

THE NEUROSECRETORY SYSTEM OF THE VENA CAVA IN CEPHALOPODA

II. *SEPIA OFFICINALIS* AND *OCTOPUS VULGARIS*

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(With Plates I–V and Text-figs. 1–3)

A system of nerves (called for short NSV system), described previously in *Eledone cirrosa*, whose only task appears to be the formation of a fine neuropile in the vena cava, is present also in *Sepia officinalis*. It has a similar disposition to that in *Eledone* but shows certain special features. It is composed of neurons the cell bodies of which are located in a layer (NSV layer) of the visceral lobe of the brain and in the paired ganglionic trunks, termed the lateral and medial NSV trunks, which emerge from the visceral lobe with the posterior infundibular and visceral nerves respectively. After accompanying these nerves for some distance these trunks take an independent course and give off some tapering branches ending blindly in the loose connective tissue.

The NSV layer and the NSV trunks are made up of unipolar cells of uniform appearance and of fibre bundles formed by their axons. The cells accumulate in cords or groups particularly abundant in the regions where the trunks give off nerves running to the vena cava. These nerves collecting the axons of all the cells of the NSV system penetrate through the muscular coat of the vein to end as a dense network under its endothelium. This neuropile extends as a continuous layer not only in a large portion of the vena cava, but also into the adjoining portions of the vena azygos, vv. ophthalmicae, vv. infundibulares anteriores and vv. infundibulares posteriores. Wherever the neuropile layer is present, the inside of the veins shows longitudinal ridges. Expansions of these ridges in the shape of tie-bars span the orifices of the ophthalmic and anterior infundibular veins, being thus particularly well exposed to the blood stream, which presumably carries away from the whole neuropile layer the products of its secretion.

In *Octopus vulgaris* the arrangement of the elements of the NSV system is similar to that of *Eledone cirrosa*, differing only in some points of minor importance.

INTRODUCTION

In the first part of this work dealing with *Eledone cirrosa* (Lamarck) (this *Journal*, Vol. 44, 1964), a system of nerves was described which forms a neuropile layer under the endothelium of the vena cava, and on the assumption that it releases some hormone into the blood it has been called 'Neurosecretory system of the vena cava' (NSV system for short). In the present account observations are recorded on the same system in two other cephalopods, *Sepia officinalis* (L.) and *Octopus vulgaris* Lamarck.

The methods of staining the nerves were the same as used previously.

*SEPIA**General arrangement of the NSV system*

As in *Eledone* the NSV system in *Sepia* is composed of neurons the cell bodies of which are situated partly in the visceral lobe¹ of the brain and partly in the ganglionic trunks which emerge from this lobe with the posterior infundibular and visceral nerves, but separate from these nerves farther along.

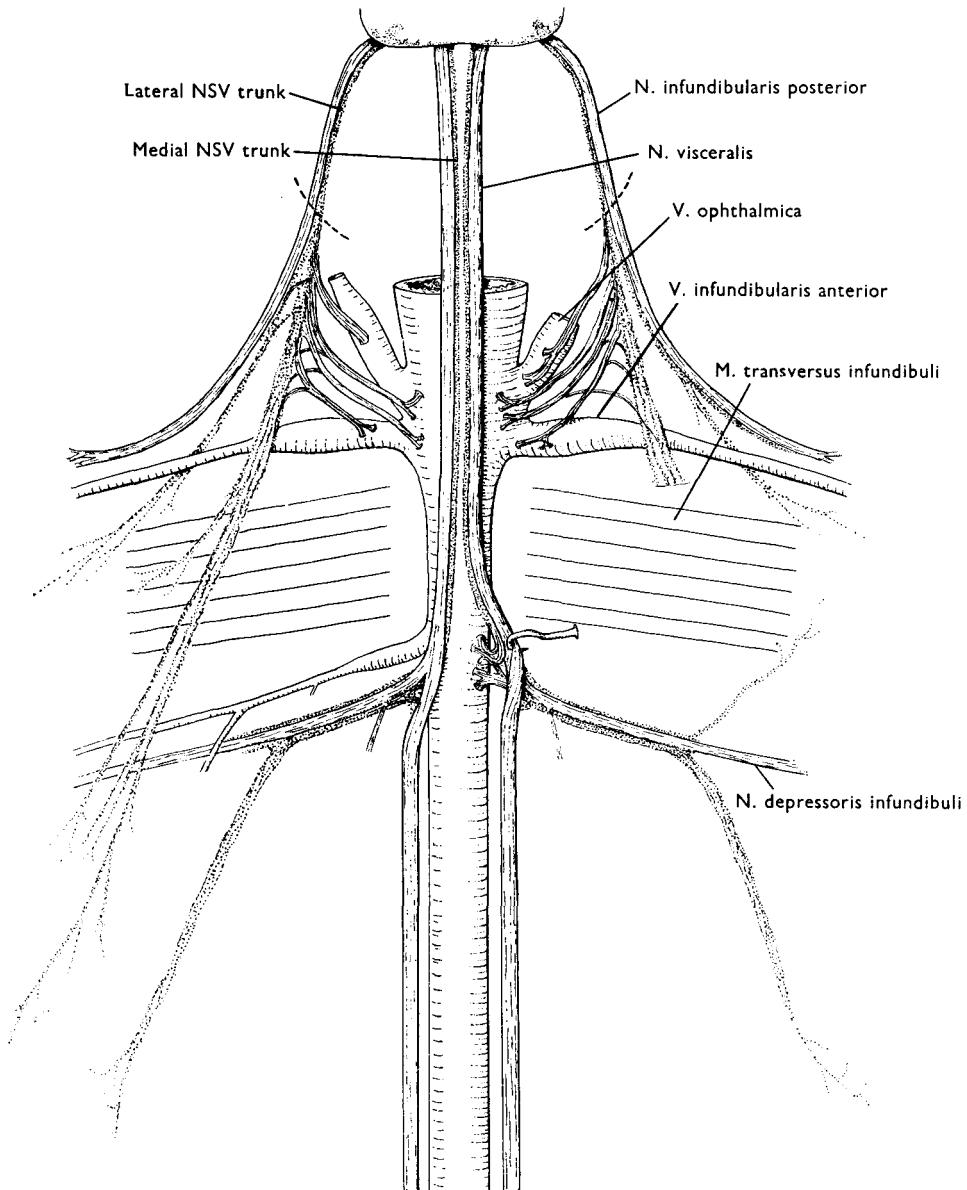
In the visceral lobe the NSV elements form a superficial layer (NSV layer) near the roots of the aforementioned nerves. Thore (1939) remarks that this layer, called by him 'extracorticales Neuropile', is much more weakly developed in *Sepia* than in octopods. This statement needs some correction. It is true that near the roots of the visceral nerves this layer is thin and even quite inconspicuous in places; farther laterally, however, it becomes thicker and has protuberances extending into the deeper regions of the lobe which reach considerable dimensions near the roots of the posterior infundibular nerves (Pl. I, fig. 1). Besides, the NSV layer is in many places not sharply delimited and strands of its cells penetrate between those of the neighbouring layer (Pl. I, fig. 2), so that their total number is greater than appears at first sight. This can also be deduced from the fact that the NSV trunks carry a considerable number of fibres whose origin can be traced to the visceral lobe.

The texture of the NSV layer differs from that of the neighbouring parts of the brain in the smaller size of the cells and a less orderly arrangement of the cells and of nerve fibres between them. The latter, which are the axons of the cells of the NSV layer, join into thinner or thicker bundles converging towards the exits of the visceral and posterior infundibular nerves. Some of these bundles appear to arise not in the NSV layer, but in the central neuropile of the visceral lobe. They evidently correspond to those fasciculi which in *Eledone* form distinct tracts running from the central neuropile to the NSV trunks. Their course in *Sepia* is not so easily discernible.

All the elements of the NSV layer, i.e. the strands of nerve cells and the bundles of fibres, pass into the trunks, termed lateral and medial NSV trunks, accompanying the posterior infundibular and the visceral nerves respectively. Of the two trunks the lateral one carries more fibres from this source.

The nerve cells in the NSV layer and in the trunks are unipolar, present an uniform appearance, and do not show any particular features. They look quite similar to those in *Eledone*, being only slightly larger, measuring about $18\ \mu$, with nuclei about $11\ \mu$ in diameter. The much larger cells which may be present in groups or singly in the NSV trunks belong most probably, as will be explained below, to the vasomotor system.

¹ The term 'lobe' is used here in the same meaning which is adopted in vertebrate anatomy for designation of the main parts of the brain distinguishable in their external aspect.



Text-fig. 1. *Sepia officinalis*. Disposition of the elements of the NSV system. Dorsal view. The diaphragm separating the vena cava from the visceral nerves is not shown. The anterior part of the vein is cut away. The right visceral nerve is pulled aside and twisted to show the nerves running from the medial NSV trunk to the v. cava. On the right side of the drawing the posterior infundibular vein and the prolongation of the lateral NSV trunk are not shown. The interrupted lines across the posterior infundibular nerves indicate the limit of the cephalic cartilage. Some variations of the course of the offshoots of the NSV trunks are shown on the right side of the drawing.

The nerve elements in the NSV trunks are much less regularly arranged than in *Eledone*. The columns of cells may be separated from each other and moreover these columns may be interrupted; the fibres unite into fascicles which may take a more or less independent course. Strictly speaking, the term 'trunk' which appeared appropriate in the description of the NSV system in *Eledone*, is here not so suitable, but it is nevertheless retained for these undoubtedly homologous structures.

As in *Eledone*, the most remarkable feature of the NSV trunks is their extension in the caudal direction far beyond the area where their nerves passing to the vein arise. The general aspect of their disposition can be seen in Text-fig. 1. The lateral trunk associating with the posterior infundibular nerve runs close to it during the passage through the cephalic cartilage and also some distance farther down. It then splits into two parts: the thinner one continues to run alongside the nerve taking a gradually diverging course; the other, which is the main part of the trunk, separates from the nerve at an acute angle and, after crossing the anterior infundibular vein on its dorsal side (Pl. I, fig. 6), runs in the postero-lateral direction. Before crossing, it sends several nerves to the vena cava, and, after crossing, a laterally deviating offshoot. Continuing its course the trunk passes close to the dorsal surface of the m. transversus infundibuli and running still farther in the same direction crosses the posterior infundibular vein and the nerve of the m. depressor infundibuli. The cords of cells of the trunk, which are already in a loose formation before crossing the anterior infundibular vein, diverge gradually during their backward course, diminishing at the same time in thickness. They split finally into a few tapering branches the hindmost of which appears to end at approximately the level of the anus.

The slender part of the lateral trunk, which at first keeps to the posterior infundibular nerve, later deviates from it, crosses the anterior infundibular vein on its ventral side and runs not far from the main trunk in nearly the same direction to split in a similar way into branches ending at the level of the transverse infundibular muscle. The course of this part of the trunk may look different in various preparations owing perhaps partly to deformations produced artificially, but there seem to be real variations also: in some specimens, for instance, the trunk appeared to run with the nerve for a much longer distance than in others, or it was split into two parts, one remaining with the nerve, and the other separating from it (Text-fig. 1, on the right side).

Some differences in the constitution of the trunk at various levels may be observed in sections. Its anterior portion, from the visceral lobe up to the point of departure of the first nerve to the vein, contains a comparatively small number of nerve cells, but a considerable amount of fibres running from the visceral lobe (Pl. I, figs. 3, 4); they form stout fascicles which pass farther back into the nerves to the cava vein. In the region where these nerves arise

the cells increase greatly in number, forming several stout cords (Pl. I, fig. 7; Pl. II, fig. 9). After crossing the anterior infundibular vein these cords diminish gradually in thickness and the number of their cells goes on decreasing notably.

It is self-evident that, as the nerves given off by the trunk arise in the same region, the axons of all the cells must travel towards this region and consequently one part of them runs anteriorly and the other posteriorly; they unite in thinner or thicker bundles which finally pass into one of the nerves running to the vein.

The medial NSV trunks accompany the visceral nerves up to the point where they pass through the cartilaginous diaphragm which stretches between the muscles of the opposite sides separating the visceral nerves from the anterior portion of the vena cava (Pl. III, fig. 13). The NSV trunks usually lie on the ventro-medial side of each nerve, but their position is not always symmetrical and occasionally one of the trunks may even be shifted to the lateral side of the nerve. In cross-sections they present a crescentic appearance (Pl. I, fig. 5). At first sight the elements of the trunks seem to be completely delimited from the visceral nerves; but, on closer examination of sections, groups of nerve cells may be found separated from the trunks and situated somewhere on the periphery of the visceral nerves and even amidst their fibres. The whole trunk may split into several parts and its bundles may run in the space between the visceral nerves.

In that area where the visceral nerves pierce the diaphragm each medial trunk sends its fibres to the vena cava, most of them curving round the cartilaginous plate of the diaphragm (Pl. II, fig. 10). Nerve cells accumulate in this region in great numbers, forming strands of irregular shapes coalescing at places into larger masses (Pl. II, fig. 11). Some of them lie in the visceral nerve, others in the corner between this nerve and its laterally running branch, the *n. depressor is infundibuli* (Hillig). The latter nerve from its point of origin is accompanied by several strands of cells situated chiefly on its ventral side; farther laterally these strands unite into stouter (most often two) trunks (Pl. III, fig. 14). One of them, which is the main prolongation of the medial trunk, deviates from the nerve (Pl. III, figs. 14, 15); running posteriorly and slightly laterally it passes into the space between the *m. depressor infundibuli* and the *m. retractor capitis* and splits into a few tapering endbranches at the level of the anus. In most instances the terminal branches of the medial NSV trunk could be followed farther posteriorly than those of the lateral trunk, but the opposite relation may also occur. As it is difficult to distinguish the finer expansions of the trunks because they do not stain well, it is possible that some of them may in fact run farther than they could be identified with certainty. It is also possible that the branches from the medial and lateral trunks may anastomose with each other in this area.

The shorter of the trunks accompanying the nerve of the depressor muscle

runs a little farther alongside it and either ends without dividing or gives some offshoots. Some variations in their course have been observed: thus, not uncommonly, one longer branch may turn in an antero-lateral direction to end near the transverse infundibular muscle (Pl. III, fig. 14). In one preparation in place of such a branch there was a fairly thick nerve which ran far forwards and entered the vena cava near the nerves from the lateral trunk. This anomaly shows that the nerve cells in the posterior extension of the medial trunk can take part in the innervation of the anterior portion of the vein.

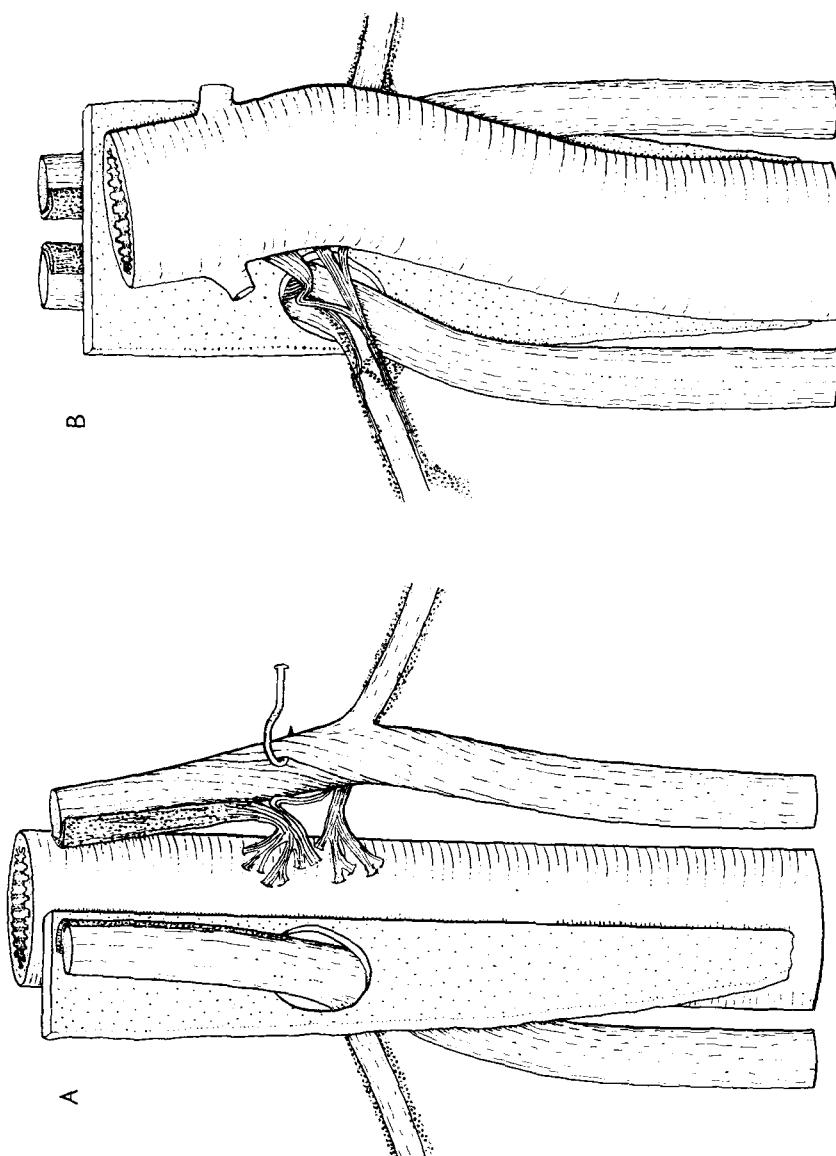
It should be pointed out that all the branches of the NSV trunks described above spread and terminate in loose connective tissue without entering into relation with any organs.

Nerves

The lateral trunk gives off nerves, three or four in number, all arising from the anterior portion of the trunk and reaching the vena cava in that region where the ophthalmic and anterior infundibular veins enter it. The first of these nerves, which is the thickest, runs close to the dorsal surface of the vena ophthalmica and as a rule penetrates into its wall before reaching the vena cava. The last of the nerves behaves likewise with respect to the vena infundibularis anterior (Pl. II, fig. 8).

As aforesaid, the nerves to the vein are made up of fibres which run in the lateral trunk in two directions: one part, arising from the cells situated in the visceral lobe and in the anterior portion of the trunk, runs in the caudal direction; the other, coming out of cells situated in the posterior prolongation of the trunk, is directed rostrally. The fibres from the opposite directions join in a common nerve as its two roots, the posterior one being thinner (Pl. II, fig. 9). Most probably each of the nerves of the lateral trunk has at least one such posterior root, but it is difficult to obtain whole mounted preparations with all of them undamaged, as well as to identify them in sections, unless they happen to be cut in the right plane so that the direction of their curving indicates their origin.

The whole mass of fibres from the medial trunk approaches the cava vein in that region where this trunk together with the visceral nerve passes through the diaphragm (Text-figs. 1, 2). Most of these fibres collect into a very stout nerve which, as mentioned above, curves round the edge of the foramen of the diaphragm. This nerve carries the axons of the cells situated in the visceral lobe and in the anterior part of the medial trunk. The axons of the cells accumulated near the foramen of the diaphragm and in the posterior extension of the trunk unite into a few nerves which may pass directly to the vein or join the nerve curving round the cartilage. In Text-fig. 2 the nerves are shown in an arrangement in which their origin is clearly distinguishable, but which



Text-fig. 2. *Sepia officinalis*. Nerves running to the v. cava from the anterior and posterior portions of the medial NSV trunk. A. Dorsal view. On the right side, the diaphragm is cut away and the visceral nerve pulled to the side and twisted. B. Ventral view. Vena cava is pulled to the side showing the nerve from the medial NSV trunk passing through the foramen of the diaphragm and curving round its edge (cf. Pl. II, fig. 10). Nerves from the posterior prolongation of the medial NSV trunk run partly directly to the vein, partly join the anterior nerve (cf. Pl. II, fig. 12). For the sake of clarity some details of the distribution of nerve elements in this area are simplified or omitted.

is met with rather rarely; more often the fibres coming from the two directions intermingle in such a way that it is very difficult to determine their destination, the more so because their course may sometimes be directly misleading as, for instance, when the fibres coming from the caudal direction appear not to turn to the vein, but to run towards the brain. It is only in sections passing exactly in a plane in which their turning back can be seen that their real destination can be ascertained (Pl. II, fig. 12).

Not all the fibres arriving at the vein from the anterior direction pass through the foramina of the diaphragm. There are some thin fibre bundles which take a more direct route, piercing the cartilage to penetrate into the vein. They seem to belong to the NSV system and it is possible that they correspond to the unpaired nerve in *Eledone* which behaves in a similar way and whose origin is in the NSV layer of the visceral lobe. In *Sepia*, however, the existence of such an individual nerve could not be ascertained because, as pointed out before, there may often be found between the visceral nerves bundles of fibres which detach themselves from the medial NSV trunk and run between the visceral nerves. Their number and arrangement may vary so much at different levels that it is practically impossible to determine whether there is one among them that might be homologous with the unpaired nerve in *Eledone*.

All the nerves given off by the NSV trunks present a characteristic appearance owing to the fact that the axons of the cells of this system are of uniform calibre and that therefore the nerves running to the vein are composed of a mass of very thin fibres (Pl. III, fig. 19). There are also some thicker fibres among them, but only a small number. Their origin does not appear to be in the NSV trunks, but in the visceral lobe, and there is some evidence that they are carried by those fascicles which arise from the central neuropile of the visceral lobe and join the NSV trunks. The nuclei scattered abundantly between the nerve fibres presumably belong to the neurolemmal sheaths.

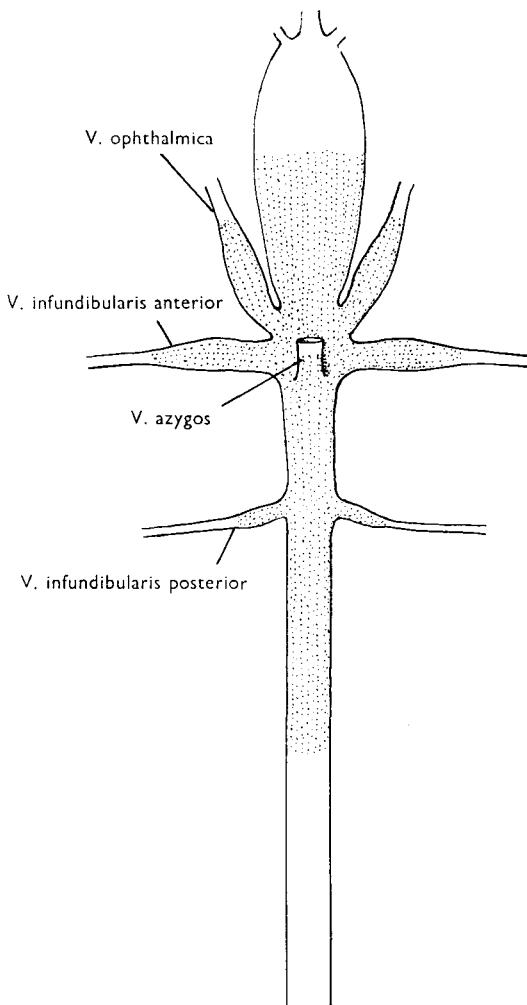
Some of the components of the NSV system were known to Hillig (1912). According to him, one nerve which he called 'N. anterior venae cavae', runs from the visceral lobe alongside the n. posterior infundibuli to end with several branches on the vein, and two or three other nerves ('Nn. venae cavae posteriores') arise from the visceral nerves and branch off in the vein. In the light of the description of the NSV system given above, Hillig's 'Nervus anterior', as far as it associates with the posterior infundibular nerve, is in fact the lateral NSV trunk.

The presence of the nerve cells in the visceral nerve was noticed by Thore (1939), but besides recording this fact he does not say anything about it.

Distribution of the nerves in the vein

The stout nerves arising from the NSV trunks give off branches penetrating through the muscular coat of the vein (Pl. IV, fig. 20). They do not give

subdividing branches as in *Eledone*, but at once dissolve into flattening bundles of fibres (Pl. IV, fig. 21); the latter take a general longitudinal course in both directions, spreading under the muscle layer all round the vein as an almost continuous layer. The arrangement of the fibres in it is more regularly longitudinal than in *Eledone*, owing to the fact that in *Sepia* the nerves



Text-fig. 3. *Sepia officinalis*. Territory occupied by the neuropile layer in the v. cava and its tributaries.

from the trunks approach the cava vein in two areas only so that the fibres have to run a long way in both directions, whereas in *Eledone* they spread from many nerves entering the vein at short distances one from another.

In the whole area of expansion of the nerve fibres some of them gradually turn towards the endothelium and break up into fine filaments intertwining with each other, thus forming a continuous neuropile layer. Its anterior limit is approximately midway between the anterior end of the vena cava and the orifices of the venae ophthalmicae, and the posterior one well behind the entrance of the posterior infundibular veins at a distance about one and a half times greater than that between the anterior and posterior infundibular veins (Text-fig. 3). The neuropile layer extends moreover into several tributaries of the cava vein, viz. v. azygos, vv. ophthalmicae, vv. infundibulares anteriores and posteriores, all of which are supplied by fibres deriving from the nerves of the cava vein. In the ophthalmic and the infundibular veins the neuropile layer extends up to the point where the bottle-shaped enlargement of these vessels ends, while in the v. azygos it occupies only a short portion of the vein, equal approximately to its diameter. These limits can often be clearly distinguished in methylene-blue preparations because those parts of the veins with the modified structure of their inner layer, which also have a thicker muscle coat, stain more darkly than the rest of the veins (Pl. I, fig. 6, Pl. III, fig. 16).

All the parts of the veins in which the neuropile layer is extending are distinguished by the presence on their inside of longitudinal ridges, interconnected by oblique links (Pl. III, fig. 17). They look quite similar to those described previously in *Eledone*, but are more regularly parallel in their course. They are much higher on the dorsal wall of the veins and on the sides than those on the ventral wall, where they are very low near the midline.

The ridges are produced by the foldings of the neuropile layer, whose surface becomes increased in this way. The varying height of the ridges depends on the thickness of the neuropile and on the number of nerve fibres under it. The abundance of the latter and their course, which is straighter than in *Eledone*, can be best seen in tangential sections (Pl. IV, fig. 24).

A peculiar feature of the cava vein in *Sepia* is the presence of thread-like tie-bars which can be found in varying numbers on its inside. They are always present in the region where the ophthalmic and the anterior infundibular veins enter the cava vein (Pl. III, figs. 17, 18). They consist of the same tissue as the inside layer of the vein and are continuous with it at both ends. One of the bars at the vessel orifices may be markedly thicker than the other: in symmetrical arrangement the thicker bar was found spanning on each side the orifice of the ophthalmic vein and the thin one that of the anterior infundibular vein. A reverse situation was also observed. Not uncommonly, however, a bar on one side, or even on both sides, was found missing and the remaining one, which could be of either sort, crossed either the anterior or the posterior vein orifice, or was situated between them. The length of the bars is variable, even in specimens of the same size. As a rule the thinner ones are

longer, the longest observed measuring 5 mm. The thinner bars are often bifurcated and then shaped as the letter Y.

Tompsett (1939), in his monograph on *Sepia*, mentions the presence in this region of an 'unpaired tie-bar situated somewhat on the right side'. This would be the extreme case in which the set of four bars was reduced to one only.

Tie-bars of the thicker sort have been observed only at the orifices of one of these veins; the thinner ones can occur at various places in the cava vein. The greatest number of them seen in one preparation was four, but as they are randomly distributed and can be easily torn away they may be actually more numerous than that. They are usually directed longitudinally or obliquely; in one preparation a transverse bar was found. It may be added that the tissue of the inner layer of the vein is very delicate and special caution is necessary if one wants to obtain whole mounted preparations with the tie-bars, as well as the ridges, undamaged.

Neuropile layer

It is evident from the foregoing description that the neuropile layer in the vena cava, and in some tributaries, is the terminal field of the whole NSV system, since to the best of evidence the axons of all the nerve cells situated in the NSV layer of the visceral lobe and in the NSV trunks end in it, producing a network of extraordinary density (Pl. IV, figs. 22, 24, 25). Not all the nerve elements in the latter, however, are of the same origin: there are also here some fibres of another sort, distinguished by their greater calibre, which evidently derive from those thicker fibres which in small number are present in the nerves running to the vein. In the neuropile layer they give ramifications which show a tendency to end in small swellings (Pl. IV, fig. 23). Whether or not these are artefacts is an open question. The identification of the thicker fibres is difficult and uncertain because of the presence of connective tissue fibres which may stain and look like nerve elements. In this respect the study of the neuropile layer in *Eledone* is much easier, since the connective tissue fibres in it are arranged in a characteristic pattern while in *Sepia* they mix with the nerve fibres in such a way that the picture is confusing.

Another difference in the appearance of the elements seen in sections through the neuropile layer of the two species is the shape of the nuclei of the endothelium covering this layer: in *Eledone* they are oval or elongated and have even outlines, whereas in *Sepia* they are mostly of irregularly angular shape (Pl. IV, figs. 23-25)—some may even have processes longer than the nucleus itself. Here again the question arises whether these are not artefacts, although it seems doubtful that such deformations could be produced during routine handling of preparations.

It may be added that the limits between the cells of the endothelium in the vein of *Sepia*, as well as in that of *Eledone*, could never be observed. It is therefore probable that this lining has a syncytial structure.

The cells of bizarre shape described previously in the neuropile layer of *Eledone* do not show in *Sepia*.

In the meshes of the neuropile fine particles of some substance are included. In *Eledone* they could be easily distinguished because they stained selectively with paraldehyde fuchsin, but in *Sepia* they did not show such specific affinity.

The tie-bars may be regarded as parts of the ridges detached from the wall of the vessel. They consist of axially oriented thin nerve fibres with some connective tissue between them and a neuropile layer around. In the thick bars to the bundle of nerve fibres some muscle fibres are added. It is possible that the thin bars can occasionally contain muscle fibres also: they have not been observed in sections, but in one instance, in a tie-bar left *in situ*, distinct though feeble contractions could be noticed.

Some peculiar agglomerations of nerve cells

Between the visceral nerves just dorsal to their roots there is a small ganglion which is connected with the visceral lobe and may be regarded as an appendix of the latter. It contains large nerve cells similar to the large cells in the visceral lobe and small cells which have some resemblance to those of the NSV layer, but whether they belong to the NSV system is doubtful. There appears to be some variability in the size and connexions of this ganglion. It has been found in asymmetrical position, and in some instances could not be identified. The matter needs further study.

Another agglomeration of nerve cells situated a little posterior to the former is peculiar in that it grows into the small vein running between the visceral nerves (Pl. V, fig. 29). This ganglion consists of cells of the same size and appearance as in the NSV system and is likely to be one of its components. On its surface turned towards the lumen of the vessel flat nuclei can be seen probably belonging to the intima of the vein pushed inside by the ingrowing nerve cells. At some places the ganglion has irregular protuberances containing a fine network of fibres. The intima of the vein in this region shows swellings very similar to the neuropile layer of the cava vein, but confined to a limited area only.

Vasomotor system

The observation of the innervation of the muscles of the vena cava in *Sepia* corroborated the view I had previously expressed that the vasomotor nerves in cephalopods have a multiple origin, i.e. derive from cells situated in various nerves (which does not imply that there would not be some centre in the

brain regulating their action). Nerve cells presumably belonging to this category are present in the visceral and posterior infundibular nerves of *Sepia* and look exactly like those previously found in one of the stellar nerves, being arranged in the same way in small groups or rows in which they may lie not very close together; single cells are also not uncommon (Alexandrowicz, 1962). In groups or singly, they may be situated not only between the fibres of the visceral and posterior infundibular nerves, but also amidst the cells of the NSV trunks, where they can be easily distinguished by their greater size ($25\text{--}60\ \mu$). The intermingling of the cells of two systems might give rise to the assumption of some functional relation between them; but it is more probable that this situation is accidental and results from the irregular distribution of the elements of both the vasomotor and the NSV system in *Sepia*. In *Eledone* the cells of the vasomotor system are accumulated into more compact ganglia and are more distinctly separated from the cells of the NSV system.

The vasomotor nerves run to the vena cava independently of the NSV nerves. Several of them spring from the visceral nerves as might be expected, but one was found to have a different origin, being given off by the posterior infundibular nerve. This fact is noteworthy in two respects: it shows that this vein may receive nerve fibres from two sources, and it explains the occurrence of groups of large nerve cells in the posterior infundibular nerve.

The fibres of the vasomotor nerves spread on the muscles of the vein in a characteristic pattern different from that of the NSV system.

Hints on dissection

For anaesthetizing the animals chloroform can be used. The mantle should be opened along the mid-line of its ventral side and the rectum together with the ink-sac duct should immediately be constricted by a ligature. The arms can be cut away and the mantle with the shell removed. The liver capsule should be split longitudinally on its dorsal side, the oesophagus and the salivary glands cut out, and the liver removed, detaching it from the capsule with some blunt instrument or with fingers, which is easily done, but care must be taken not to damage the visceral nerves. Now all the unnecessary parts of the preparation lying well beyond the area in which the NSV system is situated can be cut away. The funnel can be removed or split longitudinally and the two halves pulled to the sides. The viscera should be cut by a transverse section passing approximately at the level of the renal papillae. Before that the rectum and the ink-sac duct should be detached and pulled backwards, or, if one wants to leave them *in situ*, they must be cut through between a double ligature. The whole preparation can now be attached to a paraffin plate with the ventral or dorsal side up and put into the solution of methylene blue (in proportion of 10–15 drops of 0·5% solution of methylene blue in distilled water added to 100 c.c. of sea water).

In a preparation with the dorsal side up it is easy to find the posterior infundibular nerves at the point where they come out of the cephalic cartilage since at this place they are not covered by the dense membrane of the liver capsule. They can be followed from this point in both directions: to arrive at their roots it is necessary to take away the cartilage piece by piece; exposing the nerve up to the visceral lobe proved to be helpful for the more exact determination of the plane of the sections through its root.

The course of the nerve in the opposite direction can be followed by cutting through the tissues covering it, i.e. the liver capsule and the muscle layer. With care it is possible to expose the lateral trunk deviating from the nerve and the nerves running to the veins. It is also possible to expose the visceral nerves beyond their passage through the diaphragm and also the medial trunk running with the nerve of the depressor muscle and diverging from it. The latter, however, as well as the prolongations of the lateral trunk, are more easily accessible from the other side.

Proceeding from the ventral side one has to remove all the tissues covering the vena cava. Before doing so it is advisable to inject some of the methylene-blue solution into this vessel, which makes it easier to expose: for this a four-times stronger solution can be used and the same can also be injected at various points of preparation where the elements of the NSV system are situated. As it is almost impossible to identify them in unstained preparations it is necessary to wait until at least parts of them become discernible. On exposing the cava vein one must be aware that there is a whole system of muscles accompanying it: most of them run longitudinally as thicker and thinner bundles (some of them look like nerves) and there are also thin muscles attached to the vein and running transversely; moreover, just anterior to the foramina of the diaphragm, there is a broad band of muscle fibres inserting into the cartilage on both sides of the vein and running in half-circles in front of it (Pl. III, fig. 13). With the ventral side uppermost the lateral trunk can be best looked for in the area where it crosses the anterior infundibular vein, the latter easily distinguished with the vena cava injected since the coloured fluid passes also into the infundibular and ophthalmic veins. The medial trunk can be identified at this point where it deviates from the nerve of the depressor muscle, sometimes identifiable even in unstained preparations. Once the NSV trunks are recognized they can be freed from the overlying tissues along their whole length. This, however, must be done by stages, interrupting the dissection and leaving the preparation in the staining solution until the nerves are visible a little farther. If some parts appear to be more easily accessible from the other side, the preparations can be turned over. The staining proceeds slowly; it is therefore advisable to leave the preparations in the methylene-blue solution for a long time, up to 24 h.

From the histological point of view the staining is as a rule not satisfactory because the nerve elements are not properly differentiated, but as the components of the NSV system stain in a different tone and differ also somewhat in their appearance from the ordinary nerves, their distribution may be more or less well determined.

OCTOPUS

The first observations about the innervation of the vena cava in the Octopoda (*Eledone*, *Octopus*) were made by Chéron (1866), who discovered a nerve running from the visceral lobe to the anterior part of the vein and called it 'nerf de la grande veine'. Pfefferkorn (1915) changed its name to Nervus venae cavae anterior to distinguish it from another nerve which he termed 'N. venae cavae posterior'. The latter, according to him, is present in *Eledone*, but missing in *Octopus*. This would be an important difference indeed, because, as shown in the first part of the present work, this nervus posterior of Pfefferkorn is a nerve in its distal portion only, while its proximal portion is in fact the ganglionic NSV trunk. Its absence therefore would mean that this trunk is missing in *Octopus* and consequently the whole disposition of the

NSV system in this species would differ not only from that in *Eledone*, but also from that in *Sepia*. This, however, proved not to be the case. Pfefferkorn's mistake was obviously due to the fact that the visceral and posterior infundibular nerves in *Octopus* run for a certain distance close together—so close in fact that the lateral and medial NSV trunks in their proximal course may not be completely separated from each other. Moreover, the nerve springing from the lateral trunk continues at first to run alongside these nerves and thus can be taken for a branch of the visceral nerve. Actually, as can be clearly seen in methylene-blue preparations, the two NSV trunks a little farther posteriorly have separate courses, associating with the visceral and posterior infundibular nerves respectively (Pl. V, fig. 27), and there can be no doubt about the existence of the nerve arising from the lateral NSV trunk and running to the cava vein (Pl. V, fig. 28). It can also be ascertained that this nerve, formed by fibres coming out of the visceral lobe and of the anterior portion of the lateral trunk, has as in *Eledone* an additional root which conveys fibres from the posterior extension of the trunk.

The general disposition of the NSV system in *Octopus* is very much the same as in *Eledone* (Pl. V, fig. 26): the lateral trunk, accompanying at first the posterior infundibular nerve, deviates later from it and runs in the postero-lateral direction to end as several tapering strands. The medial trunk, which in its anterior course up to the passage through the diaphragm is closely connected with the visceral nerve, separates from it, but both run near to each other flanking the vena cava, to which the medial trunk sends many nerves. At the point where the last of these nerves takes its origin the trunk turns outwards, crosses the visceral nerve and penetrates through the m. adductor pallii medianus to end with several offshoots. Compared with the arrangement of the NSV system in *Eledone*, the following particular features, besides those mentioned above, could be observed in *Octopus*: its medial trunks run closer to the vein and their nerves are shorter and shifted more to the dorsal side; the lateral trunks give off fewer offshoots, which are slender and do not form such plexuses as may be seen in *Eledone*; conversely, the offshoots of the medial trunks in the region where they cross the visceral nerves seem to be more numerous in *Octopus* and of considerable thickness. Since in *Eledone*, too, the aspect of the components of the NSV system is variable, all these differences are of minor importance. Otherwise, the distribution of the nerves in the vein, and the network of their terminations under its endothelium, look very much alike, and the substance included in the meshes of the neuropile layer shows also the specific affinity to the paraldehyde fuchsin stain.

Comparison of the NSV system in Sepia and in the Octopoda

The main difference between the NSV system in *Sepia* and in the Octopoda is the absence in the former of the anterior nerve which in *Eledone* and

Octopus runs from the visceral lobe in the cartilaginous wall between the statocyst capsules to branch in the anterior portion of the cava vein. Evidently in relation to its absence the neuropile layer in this vein in *Sepia* does not extend to its foremost region. However, as the territory supplied by the anterior nerve in octopods is larger than this nerveless region in *Sepia*, it must be assumed that the corresponding parts of the vein in the latter species receive nerve fibres passing from the visceral lobe by way of the lateral NSV trunks. This may be the cause, or perhaps the result, of the shifting of the larger masses of the NSV layer to the sides of the visceral lobe and the attenuation of this layer near the mid-line.

The absence of the anterior nerve to the cava vein in *Sepia* has been the cause of the unwelcome confusion in the nomenclature used in the two authoritative works on the nervous system in cephalopods (of Hillig and Pfefferkorn), for homologous nerves have been given different names. In the terminology adopted in the present work and shown in the left column of Table 1, the term 'Nervus anterior venae cavae' has been retained in the sense given to it by Pfefferkorn, while the other nerves are designated according to their origin from one or the other NSV trunk.

TABLE 1. NOMENCLATURE OF THE NSV NERVES

	Pfefferkorn	Hillig
Nervus venae cavae anterior in <i>Eledone</i> and <i>Octopus</i>	Nervus venae cavae anterior in <i>Eledone</i> , <i>Octopus</i> and <i>Argonauta</i>	—
Nerves of the lateral NSV trunk: one on each side in <i>Eledone</i> and <i>Octopus</i> ; several in <i>Sepia</i>	Nervus venae cavae posterior in <i>Eledone</i> ; absent in <i>Octopus</i>	Nervus venae cavae anterior in <i>Sepia</i>
Nerves of the medial NSV trunk in <i>Eledone</i> , <i>Octopus</i> and <i>Sepia</i>	Rami venae cavae in <i>Eledone</i> and <i>Octopus</i>	Nervi venae cavae posterores in <i>Sepia</i>

It is evident that the NSV system in *Sepia*, apart from the difference just mentioned, is built according to the same pattern and consists of the same components as in the two octopod species, but it may be said that these components in *Sepia* are arranged in a much less orderly manner. This feature shows itself in the texture of the NSV layer and of the trunks, in the course of the fibre bundles in the visceral lobe and in the formation of nerves. In one instance only is a greater regularity to be found in *Sepia*, viz. in the distribution of the nerve fibres in the vein.

The comparison of the general view of the innervation of the vena cava in the species investigated may give the impression that it is poorer in *Sepia* than in *Eledone* and *Octopus*, for in the latter two species this vein, apart from receiving the supply from the anterior nerve, is approached by many nerves from the medial trunks, whereas in *Sepia* a long stretch of the vein does not receive any nerve. This is obviously due to the more posterior position of the

foramina of the diaphragm in *Sepia* so that the nerves from the medial trunk pass on to the vein far distant from the nerves given off by the lateral trunk. The number of nerves from the medial trunks is indeed smaller in *Sepia*, but they are very thick and at any rate carry a sufficient number of fibres to form a neuropile quite similar to that in octopods. It is true that one has the impression that the whole system in *Sepia* contains fewer neurons than in *Eledone*, in which an approximate estimate of their number is over two million. Their disposition in *Sepia* is such that I did not even try to count them. If their number is indeed smaller, this may be in relation to the dimensions of the area occupied by the neuropile layer. The stretch of the vena cava containing this layer, in specimens of approximately similar weight, is definitely shorter in *Sepia*, and, even taking into account the extensions of the neuropile into the tributaries of the cava vein, its total surface appears to be smaller. In an evaluation of the total volume of the neuropile not only its surface, but also its thickness, must be taken into consideration; but the thickness of this layer is so variable that the calculations obtained on the basis of its measurements would be grossly inaccurate.

The tie-bars are in fact parts of the ridges in which as the result of their detachment from the wall of the vein the surface of the neuropile layer becomes increased. Their situation across the orifices of the veins shows that the exposure of this surface to the blood stream is of some importance and affords corroborative evidence to the previously expressed assumption that a mechanical factor is instrumental in releasing from the neuropile its products of secretion. Whether these products are identical in the three species investigated remains to be established; there is some evidence that they are not, since the same specific staining method which gave positive results in *Eledone* and *Octopus* did not succeed in *Sepia*. It is, however, possible that the presence of the presumed hormone in the neuropile layer is subject to fluctuations depending on the condition of the animal. As a matter of fact, the specimens of *Sepia* from which the preparations were made were all in a moribund state. Whether this had some influence on the negative results could be verified by examining more animals. I have not been able to do this for many months because the cephalopods have at present disappeared almost completely from Plymouth waters.

A question to be answered is whether similar networks to those in the vena cava and in some of its tributaries may occur in other places of the cephalopod body. In this respect the presence of neuropile-like structures in the small vein between the visceral nerves deserves attention. It would be worth while taking into consideration the possibility of other veins containing similarly differentiated nervous tissue, especially in those instances in which they have some unusual innervation, as, for example, the suborbital sinus in *Octopus* (Boycott & Young, 1956).

It is noteworthy that the neuropile networks exposed to the blood

current are present also in the Crustacea (Stomatopoda, Decapoda). In the Brachyura the bars of the so-called pericardial organs containing such tissue span the openings of the branchio-cardial veins in the same manner as the tie-bars in *Sepia* span the openings of the veins entering the vena cava. The neurosecretory activity of the pericardial organs in crustaceans has been already ascertained; that of the NSV system in Cephalopoda has not yet been demonstrated by physiological experiments, but there are good grounds for attributing to it such a function. It is, moreover, quite possible that in all above-mentioned instances the elaboration of the products of secretion takes place in the terminal networks. In view of the fact that such analogous structures occur in so different groups of animals as Crustacea and Cephalopoda it seems not improbable that in the circulatory system of higher animals also a similarly differentiated nervous tissue, functioning on the same principle, may exist, as yet undiscovered, or, if observed, unexamined from this point of view.

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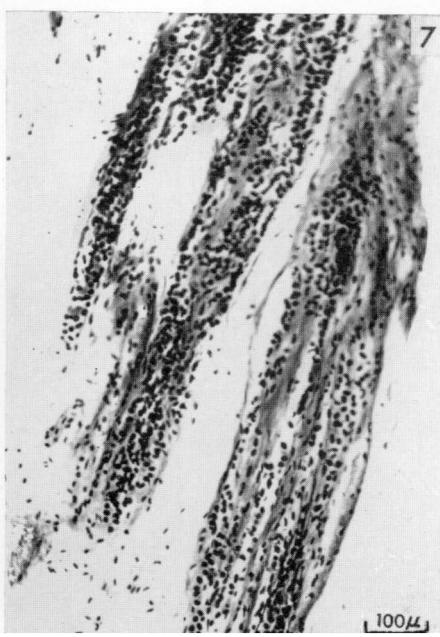
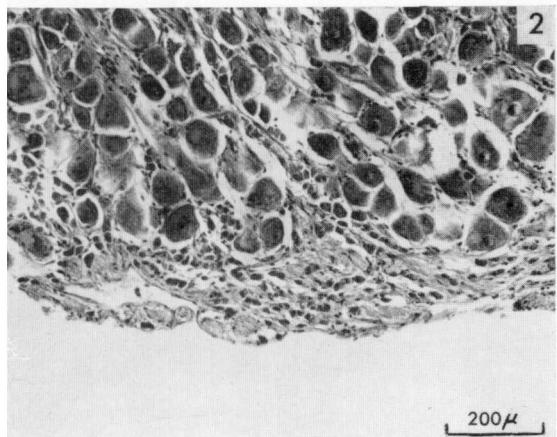
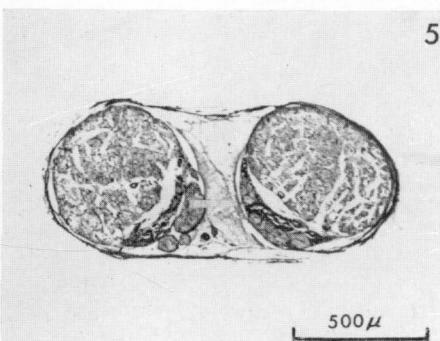
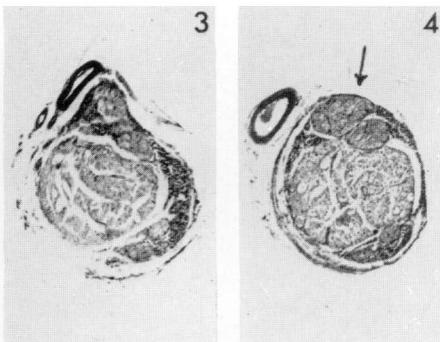
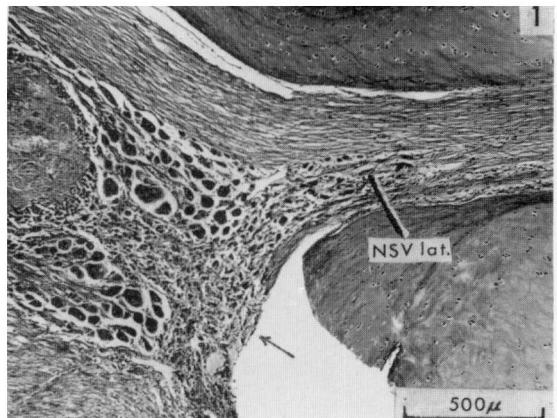
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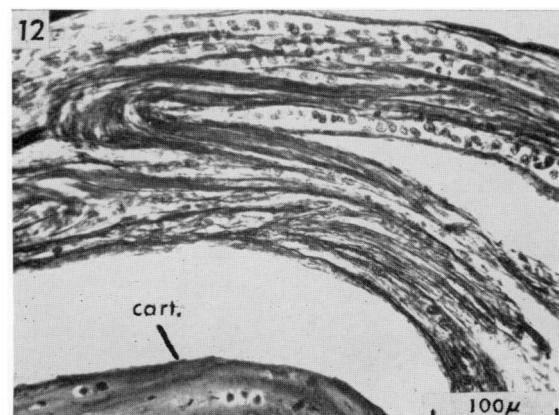
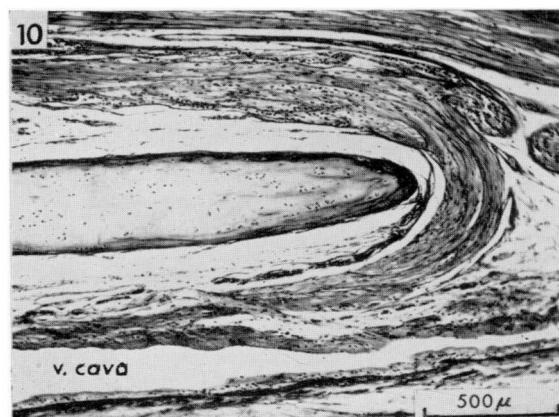
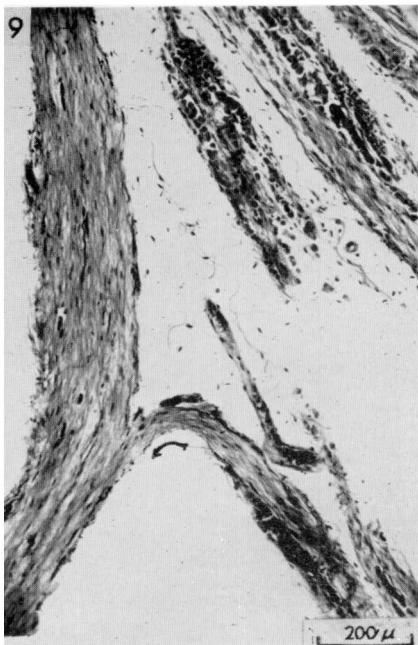
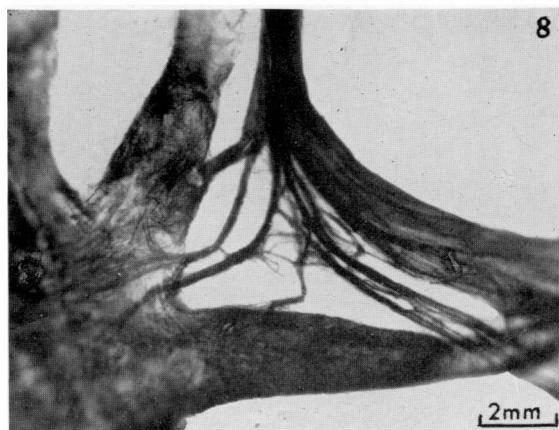
EXPLANATION OF PLATES I-V

PLATE I. *Sepia officinalis*

Fig. 1. Longitudinal section through the posterior infundibular nerve emerging from the visceral lobe showing the lateral NSV trunk continuous with the NSV layer of the visceral lobe. The arrow indicates a large protuberance of the NSV layer.

Fig. 2. NSV layer with strands of its cells passing between the larger nerve cells of the visceral lobe.





Figs. 3, 4. Transverse sections through the posterior infundibular nerve accompanied by the lateral NSV trunk a short distance after their passage through the cephalic cartilage; in fig. 4 the same trunk is divided into two parts. Note the thickness of the bundles of fibres originating in the NSV layer (arrow). Same magnification as in fig. 5. All three figures have been made from a small specimen, weighing 61 g.

Fig. 5. Transverse section through the visceral nerves accompanied by the crescent-shaped medial NSV trunks.

Fig. 6. Lateral NSV trunk crossing the vena infundibularis anterior. Photograph made from the same preparation as in fig. 8. Note the composition of the trunk of several cords, and the limit of the region of the vein with modified inner layer (cf. Text-fig. 3). Methylene blue.

Fig. 7. Longitudinal section through the lateral NSV trunk in the same region as shown in fig. 6.

PLATE II. *Sepia officinalis*

Fig. 8. Posterior infundibular nerves accompanied by the lateral NSV trunk in the region where the nerves from the trunk pass on to the v. cava. The posterior infundibular nerve with the lateral trunk have been pulled aside to show the nerve entering the ophthalmic vein (cf. Text-fig. 1). Methylene blue.

Fig. 9. Nerve to the v. cava composed of fibres running from the anterior and posterior portions of the lateral NSV trunk. Upper right, cords of the lateral trunk.

Fig. 10. Medial NSV trunk giving off the nerve curving round the cartilaginous plate of the diaphragm and running to the v. cava.

Fig. 11. Agglomerations of the cells of the medial NSV trunk in the corner between the visceral nerve and the nerve to the m. depressor infundibuli. Cells and fibres situated alongside the nerve (upper right) are also parts of the medial NSV trunk. Bodian's method.

Fig. 12. Nerve fibres coming from the posterior prolongations of the medial trunk (upper right) and turning into the nerve curving round the cartilaginous plate (*cart.*) of the diaphragm, as in fig. 10. The section passes near the periphery of this nerve. Bodian's method.

PLATE III. *Sepia officinalis*

Fig. 13. Transverse section through the v. cava and the visceral nerves anterior to the passage of these nerves through the diaphragm: *a*, longitudinal muscles; *b*, muscle band formed of semicircular fibres. Preparation from the same specimen as in fig. 5.

Fig. 14. Medial NSV trunk deviating from the nerve of the m. depressor infundibuli. Part of the trunk flanks the upper side of the nerve. Offshoot of the trunk runs in antero-lateral direction (cf. Text-fig. 1, on the right side).

Fig. 15. Longitudinal section of the medial NSV trunk at the point of its deviation from the nerve (cf. fig. 14).

Fig. 16. Posterior infundibular vein showing sharp delimitation of its region containing the neuropile layer.

Fig. 17. Part of the v. cava with the longitudinal ridges seen from inside. On the left, orifice of the v. azygos into which pass the ridges; on the right, orifice of the v. infundibularis anterior spanned by a tie-bar; the vein itself has been cut away. The irregular appearance of the ridges is chiefly due to the uneven stretching of the preparation and damaging of some of the ridges. Methylene blue. Preparation from a large specimen weighing 1060 g.

Fig. 18. Transverse section through the v. cava at the entrance of the v. infundibularis anterior with a part of the tie-bar cut longitudinally. Bodian's method.

Fig. 19. Nerve of the medial NSV trunk curving round the plate of the diaphragm. The arrow indicates two thicker fibres. Bodian's method.

PLATE IV. *Sepia officinalis*

All photographs have been made from sections stained with Bodian's method.

Fig. 20. Longitudinal section through the wall of the v. cava showing a nerve penetrating through the muscle coat and forming the neuropile layer.

Fig. 21. Tangential section through the nerve in the v. cava distributing its fibres in the space between the muscle coat and the neuropile layer.

Fig. 22. Neuropile layer in the v. infundibularis anterior. Longitudinal section.

Fig. 23. Tangential section of the ridges with a thicker fibre ending in a swelling.

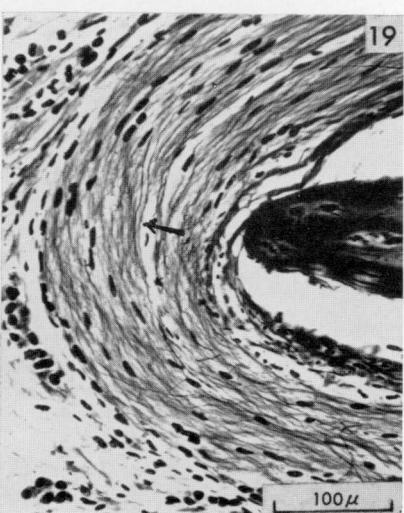
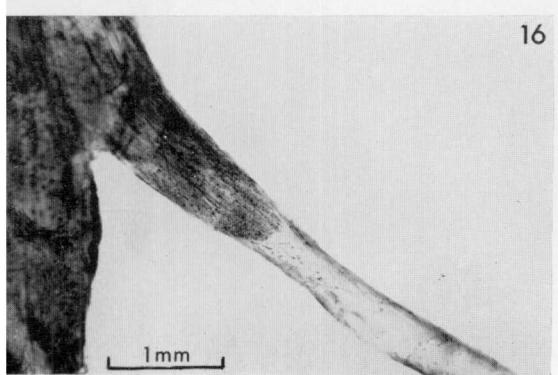
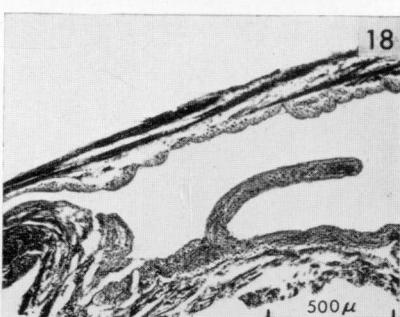
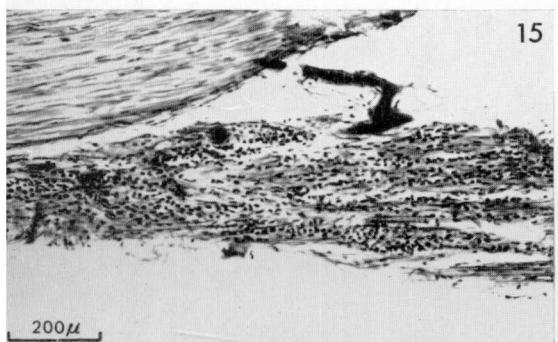
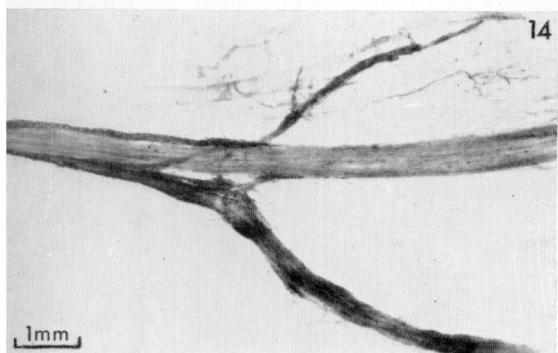
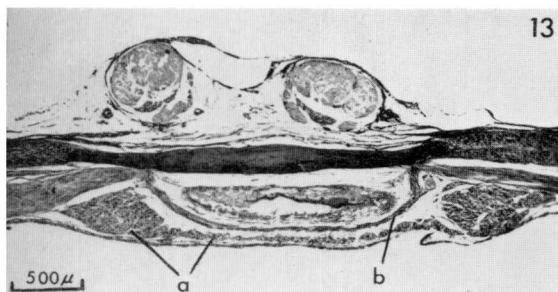
Fig. 24. Near-tangential section of the ridges. The plane of the section is inclined in relation to the inner surface of the vein so that it passes through the endothelium, seen in the upper part of the figure, and through the nerve fibres in the ridges, seen in the lower part of the figure.

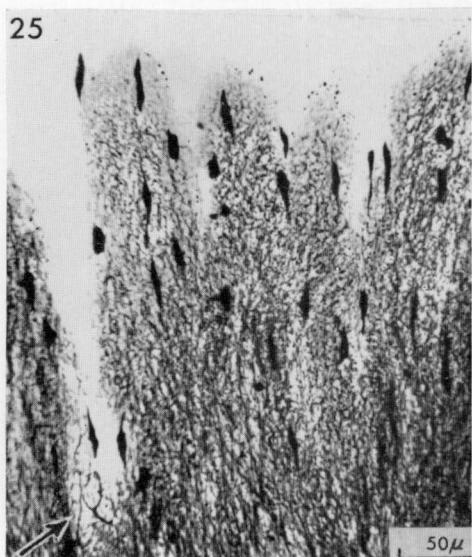
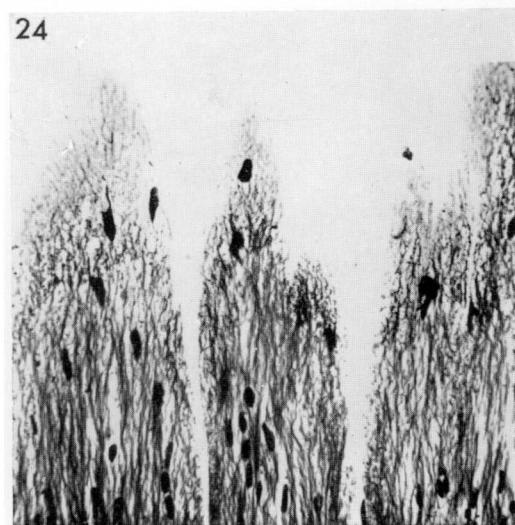
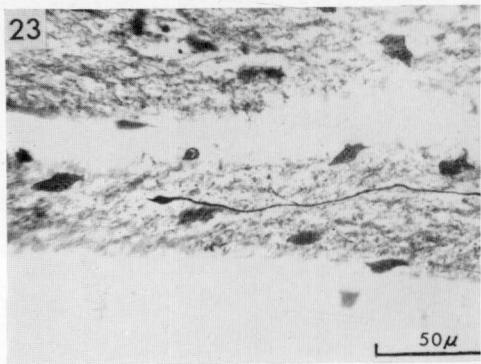
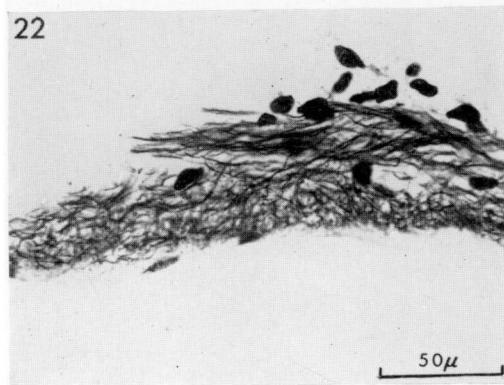
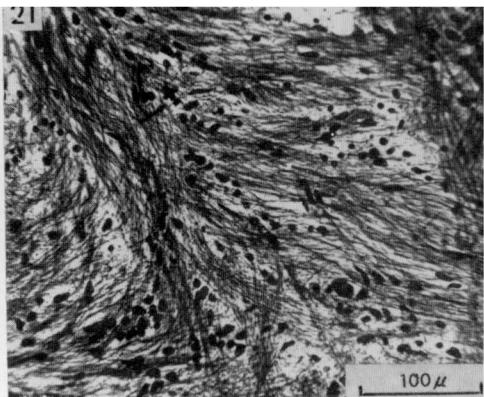
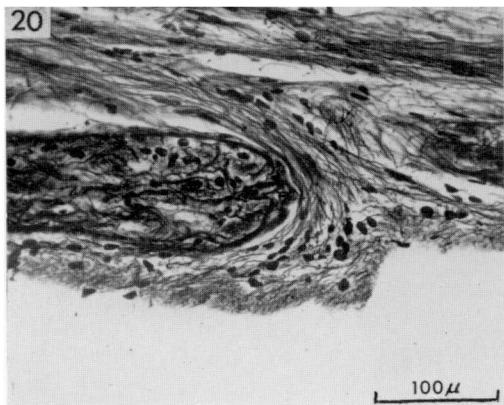
Fig. 25. Tangential section almost parallel with the inner surface of the v. cava, showing the endothelium and the fine neuropile under it. Down left, the arrow indicates thicker fibres branching in the neuropile layer.

PLATE V

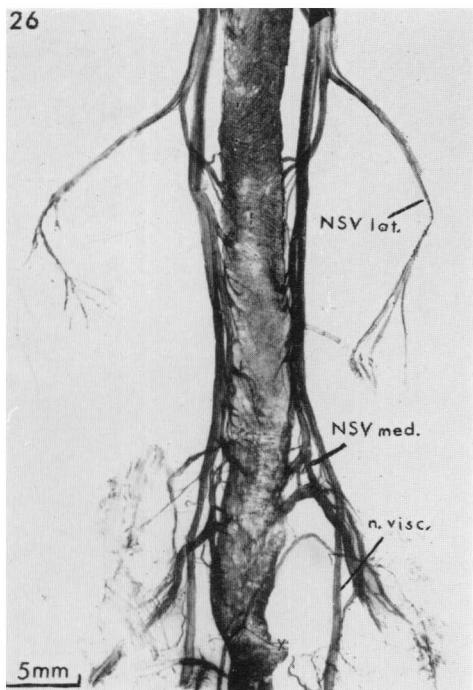
Figs. 26–28. *Octopus vulgaris*. Lateral and medial NSV trunks and their nerves running to the v. cava. All three figures made from the same preparation. In figs. 26 and 27 the two trunks (*NSV lat.*, *NSV med.*) can be clearly distinguished; *n.visc.*, nervus visceralis; *n.i. post.*, nervus infundibuli posterior. In fig. 28, the nerve from the lateral trunk is seen receiving fibres coming from the posterior prolongation of the trunk (arrow). Methylene blue.

Fig. 29. *Sepia officinalis*. Ganglion protruding into a small vein running between the visceral nerves.

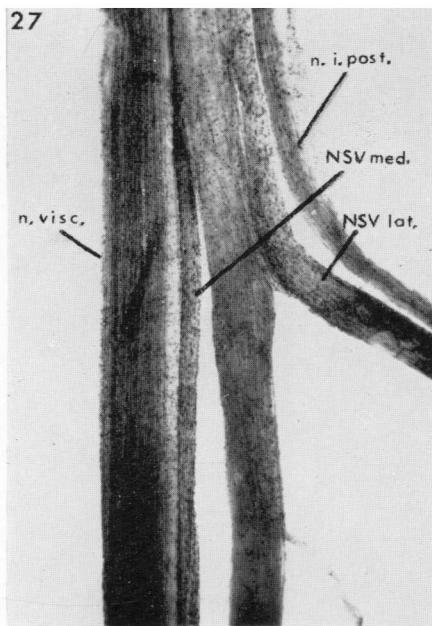




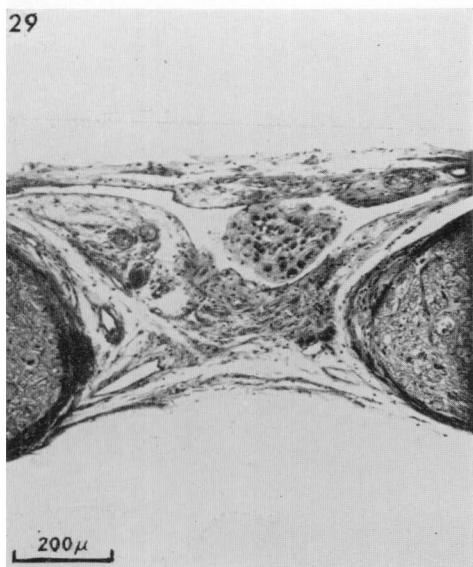
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