same through stretched and relaxed pieces. It would be interesting to try this experiment on the mantle connective. FRÖHLICH (1910) was aware of this possible source error in determining the velocity of conduction in the mantle connective, but he made no actual attempt to study the effect of stretching.

So far as can be made out there is no other covering to the nerve fibres besides the connective tissue sheath. There is no trace of a myeline

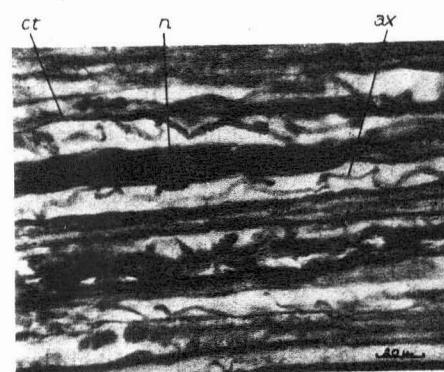


Fig. 7.—Longitudinal section of normal mantle connective. *Octopus vulgaris*. Flemming, safranin, picronigrosin. Photo. Zeiss apo. 90 1/3. *ax.*, axon; *ct*, connective tissue sheath; *n.*, nucleus of sheath.

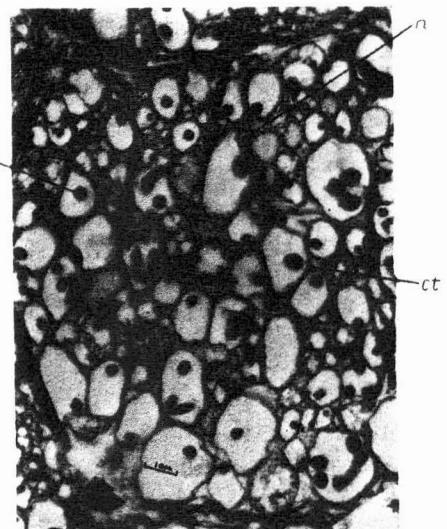


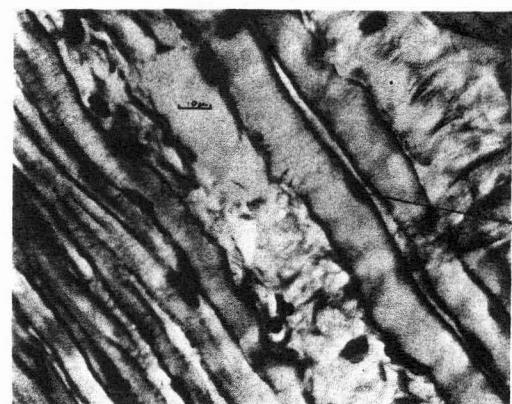
Fig. 6.—Transverse section of normal mantle connective. *Octopus vulgaris*. Flemming, safranin, picronigrosin. Photo. Zeiss apo. 90 1/3. *ax.*, axon; *ct*, connective tissue sheath; *n.*, nucleus of sheath.

sheath, and the only droplets of fat seen in the nerve were always found to belong to amoebocytes. After fixation in Ciaccio's fluid and postchroming, the normal nerve fibres do not stain with Sudan III, although after section of the nerves lipins appear in the axons.

The connective tissue tubes can be seen in Figure 8 after staining with the Bielschowsky method.



A



B

Fig. 8.—Connective tissue nerve sheaths in mantle connective. *Eleodone moschata*. Bielschowsky method, Ehrlich's haematoxylin. Photo. Zeiss apo. 90 1,3. n., nucleus of sheath.

nective tissue is to support the nerve fibres and perhaps also to pass nourishment to them. From the description of APATHY (1897) it appears that similar sheaths surround the nerve fibres of Annelids.

The mantle connectives differ in structure from the stellar nerves, the former containing a large number of very small fibres. This distinction can be well seen in *Sepia*, where not all of the mantle connective passes to the stellate ganglion. The exclusively motor part of the connective, the n. pinnae, consists almost entirely of medium sized fibres, together with a few smaller ones. The part of the connective which runs to the ganglion, on the other hand, contains a few enormous fibres, collected together at one side of the nerve and also a great number of very fine fibres. The stellar nerves contain medium-sized and small fibres and are exactly similar to the motor part of the mantle connective.

4) Histological changes following section of the nerves

I.—Stellar Nerves

After section of the stellar nerves the wound soon becomes filled with a mass of scar tissue, consisting largely of amoebocytes, which also penetrate into the ends of the nerves. The divisions of these cells, as well as the changes which take place in the connective tissues have been more fully studied after section of the mantle connective (p. 192).

Interest centres chiefly on the behaviour of the axons. As would be expected by analogy with the vertebrates the fibres of the central stumps mostly remain intact and begin to regenerate, whereas most of those of the peripheral stumps show degenerative changes (Fig. 9).

a. *Changes in the peripheral stumps.*—As in the case of Wallerian degeneration in mammals there can be distinguished two distinct sections of each degenerating axon namely a necrotic zone near the cut surface, which exhibits changes as a result of the direct traumatic effect of the lesion, and a portion further from the cut which degenerates passively as a result of separation from the cell body. This latter degeneration consists simply of a fragmentation of the axon, which first shows a coarsely granular structure and then divides up into a series of small lumps which are presently absorbed. In Fig. 10 the connective tissue tubes can be seen filled with such lumps.

While this degeneration is going on far from the cut the fibres near to the seat of injury undergo most remarkable changes. For the first few hours the cut ends remain rounded off, but after about 15-20 hours (summer) the tips proceed to swell and then to fray out into numbers of branches, just as if the neurofibrils had been released and allowed to spread themselves (Figs. 11, 12). In some cases this occurs not only at the end of the axon but also at intervals along it, producing lateral

swellings and branches (Fig. 12 A). Soon the fine fibrils are replaced by thicker prolongations which grow out, often for a considerable distance, from the swollen tip of the axon, each fibre having a small terminal swelling (Fig. 13), recalling those of the true regenerating fibres. Similar

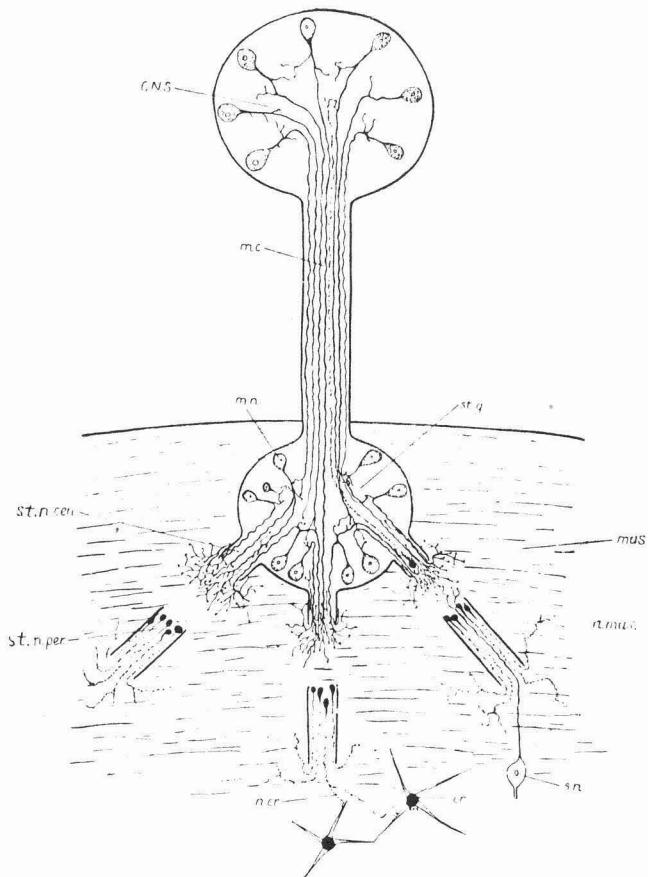


Fig. 9.—Diagram showing the changes which take place after section of the nerves, *st. n. cen.*, central stump of stellar nerve; *st. n. per.*, peripheral stump of stellar nerve. Other lettering as Fig. 1.

abortive regenerating outgrowths from degenerating axons have been described by Cajal and others.

After two or three days the axons proceed to round off again at the ends, so that curious large spheres are left, each attached to a short piece of fibre. These terminal spheres have been more fully studied in



Fig. 10.—Longitudinal section of peripheral stump four days after section of mantle connective, showing the connective tissue filled with the remnants of degenerating axons. *Eledone moschata*. Formol-Cajal stain. Photo, Zeiss apo. 90 1,3.

is sometimes hardly stained in silver preparations but is more often traversed by neurofibrils (Figs. 11 and 14).

The routine histological methods provide some evidence as to the nature of these large masses. They are well fixed by the ordinary histological reagents and especially by Flemming's fluid. They do not blacken with osmium tetroxide, so presumably contain no unsaturated fat. On the other hand they are stained by Sudan III after fixation with Ciaccio's technique, which indicates that they contain large amounts of lipin. After fixation with Flemming's fluid they stain strongly with acidic dyes, especially nigrosin (which also stains the normal axons), the central part of each sphere staining more deeply than the rest (Fig. 15).

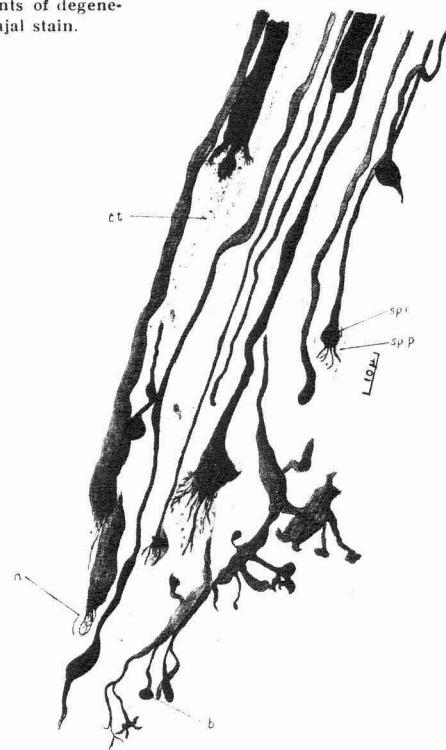


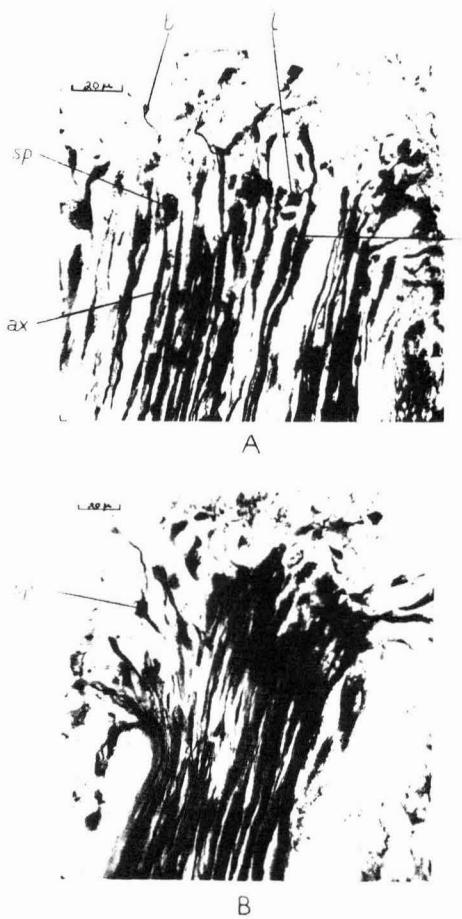
Fig. 11.—Camera lucida drawing of the ends of fibres peripheral stump, 38 hours after section of a stellar nerve *Octopus vulgaris*. Photo, Zeiss apo. 90 1,3 oc. K7. *b.*, abortive regenerative buds; *ct.*, connective tissue tubes; *n.*, neurofibrils; *sp. c.*, central portion of terminal sphere; *sp. p.*, peripheral portion of terminal sphere.

In the majority of the spheres there are no structures which stain with basic dyes but in a few cases well-defined nuclei were seen embedded in them (Fig. 15 B and C). These resemble the nuclei of the amoebocytes and it is probable that in such cases the latter have invaded the spheres and possibly come into functional relationship with them. The similarity between the nuclei of amoebocytes just outside the sphere and those within it is seen in Fig. 15 C. These nuclei are certainly not constituents of the intact nerve fibres since they are never seen in normal nerves; neither are they regularly present in the degeneration spheres which are certainly usually anucleate.

The experiments in which the mantle connective was cut at two points (p. 198) show that spheres are formed in this way at the cut surface, whenever an axon is severed from its cell body. The most remarkable point about them is that they are not absorbed but remain, indefinitely, in the nerve. They were seen in peripheral stumps of the mantle connective 180 days after the operation, long after functional regeneration had taken place by union of the two stumps (p. 204). It is impossible to say whether they are eventually absorbed

Fig. 12.—Longitudinal sections of terminal portions of peripheral stumps, 38 hours after section of the stellar nerves. *Octopus vulgaris*. Compare Fig. 16. Cajal's stain. Photo. Zeiss apo. 90 1.3 oc. K7. b., abortive regenerative bud; t., lateral bud; sp., terminal sphere; l., cut end of fibre.

or not, but it is certainly remarkable to find masses of protoplasm persisting in this way long after they have been separated from the rest of the cell body and from the nucleus. It is well known that pieces of cytoplasm (e.g., of Amoeba) may continue catabolic activities for a



time in the absence of a nucleus, but such pieces are incapable of anaabolism and eventually they die. In the present case there is no direct evidence that the spheres in question metabolise at all, but certainly they seem alive during the first period of agonal changes and there seems every reason to suppose that they remain alive, since otherwise they would be absorbed. Since they receive no stimuli there is no reason why any activities should take place in them at all and they may be regarded as masses of organic substance lying inactive in the nerve.

In many ways these bodies resemble the terminal spheres seen after the section of non-medullated nerves by PERRONCITO (1907), CAJAL (1907) and CASTRO (1930). In fact the excellent descriptions and figures given by the last author show that the early processes of degeneration of nonmedullated fibres are remarkably similar in mammals and Cephalopods and his «grands élargissements fusiformes d'un aspect homogène» resemble the lumps observed during the present work. He does not state for how long the lumps persisted in the nerves, but seems to imply that they were rapidly removed by macrophages.

While almost all the fibres in the peripheral stumps of the stellar nerves show degenerative changes in the manner described, yet there are still a very few which remain intact and send out regenerating fibres forwards. Such fibres seem to be more numerous in the large posterior stellar nerves than in the lateral ones. They are best seen after the whole stellate ganglion has been extirpated so as to remove all possibility of confusion with ingrowing fibres from the central stumps. Presumably they are afferent fibres connected with those few fibres among the muscles which remain intact after section of the stellar nerves.

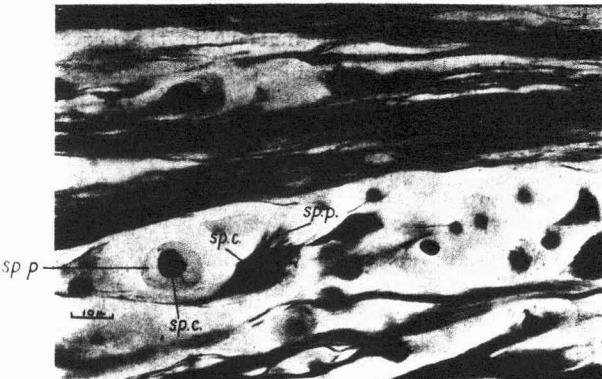
Meanwhile the endings of the fibres among the muscles also degenerate. The earliest change seen was a swelling of the fibres, which then proceeded to break up into series of lumps which were quickly removed leaving no apparent traces though it has been shown by phar-



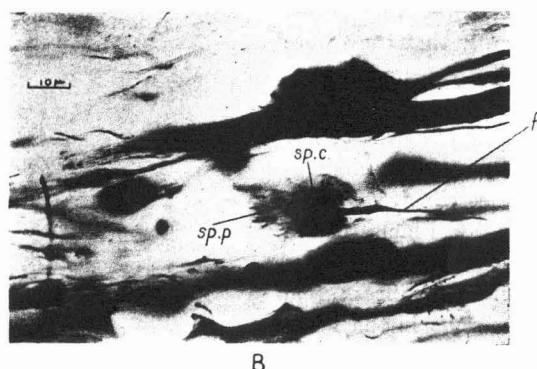
Fig. 13.—Camera lucida drawing of ends of fibres from the posterior end of an isolated piece of the mantle connective, eleven days after the operation. *Octopus vulgaris*. Cajal's stain. Photo. Zeiss apo. 90 1.3 oc. K7. b., abortive regenerative bud; ct., connective tissue tube; sc., scar; sp., terminal sphere.

macological methods (SERENI 19302) that some part of the mioneural junction persists for a long time and eventually atrophies only for want of use.

A very few fibres remained intact in the muscles even for long periods after section of the stellar nerves. They were presumably afferent fibres connected with the axons which regenerate from the peripheral stump.



A



B

Fig. 14.—Terminal portions of fibres in the peripheral stump of mantle connective, 18 days after section. *Octopus vulgaris*. Formol-Cajal stain. Photo. Zeiss apo. 90 1.3. f., preserved portion of axon; sp. c., central portion of terminal sphere; sp. p., peripheral portion of terminal sphere, showing neurofibrils.

The time taken by these changes varies, like all the processes of degeneration and regeneration, with the age of the animal and the time of year, that is to say with the temperature of the water. During the summer they are remarkably rapid; in July at Naples the fibres in the nerve trunks began to show granulation 15 hours after the operation

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and from 24 hours onwards the tubes were full of rubbish which was all cleared away by about 7th day. The first changes in the fibres in the muscles appeared during the 2nd day and degeneration was complete by the 4th day. In mammals CASTRO (1930) showed that dege-

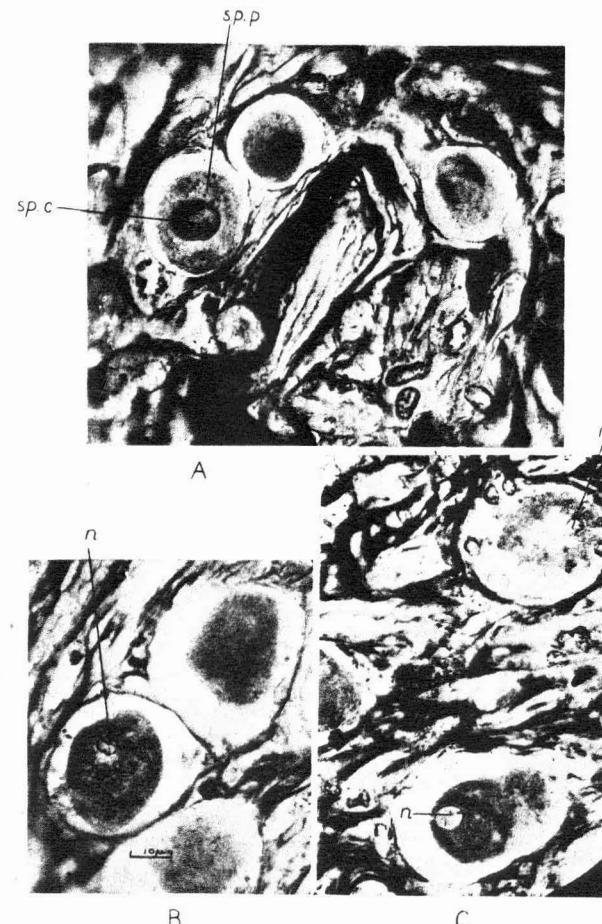


Fig. 15.—Terminal spheres from peripheral stump of mantle connective 171 days after section. *Octopus vulgaris*. Flemming, safranin, picronigrosin. Photo. Zeiss apo. 90 1.3. n., nuclei of spheres; sp. c., central portion of sphere; sp. p., peripheral portion of sphere.

neration of non-medullated (sympathetic post-ganglionic) fibres began about 48 hours after the operation and was complete after 6-8 days. It is remarkable to find equally rapid degeneration in Cephalopods since it is usually supposed that such processes take place more slowly in cold-blooded animals. Clearly the high summer temperature of the water (of-

ten 25° C. or more at Naples) enables processes to go on as rapidly in these poikilothermous animals as in the homothermous mammals.

b) *Central stumps of the Stellar Nerves.* The investigation of the changes which take place in the central stump is difficult since the intact axons do not stain readily with silver nitrate. So far as can be made out the first reaction to cutting on the part of a fibre which remains in connection with its cell body is a fraying out into a number of fine fibres, each furnished at its tip with a lump which stains very deeply with silver nitrate. The best impregnations therefore show the central stump as consisting of a more or less unstained trunk from whose cut end there sprout very large numbers of black fibres ending in small dark swellings (Figs. 16 e 17). This greater affinity of young fibres for silver nitrate has been noticed by many workers; possibly it is the result of an unusual concentration of chlorine ions in such fibres.

One of the most remarkable features of the regeneration is the great speed with which the fibres move through the scar. The outgrowth is more rapid in young animals than in adults and very much more rapid in summer than in winter. Measurements are not easy to make accurately but the following were the greatest lengths of the regenerating fibres in a series of medium-sized (100-500 gm.) *Octopus vulgaris* studied in August 1931, when the temperature of the sea water circulating in the tanks averaged about 25° C.

Section of Stellar Nerves.

Time after operation, hours.	Length of New Fibres, m. m.	Rate of Growth, m. m. per hour.
15	0.1	0.007
24	0.2	0.008
38	0.3	0.008
78	0.5	0.006
96	1.0	0.014

Section of Mantle Connective.

Time after operation, hours.	Length of New Fibres, m. m.	Rate of Growth, m. m. per hour.
13	0.1	0.008
15	0.1	0.007
22	0.2	0.009
24	0.25	0.009
38	0.7	0.018
51	0.8	0.016
168	2.0	0.012

From these figures it will be seen that the fibres grow out rather more rapidly after section of the mantle connective than after section of the stellar nerves. In both cases the rate of growth appears to in-

crease up to the 2nd or 3rd day, though the rates, as calculated, make no allowances for a possible initial latent period which would cause growth to appear slower in the early stages.

The above rates of between 7 and 18 μ per hour may be compared with the 2.8-9.9 μ per hour found by WILLIAMS (1930) for regenerating medullated nerves in amphibian larvae at 20°C and 7-24 μ per hour found by LEWIS and KIRK

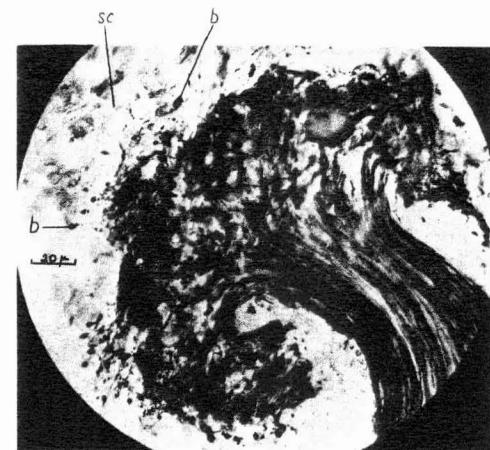


Fig. 16. — Central stump of stellar nerve 38 hours after section *Octopus vulgaris*. Compare peripheral stumps of same animal, fig. 12. Cajal's stain. Photo. Zeiss apo. 90 1.3. b., regenerating buds; sc., scar tissue.



Fig. 17. — Camera lucida drawing of ends of fibres from central stump, 22 hours after section of a stellar nerve. *Octopus vulgaris*. Cajal's stain. Photo. Zeiss apo. 90 1.3. oc. K7. ax., axon; b., regenerating buds; sc., scar tissue.

in mammals. Both authors noticed that the growth was much slower through the scar than in the peripheral stumps and the present figures all refer to fibres in the scar. No very accurate figures for the rate of regeneration of non-medullated fibres in vertebrates are available but from the results of CASTRO it would appear that the process is actually slower than in Cephalopods. In general it would appear that the rate of outgrowth depends on the opposition offered by the surrounding tissues, on the temperature and on the age of the animal, but that it is not greatly influenced by the myelination of the fibres.

Young fibres are formed in very great numbers in this way and they grow out in all directions from the severed stump. Besides those which cross the scar towards the peripheral stumps others grow out laterally

and some back up the trunk, so that some of them arrive in the stellate ganglion, and great whirls of nerves are produced round the end of the central stump. The fibres seem to take simply the lines of least resistance through the tissue and there is no evidence, at least in the early stages, of definite orientation in particular directions. Fibres can frequently be seen to deviate round obstacles and since the easiest path is provided by the scar tissue, most of the fibres grow through it. The strongest evidence that the fibres do not follow specific paths is provided by the observation that they do not necessarily re-enter the peripheral stumps but may instead grow down directly into the mantle muscle by the shortest available route. Such fibres as do find their way to the peripheral stumps penetrate them readily.

Although the great majority of the cut axons of the central stumps regenerate in this way yet there are undoubtedly a few which degenerate with the formation of large lumps similar to those described above from the peripheral stumps (Figs. 9 and 18). These degenerating fibres are presumably the peripheral portions of the axons which were observed to regenerate from the peripheral stumps. They are very few in number compared with the motor fibres.

II.—Mantle Connective

a. *General.* On account of its greater size and more ready accessibility the mantle connective is more suitable for study than are the stellar nerves. Besides the preparations with specific neurological stains for the study of the axons, the changes in the connective tissue which follow section of the nerve have also been studied with routine histological methods.

As after section of the stellar nerves, there is a very great infiltration of amoebocytes into the cut stumps, especially into the peripheral stump, where they appear to phagocytose actively and can be seen to be filled with droplets of fat. During the first few days after the operation these amoebocytes divide very frequently while actually in the stumps, the nucleus dividing amitotically. All stages in the elongation and constriction of the nuclei can readily be seen so that there can be no doubt that the division is truly amitotic (Fig. 19). The nuclei of vertebrate leucocytes commonly divide amitotically. Mitoses were never seen.

The connective tissue shows changes chiefly in the peripheral stump. Near to the cut the sheaths become completely disorganised and their nuclei distorted into many curious shapes. Further away the outline of the tubes remains intact but the inner margin becomes obscured and the lumen filled with strands of connective tissue (Fig. 20).

b. *Central Stump.* Most of the axons of the central stump remain intact and regenerate, the process being similar to that observed in the central stumps of the stellar nerves. The fibres split up into many bran-

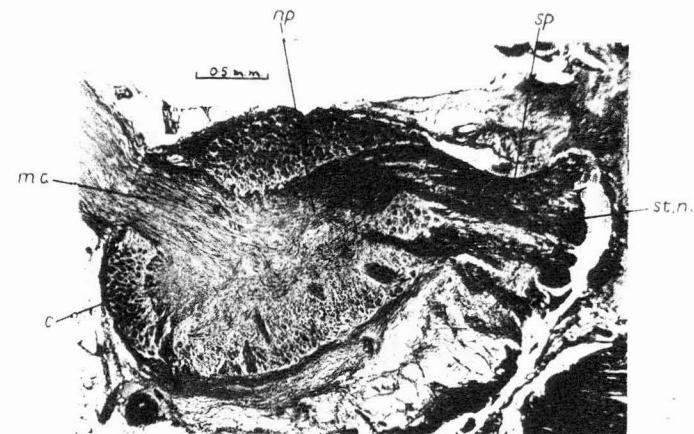


Fig. 18.—Stellate ganglion and central stumps, 5 days after section of stellar nerves. *Octopus vulgaris*. Photo. Zeiss planar. c., cell layer of ganglion; m.c., mantle connective; sp., degeneration spheres; st. n., cut surface of stellar nerve.

ches, each ending in a small, deeply staining swelling and these grow out in all directions. Many of them pass backwards up into the stump and others grow out laterally into the muscular bridge which accompanies the nerve. Since these muscle fibres are already innervated such wandering fibres soon die off. The easiest path for the regenerating fibres is through the scar tissue and by this avenue they quickly reach the peripheral stump which they penetrate. It is clear, therefore, that after section of the mantle connective the majority of the fibres of the central stump remain connected with their cell bodies which must lie in the circumoesophageal ganglia (Fig. 21). There are, however, a few fibres which

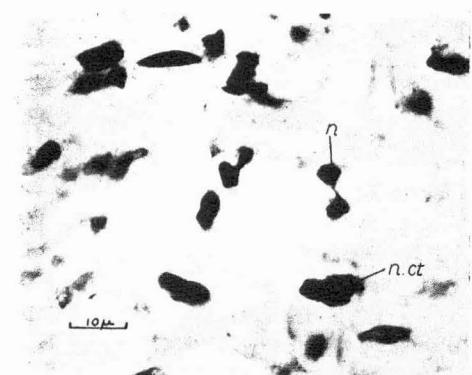


Fig. 19.—Longitudinal section of terminal segment of peripheral stump, 10 days after section of mantle connective. *Eledone moschata*. Bouin's fluid, iron haematoxylin. Photo. Zeiss apo. 90 1,3. n., nucleus of amoebocyte, dividing amitotically; n.ct., nucleus of connective tissue.

degenerate, and if sufficient time is allowed to elapse after the operation a few of the large spheres characteristic of the necrotic zone of degenerating fibres can be seen in the central stump. Correspondingly there is always some regeneration from the peripheral stump (see below) and there must therefore be a small centripetal pathway. Since no retrograde degeneration was observed in the cells of the stellate ganglion after section of the mantle connective it is concluded that none of the fibres of that ganglion send fibres forwards and the centripetal pathway therefore presumably consists of afferent fibres whose cell-bodies lie at the periphery.

c. *Peripheral Stump.* The great majority of the fibres in the distal segment of a cut mantle connective undergo a degeneration which is similar in every way to that observed after section of the stellar nerves. A few fibres however remain intact (fig. 20) and give rise to a considerable bundle of forward-growing regenerating fibres, most of which grow through the scar tissue and penetrate the central stump. Occasionally, however, they grow into the muscles (fig. 22), this being further evidence that there is no specific path laid down for regenerating fibres (p. 192).

In the degenerating fibres of the peripheral stump there is a necrotic zone showing agonal changes during the early period and then later the curious spheres which persist indefinitely in the nerve (Figs 22 & 23). Further away from the cut the axon breaks up into a number of small lumps which appear in Cajal preparations as rows of darkly-staining granules in the connective-tissue tubes. After fixation with fluids containing osmium tetroxide numerous droplets of fat could be seen in the tubes.

The degenerating fibres can be traced throughout the distal segment of the mantle connective, into the neuropil of the stellate ganglion and indeed up to the boutons which represent the endings of these fibres on

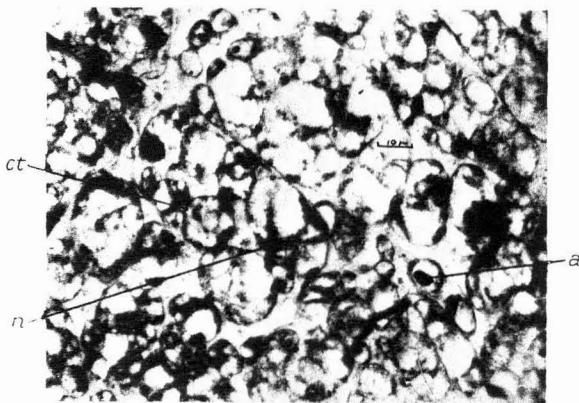


Fig. 20.—Transverse section of peripheral portion of mantle connective, 23 days after pinching. *Octopus vulgaris*. For control see fig. 6. Flemming, safranin, picronigrosin. Photo. Zeiss apo. 90 1.3. *ax.*, intact axon; *ct.*, connective tissue sheath; *n.*, nucleus of connective tissue.

the cells of the ganglion. These boutons remain intact until about the 13th hour after the operation (July), after which they begin to swell up and to stain much more deeply than the normal boutons (Fig. 24). At about this time the whole ganglion becomes filled with a great mass of small deeply-staining granules representing the degenerating axons (Fig. 25). These granules extend throughout the neuropil but never into

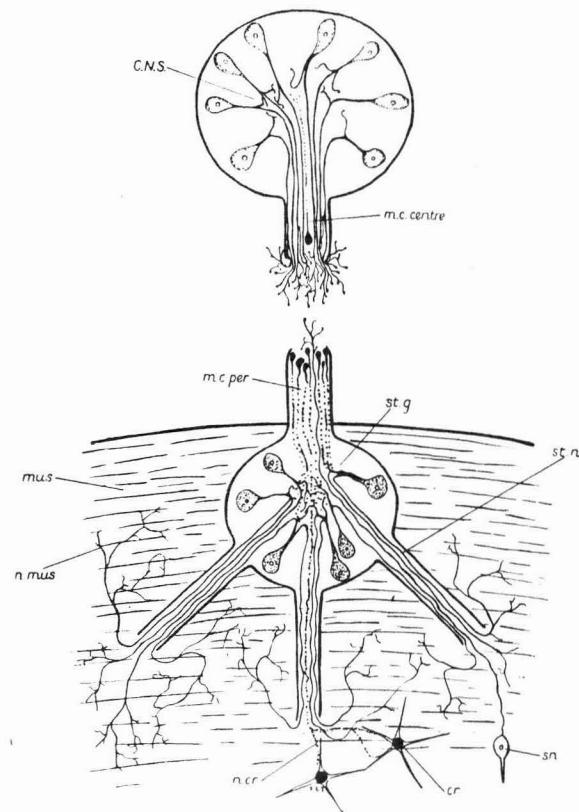


Fig. 21.—Diagram showing the changes which take place after section of the mantle connective. *m. c.*, centre, central stump of mantle connective; *m.c. per.*, peripheral stump of mantle connective. Other lettering as in fig. 1.

the cell layers, showing that the connections are all made with the dendrites of the cells, and never on the cell body itself. The granules are all absorbed by about the 3rd day, after which only the axons of the cells of the ganglion remain in the neuropil. Some granules were present in the stellar nerves and these presumably represent the degenerating fibres for the innervation of the chromatophores, which fibres have al-

ways been supposed, on physiological evidence, to run through the ganglion without synapse. It is interesting to find histological evidence that some fibres run straight through the ganglion. Prof. Sereni was also able to show at Woods Hole that certain fibres seen after staining with methylene blue attached to the muscles of the chromatopores of *Loligo pealei*, disappeared after section of the mantle connective. We did not succeed in staining these fibres in *L. vulgaris* at Naples but they are presumably the endings of the motor nerves (see HOFMANN, 1907) and we have therefore some histological evidence that they degenerate after section of the mantle connective.

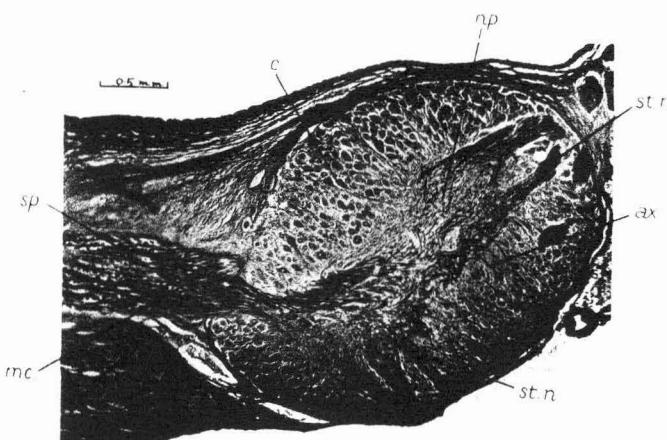


Fig. 22.—Longitudinal section of stellate ganglion and mantle connective, eleven days after section of the latter *Octopus vulgaris*. Cajal's stain. Photo, Zeiss A. c., cell layer in ganglion; m.c., peripheral stump of mantle connective; np., neuropil of ganglion; reg., regenerating fibres; sp., degeneration spheres.

While the incoming fibres of the mantle connective degenerate after cutting as described above, yet the cells of the ganglion itself remain intact and in fact their branches can be more readily traced after removal of the incoming fibres. A few boutons can still be found in the ganglion some time after the majority have undoubtedly degenerated and these are presumably the endings either of collateral branches of the motor cells or of the intercalary neurons which are known to lie in the ganglion (Young, 1932).

At no time after section of the mantle connective were any changes observed in the nerve endings in the mantle muscles although these fibres rapidly degenerate after section of the stellar nerves (p. 189). It seems evident, therefore, that after section of the mantle connective the centrifugal fibres degenerate only up to their terminations in the stellate ganglion and that the degeneration does not extend into the cells of the

ganglion itself which constitute the next link in the nervous chain. These experiments thus provide evidence of the discontinuity of neurons at synapses as direct as that obtained by similar methods in vertebrates.

The controversy as to the continuity of neurons has recently been re-opened by the work of MARUI (1919), TIEGS (1931, etc.) and BOEKE (1929) who claim to have observed prolongations from the endings of axons into the dendrites of other cells. HOFF (1932) has recently however, brought very strong evidence showing that no such continuity is demonstrable histologically and that after section of the dorsal roots of cats

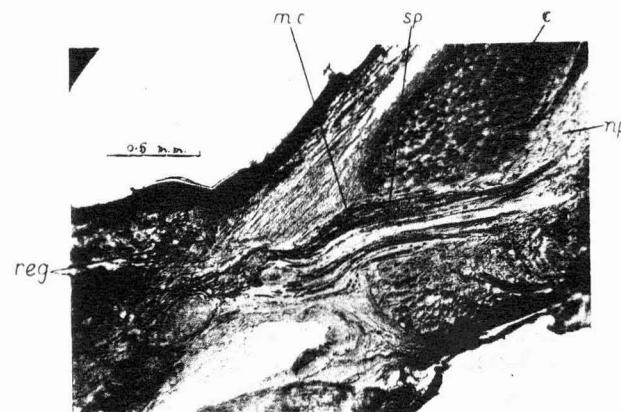


Fig. 23.—Longitudinal section of stellate ganglion and mantle connective, 18 days after section of the latter. *Octopus vulgaris*. Formol-Cajal stain. Photo, Zeiss planar. az., bifurcating axon of cell of ganglion; c., cell layer of ganglion; m.c., peripheral stump of mantle connective; np., neuropil of ganglion; sp., degeneration sphere; st. n., stellar nerve.

the boutons which constitute the endings of the afferent fibres degenerate, although the cells on which they terminate remain intact.

In view of the above description of the nerve endings and their degeneration it seems reasonable to conclude that the nervous system of Cephalopods, like that of vertebrates, is built up of chains of discontinuous neurons and not of a non-synaptic network. The work of BOZLER (1928) has thrown considerable doubt on the occurrence of non-synaptic nervous systems even among the Coelenterates, and it is now very doubtful whether they exist at all in any animal. The experiments of ALVERDES (1925) seemed to show that nervous degeneration in insects extended across several synapses after removal of the eye, but his preparations were made without specific neurological methods and the identification of degeneration depended only on the presence of masses of granules in the centres in

question. The present experiments show clearly that in the molluscs studied degeneration does not extend across synapses.

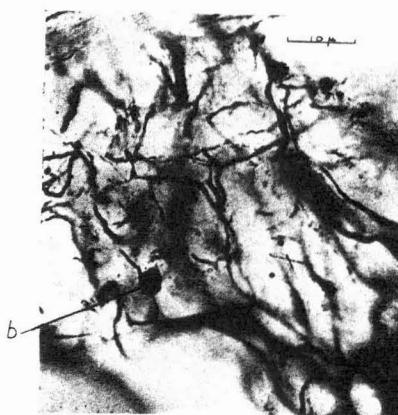


Fig. 24. — Degenerating terminal boutons from neuropil of stellate ganglion, 15 hours after section of mantle connective. Cajal's stain. *Octopus vulgaris*. Zeiss apo. 90 1,3.

cesses leading to the formation of degeneration spheres were seen in all the fibres at both ends of the isolated piece of nerve (Figs 13 and 26) as well, of course as in the peripheral stump which remained attached to the ganglion.

III. — Conclusions from Histological Changes

The histological examination of the nerves and ganglia after section of the mantle connective and stellar nerves has shown that the nervous system of Cephalopods is built of separate units, so that after section of an axon the isolated portion degenerates up to its ending on another neuron or muscle cell, the degeneration not extending into the next cell of the chain.

It is also possible to make several deductions as to the

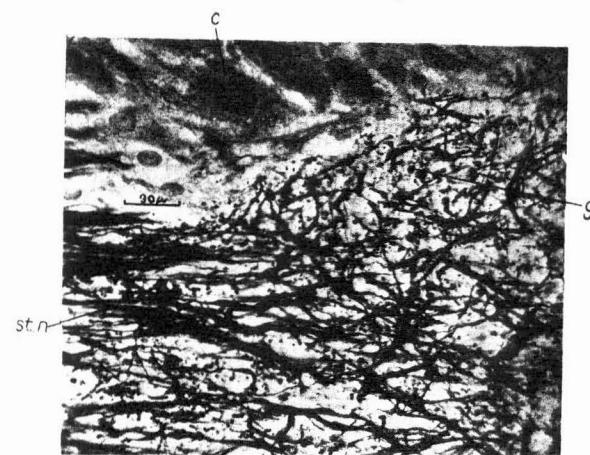


Fig. 28. — Granules in neuropil of stellate ganglion, 22 hours after section of mantle connective. *Octopus vulgaris*. Cajal's stain. Photo. Watson. 1/6 in. c., cell layer of ganglion; g., degeneration granule; st. n., stellar nerve.

arrangement of the neurons which innervate the mantle. After section of the mantle connective there is chromatolysis of cells in the circum-oesophageal centres but not in those of the stellate ganglion (YOUNG, 1929): there is much outgrowth of fibres from the central stump, less from the peripheral stump and correspondingly, many fibres degenerate in the peripheral stump, few in the central stump (Fig. 21). Accordingly there must be a small forward tract in the mantle connective which does not originate in cells of the stellate ganglion but consists of afferent fibres whose cell bodies lie at the periphery.

After section of the mantle connective most of the fibres in the stellar nerves remain intact but some degenerate, so that the large backward tract in the mantle connective consists of a) fibres which end in the stellate ganglion and b) fibres which run through the ganglion into the stellar nerves. Since after section of the mantle connective there is degeneration of the motor endings on the muscles of the chromatophores but not of those of the mantle muscles it is concluded that fibres b) innervate the chromatophores and this agrees with the physiological evidence.

After section of the stellar nerves there is chromatolysis in the majority of the cells of the stellate ganglion: most of the fibres in the central stumps regenerate but a few degenerate and correspondingly in the peripheral stump most of the fibres degenerate but a few regenerate, and nearly all the nerve endings disappear from the mantle muscles (Fig. 9). It is therefore concluded that most of the cells of the stellate ganglion send out fibres which end on the mantle muscles and that the centripetal path in the stellar nerves consists of afferent fibres, including possibly a few proprioceptors.

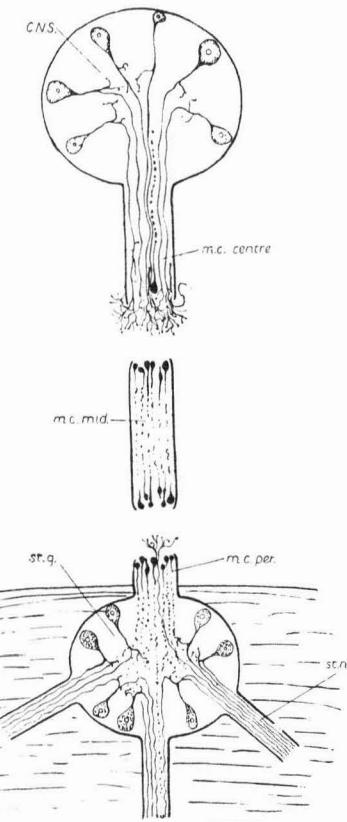


Fig. 26. — Diagram showing the changes which place after double section of the mantle connective. m. c. centre., central stump of mantle connective; m. c. mid., isolated middle piece; m. c. per., peripheral stump of mantle connective.

5) Physiological changes following section of the nerves

I. — Functional connections of the stellate ganglion

The stellate ganglion is usually considered to be a relay station for the nerves responsible for the respiratory movements, having presumably some co-ordinating function. There has been considerable controversy as to whether or not the ganglion acts as a reflex centre. V. UENKÜLL (1894), BAGLIONI (1905) and BOZLER (1927) were unable to obtain reflexes through the ganglion after it had been isolated from the C. N. S.; whereas A. FRÖHLICH and LOEWY (1907) noticed movements of distant parts of the mantle after stimulation of the central stumps of the stellar nerves, especially after the ganglion had been made more excitable by the application of nicotine. W. F. FRÖHLICH (1910) confirmed these results and TEN CATE (1929) was also able to obtain the reflexes in *Sepia*.

Experiments made to settle this point (E. S.) have shown that, as claimed by A. FRÖHLICH and LOEWY, W. F. FRÖHLICH and TEN CATE, if a piece of the mantle is divided into two parts connected only by means of the stellar nerves and stellate ganglion, then it is possible, by stimulation of one part, to obtain reflex contractions of the other. The effect could not be observed in all cases, however, and was very much less pronounced than the contractions observed in similar experiments in which the stellate ganglion was left in connection with the peri-oesophageal centres by way of the mantle connective. Further it was observed that the effect of local stimulation of the muscles was much more diffuse when the stellate ganglion was present (even though isolated from the central nervous system) than after its removal.

There is, therefore, considerable evidence that most of the sensory fibres run right through the ganglion and up to the central nervous system (see p. 194). Possibly the reflex movements recorded above are of the nature of axon reflexes. This would explain why it is not always possible to elicit the reflex, since perhaps only in some cases does a single neuron send fibres to more than one stellar nerve. TEN CATE, however, is of the opinion that a true reflex arc with three neurons is involved.

II. — Changes following section of the Mantle Connective

After section of one of the mantle connectives the whole of the mantle of the corresponding side became paralysed. The muscles no longer took part in the respiratory movements and the muscles of the chromatophores were at first relaxed, so that this side appeared pale. Later the

chromatophores gradually re-expanded (as was observed by HOFMANN (1910), but, until regeneration had taken place, neither they nor the muscles of the mantle made any further controlled movements.

Some days after the operation (the time relations vary very much with the season, that is to say with the temperature of the water) the stellate ganglion and muscles began to show peculiar conditions of excitability. Electrical or mechanical stimulation of the ganglion now very readily caused contraction of the muscles of the mantle but not of the chromatophores. The contraction of the muscles differed from the normal contraction very markedly, being delayed, much slower, more intense and above all enormously prolonged so that, as a result of a single stimulus, there often followed a contraction lasting for several minutes, from which the muscle only gradually relaxed. Stimulation of the muscles themselves at this time, while they were still in connection with the stellate ganglion, very readily produced the same delayed, intense, prolonged contraction. This is further evidence that reflex action of some sort (whether normal or axon-reflexes) can take place through the stellate ganglion.

The chromatophores at this period also showed increased excitability to mechanical stimuli but in this case the primary effect of the stimulus was a delayed contraction of the muscles of the chromatophores over a limited area round the point stimulated. This initial response then gave rise to a secondary, diffuse effect, in the form of waves of contraction passing over the skin. These waves are probably a result of the increased mechanical excitability of the muscles, the contraction of one fibre imparting a stimulus to the neighbouring fibres (HOFMANN 1910, SERENI 1930).

In a number of experiments the mantle connective was only partially cut, and in these cases the phenomena of great mechanical excitability and prolonged contraction of the mantle muscles appeared just as after section of the entire nerve. Probably every connecting fibre which runs backwards into the stellate ganglion divides many times and makes connections with many cells, so that cutting part of the nerve affects the whole ganglion. After such partial severance of the mantle connective often only the chromatophores over a small area of the mantle were affected.

In the case of the Decapods the experiments were varied by cutting either both parts of the mantle connective or else only that part which passes to the stellate ganglion, leaving the n. pinnae intact. In the former case the chromatophores were found to be paralysed over the whole surface of this side of the mantle, the mantle muscles were paralysed and the fin hung down motionless and somewhat curled underneath the body. On the other hand when only the part of the connective which passes to the ganglion had been cut, then the only chromatophores affected were

those of the lower surface and of the anterior quarter of the upper surface; that is to say that the rest of the chromatophores receive their innervation from the n. pinnae. The fin itself remained normal after this latter operation and would seem to receive all its nerves via the n. pinnae and not, as supposed by TEN CATE (1929), some also through the stellar nerves (see YOUNG, 1932).

III.—Changes following section of the Stellar Nerves

After section of the stellar nerves the changes in the conditions of the chromatophores were identical with those seen after section of the mantle connective, thus confirming the supposition that the fibres responsible for their innervation run through the stellate ganglion without having a synapse. The mantle muscles on the other hand were found to be in a very different condition. Local electrical or mechanical stimulation now only produced a slow, delayed contraction which was at first very local but then proceeded to spread through the muscle so that the latter showed ripples over its surface for quite a considerable distance round the point stimulated. This effect is in every way similar to the 'wave effect' seen in the chromatophores after denervation and is to be attributed to the same cause, namely extreme hyperexcitability to mechanical stimuli, so that the contraction of one fibre stimulates the neighbouring fibres and a wave of contraction is propagated through the muscle. A result of this excitability can be seen when the contractions of a denervated piece of muscle are recorded on a kymograph. After a stimulus has ceased, the muscle does not return to its previous length but continues to give secondary contractions for a considerable time.

A few experiments were made to determine whether there is a reversal in the degree of contraction at the anode and cathode in the denervated muscle. In the normal mantle muscles the relations are as in vertebrates, that is to say CCC > ACC > AOC > COC. After denervation ACC became greater than CCC (10 days after section of the nerves), probably COC is also greater than AOC though this appeared less clearly.

Clearly, then, the fibres responsible for the control of the respiratory movements of the mantle pass backwards in the mantle connective and are interrupted in the stellate ganglion, so that the muscle only becomes completely denervated after section of the stellar nerves and not after section of the mantle connective. This hypothesis is confirmed by the histological studies, since after section of the mantle connective no changes appeared in the nerve endings in the mantle muscle, whereas after section of the stellar nerves all the endings disappeared within a few days.

At the same time there were retrograde changes in the cells of the stellate ganglion which are separately described (YOUNG, 1932).

IV.—Post-mortem changes

In a previous communication (SERENI, 1929) it was stated that after section of the mantle connective the stellate ganglion of the operated side remained excitable for a longer period after death than the normal ganglion. This phenomenon was observed constantly in all the subsequent experiments and has also been confirmed histologically by the fact that the curious migration of the Nissl substance which occurs after death in the cells of the stellate ganglion, takes place more slowly after previous section of the mantle connective (YOUNG, 1932).

The effect was particularly striking in *Loligo* since the living muscles of this animal are almost transparent but after death they quickly become white and opaque. If the mantle connective was cut on one side before the death of the animal, then the muscles on this side remained transparent and excitable long after the normal muscles had become completely opaque and inexcitable, and a very sharp line could be recognised between the two sides. This line ran almost straight down the back, and it was very clear that the areas innervated by the different nerves did not overlap. A corresponding line is seen in the pattern of the chromatophores of the back after section of the mantle connective.

When the section of the mantle connective was made directly after the death of the *Loligo* (that is to say after the heart had ceased to beat) then the muscles of the two sides became opaque and inexcitable simultaneously. Evidently the fact of being in connection with the central nervous system at the moment of death in some way accelerated the process of death of the separate tissues, or rather release from such central influences delayed the onset of the process.

At first it was supposed that the greater viability of the ganglion on the operated side was due to its isolation from the stream of tonic impulses, thus allowing its cells to build up reserves which are available after death and possibly might also be responsible for the very prolonged nervous discharge which was seen on stimulating the ganglion some days after section of the mantle connective. If this were the case, however, it might be supposed that there would only be time for such an accumulation of substances to take place if the mantle connective were cut a considerable time before death; whereas in fact the survival effect was just as marked whether the nerve was cut one minute or one week before death, but was absent when the cut was made directly after death. Moreover in *Loligo* the same effect could be seen after section of the

stellar nerves. If a few of these were cut before death, then the corresponding portion of the mantle muscles remained transparent much longer than the rest of the mantle after death. In this case there is no question of an accumulation of reserve substances in the cells of the stellate ganglion.

On the other hand the prolonged contractions seen as a result of stimulating the stellate ganglion, were not manifested immediately after section of the nerves but only after the lapse of some days. It would seem, therefore that there are two separate processes :

1. The gradual acquisition of the property of hyperexcitability (accumulation of unused reserves?) due to isolation from the central nervous system ;

2. Some sudden nervous discharge at the moment of death which exhausts the tissues.

Since the mantle is concerned primarily with respiration, it might be expected that when the circulation is stopped asphyxia of the respiratory centres in the peri-oesophageal ganglia would cause very violent stimulation of the stellate ganglion and mantle musculature.

V.—Functional regeneration after section of the mantle connective

As is described above (p. 193) the process of outgrowth of nerve fibres begins soon after the mantle connective has been cut and continues more or less rapidly according to the temperature. Changes in the physiological conditions of the ganglion and muscles only begin to be apparent when the process of growth is complete and functional connection re-established. This stage was reached in all in six cases.

The earliest signs of regeneration were seen in a young *Octopus* (170 gms.) in which the mantle connective had been cut on the 30th July. After its death on the 9th day of September (i.e. after 65 days) it was found that local electrical stimulation of the skin on the operated side was followed by an immediate, somewhat diffuse expansion of the chromatophores, instead of the delayed local effect which is characteristic of the denervated chromatophores. The effect was however always less diffuse on the normal than on the operated side and only appeared over certain parts of the skin. Stimulation of the stellate ganglion of this animal on the operated side caused an immediate contraction without any sign of the great prolongation which is characteristic of the responses of the ganglia which have been severed from the central nervous system.

Another *Octopus* operated on 24.6.30 died on 3.9.30 (i.e. after 71 days) and the chromatophores of the cut side were found to respond

with an immediate somewhat diffuse expansion ; stimulation of the stellate ganglion was followed by a rapid, brief contraction of the muscles.

In the other four cases (survivals of 111, 126, 134, and 139 days) signs of regeneration were observed before death. First spots of darker colour appeared on the skin and then later the chromatophores of the operated side were seen to change colour synchronously with those of the normal side. In these cases stimulation, after death, of the mantle connective central to the cut produced movements of the mantle and contraction of the muscles of the chromatophores. In all cases the area of diffusion of the effect of a stimulus applied to the skin remained smaller on the operated side than on the normal sides. Probably the diffusion depends on the nerve plexus below the skin and this is at first simple and only later gradually increases in complexity, allowing of a wider diffusion of the stimulus. The exact nature of this plexus is not known.

All the cases of functional regeneration took place in the summer ; during the other seasons regeneration was much slower. For instance an *Eledone* was operated on Nov. 11th 1929 and after its death on 30th April 1930 it was found that the regenerating fibres had only grown out a very short distance from the stumps and had not yet met in the scar. In these cases the physiological conditions of the mantle and chromatophores remained those typical of the denervated organs.

6) Summary

1. The synaptic contacts between neurons in the stellate ganglion of Cephalopods are in the form of terminal boutons comparable to those found in Vertebrates. There is no evidence of neurofibrillar continuity across the synapse.

2. Every nerve fibre in a nerve trunk is enclosed in a separate sheath of connective tissue ; when the nerve is relaxed the fibres may run spirally inside the connective tissue tubes.

3. After section of a nerve trunk the isolated portions of the axons show Wallerian degeneration very similar to that seen in Vertebrates and extending only as far as the end of the fibre and not into the next cell of a chain. The system therefore consists of discontinuous neurons.

4. Although the distal parts of isolated fibres disappear completely, yet certain large spheres in the necrotic region near to the cut surface remain indefinitely in the nerve.

5. The processes of degeneration and regeneration are as rapid in Cephalopods during the summer as in Vertebrates, outgrowth of new fibres being at the rate of 7-18 μ per hour.

6. The amoebocytes which invade the cut stumps divide amitotically.

7. The degeneration experiments show that of the backward running fibres in the mantle connective most end in the stellate ganglion but some, namely those for the chromatophores, pass through the ganglion into the stellar nerves.

8. There is also a forward path in the mantle connective, consisting of afferent fibres whose cell bodies lie at the periphery.

9. The cells of the stellate ganglion send out fibres to the mantle muscles via the stellar nerves, in which there is also a small centripetal path of afferent fibres.

10. After section of the stellar nerves the regenerating fibres may grow down directly into the muscles without penetrating the peripheral stumps.

11. Reflexes through the isolated stellate ganglion were sometimes observed and may perhaps be interpreted as axon reflexes.

12. After section of the mantle connective the stellate ganglion and mantle muscles showed great excitability and prolongation of response. The chromatophores became paralysed and on direct stimulation gave a local response from which waves of contraction were then propagated.

13. After section of the stellar nerves the mantle muscles showed delayed response to mechanical and electrical stimuli. Though at first localised the response later spread as waves of contraction comparable to those observed in the chromatophores.

14. After isolation of a stellate ganglion or some of the mantle muscles from the central nervous system then the onset of post-mortem changes was delayed, so that the isolated tissues survived much longer than the normal tissues.

15. Functional regeneration after section of the mantle connective was observed in six cases.

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Le basi fisiomorfologiche dello sviluppo embrionale dei Selaci. - Parte I.

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(con 45 figg. nel testo)

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Il problema

Le ricerche sull'accrescimento embrionale dei Cefalopodi (RANZI 1929, 1930 I) — che mi permisero di porre in evidenza come l'embrione assorba, prima di uscire dall'uovo, sostanze minerali dall'ambiente — altro non erano che un primo capitolo di un programma molto più vasto di ricer-