STUDIES ON THE BRAIN OF NEPHTYS

(ANNELIDA, POLYCHAETA)

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INTRODUCTION

Most families of polychaetes are homogeneous, but there is none more so than the Nephtyidae. The smallest species is only a few millimetres long, the largest a foot long, but apart from this size difference, one member of the family looks much like another. Most systematists (e.g. McIntosh, 1908; Fauvel, 1923) have regarded the family as monogeneric, though in the most recent monograph (Hartman, 1950), it has been divided into three genera, one of them comprising a single species. Whether the family should be regarded as mono- or trigeneric, the differences separating genera and species are trivial. The only characters on which taxonomists can rely are the number and disposition of the branchiae, the form of the parapodial lobes and chaetae, and the number of papillae on the proboscis. This uniformity of external morphology is undoubtedly a reflection of the fact that all the species live in much the same habitat and, from what little is known of them, have similar habits. Nephtys lives in intertidal or sublittoral sand or mud; it does not occur among rocks or debris. It probably does not form a permanent burrow, but lives in the surface of the substratum which it may leave for spawning or other excursions since it is an active swimmer.

The structure which *a priori* one would expect to vary least in such a family is the nervous system. Not only is this regarded as a comparatively conservative part of the animal, so far as evolution is concerned, but, if the behaviour is more or less the same from one species to another, one would expect that even in detailed structure the brains of all nephtyids would be the same. This is not the case however. Even in gross morphology the supra-oesophageal ganglion and its associated structures are extremely variable and there are several obvious differences in the minute structure of the ganglion in different species. In this study attention has been directed primarily towards a pair of posterior lobes of the supra-oesophageal ganglion which are found in some species but not in others.

From the brief consideration of the structure of the ganglion which precedes the account of the posterior lobes, it appears that, apart from the latter, the posterior half of the ganglion is relatively constant throughout the family and that it bears a strong resemblance to the posterior part of the nereid brain. The most important features of this region of the brain are the eyes and groups of neurosecretory cells. Morphological and experimental evidence will be presented which suggests that these structures are homologous in the two families. In particular, one group of neurosecretory cells in the brain of *Nereis* appears to be

homologous with the posterior lobes of Nephtys, and this fact provides a basis for a re-examination of the current concepts about the fundamental nature of neurosecretory cells.

HISTORICAL.

HISTORICAL

There have been some half-dozen accounts of the morphology of the brain of the Nephtyidae, all but one of them nineteenth century works. Although they deal with only three species, Nephtys caeca Fabricius, N. hombergi Aud. & Edw., and N. cirrosa Ehlers, the accounts differ in almost every detail. Certainly none of the nineteenth century authors anticipated great inter-specific variation, and indeed, most of the controversy that was aroused was based essentially on the supposition that the supra-oesophageal ganglion would be identical in all species of Nephtys. However, although inter-specific differences account for some of the differences between the descriptions of various authors, they do not account for them all; several of the accounts are concerned with the same species and still conflict.

The first account of the brain of Nephtys was that by Delle Chiaje (1825) who described and published drawings of the nervous system of N. hombergi (under the name of Nereis scolopendroides). It is now only of historical interest, for the illustration (fig.1A) shows an enormous, heart-shaped sub-oesophageal ganglion, a quadrilobed supra-oesophageal ganglion, and circum-oesophageal connectives with a pair of ganglia on each, such as never existed in any

polychaete. However, Delle Chiaje must be given credit as one of the first invertebrate zoologists to describe the supra- and sub-oesophageal ganglia in any annelid. He failed to notice that the ventral nerve cord was a double structure, but this is difficult to observe in Nephtys without cutting sections of the animal.

The earliest account of any significance was that by Quatrefages (1850), who described a remarkable brain in Nephtys bononensis (= N. caeca). His illustration is shown in fig.1B. In the posterior part of the prostomium, he found an elongate, oval structure, evidently the supra-oesophageal ganglion, from the anterior margins of which arose the circum-oesophageal connectives. In addition, he described a further nervous mass in the anterior part of the prostomium. This consisted of a transverse chain of seven ganglia, a group of three on either side near the bases of the anterior antennae, and a seventh, transverse ganglion joining them. The only connection between these anterior ganglia and the supra-oesophageal ganglion was by way of two small nerves running from the most lateral of the anterior ganglia to the circum-oesophageal connectives on each side. The antennary nerves were described as arising from the anterior chain of ganglia. Quatrefages also described a pair of eyes in the posterior part of the supra-oesophageal ganglion. While there are eyes in approximately the

position he shows, they are embedded within the ganglion and are not visible unless the ganglion is cut open. Quatrefages did not suggest this and he may well have confused the nuchal organs with eyes, a mistake made by some later authors also.

Claparède (1868) re-examined N. hombergi. He was unable to find any structures resembling the anterior chain of ganglia described by Quatrefages in N. caeca. Although he examined a different species, he concluded that Quatrefages had been mistaken and that the anterior ganglia did not exist in any Nephtys. He found that the antennary nerves arise from the anterior margin of the ganglion (Quatrefages had described them as emerging from the anterior ganglionic chain) and also noticed that the posterior margin of the ganglion was bifurcate (fig.1C).

Ehlers (1864-8) described the brain of N. caeca and was the first to record the two cylindrical processes which extend caudally from the posterior margin of the supra-oesophageal ganglion to the fifth segment (fig.1E). He agreed with Claparède that the anterior ganglia described by Quatrefages did not exist, but disagreed with him on several other points. Claparède had described the brain as elongate and bifurcated posteriorly; Ehlers observed that it was short, trapezoidal and had two long posterior processes. The two authors studied different species and

certainly, the differences between the two descriptions of the supra-oesophageal ganglia were too great to be attributed to faulty observation; they could only be due to inter-specific differences in the morphology of the ganglion. Ehlers evidently mistook the nuchal organs for eyes, for he described them as being located at the posterior margins of the prostomium, in the position actually occupied by the nuchal organs, and as having a lens, which they lack.

Ehlers' observations were substantially confirmed by Schack (1886) in his study of the same species. Schack also described briefly, and incorrectly, the histology of the posterior lobes of the brain and stated that they were filled with large cells which he supposed to be neurones. His examination was superficial, however, for he failed to notice the eyes embedded in the ganglion and repeated Ehlers' mistake of confusing the nuchal organs with them. He also failed to notice the very obvious tract of processes running from the cells in the posterior lobes along the sides of the ganglion to the lateral walls of the prostomium.

All the earlier work was reviewed by Pruvot (1885) who gave a detailed account of the central nervous system of N. hombergi (figs.1D and F) and attempted to reconcile the conflicting views of the previous authors. He described a flattened mass of connective tissue which lay between the circum-oesophageal connectives in the anterior

part of the prostomium, and from which two fairly thick bundles of connective tissue ran into the antennae. There can be no doubt that Pruvot was right, up to a point, in suggesting that this mass represented Quatrefages' seven anterior 'ganglia'. In fact the tissue is not connective, but is composed of epidermal mucus cells, but once Quatrefages had made the initial mistake of supposing it to be nervous tissue, the discerning of ganglia in it followed almost as a matter of course. Pruvot also suggested that Ehlers too had mistaken this anterior mass for nervous tissue. He was unable to find the posterior lobes of the supra-oesophageal ganglion of N. hombergi and supposed that there were none in any species of Nephtys. He proposed that what Ehlers had called the supra-oesophageal ganglion was, in fact, the mass of glandular tissue in the anterior part of the prostomium and what he had called the posterior lobes was the real supra-oesophageal ganglion, which in N. hombergi has a fairly well-marked groove along its ventral surface. While Pruvot was certainly right in his criticism of Quatrefages, the observations of Ehlers could not be dismissed so lightly, the more so after Schack had described in some detail precisely the same structures that Ehlers had seen.

Pruvot had examined the nervous system of N. hombergi and Ehlers and Schack that of N. caeca, so that the dis-

crepancies may well have been due to specific differences in brain structure in the two worms. This possibility does not seem to have occurred to Pruvot. However, in 1894 Baron de Saint-Joseph published descriptions of the nervous systems of N. caeca, N. hombergi and N. cirrosa which should have decided the conflict as he was the first person to examine both N. caeca and N. hombergi (fig.1G). However, he failed to find the posterior lobes in any species and agreed in the main with Pruvot. In his description, he says that the ganglion of N. caeca extends to the boundary between the first and second segments where it bifurcates, i.e. it is essentially the same as the supra-oesophageal ganglion of N. hombergi.

From the work of the latter half of the nineteenth century we are left with a series of contradictory statements about the structure of the brain of Nephtys. Some, though not all, of the discrepancies can be attributed to the fact that different observers studied different species. It is a little surprising to find Pruvot, who gave a detailed and careful account of the brain of N. hombergi, suggesting that Ehlers, who was usually a very exact observer, mistook a mass of connective tissue for the supra-oesophageal ganglion, quite overlooking the fact that Ehlers was working with N. caeca. The fact that some authors

assumed, incorrectly, that the nervous system was the same in all species of Nephtys, does not account for all the discrepancies however. The descriptions of the nervous system of N. hombergi given by Clarparède, Pruvot and Saint-Joseph are all more or less congruent and are essentially correct. But the descriptions of N. caeca by Quatrefages and Saint-Joseph differ in important respects from those given by Ehlers and Schack. The French authors describe the supra-oesophageal ganglion as being elongate, extending back to the boundary between the first and second segments, and being bifurcate posteriorly, whereas Ehlers and Schack agree that it is relatively short, confined to the prostomium, and that there are two long, cylindrical processes extending from the posterior margin of the ganglion.

Rullier (1947) re-examined N. caeca and N. hombergi and found that there are indeed posterior lobes of the supra-oesophageal ganglion of N. caeca and that the descriptions of the brain of that species by Quatrefages and de Saint-Joseph bear a much greater resemblance to the brain of N. hombergi. Rullier also examined the brains of N. cirrosa and N. hystricis and found that they, too, were provided with posterior lobes. He concluded therefore that posterior lobes were a normal feature of the brain of Nephtys and that the inter-specific variation resided solely

in the degree to which the lobes were developed; it will be recalled that the ganglion of N. hombergi is slightly bifurcated posteriorly although there are no elongated lobes such as are found in N. caeca. This analysis is not quite true, as we shall show, for the posterior part of the brain of N. hombergi is composed of neuroglial fibres, while the posterior lobes of N. caeca are filled with mucus cells.

It appears, as Rullier suggested, that both Quatrefages and Saint-Joseph mis-identified the worms they studied and did not examine N. caeca at all. The alternative explanation is that there is considerable intraspecific variation in this species and that the North German specimens have posterior lobes and the French ones have not. Since specimens of N. caeca from the east and west coasts of Scotland, the south of England and northeast Bacific all have posterior lobes which are as Ehlers described them, this seems most unlikely.

CAPTIONS TO FIGURES

Figure 1. A. The anterior nervous system of Nephtys hombergi according to Delle Chiaje (1825).

B. The anterior nervous system of Nephtys caeca according to Quatrefages (1850).

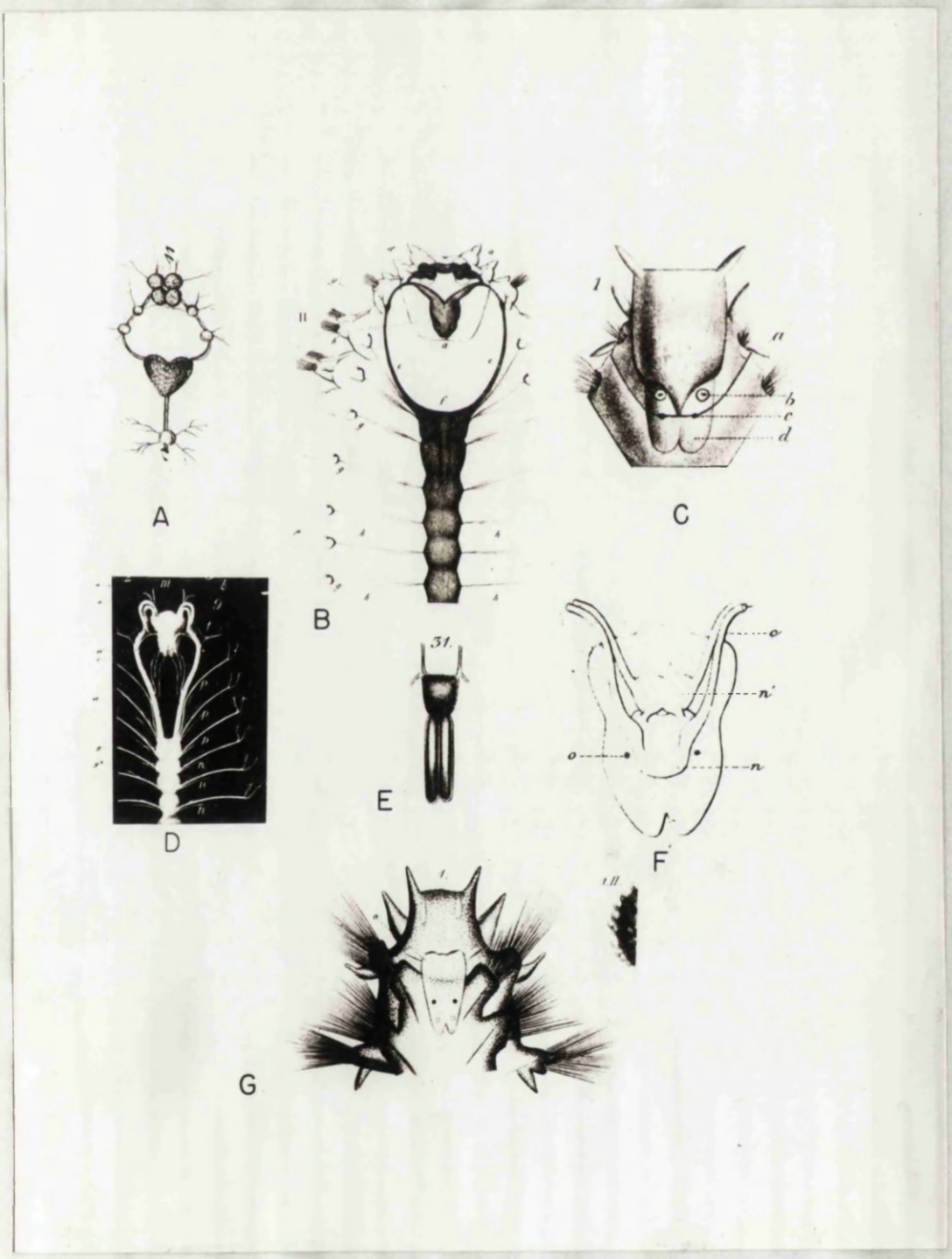
C. The prostomium of Nephtys hombergi, showing the supra-oesophageal ganglion by transparency. Claparède (1886). b. nuchal organs, c. eyes.

D. The anterior nervous system of Nephtys hombergi according to Pruvot (1885).

E. The supra-oesophageal ganglion of Nephtys caeca according to Ehlers (1864-8).

F. The supra-oesophageal ganglion of Nephtys hombergi according to Pruvot (1885). n, n' are two areas of neuropile from which the circum-oesophageal connectives arise. o. eyes.

G. The anterior end of Nephtys caeca according to Saint-Joseph (1894) showing the supra-oesophageal ganglion by transparency.


A
B
C
D
E
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G
31.

MATERIALS AND METHODS

MATERIALS AND METHODS

The histological study has been based on an examination of the species of Nephtys and Aglaophamus listed below. The two genera are regarded as sub-genera by some taxonomists. I have had no opportunity to examine Micronephtys, the third genus in the family. The species marked with an asterisk have been taken from museum collections and are therefore inappropriately fixed for careful histological work.

N. caeca (Fabricius)
N. caecoides Hartman
N. californiensis Hartman
N. cirrosa Ehlers
*N. cornuta Berkeley & Berkeley
N. cornuta franciscana Clark & Jones
N. ferruginea Hartman
*N. glabra Hartman
N. hombergi Aud. & Edw.
N. incisa Malmgren
N. longosetosa Oersted
*N. magellanica Hartman
N. parva Clark & Jones
N. picta Ehlers
N. punctata Hartman
*N. rickettsi Hartman
*N. squamosa Hartman
*Aglaophamus dicirris Hartman
*A. erectans Hartman
*A. virginis (Kinberg)

N. rickettsi is synonymous with N. discors Ehlers according to Pettibone (1954).

The gross morphology of the anterior nervous system with its associated structures has been determined by

dissection. As the entire dorsal part of the supra-oesophageal ganglion is in contact with the cuticle of the prostomium, it is most convenient to make lateral incisions of the body wall as far forward as the first segment, to reflect the dorsal body-wall forwards and to dissect the brain from its ventral surface.

Except for the museum specimens, worms have been fixed in Bouin's fluid, made up in sea-water, and transverse and frontal serial paraffin sections have been prepared at 7 or 10 μ thickness. They have been stained as a matter of routine with paraldehyde fuchsin. This technique has been developed by Gomori (1950), Halmi (1952) and Dawson (1953), for the study of neuro-secretory products. I have used Gabe's (1953b) method of preparing the paraldehyde fuchsin. The final method is thus:

1. Remove paraffin and hydrate sections.
2. Refix in Zenker-formaldehyde (Helly), 1-2 hours.
3. Lugol's solution, 5 minutes.
4. Rinse in water.
5. 5% sodium thiosulphate, 2 minutes.
6. Rinse in water.
7. Oxidise in acid permanganate, 1 minute

Potassium permanganate	3 gm.
Conc. Sulphuric acid	3 ml.
Distilled water	1000 ml.

8. Rinse in water.
9. Decolorise in 2.5% sodium bisulphite.
10. Wash in water.
11. Stain in paraldehyde fuchsin, 10 minutes.
    The paraldehyde fuchsin is made according to the directions given by Gabe (1953b). The stock solution consists of a 0.75% solution of paraldehyde fuchsin in 70% alcohol. The staining solution found best for polychaete material

is:

Stock solution	15 ml.
70% alcohol	150 ml.
Glacial acetic acid	2 ml.

(For vertebrate material the stock solution should be diluted only 3 or 4 times with 70% alcohol.)

12. Wash in two changes of 95% alcohol.
13. Rinse in 0.25% hydrochloric acid in absolute alcohol, 15 seconds.
14. Rinse in distilled water.
15. Mordant in phosphotungstic-phosphomolybdic acid, 10 minutes.

Phosphotungstic acid	4 gm.
Phosphomolybdic acid	1 gm.
Distilled water	100 ml.

16. Rinse in water.
17. Counterstain, 1 hour.

Light green (fast green)	0.4 gm.
Orange G	0.5 gm.
Glacial acetic acid	1.0 ml.
Distilled water	100 ml.

18. Rinse in 2% acetic acid in 95% alcohol.
19. Dehydrate rapidly, clear and mount.

Best results have been obtained with material fixed in Bouin's fluid, but sublimate fixatives are also suitable, though they usually require a stronger solution of paraldehyde fuchsin (dilute the stock solution only 4 or 5 times with 70% alcohol instead of 10 times). The times suggested above are for Bouin-fixed material. Steps 2-5 may be omitted, but mordanting with Zenker-formaldehyde improves counterstaining.

More detailed histological and histochemical studies have been made on N. californiensis, N. cirrosa and N. hombergi. Specimens have been fixed in Zenker, Helly, Bouin, Heidenhain's 'Susa', formaldehyde and 95% alcohol.

All but the last two fixatives were made up in sea-water as a means of reducing shrinkage and improving fixation. A great variety of staining techniques have been used of which the following have been found the most rewarding.

General methods for the nervous system: Azan, Heidenhain's iron haematoxylin, Holmes' silver impregnation technique (Nicol, 1948a).

Neurosecretory products: by far the best results have been obtained with paraldehyde fuchsin following Bouin fixation. Gabe's (1953b) method, using picro-indigocarmine as a counterstain, is rapid and stains most neurosecretory products sharply and distinctly. The method described in detail above takes much longer, but it has the advantage of differentiating tissues in the brain so that the same preparations can be used for microanatomical studies of the ganglion. For an examination of the secretory cycles of the cells it has been found preferable to fix the material in Zenker and stain by the longer technique. This method results in much less intense coloration of the neurosecretory products than when the specimens have been fixed in Bouin's fluid, which is something of an advantage, for in Bouin- and Helly-fixed material the staining of the secretion is often so intense as to obscure the cell structure. Neither of the other two techniques commonly used in the study of

neurosecretion, acid chrome haematoxylin and phloxin (Gomori, 1941) or Altmann's fuchsin and methyl green picrate (Gabe, 1947), have been found particularly useful with this material.

Lipids: material has been fixed in formaldehyde, refixed in 2% osmic acid solution and counterstained with nuclear fast red, or else, fixed in formaldehyde, post-chromed for 24 hours at 37°C in saturated potassium dichromate solution and stained with sudan black B. This is essentially Ciaccio's method as described by Pearse (1953).

Glucids, etc.: Material fixed in Bouin and also in Heidenhain's 'Susa' was subjected to the following tests: periodic acid schiff, with and without previous digestion with diastase, and following acetylation, with and without saponification with KOH (MacManus & Cason, 1950). Mucicarmine (Southgate, 1927), dialysed iron (Hale, 1946), alcian blue 8GS (Steedman, 1950) and toluidene blue have been used to demonstrate acid mucopolysaccharides.

Experiments have been performed on N. caeca, N. californiensis, N. cirrosa, N. cornuta franciscana and N. punctata. Details of the experimental techniques can more suitably be described in the appropriate sections.

THE SIZE, FORM AND STRUCTURE OF THE SUPRA-OESOPHAGEAL GANGLION.

THE SIZE, FORM AND STRUCTURE OF THE SUPRA-OESOPHAGEAL GANGLION

Factors Influencing the Form of the Ganglion

The supra-oesophageal ganglion of Nephtys is extraordinarily variable in both its gross morphology and its fine structure. By far the most conspicuous of the variations that are encountered is the development of a pair of lobes at the posterior margin of the ganglion, which in some species are many times larger than the ganglion itself, but in others may be missing altogether. As we shall show, these lobes are usually filled with mucus-cells, but there are also important variations in the nervous part of the ganglion. These are attributable, in the main, to two factors: the great size range of members of the Nephtyidae, and the presence of a large, eversible pharynx.

The smallest species of Nephtys, N. cornuta, is 5 mm long, the largest species, N. californiensis, is 250-300 mm long. The size of the cells does not vary to anything like the same extent, and, as a general rule, smaller animals are composed of fewer cells than large ones. While this is possible in most tissues of the body, it is not possible in the brain. It is axiomatic that brains capable of the same functions must contain approximately the same number of cells. As a result of this, the supra-oesophageal ganglia of small species are relatively larger

than those of big ones. Because the external body proportions are roughly constant in members of this family, the relatively larger brain of the small species cannot be accommodated completely within the prostomium. In fact, the ganglion cannot become very much wider or thicker, it can only become longer, and so it tends to project into the anterior body segments. In N. cornuta and N. parva, for instance, it extends from the first to the third body segments.

The influence of the proboscis is less obvious. The pharynx of Nephtys is very long and very muscular and it is commonly greater in diameter than the anterior segments through which it must pass as the proboscis is everted. The mouth is accordingly modified. Externally it is T-shaped and extends to the anterior margin of the fifth segment. It is bordered by lateral lips which are connected ventrally by a folded, muscular, gular membrane that is tucked between the lateral lips when the proboscis is inverted. As it is everted, the lateral lips are thrust aside and the gular membrane is stretched. The sub-oesophageal ganglion lies at the posterior end of the mouth, in the fifth segment, and the circum-oesophageal connectives and the circum-oral blood vessels run in the lateral lips (see Appendix II) and so are displaced, but not stretched when the proboscis is everted. The result of this

structural adaptation to the presence of a large pharynx is that the circum-oesophageal connectives are very long, and there has been a tendency for them to be shortened by the movement backwards of the supra-oesophageal ganglion.

The posterior part of the prostomium is fused with the first segment, but its hinder limit is indicated by the nuchal organs (Racovitza, 1896; Holmgren, 1916; Rullier, 1950). That part of the supra-oesophageal ganglion, if any, which is in the prostomium is epidermal, i.e. it is in contact with the prostomial cuticle. Any part of the ganglion which extends into the body segments is suspended beneath the epidermis by extensions of the epidermal basement membrane which bound the whole ganglion, including the posterior lobes. Only in N. incisa and N. picta is the ganglion found in its primitive position in the prostomium (figs. 2 & 3), and even in these species the posterior tip of it extends into the first segment. In all the other species, part, or even the whole of the brain lies posterior to the prostomium. Thus in N. caeca, N. californiensis and N. cirrosa (figs. 4, 19 & 27) only half the ganglion is in the prostomium; in N. caecoides, N. ferrugines, N. hombergi and N. punctata the extreme anterior tip alone is in the prostomium (figs. 5, 6, 7, 8), while in the two small species N. cornuta and N. parva the entire ganglion lies in the segmental part of the body (figs. 9 & 10).

The Size of the Ganglion

The supra-oesophageal ganglion of a small species of Nephtys is not a replica in miniature of that of a large species. The ganglion cells are certainly smaller, but those in the smallest species are only one-tenth to one-fortieth the volume of corresponding cells in the largest species, whereas the ganglion is two-hundred times smaller (figs.11, 12 & 13). And the smaller the worm, the larger the fraction of the brain occupied by the neuropile which is composed of the axons of these cells (fig.14). So, not only are the ganglion cells relatively larger in a small worm, but there is less space around the neuropile in which to accommodate them.

This situation is mitigated to some extent by a reduction in the amount of neuroglia and a closer packing of the nerve cell bodies. It is impossible to measure the volume occupied by glia cells, but even on a superficial examination of a large worm like N. californiensis it is obvious that a quarter or a third of the brain is occupied by neuroglia. In N. parva and N. cornuta there is virtually none. The ganglion cells are not arranged in discrete groups, or nuclei, in any species; they tend rather, to be uniformly distributed in the ganglion. But in N. californiensis, and several other large species, discrete nuclei can be discerned, though they are never as

distinctive as those in the brain of Nereis, for example. In the smallest species of Nephtys even this slight structural organisation in the brain is lacking and the cells are packed uniformly and closely together. The loss of neuroglia and of structural organisation from the brain of small species of Nephtys achieves some economy of space and compensates to some extent for the relatively larger nerve cells that they possess. In spite of this, small species have a relatively larger brain than big species (fig.15), and the brain becomes elongated.

We have assumed so far, that the number of nerve cells in the brain is constant whatever the size of the worm. So far as the posterior part of the ganglion is concerned, this appears to be justified. A count of nerve cells in the groups posterior to the eyes in N. picta (ganglion 0.016 cu. mm. in volume) and the cells in the same groups in a brain of N. californiensis (0.273 cu. mm.) gave totals which agreed remarkably. But in the anterior part of the brain this is not so. Independently of any effect on the brain caused by differences in the size of various species, corpora pedunculata have evolved within the family. They are composed of a very large number of extremely small cells and, as in other polychaetes, they lie just above the origin of the circum-oesophageal connectives. They are missing from N. cornuta, N. incisa, N. parva and N. picta,

but they are moderately well developed in N. caeca, N. cirrosa and N. longosetosa. In N. caecoides, N. californiensis, N. ferruginea and N. punctata there appears to be an intermediate condition in the development of thse structures, for there are numerous ganglion cells in the appropriate part of the brain, but they are not very different in size from other small nerve cells in the ganglion and they are not organised into mushroom bodies.

The Structure of the Posterior Part of the Ganglion

The arrangement of groups of ganglion cells in the posterior part of the brain, i.e. posterior to the photoreceptors, can best be understood in a large worm like N. californiensis. While the nerve cell-bodies do not form distinct and separate nuclei, the axons enter the neuropile at certain loci, and by tracing the axons back to the cell bodies it is possible to find the approximate boundary of each group. In smaller species this is much more difficult because of the close packing of the cells. However, once the structure of the brain of a large species has been understood, any serious discrepancies in the arrangement of the cells can be detected even in the smallest worms. In fact, the same features occur in all the species I have examined, and the supra-oesophageal ganglion posterior to the eyes is invariable in the essentials of

its minute structure.

The following nuclei have been identified. They are illustrated in a slightly simplified form in fig.16.

Nucleus 15. The optic nucleus. This group of cells extends above and below the ganglionic photoreceptors and is immediately posterior to them. The dorsal part of the nucleus is composed mainly of small, pyriform cells; at the level of the eyes, and ventral to them, large, almost spherical cells predominate. The axons from the photoreceptors and from all the cells in the optic nucleus enter the neuropile in a single group and are connected with the nucleus of the other side by the optic commissure running through the neuropile.

Nucleus 18. The nuchal nucleus is composed of small, pyriform cells and lies at the level of, and ventral to the eyes. Axons run into the neuropile in a compact bunch where they form the nuchal commissures, the most posterior of the commissures in the ganglion. There is a connection between the optic and nuchal commissures. The nuchal nerve runs dorsally from the nuchal nucleus to the nuchal organ. Since the latter is always at the posterior margin of the prostomium where the ganglion is in contact with the cuticle, the nuchal nerve consists of a tract of axons running through the neuroglia for most of its course, and emerges from the ganglion immediately below the nuchal organ

(fig.17). The elongation of the brain of small worms and the backward movement of it in others, have the effect of increasing the distance between the nuchal nucleus and organ, so the nuchal nerve becomes very long; but even so, it emerges from the brain only at the level of the nuchal organ in every species.

Nucleus 16. A small group of cells lie above and between the optic and nuchal nuclei and close to the neuropile.

Nuclei 17 and 20. The postero-lateral and posterior parts of the ganglion contain two important groups of cells which are also neurosecretory (see later section). Nucleus 17 is the more dorsal but both are quite extensive and their constituent cell-bodies are found in both dorsal and ventral parts of the brain. It is difficult to distinguish between the nuclei because the cells of each have the same appearance and are contiguous, but there are two loci at each side of the neuropile where the axons enter it.

Nucleus 22. A few cells in the posterior ventral part of the brain appear to send axons into the neuropile separately from those of nucleus 20, their immediate neighbour. They perhaps constitute a separate nucleus.

There are several excellent accounts of the minute structure of the supra-oesophageal ganglion of nereids, members of a family closely related to the Nephtyidae.

From the descriptions of the brain of Nereis by Holmgren (1916) and Defretin (1955), it is apparent that so far as the posterior part of it is concerned, there is a considerable correspondence between it and the brain of Nephtys (figs.16 and 18). The optic nucleus of Nereis lies immediately behind the posterior optic nerve, and, according to Defretin, is composed mainly of large, spherical cells; this is true of the ventral part of the nucleus in Nephtys. The nuchal nucleus of Nereis is posterior and somewhat ventral to the optic nucleus, as in Nephtys. The small nucleus 16 lies dorsal and internal to the optic nucleus in Nereis, but in Nephtys it is at the same level as the eyes and is between the optic and nuchal nuclei. There appears to be a slight difference in the relative positions of the nuclei 15, 16 and 18, but the axons enter the neuropile in the same positions in the two worms. Nuclei 17 and 20 are extensive in Nereis and they are composed of neurosecretory cells (Scharrer, 1936), as they are in Nephtys. They occur in the same relative positions in the two worms. Defretin was unable to identify Holmgren's nucleus 22 in Nereis. It is impossible to be certain that the cells labelled as this nucleus in fig.16 should be separated from nucleus 20, but they occur in the appropriate position and their axons run into the neuropile slightly medial to those from nucleus 20. Holmgren also described

a further nucleus (21) in the dorsal part of the ganglion and Defretin suggests that it is composed of neurosecretory cells. There is no discrete group of cells in a corresponding position in the brain of Nephtys, though there are several large neurosecretory cells in the dorsal part of the ganglion which have not so far been accounted for. It is possible that they represent a rather diffuse 21st nucleus.

Apart from a slight uncertainty about the identity of nuclei 21 and 22, and some shifting of the relative positions of other nuclei, there is an almost exact correspondence between the posterior halves of the brains of Nephtys and Nereis. The most striking difference is the absence of an epidermal nucleus (19) from the brain of Nephtys, although it forms the most extensive single nucleus in the nereid brain. The nerve from this nucleus supplies the lateral and posterior walls of the prostomium of Nereis, but in Nephtys the brain is epidermal, so it is not surprising that this nucleus and its nerve should be missing. The other obvious difference is that there are posterior lobes on the brain of some species of Nephtys, but none in Nereis. An examination of these differences will form the main part of this study of the posterior part of the brain of nephtyids.

CAPTIONS TO FIGURES

Figure 2. The supra-oesophageal ganglion of *N. incisa*, dissected from the ventral side. NO. level at which the nuchal organs are found.

Figure 3. The supra-oesophageal ganglion of *N. picta* from the ventral side.

Figure 4. The supra-oesophageal ganglion of *N. caeca* from the ventral side.

Figure 5. The supra-oesophageal ganglion of *N. caecoides* from the ventral side.

Figure 6. The supra-oesophageal ganglion of *N. ferruginea* from the ventral side.

Figure 7. The supra-oesophageal ganglion of *N. hombergi* from the ventral side.

Figure 8. The supra-oesophageal ganglion of *N. punctata* from the ventral side.

Figure 9. The supra-oesophageal ganglion of *N. cornuta* from the ventral side.

Figure 10. The supra-oesophageal ganglion of *N. parva* from the ventral side.

Figure 11. The relationship between the volume of the photo-receptors and the size of the supra-oesophageal ganglion in various species of *Nephtys*.

Figure 12. The relationship between the volume of the largest nerve cell bodies in the supra-oesophageal ganglion in various species of *Nephtys*, and the size of the supra-oesophageal ganglion.

Figure 13. The relationship between the mean volume of the cell bodies in nuclei 17 and 20 and the size of the supra-oesophageal ganglion in various species of *Nephtys*.

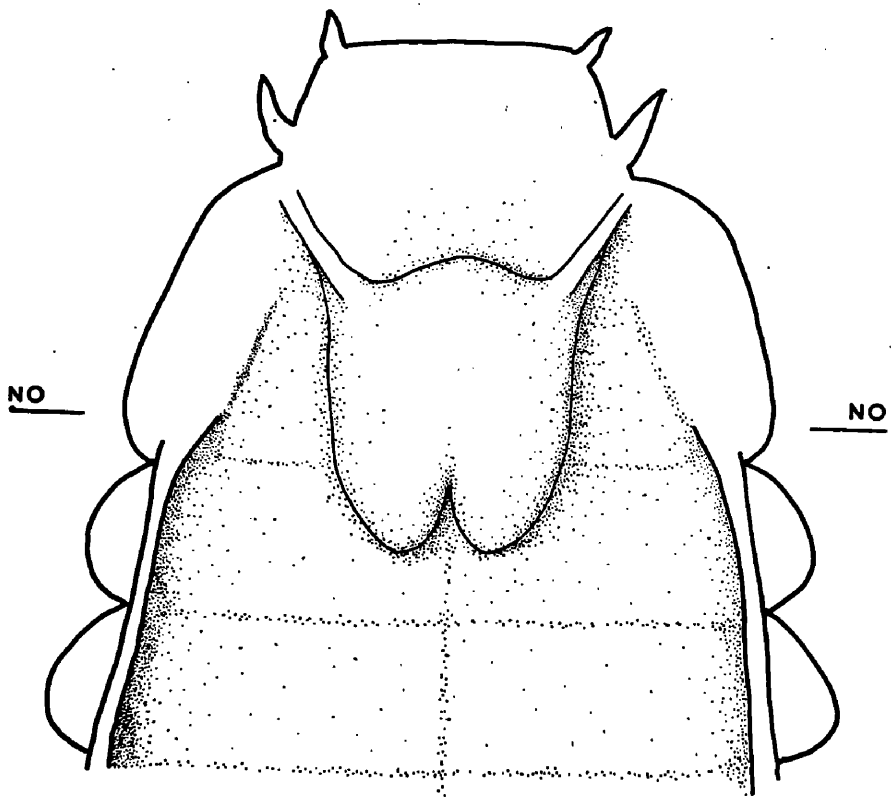
Figure 14. The relationship between the fraction of the supra-oesophageal ganglion occupied by neuropile and the brain volume in various species of *Nephtys*.

Figure 15. The relationship between the relative size of the brain and the size of the worm. (The relative size of the brain is the volume of the ganglion divided by the cube of the diameter of a body segment.)

Figure 16. A slightly simplified plan of the supra-oesophageal ganglion of N. californiensis to show the arrangement of the nuclei in the posterior part of the brain.

Figure 17. Transverse section through the supra-oesophageal ganglion and the nuchal organs of N. caeca.

Figure 18. The arrangement nuclei in the supra-oesophageal ganglion of Nereis. (From Hanström, 1928, simplified from Holmgren, 1916).
A. view from above; B. view from below.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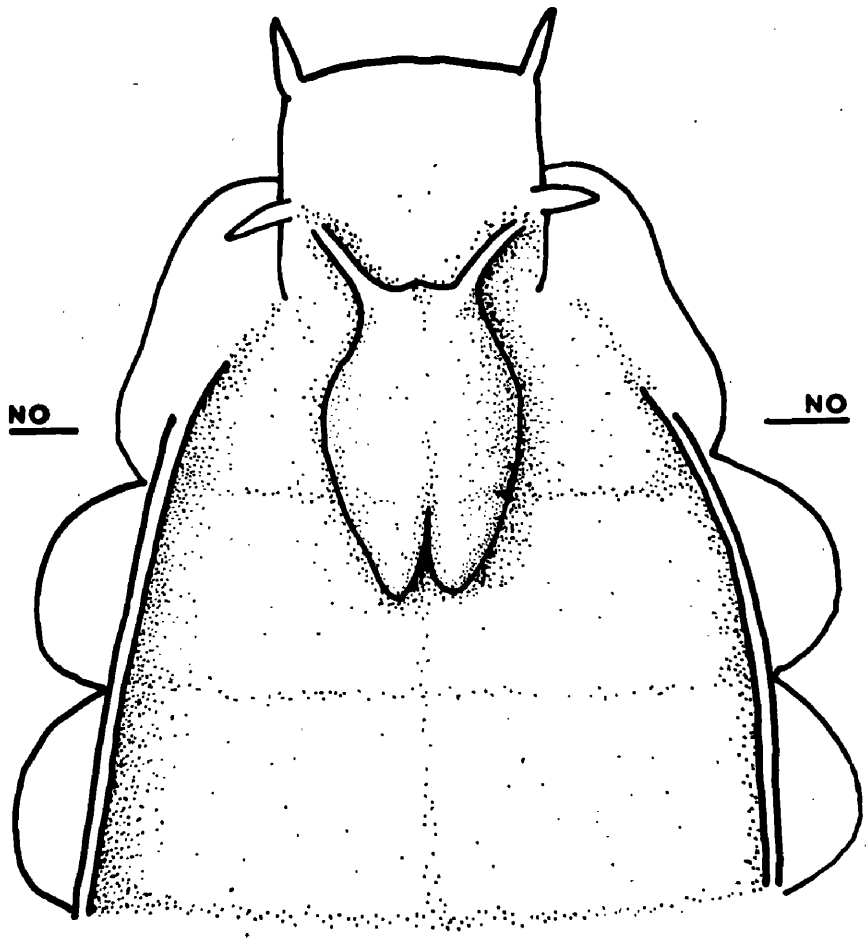
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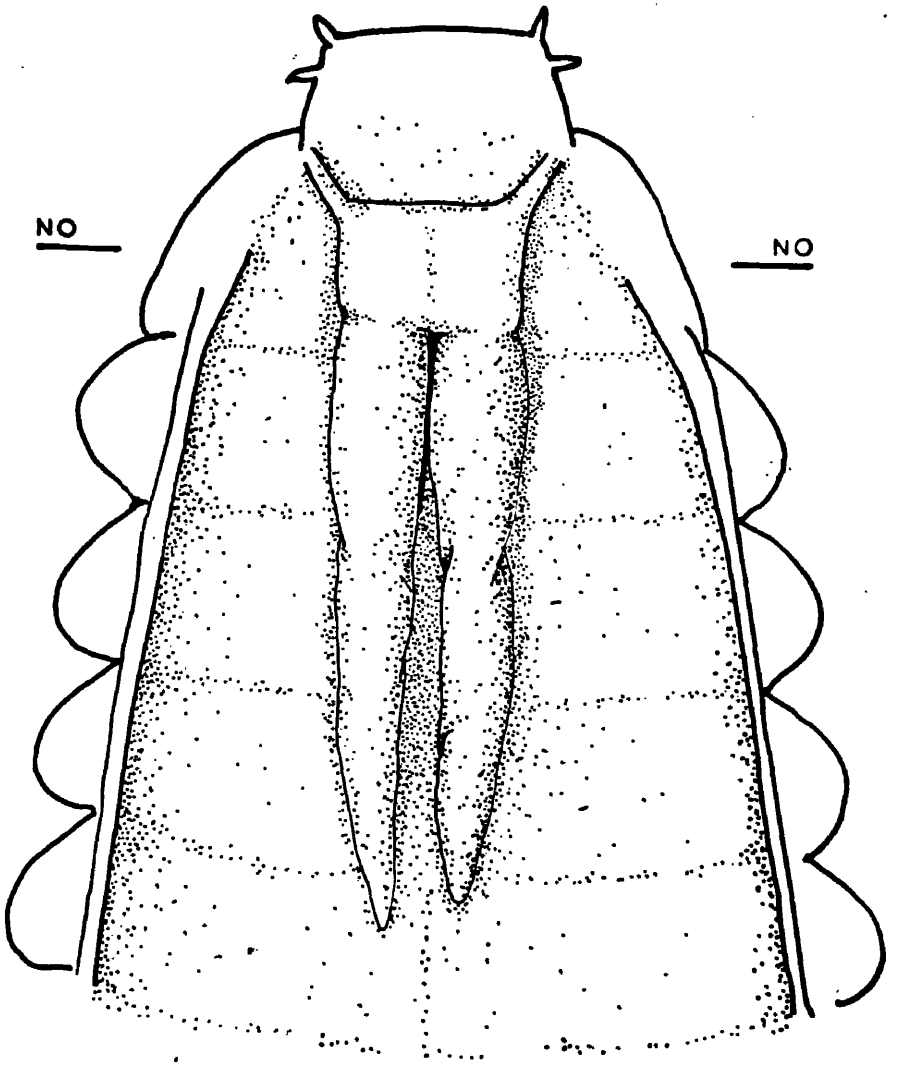
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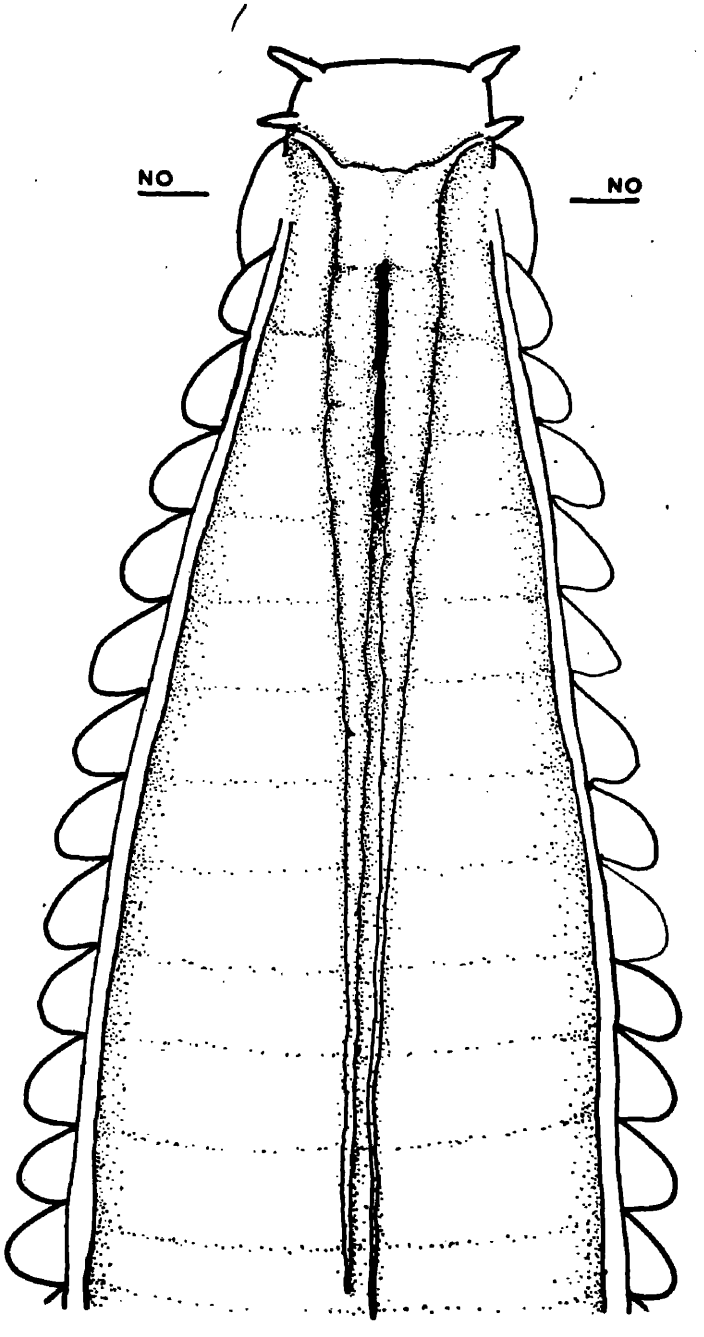
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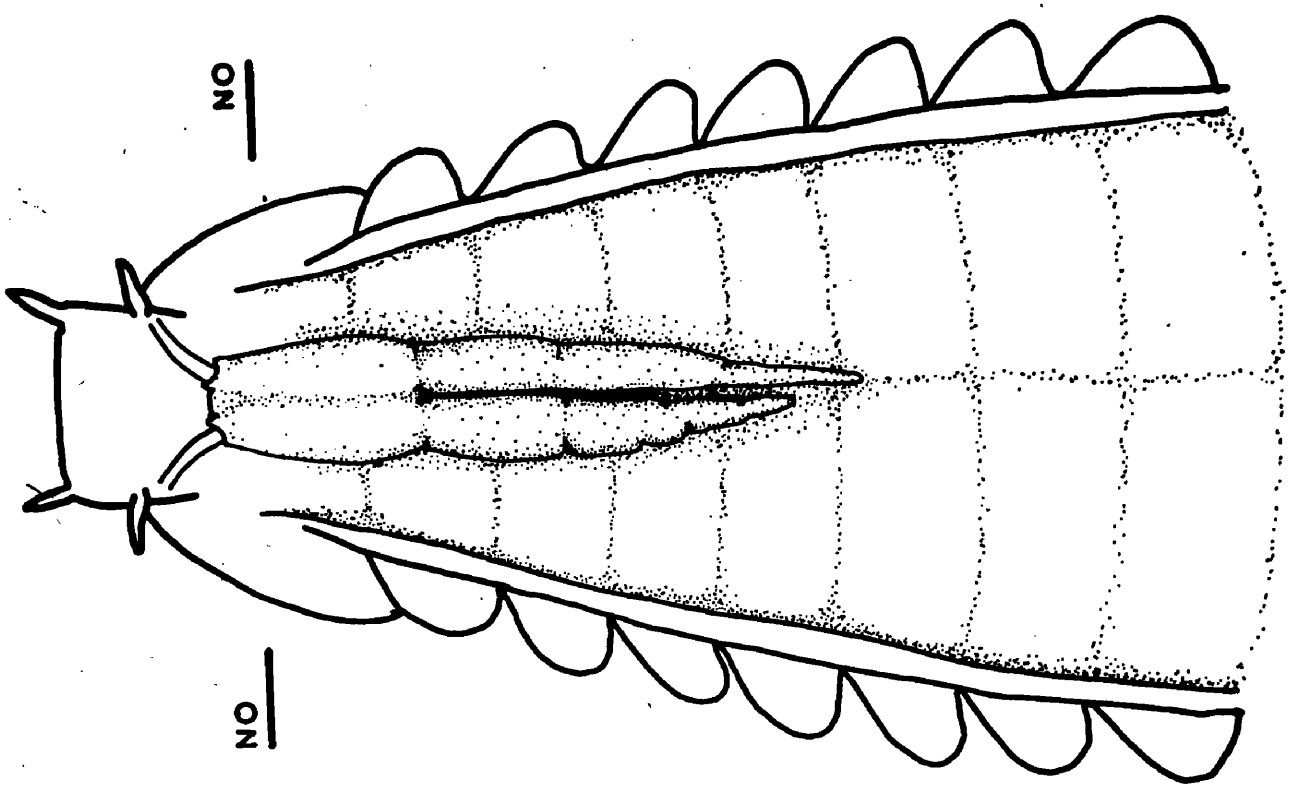

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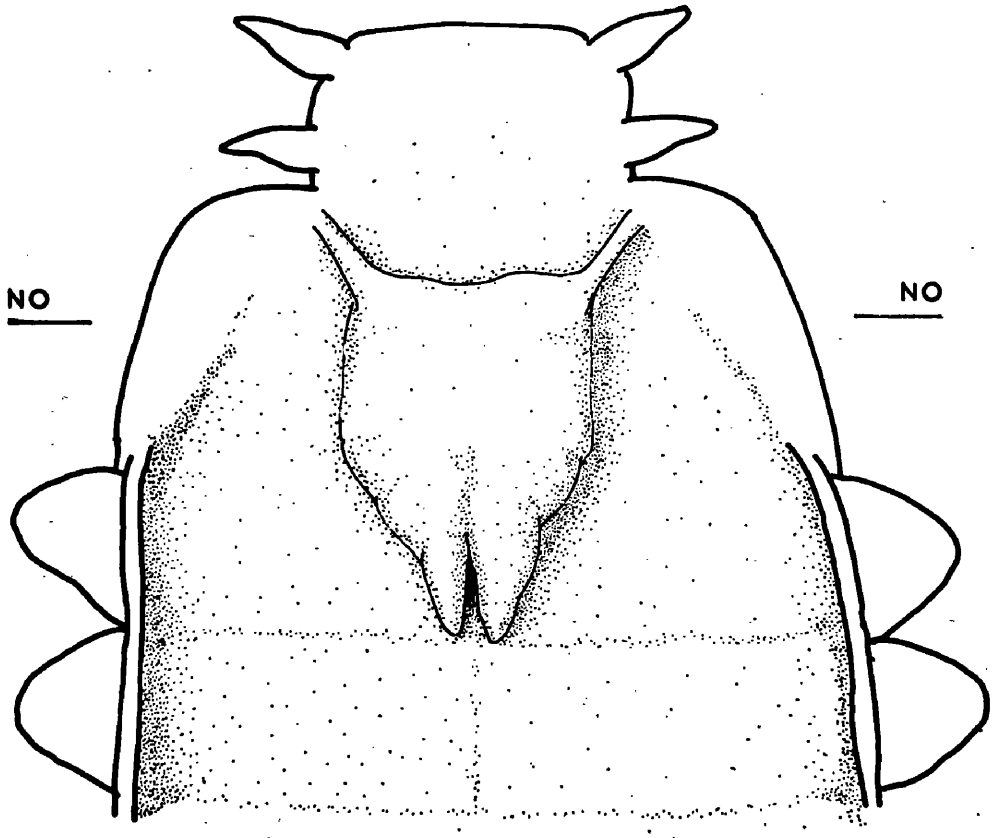

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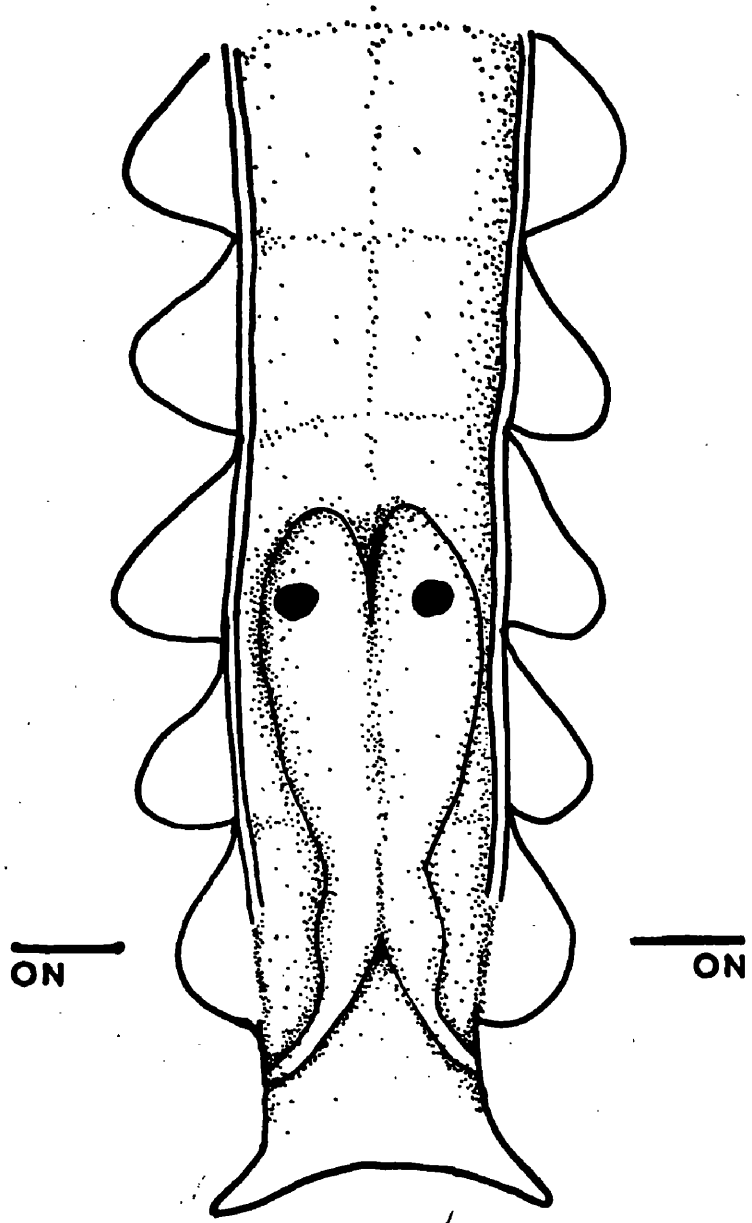
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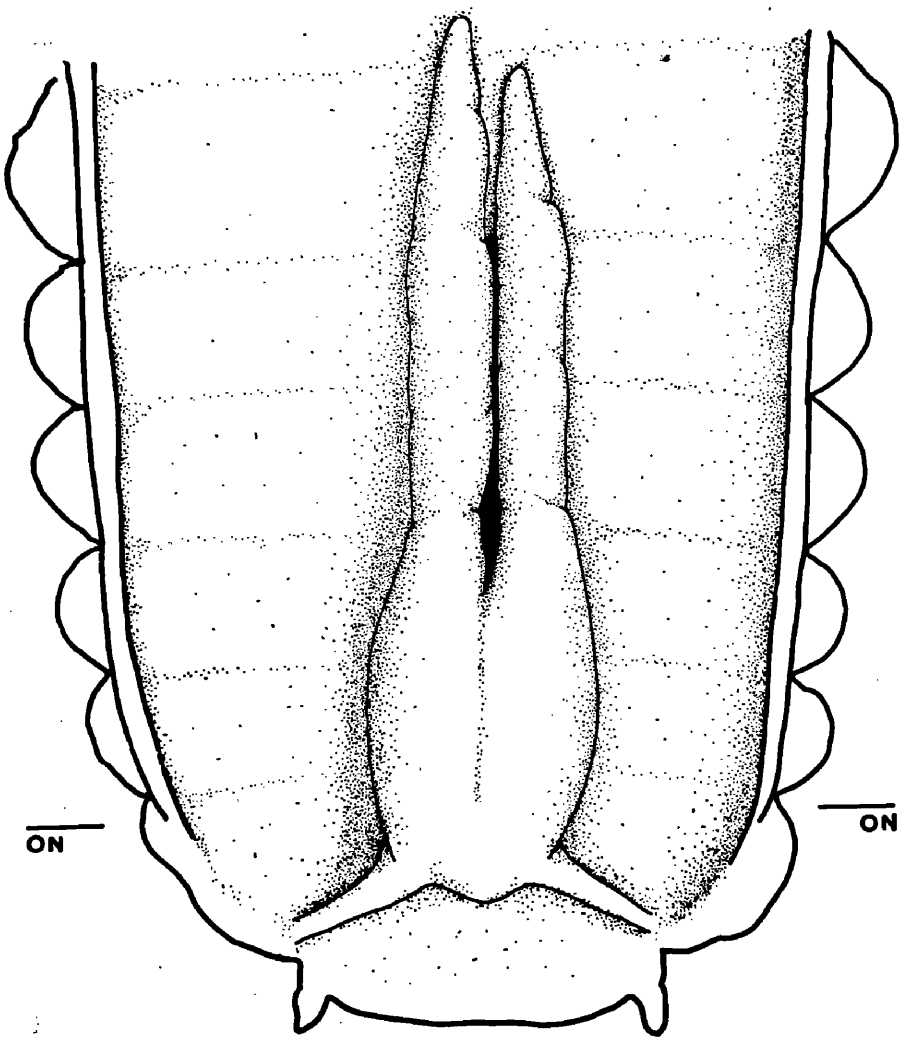
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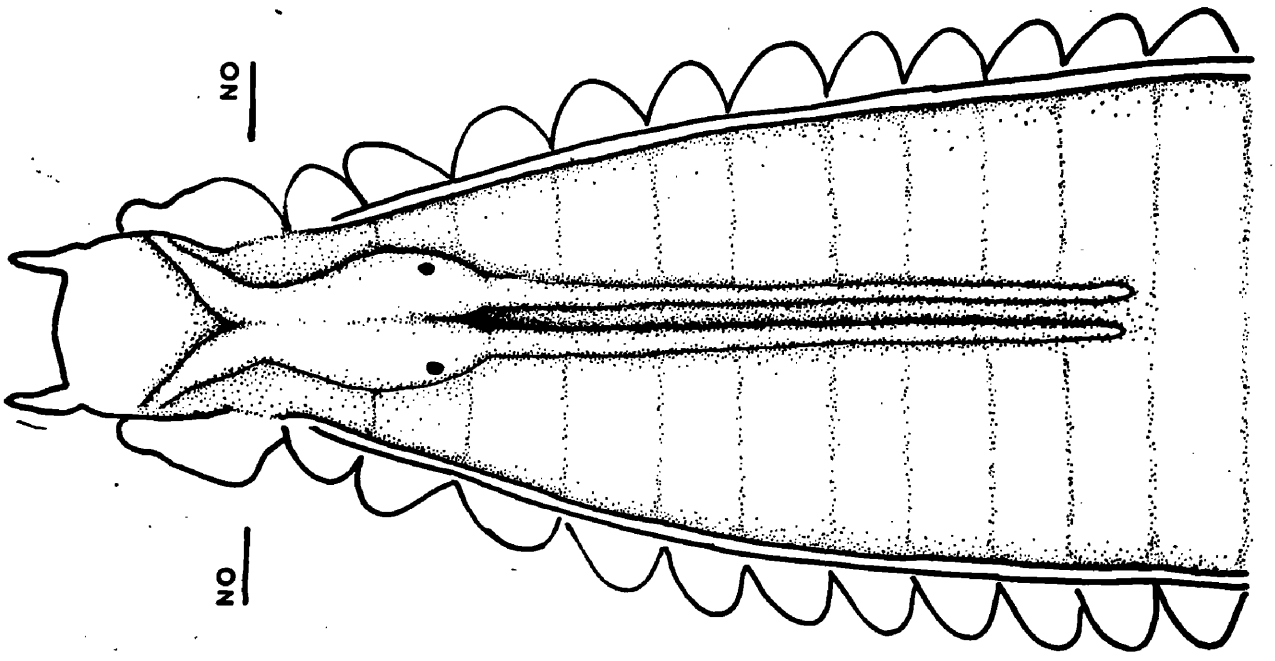
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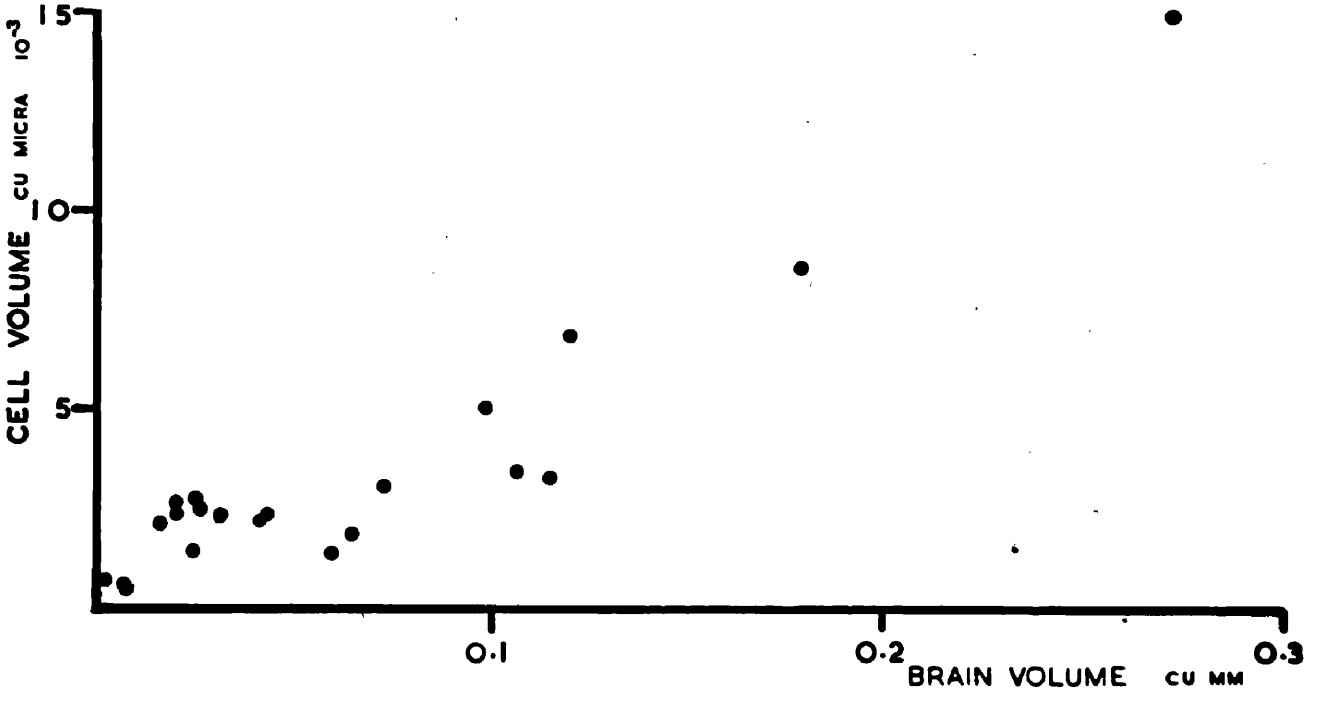
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10
5
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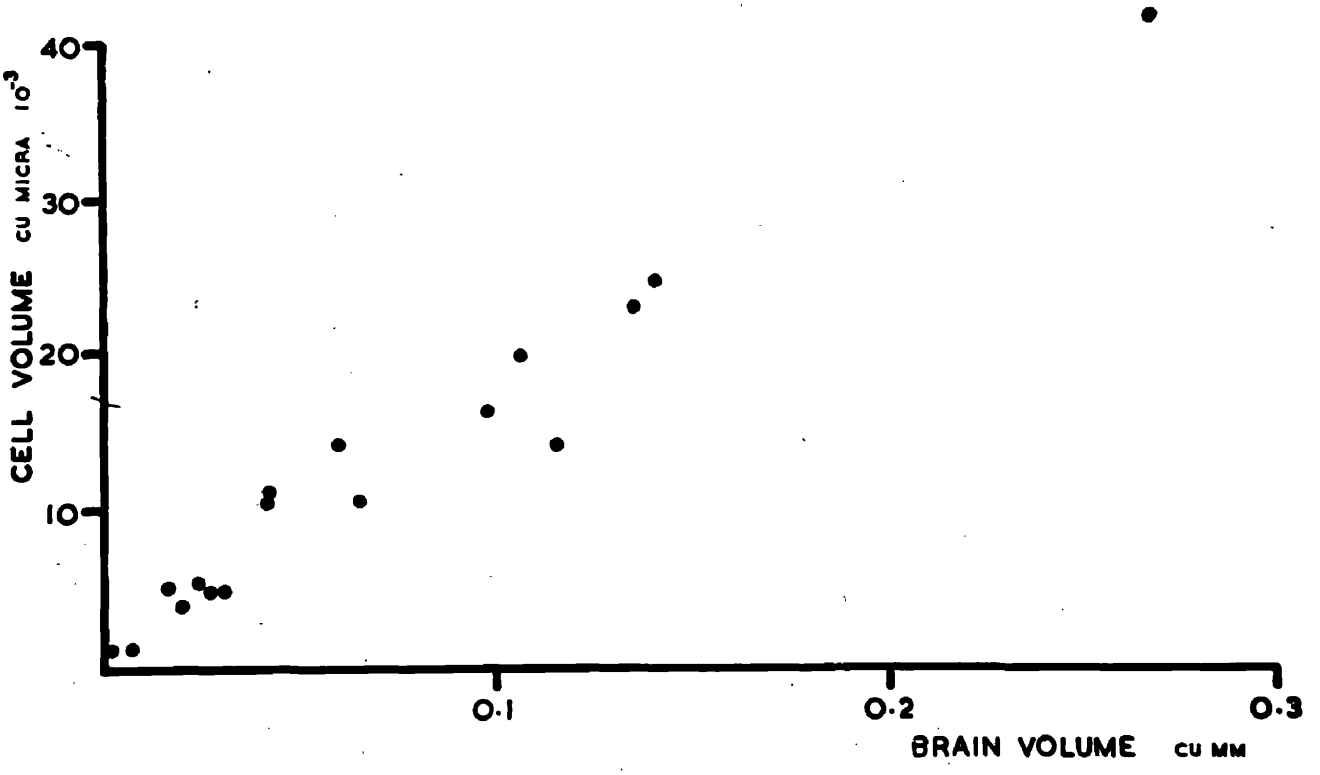
BRAIN VOLUME CU MM

11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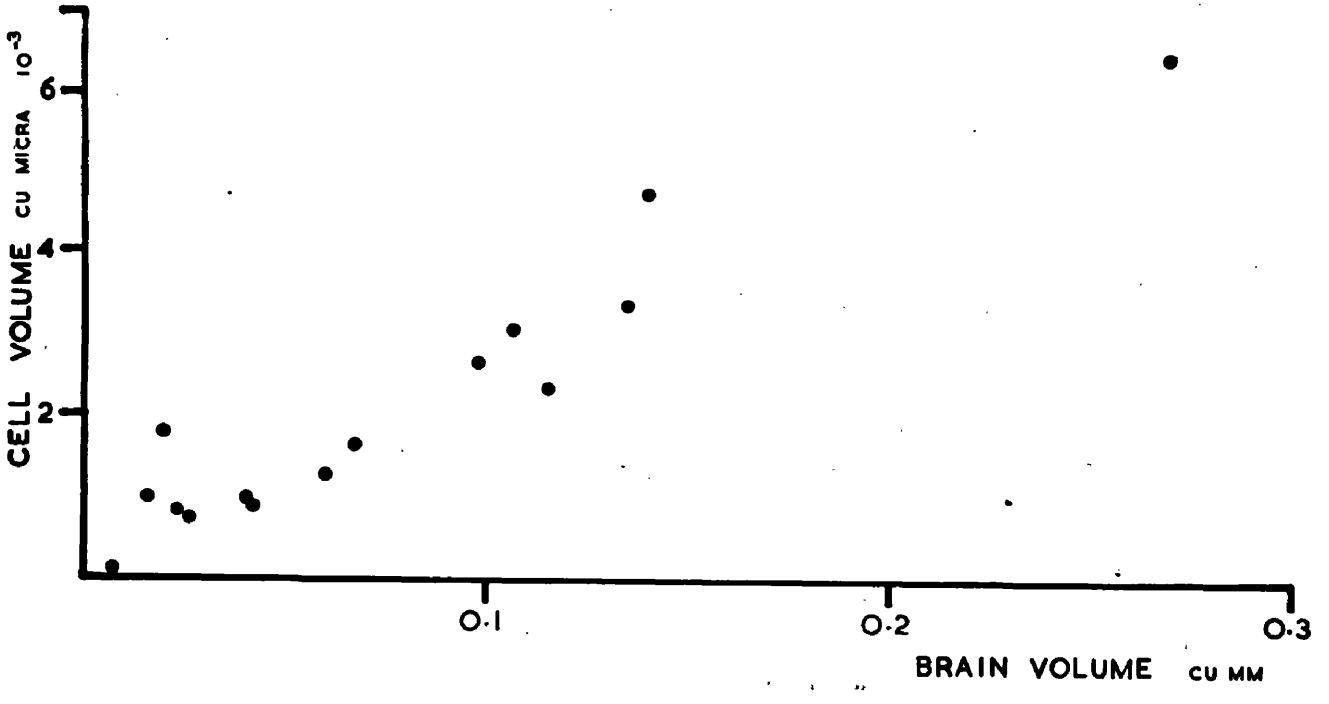
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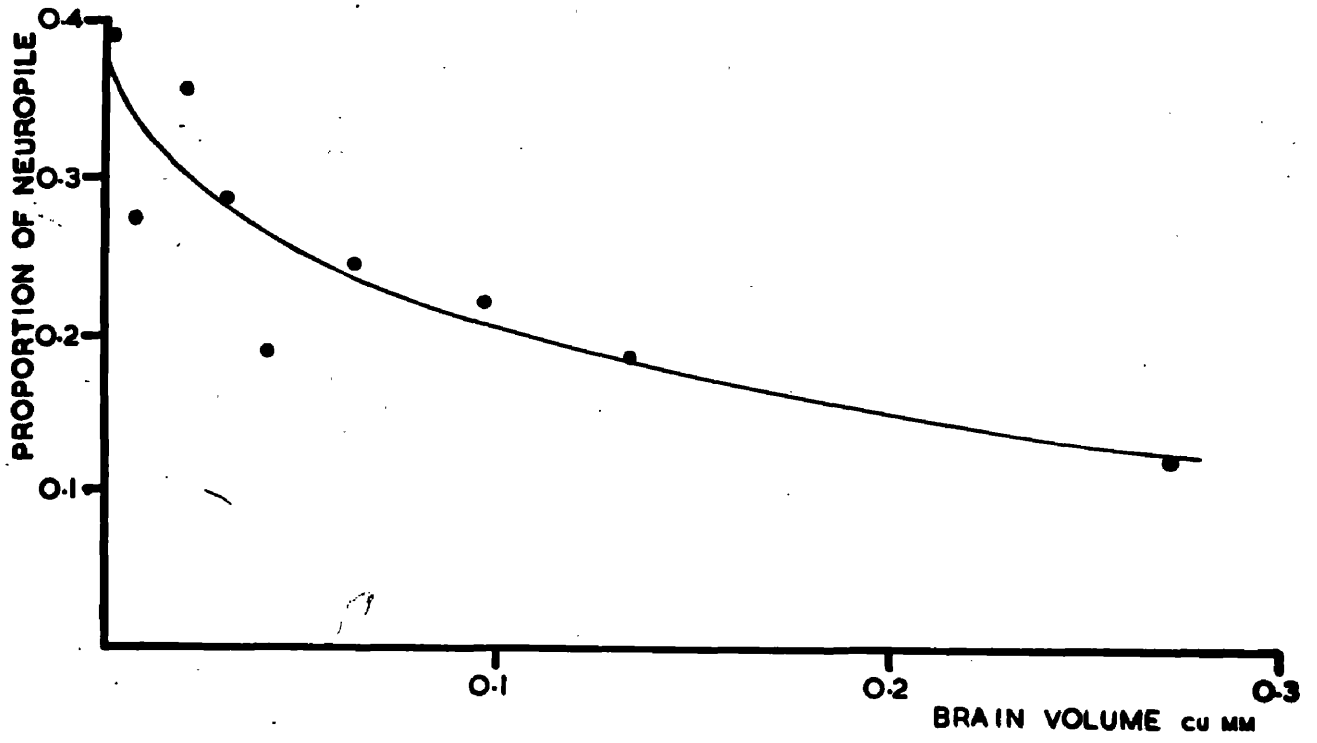
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BRAIN VOLUME cu mm

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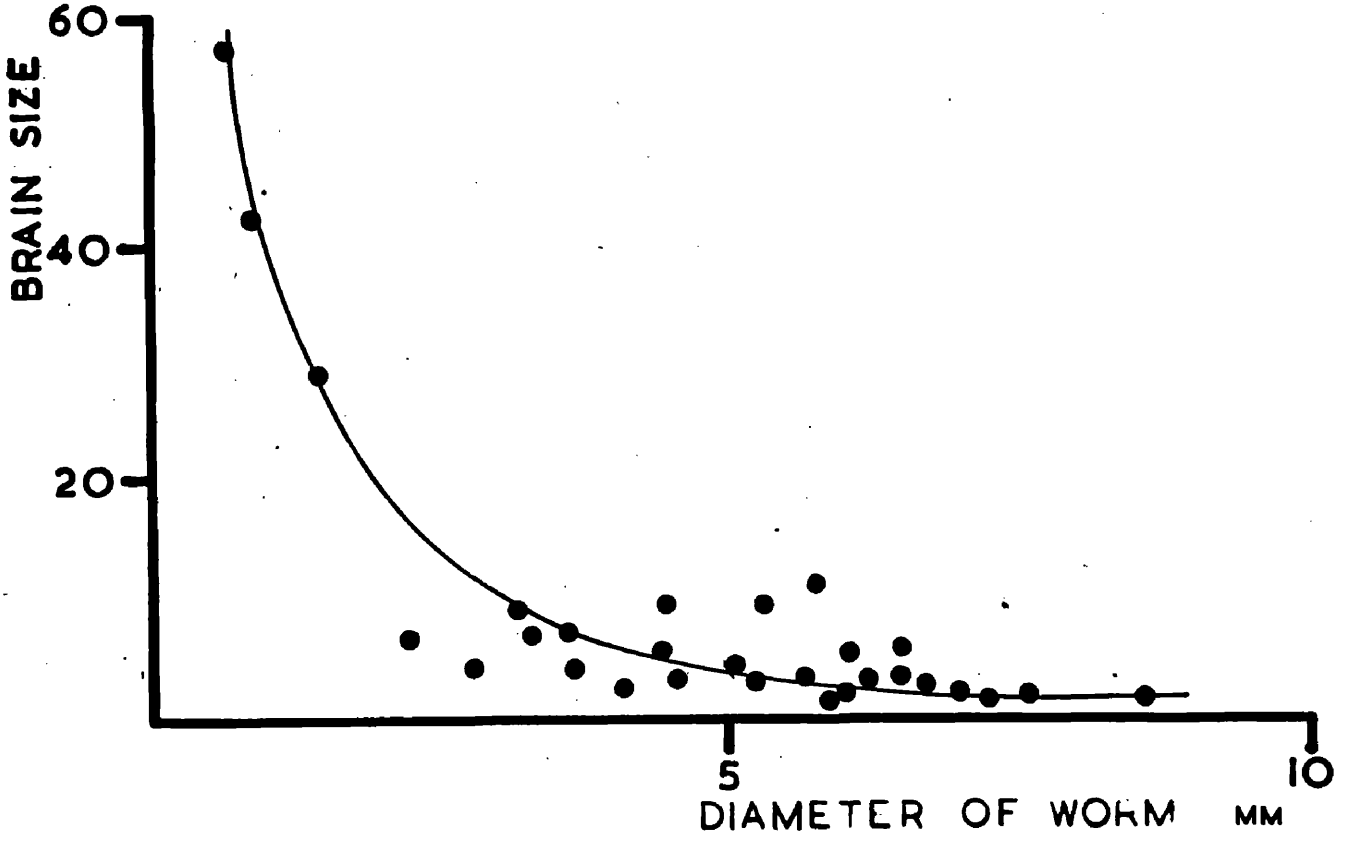
BRAIN SIZE
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40
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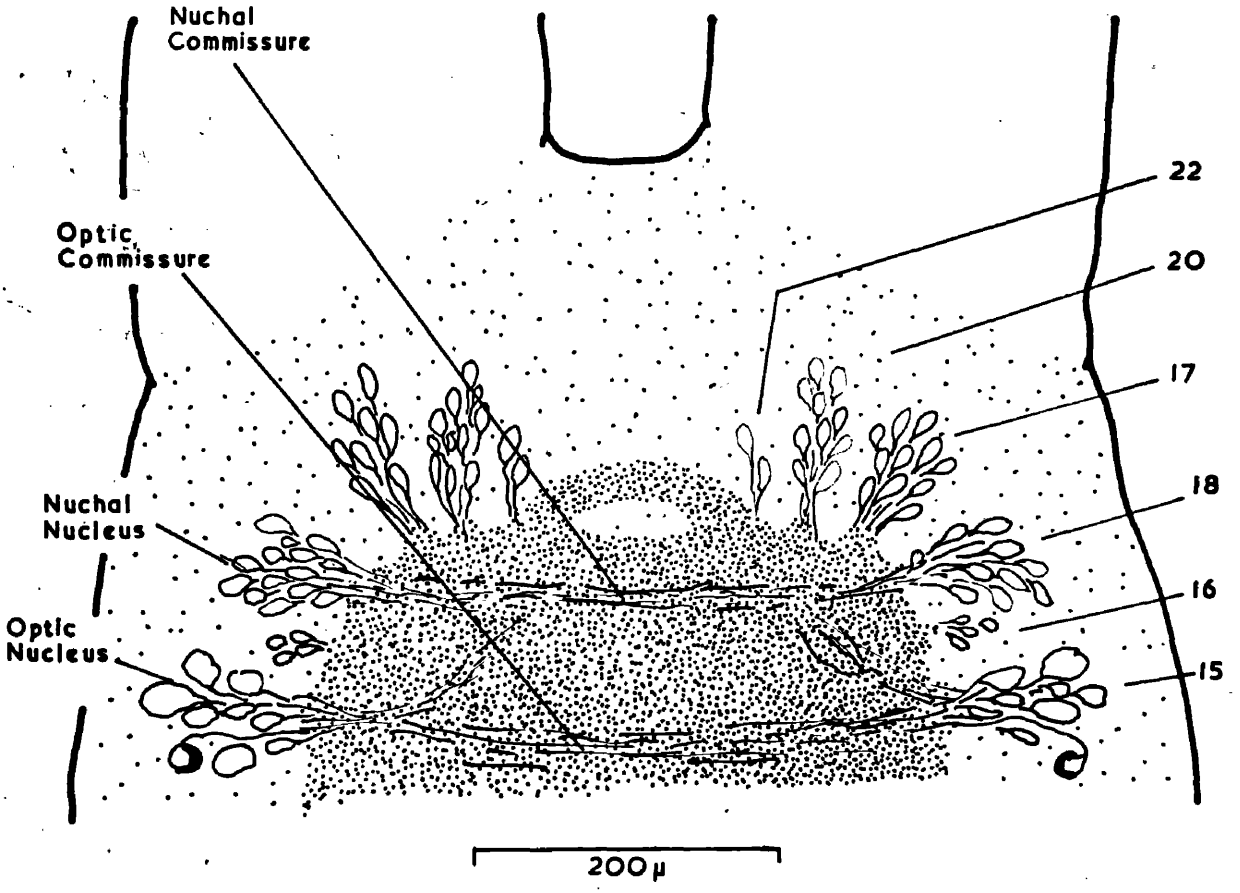
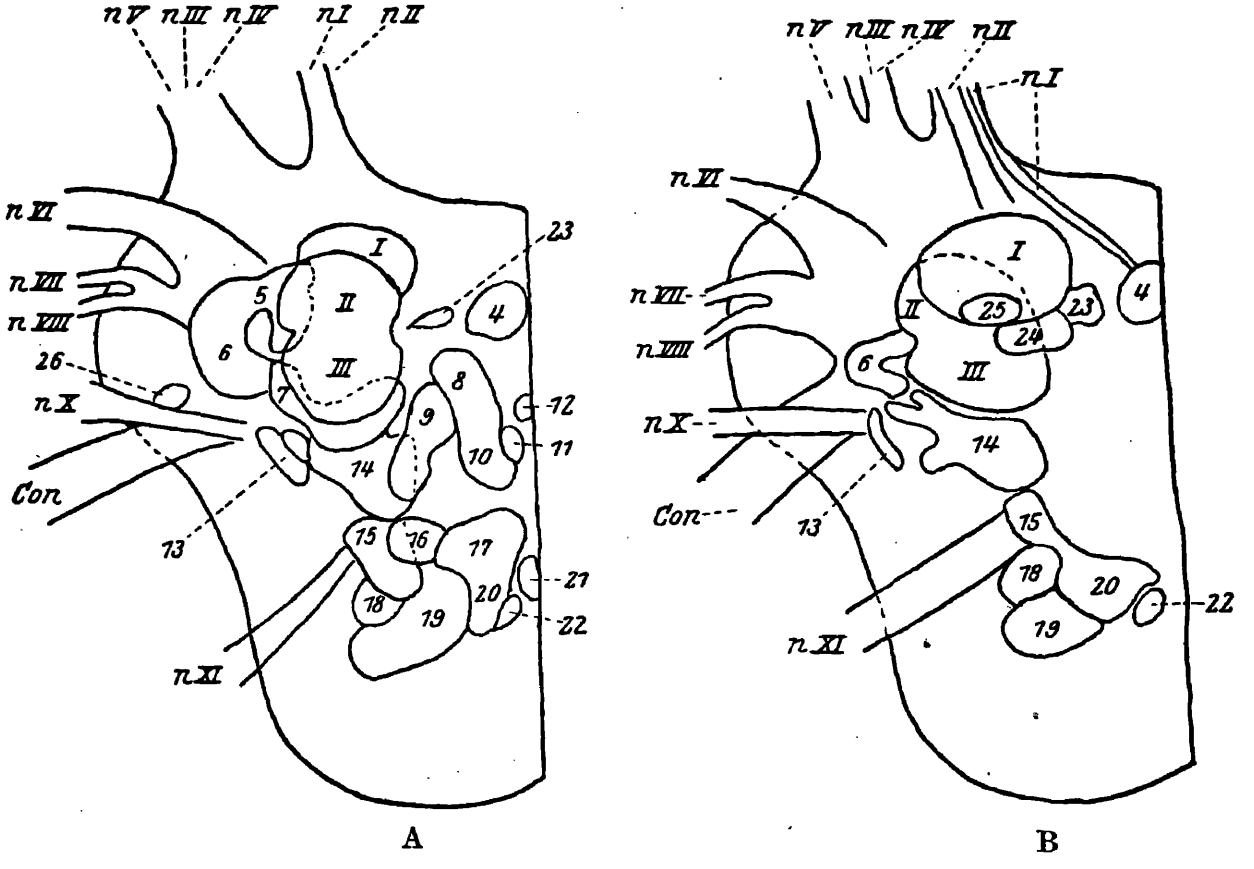
DIAMETER OF WORM mm

15


Nuchal Commissure
Optic Commissure
Nuchal Nucleus
Optic Nucleus
22
20
17
18
16
15
200μ

16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THE POSTERIOR LOBES OF THE SUPRA-OESOPHAGEAL GANGLION.

THE POSTERIOR LOBES OF THE SUPRA-OESOPHAGEAL GANGLION

Anatomy and histology of the posterior lobes of Nephtys californiensis

The supra-oesophageal ganglion of N. californiensis is a trapesoidal structure lying in the posterior part of the prostomium and the anterior part of the first segment (fig.19). It is in contact with the prostomial cuticle and in segment I is suspended beneath the epidermis. It is bounded by a connective tissue sheath which is continuous with the basement membrane of the epidermis (fig.20). Attached to the posterior margin of the ganglion, continuous with it and enclosed within the same membrane, there are two tapering cylindrical processes, the posterior lobes. They extend caudally as far as segment VII or VIII. Occasionally they extend only into segment VI and sometimes they reach segment IX. In living or freshly killed worms the posterior lobes are translucent and whitish; the supra-oesophageal ganglion is dark. In preserved material the ganglion becomes opaque, dull white. In all events, the lobes can be clearly distinguished from the ganglion proper, even in a cursory examination.

The posterior lobes are filled with large, irregularly shaped and highly vacuolated cells (fig.21). These cells vary in size, but are usually about 100 μ long and 40 μ wide.

The vacuoles may be small and numerous, as in fig.21, or they may apparently coalesce to form one or two large vacuoles which occupy most of the cell. When this is so, the nucleus is frequently to one side and thin strands of cytoplasm cross the cell between the vacuoles. Alternatively, there may be a single vacuole, opening into the neck of the cell. The vacuole contains granules and the cell therefore has the same appearance as the prostomial mucus-cells (fig.22A). Because of their high degree of vacuolation, these cells in the posterior lobes are difficult to fix satisfactorily. After sublimate fixation the material in the vacuoles has a reticulate appearance, but after Bouin fixation the vacuoles can be seen to be filled with numerous granules or globules of secreted material (fig.22B).

The nuclei of posterior lobe cells are roughly oval, approximately 10 x 15 μ and are frequently irregular in outline. Often the nuclear surface has a number of projections or bumps on it. This does not appear to be a fixation artefact because the nuclei have the same appearance whatever fixative is used. It is sometimes found that nuclei of cells undergoing great activity are irregular in outline, as for example in some neurosecretory cells (Scharrer & Scharrer, 1954a). There is usually a single large nucleolus which is very conspicuous, but in a minority

of cells (possibly 5% of them) there are two nucleoli, although in other respects these cells resemble the others in the posterior lobes.

The posterior lobe cells are drawn out and have long necks. These cell processes collect together and run in a tract along each side of the supra-oesophageal ganglion (figs. 20 and 22D) to the anterior part of it where the circum-oesophageal connectives originate. There they turn sharply through a right angle and run perpendicularly to the lateral walls of the prostomium (fig.23). The cells processes running to the prostomial walls are very distinctive and will be called the lateral organs for the sake of convenience, though they cannot properly be regarded as constituting an organ any more than, for instance, can the sinus gland of the crustacean eyestalk. The ends of the processes penetrate into and possibly through the cuticle overlying them. The prostomial cuticle is fairly thick (16 μ) in most places, but over the lateral organs it is reduced to a thickness of 2.5 μ and over the terminations of the cell processes is either absent altogether, or else is so thin that it cannot be detected by ordinary histological methods. When the cuticle is stripped from the sides of the prostomium it is seen to be peppered with perforations marking the ends of the cell processes of the lateral organ (fig.22E). The lateral organs extend along

the sides of the prostomium in front of the supra-oesophageal ganglion, almost to the anterior antennae at the antero-lateral corners of the prostomium (fig.24).

The lateral organs are not, in this species, derived entirely from the posterior lobe cells. A second group of vacuolated cells of exactly the same form and with the same staining properties as those in the posterior lobes, are situated in the anterior part of the prostomium (figs.22A and 25). A fine bundle of cell processes extends from these to the anterior part of the lateral organ, though the bundles of processes are not as distinct as those running along the sides of the supra-oesophageal ganglion from the posterior lobe cells. Further, there are vacuolated cells located along the tract of cell processes, so that nowhere is there a distinct demarcation between the cell body region and the tract of processes as in the posterior lobes. There are thus anterior and posterior lateral organs, continuous with each other, but derived from prostomial and posterior lobe cells respectively.

The entire system of vacuolated secretory cells and their processes is epidermal, as is the nervous system. They are all bounded internally by the basement membrane of the epidermis or by extensions of it. The posterior lobes and the lateral tracts formed by the processes of the cells filling them are enclosed within the same connective tissue

membrane as the supra-oesophageal ganglion and have the same thin, cellular, pericapsular sheath on their outer surface. The vacuolated cells are sharply marked off from the nervous tissue of the ganglion by a dense mass of neuroglial fibres, and a similar but thinner layer separates the lateral tracts from the ganglion, although they are all within the same connective tissue sheath (fig.22D). Neuroglial fibres penetrate between the cell processes in the lateral tracts and occasional neuroglial cell bodies can be seen scattered among the cell processes in the lateral tracts and the lateral organs. Neuroglial fibres also penetrate into the posterior lobes, though they are not numerous, and a few of the cell processes from the posterior lobes run through the neuroglial mass at the posterior end of the ganglion. The neuroglia separating the posterior lobes and the lateral tracts appears to be of a different constitution from the neuroglia in the ganglion, though no morphological differences can be seen. Under some (undetermined) conditions of fixation or staining, the neuroglia at the posterior end of the ganglion stains with the orange G while the neuroglia in the ganglion proper takes up light green in the counterstain, but usually both stain in the same way.

From anatomical considerations alone it would be reasonable to suppose that secretions produced in the

vacuolated cells in the posterior lobes and the anterior prostomial cells reach the exterior by way of the lateral organs. This is indeed so, for if the worms are roughly handled in the process of fixation, the cell contents are frequently extruded. Some worms were narcotised and most of the body wall was trimmed away in the hope of improving the fixation of the posterior lobes. The posterior lobes and the lateral organs were found to be empty and only a small quantity of fuchsinophil material was found on the cuticle over the lateral organs. Other worms which have been less severely handled before fixation have shown empty posterior lobe cells, but with the secretion concentrated in the lateral organs and penetrating through the cuticle on to its outer surface.

The Nature of the Secretion

The secretory cells of the posterior lobes, the lateral organs and the anterior prostomial group of cells are all PAS-positive. They therefore fall into the group of tissues containing polysaccharides, mucopolysaccharides, mucoproteins and glycolipids (Pearse, 1953). The presence of glycogen can be ruled out by preliminary digestion with diastase, which does not change the PAS reaction. The cells also give a positive reaction with paraldehyde fuchsin, mucicarmine, dialysed iron and alcian blue 8GS, and show

metachromasia with toluidene blue. These techniques were carried out on paraffin sections of N. californiensis fixed with formalin and also on sections fixed with absolute methyl alcohol. The results were identical with both fixatives.

These histochemical tests suggest that the posterior lobe cells produce a mucoid material of some sort which is probably an acid mucopolysaccharide. The epidermal mucus-glands of the parapodia have an appearance similar to those of the posterior lobes and the anterior prostomial group of cells, and have the same staining reactions when these tests are applied to them. Thus, although the posterior lobe mucus-cells are anatomically specialised, they do not appear to produce a secretion different from that of the simple epidermal mucus-cells of other parts of the body. This is not the general experience, for specialised mucus-cells in various parts of the body of an animal frequently produce different sorts of mucus (see, e.g., Gomori, 1954) and the differences are commonly sufficiently great to be detected by the methods employed here.

The Comparative Anatomy of the Posterior Lobes and the Prostomial Mucus-glands of the Nephtyidae

Posterior lobes, similar morphologically and histologically to those described in detail in N. californiensis,

also occur in N. caeca, N. caecoides, N. ferruginea, N. longosetosa, N. parva and N. punctata. The degree of development of the lobes varies considerably from one species to another. In N. parva, a small worm, the lobes are so narrow that only three or four cells can be séen in cross-section, but the lobes extend to segment XI. In N. longosetosa they extend to segment VII, in N. caeca only to segment V. The longest lobes I have seen are those of N. caecoides in which they extend from the supra-oesophageal ganglion in segment I to segment XV. In all these species the lobes are filled with vacuolated cells similar to those in N. californiensis, and the presence of lobes of this sort involves also the presence of lateral tracts of cell processes running to the lateral organs.

In a second group of worms, viz. N. cornuta, N. cornuta franciscana, N. hombergi, N. incisa, N. picta, Aglaophamus dicirris, A. erectans and A. virginis, there are no posterior lobes filled with vacuolated mucus cells. The posterior end of the supra-oesophageal ganglion may be bifurcate, however, and give the superficial appearance of lobes. This is undoubtedly the basis of Rullier's (1947) statement that 'ces lobes postérieurs existent chez toutes les Nephthys que j'ai étudiées. Ils sont très longs chez N. caeca, moins développés chez N. cirrosa et très courts chez N. hombergi et N. hystricis. Il y'a donc tous les

termes de passage.' I have not examined N. hystricis, but the supra-oesophageal ganglion of N. hombergi and all the other species in this group, whether bifurcated posteriorly or not, ends in a mass of neuroglial fibres similar to those separating nervous tissue from glandular tissue in N. californiensis and the species of the first group. N. cirrosa represents a special case which will have to be discussed in greater detail below. It has lobes unlike those of any other species I have seen.

The mucus-glands of the prostomium are as variable in their arrangement and disposition as the posterior lobes. The locations of these glands in the Nephtyidae are in a group in the anterior median part of the prostomium, along the lateral walls of the prostomium, and in the posterior lobes. The mucus-cells in the lateral walls of the prostomium can be divided into two groups, anterior and posterior, on the basis of their fate in certain species. The division between the anterior and posterior groups of lateral mucus-cells can be set at the level of the posterior antennae, though no anatomical division between the two groups can be made in those species in which both are present, because they grade into each other. The two groups correspond to the anterior and posterior divisions of the lateral organs of N. californiensis.

The anterior median prostomial group of mucus-cells is present in all species. These cells may have long necks and open to the lateral walls of the prostomium by way of the anterior lateral organs, as in N. californiensis, N. caecoides, N. longosetosa and N. cornuta, or they may open directly to the dorsal, or more usually, to the ventral surface of the prostomium. In N. caeca, N. ferruginea, N. parva, N. punctata and N. incisa, where the latter condition obtains, there are no anterior lateral organs and their place is taken by an anterior group of epidermal cells. This is almost the condition found in N. longosetosa, but a few of the anterior median mucus-cells open to the lateral walls of the prostomium, forming a small anterior lateral organ in addition to a small anterior group of mucus-cells in the lateral walls. Posterior lobe cells invariably open to the exterior by way of the posterior lateral organs. N. incisa and N. cornuta do not have posterior lobes and consequently lack lateral organs, but the place of the latter is taken by epidermal mucus-cells. N. picta and Aglaophamus spp. have very few epidermal mucus-cells in the prostomium and the only recognisable group of them is in the median anterior area. These open directly to the exterior.

The anterior and posterior parts of the mucus-gland system vary independently and the degree of development of

epidermal mucus-glands also differs markedly in different species. The various forms the system may take are summarised in Table I and in fig.26.

TABLE I

Species	Post. Lobes	Post. Lat. Organs	Post. Lat. Mucus-cells	Ant. Median Mucus-cells	Ant. Lat. Organs	Ant. Lat. Mucus-cells
N. californiensis	x	x	-	x	x	-
N. caecoides	x	x	-	x	x	-
N. longosetosa	x	x	-	x	x	x
N. caeca	x	x	-	x	-	x
N. ferruginea	x	x	-	x	-	x
N. glabra	x	x	-	x	-	x
N. magellanica	x	x	-	x	-	x
N. parva	x	x	-	x	-	x
N. rickettsi	x	x	-	x	-	x
N. pictata	x	x	-	x	-	-
N. cornuta	-	-	x	x	x	-
N. cornuta franciscana	-	-	x	x	x	-
N. incisa	-	-	x	x	-	x
N. squamosa	-	-	x	x	-	x
N. hombergi	-	-	-	x	-	x
N. picta	-	-	-	x	-	-
A. dicirris	-	-	-	x	-	-
A. erectans	-	-	-	x	-	-
A. virginis	-	-	-	x	-	-
N. cirrosa	*	*	-	x	x	-

*See separate discussion of this species.

The Posterior Lobes of *Nephtys cirrosa*

N. cirrosa represents a special case and must be described in detail. The supra-oesophageal ganglion lies in the posterior part of the prostomium and in the first segment. A pair of lobes extends from its posterior border into the anterior part of the fourth segment (fig.27). The histological appearance of these lobes differs markedly from that of any other species. The lobe itself is separated from the ganglion proper by a barrier of neuroglial fibres as in other species with posterior lobes. There is a dense penetration of neuroglia into the posterior lobes, quite different from the occasional neuroglial fibres which penetrate into the posterior lobes of a species such as *N. californiensis*. Secretory cells are scattered among the neuroglia, together with the neuroglial cell-bodies and larger matrix cells (fig.22C). The cell bodies of the secretory cells are about 15 μ long by 8 μ wide and are packed with fuchsinophil granular inclusions, which are often so numerous and dense as to obscure the structure and form of the cell. These cells are most numerous in the posterior ends of the lobes; a few are to be found along the lateral edges of the lobes as far forwards as the posterior end of the brain.

The processes from the posterior lobe secretory cells of *N. cirrosa* are much finer than those of *N. californiensis*

and can only be traced by the course of the granules. They run along the sides of the posterior lobes and the supra-oesophageal ganglion to the anterior margin of the latter. Then they run along the outer edges of the circum-oesophageal connectives, which are in contact with the epidermal cells of the lateral prostomial walls. At the point where the circum-oesophageal connectives turn sharply in a ventral direction, the fuchsinophil granules can be seen running through the epidermis to the cuticle. Morphologically, the disposition of the cells and processes that stain with paraldehyde fuchsin is essentially the same in N. cirrosa as in N. californiensis (fig.28). There are far fewer secretory cells in the posterior lobes of N. cirrosa and their processes are much narrower, so that in consequence there are no conspicuous lateral organs, but the processes from posterior lobe cells run through the epidermis in the position where lateral organs would be expected to be found. Epidermal mucus-cells of the posterior lateral group adjoin the terminations of the fibres from the posterior lobe cells. The mucus-glands of the median anterior group open to the exterior in the lateral walls of the prostomium, forming an anterior lateral organ.

Discussion

From these descriptions of the arrangement of the posterior lobes and their associated structures in the Nephtyidae, it appears that we have to deal with a system of epidermal mucus-cells which have become more or less incorporated with the supra-oesophageal ganglion. It is a simple matter to arrange the species in a series showing progressive stages in a centripetal migration of epidermal mucus-cells. In a species such as N. picta or Aglaophamus spp. there are relatively few mucus-cells in the prostomium and those that there are are embedded in the epidermis and open directly to the exterior. At the other end of the series we may place N. californiensis or N. caecoides. In these worms there are no mucus-cells in the epidermis of the prostomium; instead they are concentrated in the posterior lobes and in the anterior prostomial group and they communicate with the exterior by long processes terminating in the lateral organs. Intermediate in the series are N. cornuta, in which the anterior prostomial mucus-cells open to the exterior in the lateral walls of the prostomium and there are no antero-lateral mucus-cells and no posterior lobes, and N. caeca in which there are posterior lobes and posterior lateral organs, but cells of the median anterior prostomial group open directly to the exterior. It seems likely that the epidermal mucus-cells of the anterior lateral

walls of the prostomium have migrated into the anterior median group, and those in the posterior part of the lateral walls have migrated into the posterior lobes. Both groups of mucus-cells open to the exterior in the lateral walls of the prostomium whether they are in their original peripheral position or have migrated centrally. To judge from the disposition of the mucus-cells in the species illustrated in fig.26, the two processes have gone on independently.

The fact that 20 or so species can be arranged in such an order that a sequential elaboration and integration of the mucus-gland system can be demonstrated does not, of course, prove that the evolution of these structures has taken the same course. If it had, one might expect to find more intermediate cases in the postulated series. The hypothesis would receive strong support if a species were discovered in which there was a group of mucus-cells lateral to the supra-oesophageal ganglion in the position occupied by the lateral tract of processes from the posterior lobe cells in N. californiensis. However, this intermediate condition does not exist in any of the 21 species I have examined and the nearest approach is that found in N. californiensis, in which a few mucus-cells are scattered along the lateral tracts of cell processes.

While the mucus-cells of the posterior part of the prostomium apparently occur either in the epidermis or in the posterior lobes, but not in intermediate positions, those of the anterior part of the prostomium are not so uncompromisingly divided into peripheral or central groups. In most species those in the middle of the prostomium open directly to the exterior either dorsally or ventrally or both, and the anterior lateral mucus-cells are not related to those in the central group. In N. longosetosa a few cells in the median group do not open to the exterior ventrally, as the rest of them do, but by way of long processes to the sides of the prostomium. This species has still a small group of anterior lateral mucus-cells in addition to the incipient lateral organ. In N. californiensis and N. caecoides the anterior lateral mucus-cells are completely replaced by the anterior part of the lateral organ and all the cell bodies are located in the central mass or along the course of the cell processes. Some of the mucus-cells in the median mass of both these species still open directly to the dorsal or ventral surfaces of the prostomium. The tendency for epidermal mucus-cells in the prostomium to migrate inwards while retaining their original connection with the exterior is illustrated in N. incisa. In this species there are neither anterior nor

posterior lateral organs, but the mucus-cells in the lateral walls of the prostomium are arranged in clumps which project inwards beneath the epidermis (fig.29).

Although the posterior part of the prostomial mucus-gland system comes to have an intimate connection with the supra-oesophageal ganglion, the anterior part has no connection with any part of the nervous system. In spite of this it seems justifiable to treat both as parts of the same system, particularly in view of the fact that in what we have postulated to be the primitive condition, found in such a species as N. picta, it is impossible to distinguish between anterior and posterior groups of mucus-cells in the lateral walls of the prostomium. At first sight it may seem surprising that epidermal mucus-cells should have been incorporated in the brain to the extent that they are contiguous with the nervous tissue and are enclosed within the membranes which invest the ganglion. There is, however, a precedent for this in the evolution of the cerebral organs of nemerteans, which is strikingly similar to the postulated evolution of the posterior lobes of Nephtys.

The cerebral organs of nemerteans are partly glandular and partly ganglionic. Their structure had been known for some time, but no detailed and comparative account of them had been given until Scharrer (1941) made a study of

the structure of those of Lineus and Cerebratulus, in which they are incorporated within the brain capsule. She proposed that they had evolved from epidermal structures. In Carinella annulata the ganglion cells and glandular cells of the cerebral organ are purely epidermal and are connected with the cerebral ganglion by a long nerve running through the muscle layers of the body wall. In Drepanopus albolineatus the cerebral organ is internal to the muscle layers but is connected to the exterior by a canal. In Amphiporus marmoratus, Lineus coecineus and Cerebratulus lacteus the cerebral organ is associated closely with the cerebral ganglion and shows a progressively greater degree of incorporation within it in the three species. In the first it is in contact with the ganglion, but still appears as a separate structure. In the other two it is completely within the connective tissue sheath of the cerebral ganglion. In Cerebratulus there is an uninterrupted transition from ganglion cells to glandular cells in the posterior and antero-lateral parts of the brain.

Scharrer was able to cite embryological evidence of the epidermal origin of the cerebral organs of Lineus in support of her thesis. The hypothesis that the posterior lobes of Nephtys are derived from epidermal mucus-cells would be greatly strengthened by embryological evidence of the migration of cells from the epidermis into the lobes

and the anterior median group of mucus-cells. Unfortunately no detailed study of the embryology of any nephtyid has ever been carried beyond the larval stage. Until it has been, an analysis of the evolution of these structures must be based on a consideration of comparative anatomy alone. The evidence for this thesis, then, amounts to the following:-

1. Cells in the prostomial epidermis, in the median anterior group, in the posterior lobes, and in the parapodial mucus-glands are identical in appearance.
2. All respond in the same way to the histochemical tests discussed above and all secrete an acid mucopolysaccharide.
3. All these structures are epidermal and are bounded internally by the basement membrane of the epidermis or by extensions of it.
4. A sequence can be discerned in the species examined which is consistent with the view that a centripetal migration of these cells has taken place.
5. A strikingly similar example of the phylogenetic and ontogenetic centripetal migration of epidermal glandular cells with a subsequent incorporation in the cerebral ganglion has been reported in the nemerteans.

N. cirrosa represents a separate problem and probably illustrates a further stage in the evolution of the posterior lobes and a closer association of the cells in them with the supra-oesophageal ganglion. Only one of the three types of cell in the posterior lobes of N. cirrosa is secretory and they are much smaller than the cells in the posterior lobes of N. californiensis and have a quite different appearance. In fact, they look much more like the neurosecretory cells of the supra-oesophageal ganglion. The question arises, are the posterior lobes of N. cirrosa homologous with those of N. californiensis? There are three possible origins of the cells in the lobes of the former species:

1. They cannot be homologised with any feature of the brain of any other nephtyid and the resemblances are the result of convergence. In other words, the posterior lobes of N. cirrosa have developed de novo.
2. They represent ganglion cells of the posterior part of the supra-oesophageal ganglion which have migrated caudally into the neuroglial area at its posterior end. The whole of the posterior part of the brain has therefore hypertrophied.
3. They represent modified posterior lobe cells, and the posterior lobes of N. cirrosa can be homologised with those of N. californiensis and other nephtyids.

As to the first alternative, the appearance of a completely new nervous structure, with no hint of its existence in any other species, is not likely. While the possibility cannot be excluded, particularly since only about a third of the species in the family have been examined, to admit it on so slight evidence would be to deny the principles of comparative morphology.

The second alternative, that the posterior region of the supra-oesophageal ganglion has hypertrophied in this species, is at first sight the most attractive. However, in spite of the great variation in the fine structure of the supra-oesophageal ganglion of Nephtys, the arrangement of groups of neurones in the posterior part of the brain is one of the few constant features. There are two large groups of nerve cells in the posterior part of the brain of all species of Nephtys, which are homologous with a similar group of nerve cells in Nereis also. In some species of Nephtys they may extend part of the way into the anterior part of the posterior lobes, but they are always separated from the secretory cells in the lobes by a barrier of neuroglial fibres. These are represented in the posterior part of the ganglion of N. cirrosa, and not in the posterior lobes. In addition there are a pair of large neurosecretory cells (Type A, see later section) of a distinctive type in the posterior part of the brain. These, too, are present

in the ganglion and not in the lobes in N. cirrosa. Finally, the eyes of Nephtys which are located in this part of ganglion and bear a constant relation to other groups of ganglion cells, occur in their usual position in N. cirrosa. Thus all the recognisably constant features of the posterior part of the supra-oesophageal ganglion of other species of Nephtys occur also in N. cirrosa in their typical position and not in the posterior lobes. For this reason it is difficult to maintain that the posterior lobes of this species represent a hypertrophy of the posterior part of the supra-oesophageal ganglion.

We are thus forced to consider the third alternative, that the posterior lobes of N. cirrosa and N. californiensis are homologous. In favour of this view, the fibres from the secretory cells of the posterior lobes of the former species run in the same place and open to the exterior in the same place as they do in N. californiensis. In addition, both produce fuchsinophil granules of secreted material. On the other hand the secretory cells in the posterior lobes of N. cirrosa form but a small minority of the cells and they look nothing like those of the posterior lobes of other species of Nephtys. In fact, they look like the majority of neurosecretory cells of the supra-oesophageal ganglion.

The posterior lobes of N. cirrosa demonstrate a remarkable incorporation of epidermal glandular cells within

the central nervous system. Even in the nemerteans, the glandular tissue of the cerebral organ is recognisably of the same histological appearance whether it is epidermal, as in Carinella, or completely incorporated within the cerebral ganglion, as in Cerebratulus (Scharrer, 1941). This is also true of most nephtyids but in N. cirrosa the secretory cells of the posterior lobes appear to have become completely integrated with the nervous system. Indeed, were it not for a knowledge of the structure of the posterior lobes, and their probable evolution in other nephtyids, one would certainly not attempt to distinguish between the posterior lobes of N. cirrosa and the rest of the supra-oesophageal ganglion.

The function of the mucus-gland system of the prostomium is unknown. The cells of the posterior lobes of N. caecoides, in which the lobes reach their greatest development, produce copious quantities of mucus which appears to be readily discharged to the exterior. Whatever its function, it must be of considerable biological significance. The fact that the prostomial mucus-glands are so poorly developed in some species, e.g. N. picta, suggests that there has been a great elaboration of some activity of Nephtys in the course of the evolution of this worm. Unfortunately, practically nothing is known of the ecology of the Nephtyidae and it is impossible to speculate on the

function of this glandular system. One or two possible functions can be excluded, however. One of the commonest functions of epidermal mucus-cells in polychaetes is the secretion of a tube in which the worm lives. In most species of Nereis this is done by parapodial mucus-glands, but in Nephtys the epidermal mucus-glands of the segmental part of the body are poorly developed and the worm does not secrete a tube, nor does it consolidate its burrow in the sand with mucus. Several polychaetes lay their eggs in mucous capsules and this could conceivably be a function of the prostomial mucus-glands of Nephtys. But Augener (1912) has described epitokous forms of a number of species of Nephtys and Thorson (1946) has found pelagic larvae of the worm. There is every indication that Nephtys swarms in the surface of the sea for spawning and that it has pelagic eggs and larvae. Finally, mucus is sometimes secreted to form a food trapping net, as in the chaetopterids. This seems unlikely in Nephtys because it has no permanent burrow and is probably a carnivore. This possibility must be rejected with caution though, since Nereis diversicolor, regarded as a typical carnivorous polychaete, has been observed to secrete a mucus net for just this purpose (Harley, 1950).

CAPTIONS TO FIGURES

Figure 19. The supra-oesophageal ganglion and posterior lobes of N. californiensis dissected from the ventral side.

Figure 20. A series of transverse sections through the prostomium and first segment of N. californiensis, to show the relation between the nervous system (stippled), the posterior lobes, and the epidermal basement membrane.

Figure 21. Cells from the posterior lobes of N. californiensis.

Figure 22. A. Mucus-cells from the anterior prostomial group of N. caeca. The long necks of the cells are filled with fuchsinophil granules. Bouin; paraldehyde fuchsin; 7 μ paraffin sections. No filter.

B. Posterior lobe cells of N. longosetosa filled with strongly fuchsinophilic granules. The granules can be seen filling the cell processes which run towards the upper left-hand corner of the lateral tract. Bouin; paraldehyde fuchsin; 7 μ paraffin sections. Wratten 58 filter.

C. Posterior lobe cells of N. cirrosa showing the large non-secretory matrix cells, neuroglial fibres and, along the upper edge, small secretory cells filled with fuchsinophil material. Bouin; paraldehyde fuchsin; 7 μ paraffin sections. Wratten 58 filter.

D. Frontal section through the prostomium and anterior segments of N. californiensis. The supra-oesophageal ganglion occupies the upper right-hand half of the photograph; cell processes from the posterior lobes run along the side of the ganglion to the lateral organ in the upper left centre of the figure. Helly; paraldehyde fuchsin; 7 μ paraffin sections. Wratten 58 filter.

E. The lateral organ of N. ferruginea. The cell processes run to the dark-staining cuticle which can be seen to be perforated over the ends of them. Bouin; Mallory triple stain;

10 μ paraffin sections; Wratten 25 filter.

Figure 23. Transverse section through the prostomium of N. ferruginea, showing the lateral organs at their greatest development.

Figure 24. Lateral view of the prostomium and anterior segments of N. californiensis. Cross-hatched area indicates the attachment of the first parapodium which has been removed to expose the area occupied by the lateral organ.

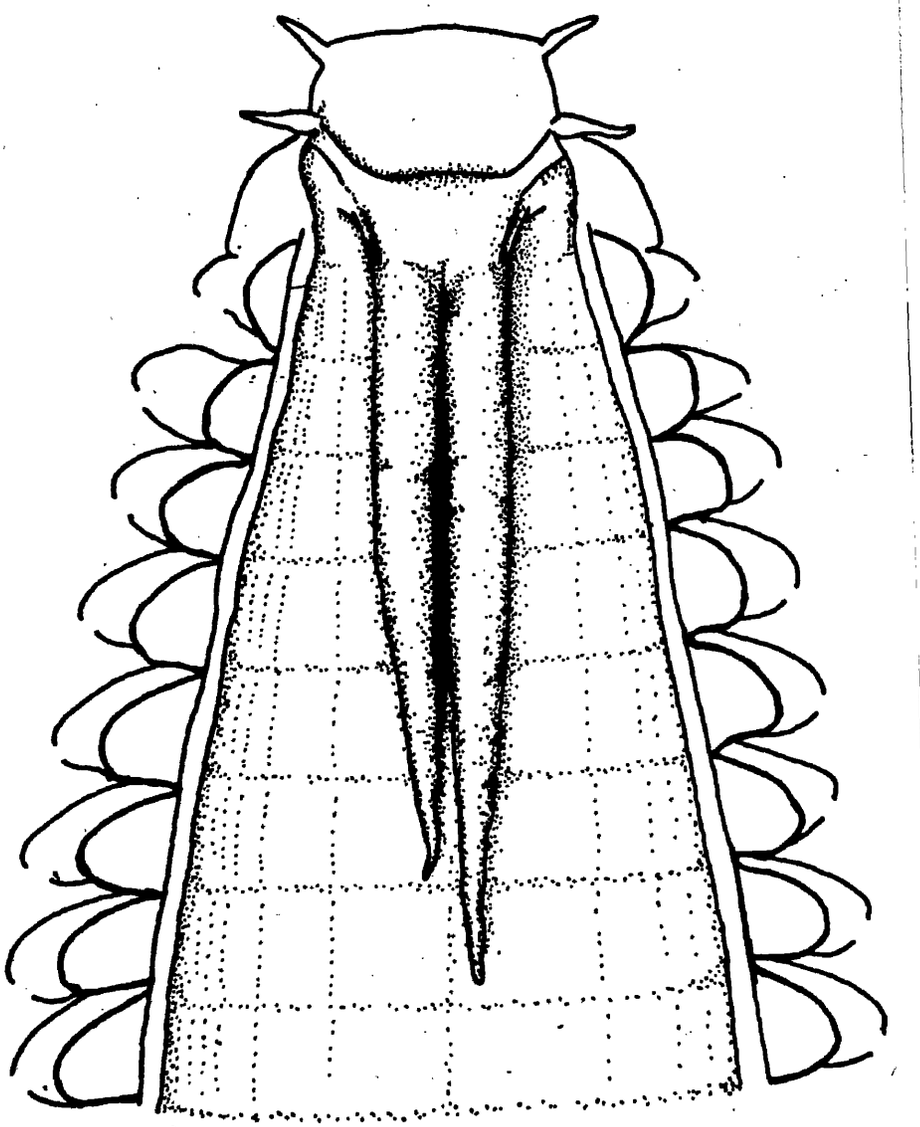
Figure 25. Composite frontal section through the anterior end of N. californiensis to show the relation between the posterior lobes, the lateral tracts, the lateral organs and the prostomial mucus-cells.

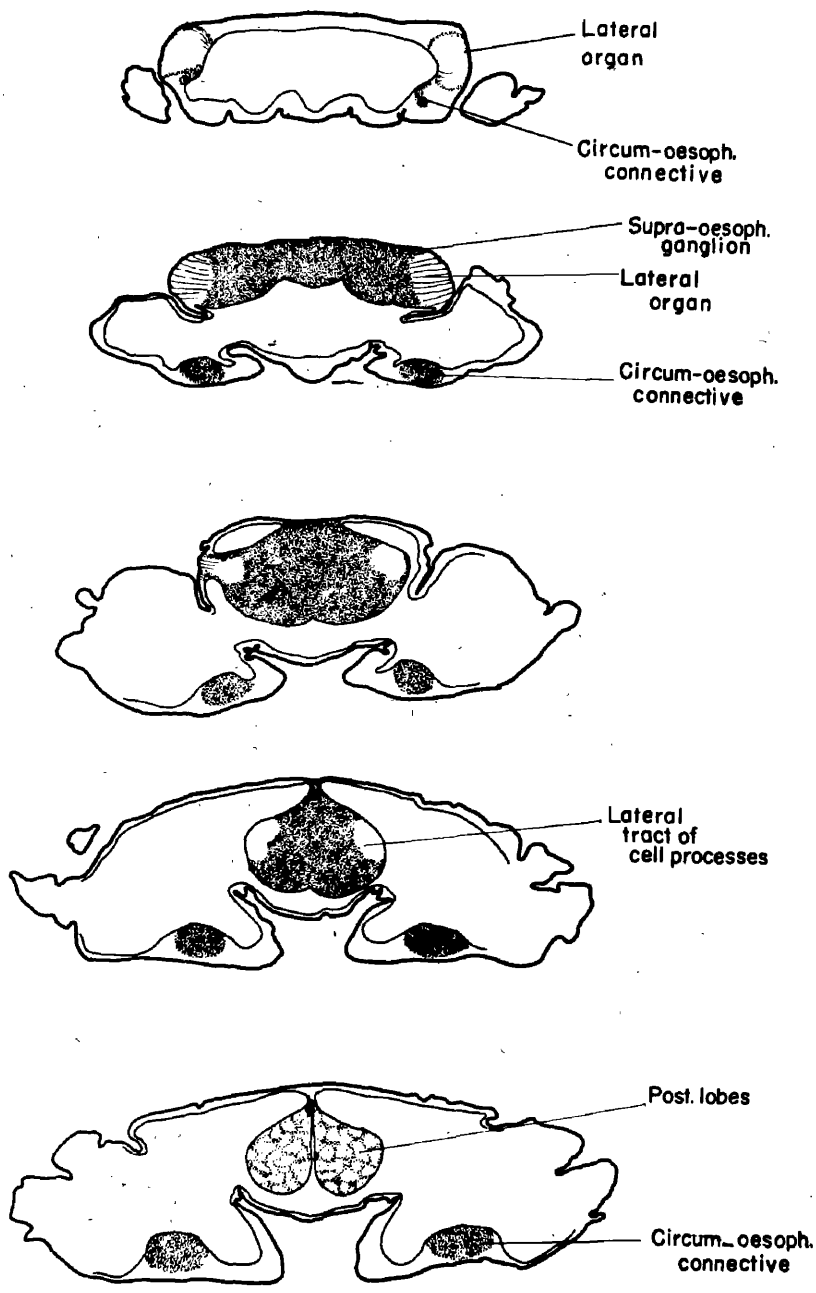
Figure 26. The various arrangements of the prostomial mucus-gland system found in the Nephtyidae. Sites of mucus-secreting cells are indicated in black. The first three species in the upper row show an increasing development of prostomial epidermal mucus-glands; the remainder indicate stages in the centripetal migration of epidermal mucus-cells into the posterior lobes and into the anterior prostomial group.

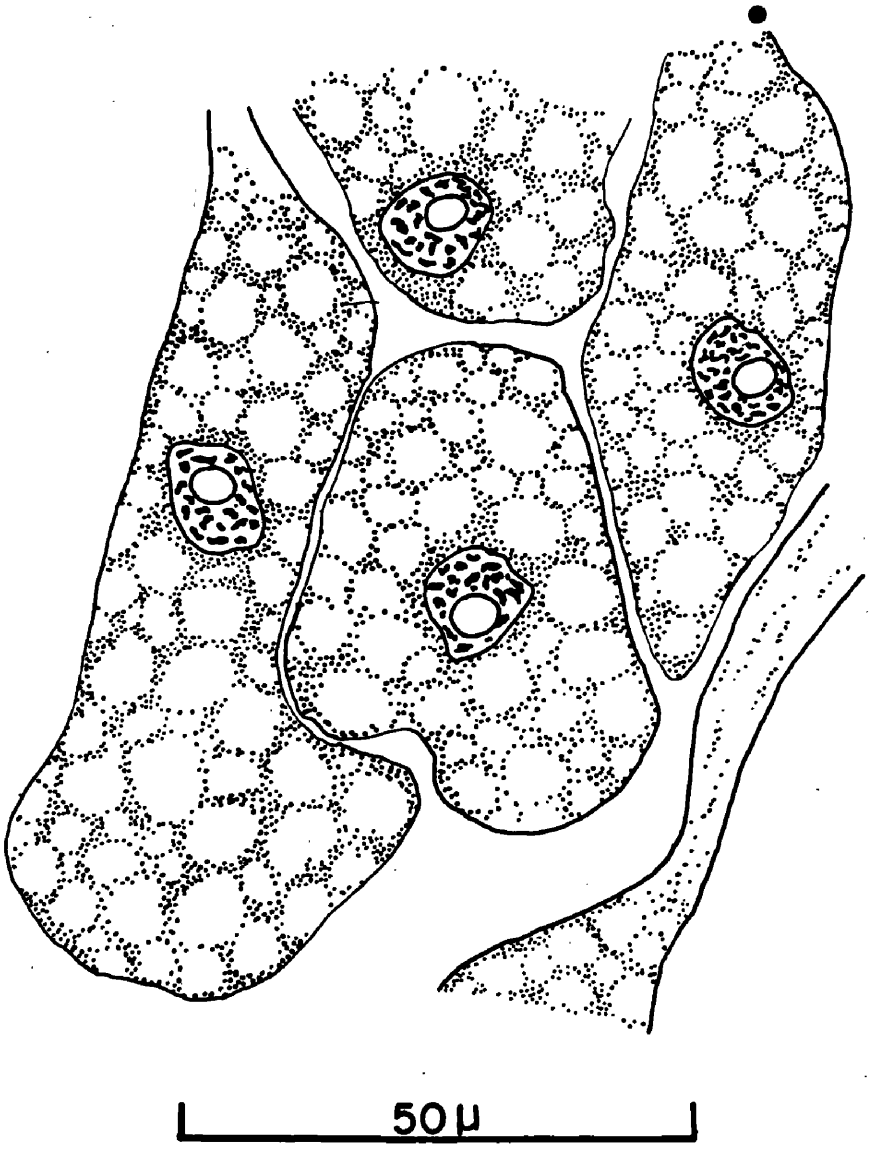
Figure 27. The supra-oesophageal ganglion and posterior lobes of N. cirrosa dissected from the ventral side.

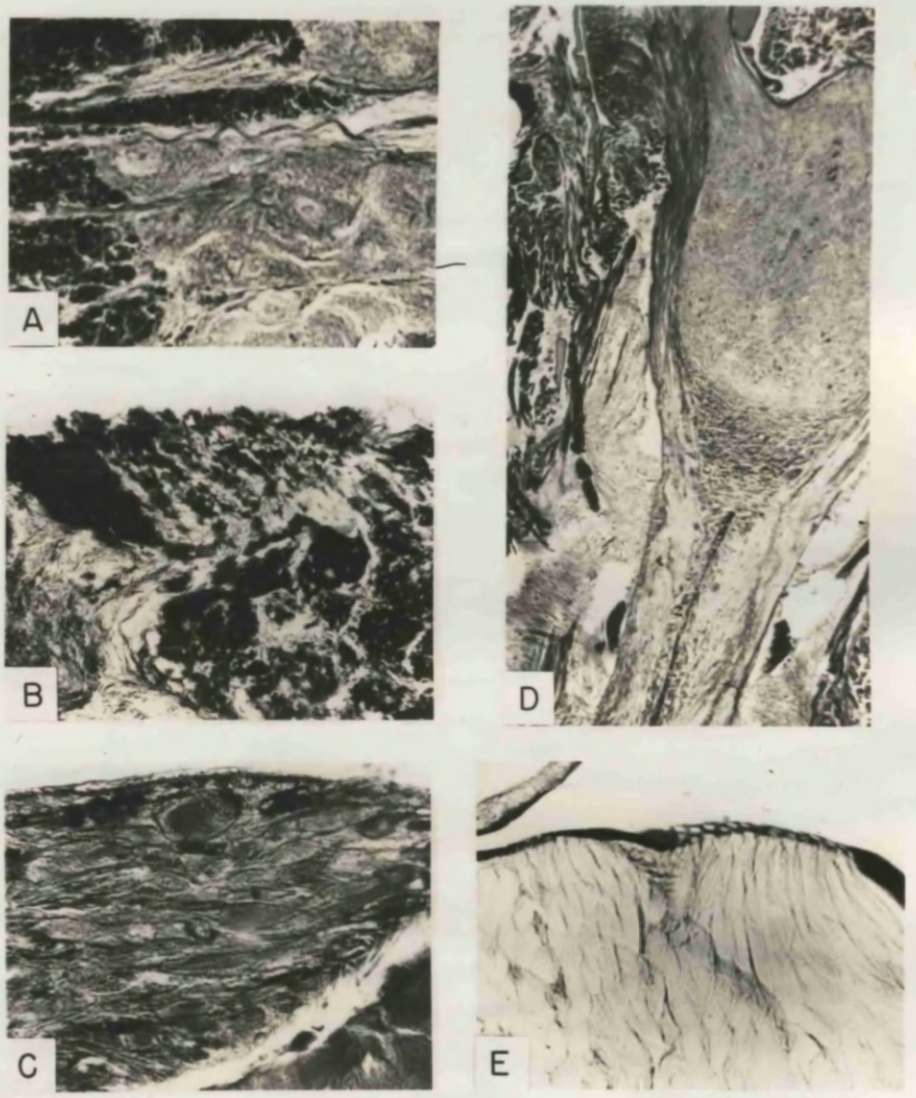
Figure 28. Composite frontal section through the anterior end of N. cirrosa to show the epidermal mucus glands of the prostomium and the path taken by the granules from the secretory cells in the posterior lobes.

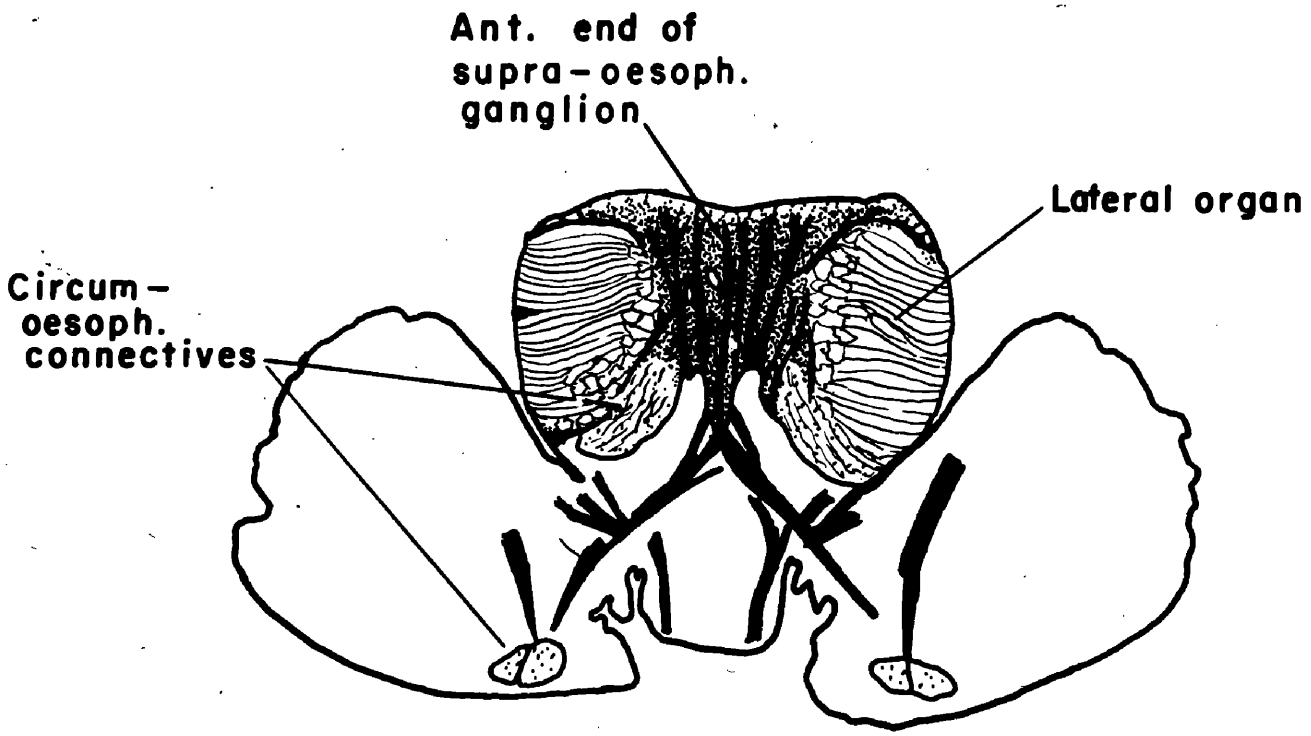
Figure 29. Transverse sections through the prostomium of (A) N. incisa and (B) N. picta to show the arrangement of the epidermal mucus-glands and their inward migration in the former species.



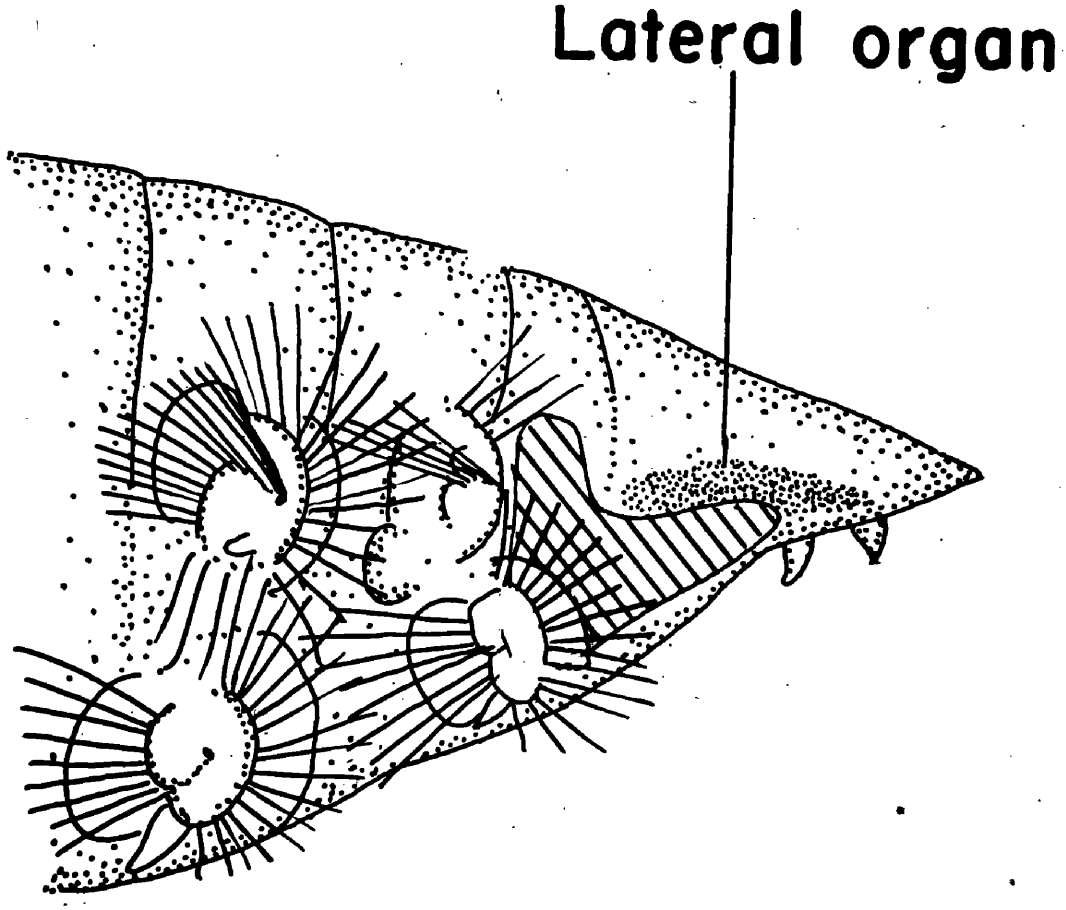

Lateral organ
Circum-oesoph. connective
Supra-oesoph. ganglion
Lateral organ
Circum-oesoph. connective
Lateral tract of cell processes
Post. lobes
Circum-oesoph. connective


50μ

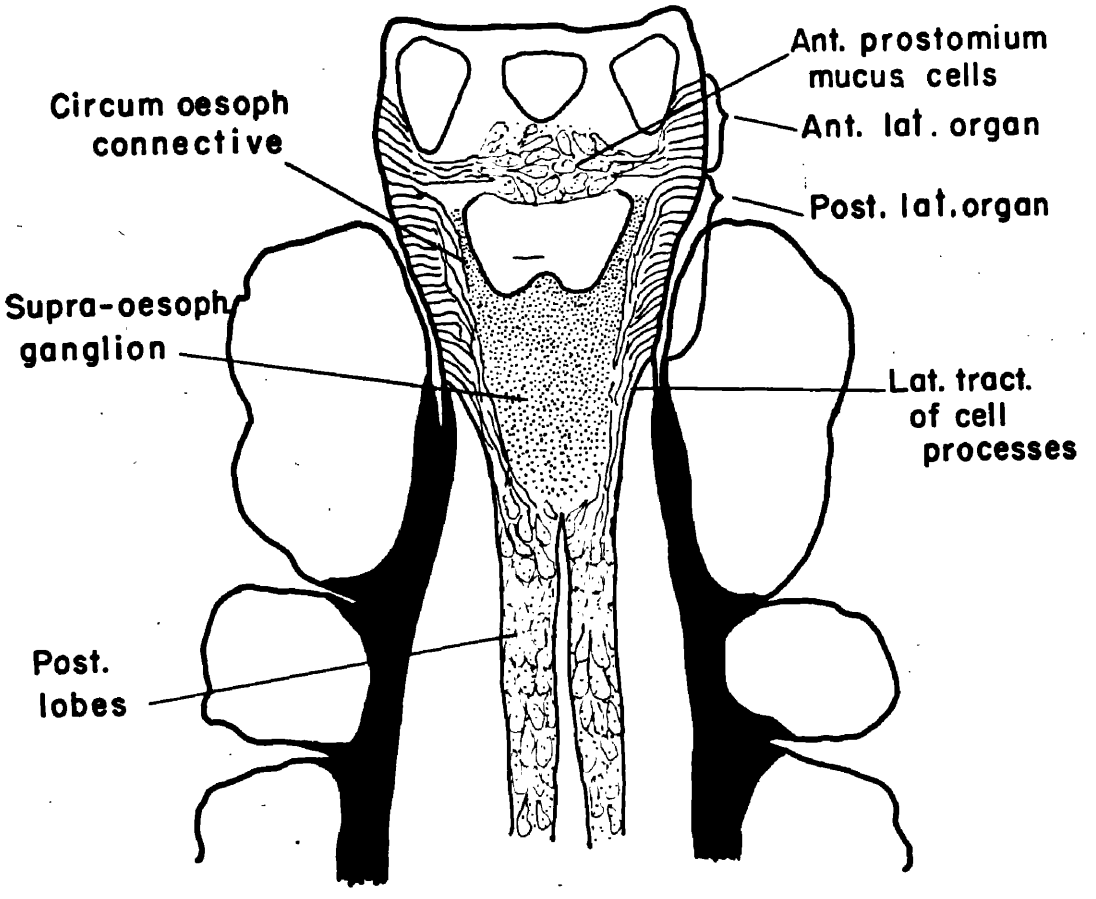

A
B
C
D
E

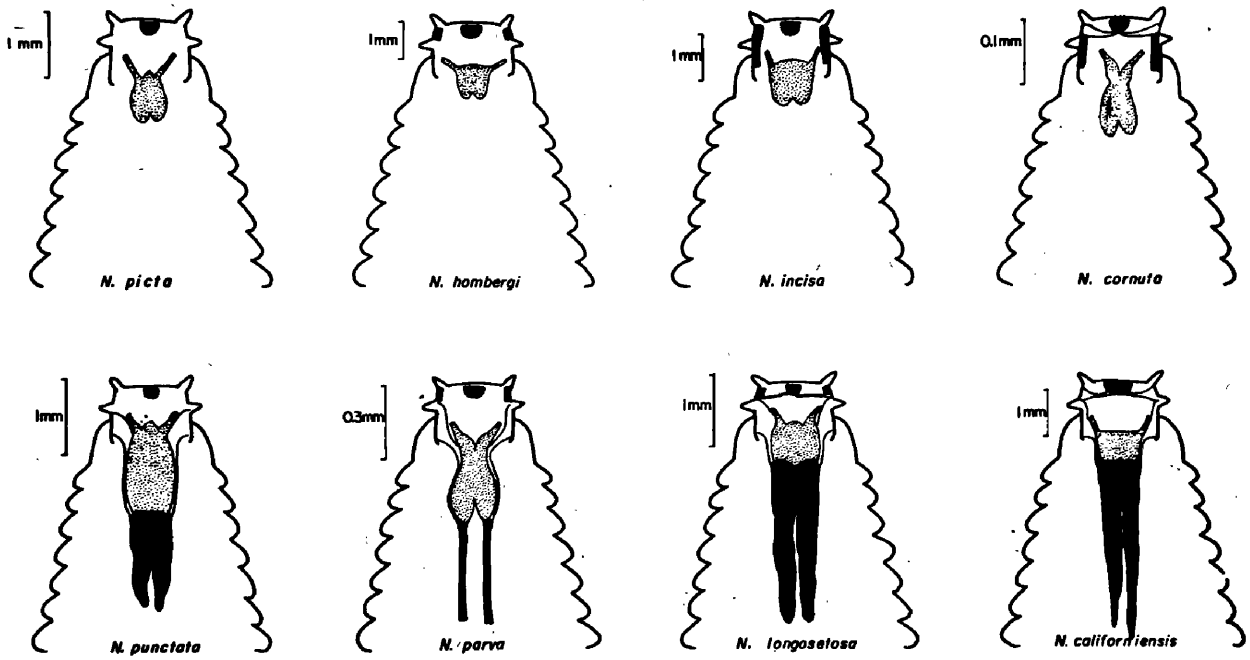

Ant. end of
supra-oesoph.
ganglion
Lateral organ
Circum-
oesoph.
connectives

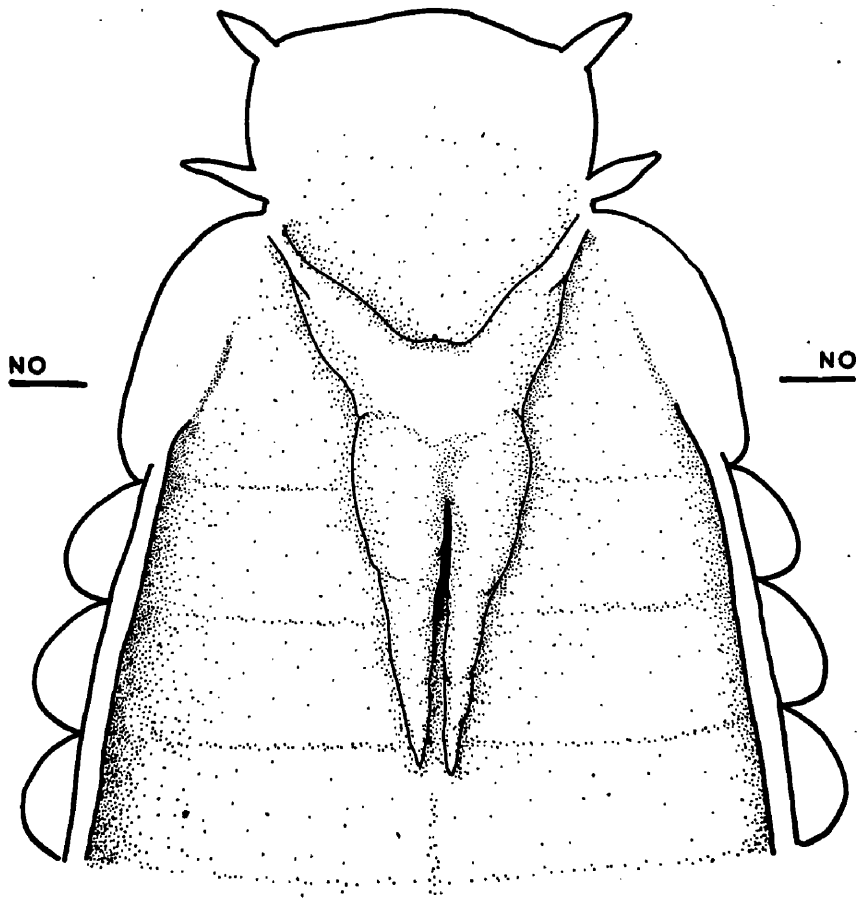
23


Lateral organ

24


Ant. prostomium
mucus cells
Circum oesoph
connective
Ant. lat. organ
Post. lat. organ
Supra-oesoph
ganglion
Lat. tract.
of cell
processes
Post.
lobes


1 mm
N. picta
1mm
N. hombergi
1mm
N. incisa
0.1mm
N. cornuta
1mm
N. punctata
0.3mm
N. parva
1mm
N. longosetosa
1mm
N. californiensis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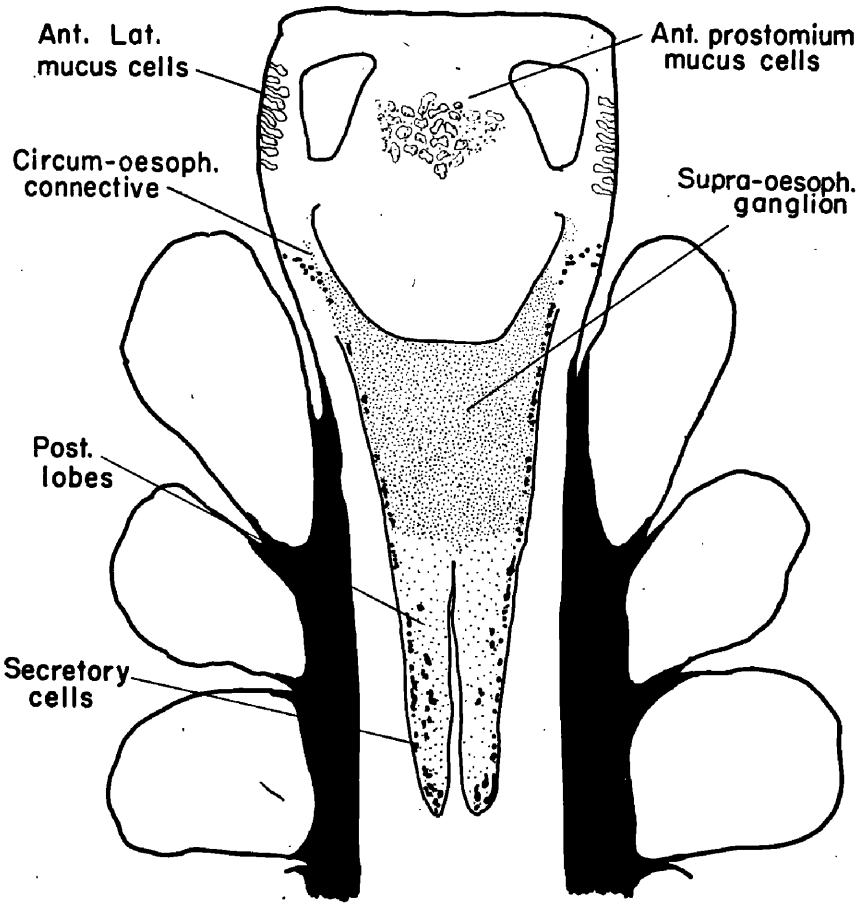
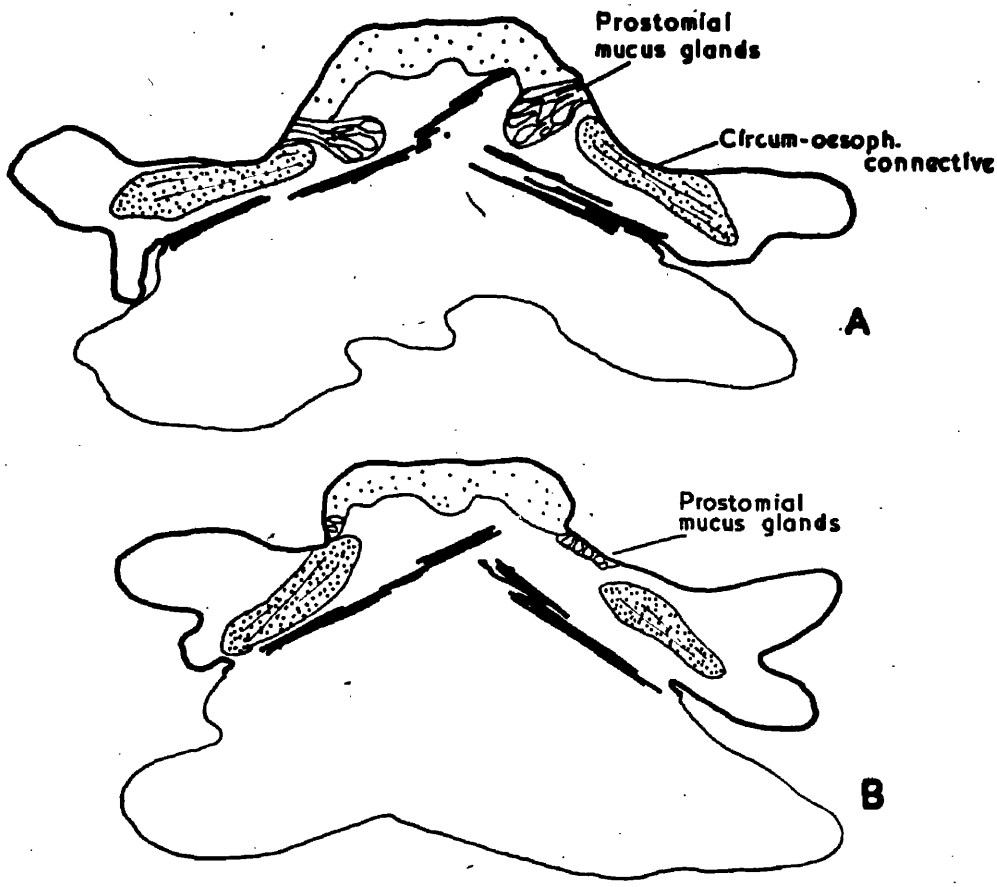
NO


Ant. Lat. mucus cells
Ant. prostomium mucus cells
Circum-oesoph. connective
Supra-oesoph. ganglion
Post. lobes
Secretory cells

28


Prostomial
mucus glands
Circum-oesoph.
connective
A
Prostomial
mucus glands
B

THE EYES AND THE PHOTONEGATIVE BEHAVIOUR

THE EYES AND THE PHOTONEGATIVE BEHAVIOUR

The Structure and Disposition of the Photoreceptors

Three types of photoreceptor are found in members of the Nephtyidae, though not all species possess all three types. They are: (a) inverted single-celled receptors lying in pigment cups and embedded in the supra-oesophageal ganglion, a pair of unit receptors on each side, (b) single-celled receptors lacking pigment cups, situated in the prostomium at the extreme anterior edge of the supra-oesophageal ganglion, (c) epidermal receptors in the body wall, probably in the pygidium of two small species and possibly in the prostomium of several species.

The photoreceptors of the supra-oesophageal ganglion

Bilaterally symmetrical photoreceptors embedded in the posterior part of the supra-oesophageal ganglion are present in nearly all species of Nephtys. Only N. incisa among the species I have examined lacks them (Table II). The eyes are not generally visible from the exterior, nor even in dissection unless the ganglion happens to be cut at the right level. However, in two small species, N. cornuta franciscana and N. parva, they are visible from the exterior by transparency. The eyes of these species are about half the size of those of a large species such as N. Californiensis, but the linear dimensions of the worms themselves are

reduced by a factor of 20-25, and the amount of tissue overlying the eyes is both relatively and absolutely very much less. In these two small species the eyes can be seen as two black spots through the dorsal side of the third segment, the supra-oesophageal ganglion extending from the prostomium to that segment. In all species of Nephtys which possess eyes, they lie in the posterior part of the brain, a little anterior to two paired groups of ganglion cells which lie at the postero-lateral corners of the neuropile (fig.30A).

Each photoreceptor consists of a single vacuolated cell lying within a pigment cup which is composed of dark brown (melanin?) extracellular granules. Since there are no other cells in the immediate neighbourhood, apart from the dense, coarse neuroglial fibres, it seems likely that the pigment granules are secreted by the sensory cell itself. The dimensions of each photoreceptor cell are about 40 x 20 μ in the largest worms, and about half that size in the smallest species (fig.11). Projecting into the vacuole of the cell is a ridge which is mushroom-shaped in cross section (figs.30B and C). Photosensitive material is probably concentrated in this ridge, particularly at its surface, since it possesses somewhat different staining properties from the rest of the cell. The almost spherical nucleus is 8 or 9 u in diameter and lies near the origin of

the axon. Two such vacuolated cells lie close to one-another and the pigment cups of each are contiguous, resulting in an H- or Y-shaped mass of pigment with the photoreceptor cells directed either dorsally and ventrally, or else dorso-laterally and ventro-laterally. The particular form of the pigment cup varies from one species to another, but appears to be constant within a species.

Closely applied to the vacuolated cell, on the side opposite the pigment cup, is a second nerve-cell body which is non-vacuolated. It is about one-third the size of the vacuolated cell and has an elongated nucleus 15 u long and 5-7 μ wide (fig.30B). I can suggest no function for this cell, though the consistency of its occurrence and its close association with the vacuolated cell, suggest that it has some functional association with it. The association between what is apparently a sensory cell in a pigment cup and an accessory cell is faintly reminiscent of the situation in some arthropodan compound eyes. In these, an eccentric cell is associated with the retinula cells and is responsible for the sensory discharge in the optic nerve when the ommatidium is illuminated (Waterman & Wiersma, 1954). Axons from both the sensory cells and the accessory cells run directly into the neuropile. Once inside the neuropile, they are lost in the general tangle of nerve fibres. An identical arrangement of photoreceptor cells,

pigment cups and accessory cells is found on either side of the brain.

Anterior prostomial receptors

Two pairs of cells, which are probably photoreceptors, lie in the anterior part of the prostomium outside the supra-oesophageal ganglion. They are not universally present, nor do they always have the same appearance. N. incisa, N. punctata and N. rickettsi appear to lack them, while in the remaining species they may be vacuolated and resemble the eyes in the supra-oesophageal ganglion closely, or they may be non-vacuolated (see Table II). There is little to show that the non-vacuolated cells are photoreceptors but whether they are functional or not, they are assumed to be homologous with the vacuolated anterior receptors of other species because they are the same size and shape, are in the same position, and bear the same relation to the brain.

The circum-oesophageal connectives leave the supra-oesophageal ganglion at its antero-lateral corners. Immediately dorsal to the connectives there is a group of small ganglion cells extending from one side of the ganglion to the other (fig.31A). The prostomial photoreceptors lie immediately anterior to these cells, in contact with, but internal to the epidermis of the lateral walls of the prostomium. They are about 40 x 20 μ in size and have an

TABLE II

Species	Anterior Eyes	Posterior Eyes
Nephtys caeca	non-vacuolated	present
Nephtys caecoides	vacuolated	present
Nephtys californiensis	vacuolated	present
Nephtys cirrosa	non-vacuolated	present
Nephtys cornuta	non-vacuolated	present
Nephtys cornuta franciscana	non-vacuolated	present
Nephtys ferruginea	vacuolated	present
Nephtys hombergi	vacuolated	present
Nephtys incisa	?	?
Nephtys longosetosa	vacuolated	present
Nephtys magellanica	vacuolated	present
Nephtys parva	vacuolated	present
Nephtys picta	vacuolated	present
Nephtys punctata	none	present
Nephtys rickettsi	none	present
Nephtys squamosa	non-vacuolated	present

oval nucleus. In those species in which they are vacuolated, a ridge projects into the vacuole of the cell and there is some indication that the photosensitive material is concentrated in the surface of the ridge (figs.31B and C). These cells invariably lack pigment cups, but otherwise the vacuolated variety is identical with the photoreceptors of the supra-oesophageal ganglion. Indeed, it is chiefly for this reason that they are presumed to be photoreceptors. The non-vacuolated cells appear to be homologous with the vacuolated ones. There is no suggestion of an accessory cell in contact with these photoreceptors, but usually two identical photoreceptor cells lie in contact with each other. The axons from the receptor cells on either side of the prostomium run laterally and caudally and enter the supra-oesophageal ganglion at its anterior margin at about the point of origin of the circum-oesophageal connectives.

Epidermal photoreceptors

Throughout the taxonomic literature of the Nephtyidae, there are numerous and repeated references to pigment spots, usually with the implication or statement that they are light sensitive. There is nothing particularly reprehensible in the assumption that single-celled photoreceptors might occur in the epidermis of Nephtys and that pigment spots indicate their presence, because epidermal photoreceptors

are well known from other annelids, though in oligochaetes rather than polychaetes. However, as far as I know, this assumption has never been justified by the discovery and description of the receptor cells themselves. After searching sections of the epidermis of the prostomium and of segments from various parts of the body of nephtyids and finding nothing which might be regarded as a photoreceptor, I directed attention to four species, N. californiensis, N. caecoides, N. parva and N. cornuta franciscana. The latter two species have a ring of pigment spots surrounding the pygidium, the former two have a well marked pigment pattern on the prostomium ending in two symmetrically placed patches of pigment and a third patch in the anterior median part of the prostomium, all suggestive of epidermal photoreceptors.

The pigment spots are formed by an accumulation of small granules of dark brown substance which are extracellular and which surround certain epidermal cells. Apart from the fact that they are surrounded by pigment granules, these cells differ in no way from other epidermal cells in the neighbourhood. They are roughly conical in shape, with the apex of the cone in contact with the epidermal basement membrane, and the base of the cone distal. Epidermal photoreceptors of other annelids are usually differentiated from structural epidermal cells by the presence of

vacuoles or refractile bodies, and the fact that some epidermal cells in Nephtys are surrounded by pigment seems insufficient reason for regarding them as photoreceptors. Particularly in the small worms N. cornuta and N. parva, pigment granules are laid down along practically all the connective tissue of the body and the epidermal cells of the pygidial ring differ from the others only in that the pigment granules surrounding them are more numerous and more concentrated. On the other hand, all four species react by swimming or by a slight contraction of the longitudinal musculature if light is shone on the posterior segments; the middle of the worm appears to be insensitive to light. The same is true of N. caeca, N. cirrosa, N. hombergi and N. punctata. If there are any photoreceptors in the epidermis, they are cells of this type and are not specialised morphologically. It is possible, though I have not been able to observe it, that fibres from these cells run through the basement membrane of the epidermis to connect with a sub-epidermal nerve plexus.

Experimental Studies

If Nephtys is dropped into a pool of water in the sand, it swims for a short time and then begins to burrow. The prostomium is thrust against the substratum and the undulatory waves passing along the body vibrate the pro-

stomium in the sand and drive it forward. As soon as the anterior segments are buried, the locomotory activity changes to alternate contractions of the longitudinal and circular muscles and the worm burrows like an earthworm. Swimming is a necessary prelude to burrowing. Usually the worm buries itself at the first attempt, but if it does not do so, it remains inactive on the bottom of the pool for a time, perhaps a minute, and then swims and attempts to burrow again. The same pattern of behaviour is invariably followed: the worm swims at intervals and at the end of each swimming excursion attempts to burrow; the attempts are repeated until the worm does eventually bury itself. The experiments described below have been designed to provide at least a partial analysis of this behaviour on the supposition that one of the factors controlling it is the illumination of the worm, and to determine the functions of the photoreceptors.

Two reactions to illumination are found in the polychaetes; a synergic contraction of the longitudinal muscles mediated by giant axons, or locomotion. The former is characteristic of the tubicolous worms, the latter of the errant worms. These responses are not limited to those families conventionally regarded as 'sedentary' and 'errant' respectively, but the sudden contraction response is more marked in worms living in permanent or semi-

permanent burrows or tubes, and the locomotory response is more marked in worms which do not inhabit burrows. The phenomenon of contraction is the more spectacular and the more amenable to experimentation, and it has accordingly attracted more attention than the locomotory response. The literature on the responses of tubicolous worms to stimulation by light has been discussed and reviewed by Nicol (1948b, 1950). Among the polychaete families usually regarded as 'errant', the Nereidae is the only one in which the response to illumination has been studied in detail. Herter (1926) found that Nereis diversicolor is negatively phototactic. However, even the nereids usually live in consolidated burrows or secreted tubes of a more or less permanent character and they contract on a sudden change of light intensity, whether it be an increase or a decrease. Several polychaete families include species which are pelagic during the breeding season and numerous observations scattered in the literature suggest that these worms are positively phototactic at this time, at least, insofar as they are attracted to lights in the water. This represents a reversal of their usual photic response, but there appears to have been no systematic investigation into the precise mechanism of this behaviour.

Nephtys differs from those worms which have been studied in detail in this context, because although it

burrows in the sand, it does not form a consolidated, permanent burrow, and the sudden withdrawal reflex on stimulation is not found to any marked degree in the Nephtyidae. Both its habits under natural conditions and its behaviour in the laboratory suggest that it is much closer to the generalised 'errant' polychaete than any that has been studied before.

The experiments fall into two groups: first a series of investigations into the nature of the photonegative reactions of a single species, N. cirrosa, and second, an investigation of the function of the anterior prostomial and the ganglionic photoreceptors. Ideally the latter investigation should be carried out by removing one or other pair of eyes and observing the resulting changes of behaviour in the manner in which Herter (1926) experimented with Nereis diversicolor. This is impossible in Nephtys, for the single-celled receptors cannot be removed individually and could be extirpated only by doing considerable damage to the supra-oesophageal ganglion. Instead, species have been selected for these studies which possess one or other set of receptors, or both of them. In other words, by a fortunate interspecific variation of these eyes, the extirpation has already been accomplished by the investigator. For such experiments to be valid, it is necessary to assume that the brain structure is identical in all

species of Nephtys and that the only variable in these experiments is the presence or absence of eyes. Since it is impossible to follow the interneural connections within the supra-oesophageal ganglion, this assumption is gratuitous, even if, at first sight, reasonable in a group of closely related species of a single genus. Even on a cursory examination of the supra-oesophageal ganglion of a number of species of Nephtys, it is apparent that the minute structure varies considerably from species to species, particularly in the anterior part of the brain. In these circumstances, the results of the experiments must be interpreted with caution. A consideration of their validity will be deferred until the results are analysed and discussed. Because of sexual maturity there may well be changes in the behaviour of the worms when they are illuminated, only immature specimens have been used.

Experiments with Nephtys cirrosa

a. The light-dark choice experiment. When several worms are placed in a rectangular dish of water, one-half of which is illuminated and the other half covered with a black screen, they eventually move into the darker half. This is a surprisingly lengthy process for so active an animal as Nephtys, and it is evident that the number of worms in each half fluctuates because the worms re-enter the lighted half from the dark. If each worm is placed in a separate dish,

but otherwise under the same experimental conditions as before, the numbers returning to the light half are reduced because in this case quiescent worms in the dark half are not disturbed by active worms as they accumulate there. Even so, although worms spend more time in the dark compartment than before, they still reappear in the light; it is only exceptionally that they are all in the dark compartment at the same time and in no case do they remain permanently in the dark. The results of the two experiments are illustrated in fig.32. The results show that Nephtys is photonegative, though they provide no information about the mechanism of this process. Further, they show that under these experimental conditions, the orientation movement is imperfect because the worms do not stay in the dark when they reach it.

b. The effect of illumination on the activity of Nephtys. If Nephtys is placed in a jar of sea-water and is illuminated, it swims around the vessel for a short time at fairly regular intervals. Other forms of activity in these circumstances have been observed but rarely, and it is concluded that normally the worms will exhibit alternating periods of rest and activity. This periodic behaviour is discussed further in Appendix I, and it has been used to study the effect of illumination on the worm.

Illumination was provided by eight 60-watt 'Metrovik' electric light bulbs, rated at 250 volts and run at 245 volts from the public mains supply. The intensity was adjusted by varying the number of bulbs and the distance between them and the jars containing the worms. In this way the intensity could be varied without affecting the spectral quality of the light. During the period of the experiments the voltage of the mains supply varied by less than 3% which is insufficient to affect either intensity of spectral quality of the light appreciably. According to information supplied by the manufacturers, the spectral output of the bulbs is as shown in Table III.

TABLE III

Spectral output of the light source compared with natural daylight

Wavelength A.	Incandescent 60-watt Tungsten filament lamp. 2848°K.	Natural daylight 6500°K
3800-4200	0.0054	0.032
4200-4400	0.0585	0.26
4400-4600	0.249	0.83
4600-5100	5.39	10.65
5100-5600	33.52	41.8
5600-6100	42.68	35.8
6100-6600	16.5	9.9
6600-7600	1.52	0.68

A diffusing screen of a single sheet of tracing paper was interposed between the light and the worms. This affects slightly the spectral quality of the light falling upon them, but this has not been measured. The intensity of the light was measured with an Everett-Edgcombe photocell and meter and was adjusted in equal logarithmic steps between 0.45 and 14.4 candles per square foot. The worms were between 70 and 85 mm long and were collected at low tide. Each was immediately placed in a glass jar containing about 700 ml of fresh, aerated sea-water. The jars, with the worms, were kept in complete darkness for $2\frac{1}{2}$ hours until the experiment started. After this period of dark adaptation, the light was switched on and for the following two hours the activity of each worm was measured by counting the number of times it swam during that period. At the end of the experiment the water was changed in preparation for the next period of dark adaptation and observation. All the experiments were carried out on the same individuals and were completed within three days of collecting the worms. The experiments were carried out in a concrete floored room, free from vibration and sudden noise, with the water temperature constant at $14^{\circ}C$. The oxygen concentrations were measured by the Winckler technique and did not vary appreciably during the course of an experiment.

Observations were made on five worms simultaneously and the results are shown in fig.33. It will be seen

that there is a sigmoid relationship between the swimming activity of the worms and the logarithm of the light intensity, so that within limits, the activity is approximately a linear function of the logarithm of the light intensity, and outside these limits there is no change in activity with a change in light intensity. No lower limit of light intensity, below which the worms are inactive, has been found. Even in dim red light they are still active.

c. Orientation in a light beam. A single worm was placed in a large glass tank of sea-water, lined on three sides and the bottom with black matt paper to reduce light reflection to a minimum. The worm was dark adapted for half an hour and then illuminated from one end of the tank by a beam of parallel light from a 'Pointolite' microscope lamp and the behaviour of the worm was recorded. As soon as the worm reacted the light was extinguished and the worm left in darkness for a further period of half an hour. After eight or nine such tests the worm was discarded and a fresh one used. The light was always at the same end of the tank and always projected along the bottom of it. The direction at which the light beam impinged on the worm depended entirely upon the position taken up by the latter during the period of darkness. The conditions of the experiments are summarised in Table IV.

TABLE IV.

Direction of incident light beam	Worm A	B	C	D	E	F	Total exposures
Anterior	8	2	0	1	1	1	13
Posterior	2	1	1	2	4	2	12
Lateral	0	5	1	2	5	1	14
Post. diagonal	0	1	1	1	0	0	3
Ant. diagonal	0	0	0	0	0	0	0
Total exposures	10	9	3	6	10	4	42

With one exception, the reaction of the worm was always the same; it swam forward even on the occasions when this involved swimming directly up the light beam towards the light source. In the single exceptional case the worm failed to react at all. The results of these experiments clearly indicate that whatever the direction of the incident light beam, Nephtys cirrosa does not orient itself in it.

d. Inhibition of the photonegative reaction. Five worms were placed in jars of sea-water containing enough sand to cover the bottom to a depth of 2-3 mm; the sand was not uniformly distributed, but was heaped in some places to a depth of 5 mm. The worms were then exposed to light. Four of them, when they had burrowed as far as possible into

the sand, showed no further activity, although most of the body was still exposed to light. In one case, where the sand was not heaped up and therefore nowhere deep enough for effective burrowing, the worm was more active than the others. The same worms, under the same conditions, in jars without sand, were active during the whole period of two hours during which they were under observation. The results are summarised in Table V.

TABLE V

Number of swimming excursions during two hours exposure to light following two hours of dark adaptation.

Worm	With sand	Without sand
A	1	29
B	10*	37
C	1	47
D	1	65
E	3	29
Average	1.2	43

*Worm B was in a jar with insufficient sand for effective burrowing. The number of its swimming excursions has been omitted from the average figure.

Four experiments were then carried out to test whether this inhibition of the reaction was brought about by the

contact of the worms with the sand or by the obscuring of the light by it.

1. Four worms were placed in glass tubes 50 cm long and 4 mm internal diameter in a tank of water and then illuminated.

2. Six worms were provided with short lengths of glass tubing (15 mm long), placed in water and illuminated.

3. Six worms were placed between glass plates in a tank so that the dorsal and ventral surfaces of the worm were in contact with the plates, and then illuminated.

4. Six worms were provided with dorsal contact over a short length of the body by placing them under microscope slides raised on supports.

In the first three experiments all activity was inhibited while the worms were illuminated for a period of two hours, following two hours dark adaptation. The fourth experiment was inconclusive because the worms did not stay in position beneath the microscope slides for any length of time. It follows that if part, at least, of the dorsal and ventral surfaces of the body are in contact with some solid object, the reaction to illumination is inhibited.

In the light-dark choice experiment, it was observed that three worms were inactive and remained in the light so long as they were upside down, i.e. ventral side uppermost. When they were righted, they swam into the dark compartment

within a short time. In another experiment of this kind, the worms lying upside-down were left undisturbed and the time they took to right themselves and swim into the dark compartment was observed and compared with the time taken by worms lying ventral side downwards (Table VI).

TABLE VI

Time taken by worms to reach the dark compartment in the light-dark choice experiment.

Lying ventral side down	Lying ventral side up
0.5 mins	95 mins
0.5	117.5
2.0	225
	11.5
	15
Average: 1.0 mins.	92.8 mins.

In both these experiments the worms were in separate dishes, so that disturbance by active worms swimming into quiet ones did not affect results. Although the inhibition of the photonegative behaviour is only partial, and in any case Nephtys does not normally lie upside-down unless it is moribund, there is a suggestion in these results that dorsal contact alone is sufficient to inhibit the activity of the

worm in light and that ventral contact with the substratum, while usual, is superfluous. But the possibility remains that since the illumination was from above, the photo-receptors, which are nearer the dorsal than the ventral surface, were not adequately stimulated when the worm was upside-down and the light struck the ventral surface. A series of experiments was therefore carried out in which the worms lay on their ventral surface, but were illuminated from below. If it is true that the opacity of the body is sufficient to prevent adequate stimulation of the photo-receptors, the worms should remain inactive although the dorsal surface of the worm is not in contact with any solid object.

Single worms were placed in a glass tank of sea-water, half the bottom, the sides, and most of the top of which were covered with matt black paper to reduce reflection and to provide light and dark compartments when the tank was illuminated from below. The worms were left in darkness for half an hour and then the light below the tank was switched on and the time taken for the worms to reach the dark compartment was recorded. All the worms were ventral side downwards during the experiments. The worms reacted to illumination by swimming and in all but two cases reached the dark compartment in a single swimming excursion. The two exceptional cases represent occasions when two swimming

TABLE VII

Time, in seconds, taken for worms to reach the dark compartment when suddenly illuminated from below.

Worm	Test No. 1	2	3	4	5	Average
A	14	17	12	23	23	18 secs.
B	9	20	16			16
C	173*	17	295*	22		127
D	12					12
E	17	12	15	5	9	12
F	5	6	13	10		9
G	10	9				10

*Two swimming excursions before entering dark compartment.

excursions were necessary. Thus the direction of incident light is found to be irrelevant and the worms react as readily when they are illuminated from below as from above. This finding conforms with the possibility that dorsal contact alone is sufficient to inhibit the reaction to illumination. In addition, single-celled receptors, with a fine sensory hair projecting through the cuticle have been detected on the dorsal surface of the worm. There are others on the ventral surface, but those on the dorsum are more numerous. These are presumably contact receptors and thus the worm has the sensory equipment to detect contact between

its dorsal surface and the sand.

Experiments with Nephtys cornuta

Nephtys cornuta franciscana has the same complement of photoreceptors as N. cirrosa, that is, there are paired receptors in pigment cups embedded in the supra-oesophageal ganglion and a pair of non-vacuolated cells without pigment cups in the anterior part of the prostomium. However, it is a very small worm and the eyes lie close to the surface of the dorsum. In N. cirrosa, the eyes are so deeply embedded in the ganglion that it is difficult to imagine that any but very diffuse light reaches them at all. This being so, it is not surprising that although N. cirrosa has bilaterally symmetrical photoreceptors, which by virtue of their pigment cups are directional, it should not be able to orient itself in a light beam. In N. cornuta, on the other hand, the eyes are so close to the surface, that there is a likelihood that they are more perfectly functional. The following experiments were designed to test whether this is so or not.

Single worms were placed in a glass tank of sea-water and illuminated from one end of the tank by a parallel beam of light from a microscope lamp. The worms were dark adapted for half an hour before each test and some seventy tests were carried out on twelve specimens. As before, the direction of the incident beam depended upon the position

taken up by the worm between tests, but sufficient observations were made to cover all possible directions of the incident light relative to the worm. Two types of reaction were observed. The worms swam forwards, gradually turning into the beam until they were orientated longitudinally with respect to it, and then swam down-beam. This was most often observed when the incident light was lateral or posterior. Usually when the worms were facing the light when it was switched on, they swam once or twice in a tight circle in the beam and then swam down it until they reached the end of the tank furthest from the lamp. On eighteen occasions the worms failed to react at all, on eight the worms swam out of the beam and did not appear to orient themselves, while on two occasions, the worms circled and then swam into the beam towards the light. If the worms were dropped from a pipette into the light beam, they invariably oriented themselves in it and swam down-beam. All this is clear evidence that N. cornuta is able to orient itself in a light beam and suggests that N. cirrosa does not do so because the eyes are too deeply embedded in the ganglion to be completely functional.

Experiments with Nephtys californiensis and Nephtys punctata

Nephtys californiensis and N. punctata both differ from the two foregoing species in the structure of the eyes.

N. californiensis has both anterior prostomial and ganglionic eyes, but the former are vacuolated. N. punctata has ganglionic, but no anterior prostomial eyes. Both species when placed in a tank with light and dark compartments find their way into the dark compartment, but reappear periodically into the light, as N. cirrosa does under the same circumstances. Tests were made on six specimens of N. punctata and eight of N. californiensis to find if either worm was able to orient itself in a light beam. The experimental technique was the same as that described previously. In ten of the forty-five tests made on N. punctata, the worms failed to react when illuminated; on all the other occasions they swam forwards regardless of the direction of the incident light beam. The worms showed no sign whatever of orientation. The same results were obtained in 36 tests on N. californiensis; on three occasions the worms failed to react, on the rest they swam forwards and did not orientate themselves. Thus, although exhaustive tests have not been made, it is safe to conclude that the morphological differences between the eyes of N. cirrosa and N. californiensis do not affect their function and that even when the anterior eyes are missing, as they are in N. punctata, the behaviour is not altered.

Conclusions

From the foregoing experiments, the following preliminary conclusions can be drawn about the function of the eyes:

1. No differences in behaviour can be detected between species with vacuolated and non-vacuolated prostomial photoreceptors (cf. N. cirrosa with N. californiensis), nor between species with anterior receptors of either type and those lacking them altogether (cf. N. californiensis and N. cirrosa with N. punctata).

2. The photoreceptors of the supra-oesophageal ganglion, although theoretically adequate to permit the animal to orient itself in a light beam, are apparently too deeply embedded in the brain for this in most species. When the ganglionic photoreceptors are close to the surface, however, the worm can orientate itself (cf. N. cornuta with N. californiensis, N. cirrosa and N. punctata).

3. Even when deeply embedded in the supra-oesophageal ganglion, the posterior eyes apparently receive sufficient stimulation by diffuse light for the animal to perform kinetic orientation movements (cf. N. punctata with N. californiensis and N. cirrosa).

4. No experiments have been performed on a species of Nephtys which possesses anterior eyes, but no ganglionic eyes, nor is it known certainly if any such species exists. Thus

it is not clearly established whether the anterior prostomial receptors are functional or not. There are two possibilities. Either the anterior receptors are completely non-functional and the stimulation of the ganglionic receptors by diffuse light evokes kinetic movements in the worm, or else they are functional and are involved in the kinetic movements, but they can be functionally augmented or even replaced by the posterior eyes.

Discussion

The photoreceptors of the brain and the prostomium of Nephtys resemble those found in other annelids. The photoreceptors in the epidermis of Lumbricus consist of single, vacuolated sensory cells without pigment cups (Hess, 1925), while in Stylaria there are several such cells in a group on either side of the prostomium, each partly invested by small pigment cells (Hesse, 1902). The unit photoreceptor of leeches also consists of cells of this type, though usually several are grouped within the same pigment cup (Scriban & Autrum, 1932-4). Polychaete eyes are frequently more complicated and may include more than one type of cell. However, the lateral ocelli of Polyophthalmus (Opheliidae) take the form of single, vacuolated cells of the type found in Nephtys, but with digital processes projecting into the vacuole (Hesse, 1896).

Judged by their disposition and their relationship with groups of ganglion cells in the brain, the eyes of Nephtys are homologous with those of Nereis. The sensory axons of the posterior receptors of Nephtys enter the neuropile immediately anterior to the antero-lateral groups of ganglion cells (figs.16 and 30), exactly as the posterior optic nerves do in Nereis (Scharrer, 1936, and personal observation). These ganglion cells are homologous in the two worms and are both important neurosecretory centres. The anterior eyes are less certainly homologous. The anterior part of the supra-oesophageal ganglion of Nephtys is more variable in its minute structure than the posterior part, and there are no conspicuous or constant groups of ganglion cells which can be used as landmarks. The anterior optic nerves of Nereis enter the brain between the two roots of the circum-oesophageal connectives; in Nephtys the sensory axons enter the neuropile lateral to the connective roots, though immediately beside them (fig.31). It is impossible to say how important this difference is, but the receptors themselves are in almost the same position in the two worms.

The suggestion that the eyes of the two worms are homologous is strengthened by a comparison of the experiments of Herter (1926) and Ameln (1930) on the photonegative behaviour of Nereis diversicolor, with those on Nephtys.

Herter showed, as a result of extirpation experiments, that Nereis was able to orient itself in a light beam, i.e. exhibit a phototaxis, only if the posterior eyes were intact. If they are removed the worm exhibits only a photokinesis, despite the bilateral and directional arrangement of the anterior eyes. Since the sensory data provided by the anterior eyes is theoretically sufficient to permit the worm to orient itself in a light beam, its inability to do so is interpreted as meaning that the deficiency lies in the central nervous system. The anterior eyes of Nephtys are not provided with pigment cups and are not directional. The posterior eyes are both, but the worm exhibits a phototaxis only if they are close to the surface, as they are in N. cornuta. It is likely that the brains and photoreceptors of Nereis and Nephtys are essentially the same as far as the photonegative behaviour is concerned, save for two things. The anterior eyes of Nereis are directional although the sensory data they provide is not used, whereas in Nephtys they are not. The posterior eyes of Nephtys, although directional receptors, are, in most species too deeply embedded in the ganglion to function as such.

This conclusion is based on the assumption that the structure of the supra-oesophageal ganglion is essentially the same in all species of Nephtys. This is not strictly true, but the arrangement of the ganglion cells in the

posterior part of the brain with which we are chiefly concerned, is the one constant feature of the ganglion. The conclusions are not proven, but are a reasonable interpretation consistent with the facts at our disposal.

Fraenkel and Gunn (1940) defined an orthokinesis as a dependence of the average linear velocity of an animal upon the intensity of the stimulus. Within limits, the frequency of swimming of Nephtys cirrosa is a linear function of the light intensity (fig. 33). Since the duration of swimming excursions and the speed of swimming are approximately constant whatever the light intensity (see Appendix I), the conditions for an orthokinesis are apparently satisfied. But the worm does not swim faster in brighter light as the definition suggests, it merely swims more often, and, under natural conditions, it does not escape from the light by moving into a shadow but by burrowing out of it. Each attempt at burrowing is preceded by swimming and the first attempt may be unsuccessful, so the more frequently it swims, the sooner it is buried. The orientation movement is therefore not orthokinetic in the proper sense of the term, but is some other kind of kinetic movement. Klinokinesis, such as Ullyott (1936) described in the triclad Dendrocoelum, has never been observed to form a part of the behaviour of Nephtys.

A discussion of the biological significance of this

behaviour is hampered by an almost total lack of knowledge of the habits of Nephtys. The animals lie half buried in the sand when covered by water and may leave it temporarily to seize their food, but they must bury themselves again immediately. The stimulus initiating the last behaviour is either the exposure of the worms to light, or,more likely since they are active even in the dark if unburied, the absence of contact between the sand and the dorsum of the worm. But the time it takes to get buried depends upon the light intensity. Even so, it does not seem possible that the photonegative response can play a very important role in the normal behaviour of the worm, particularly as the photoreceptors are so primitive. Why the posterior eyes should be directional when the worm does not use them, can be explained if it is assumed that the larger species have evolved from smaller ones in which much less tissue covered the receptors. They would then have been in much the same situation as N. cornuta.

CAPTIONS TO FIGURES

Figure 30. A. Frontal section through the supra-oesophageal ganglion of *N. californiensis* showing the position of the posterior eyes.

B. Sagittal section through a single element of the photoreceptors of *N. californiensis*.

C. Transverse section through the photoreceptors of *N. ferruginea*.

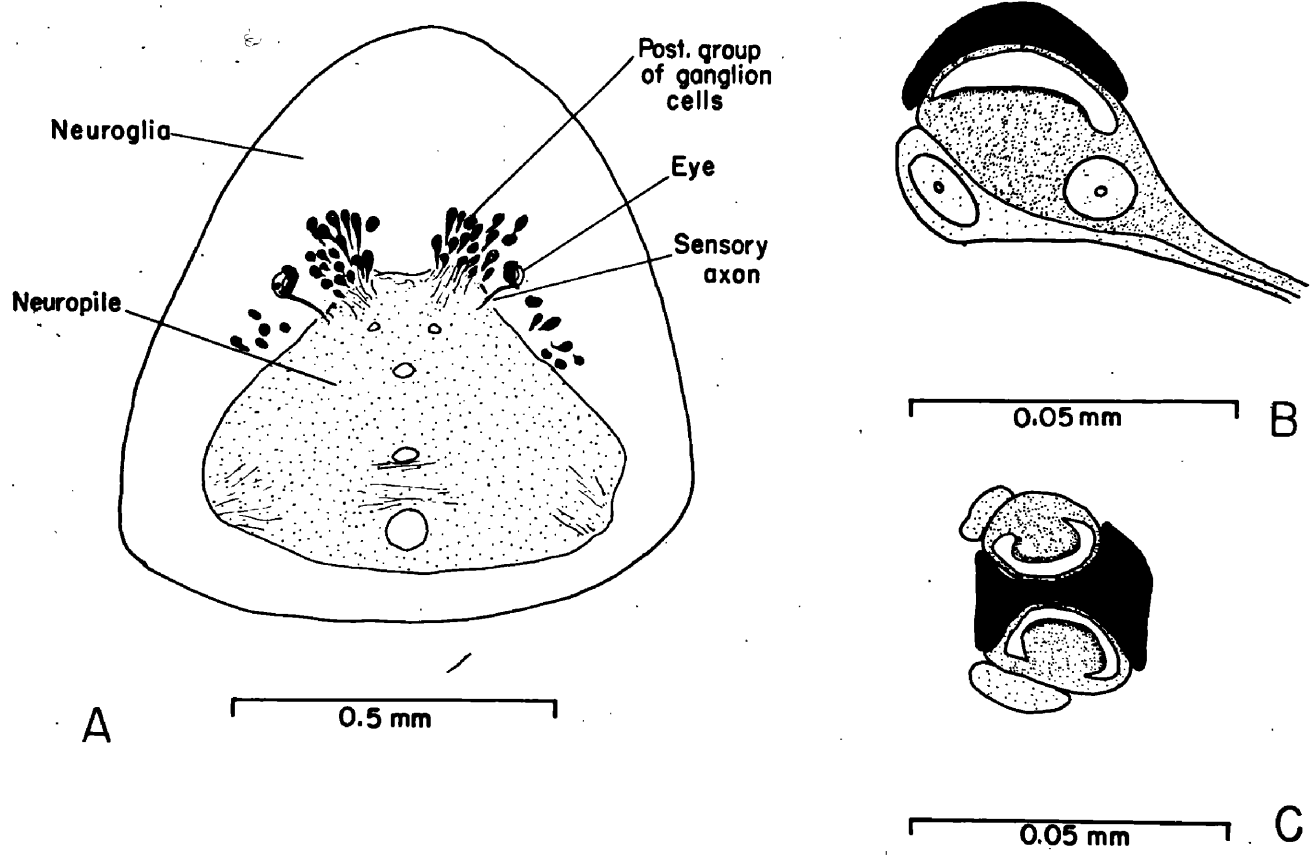
Figure 31. A. Frontal section through the prostomium and anterior segments of *N. cirrosa* showing the position of the anterior eyes.

B. Sagittal section through a single element of the anterior photoreceptors of *N. californiensis*.

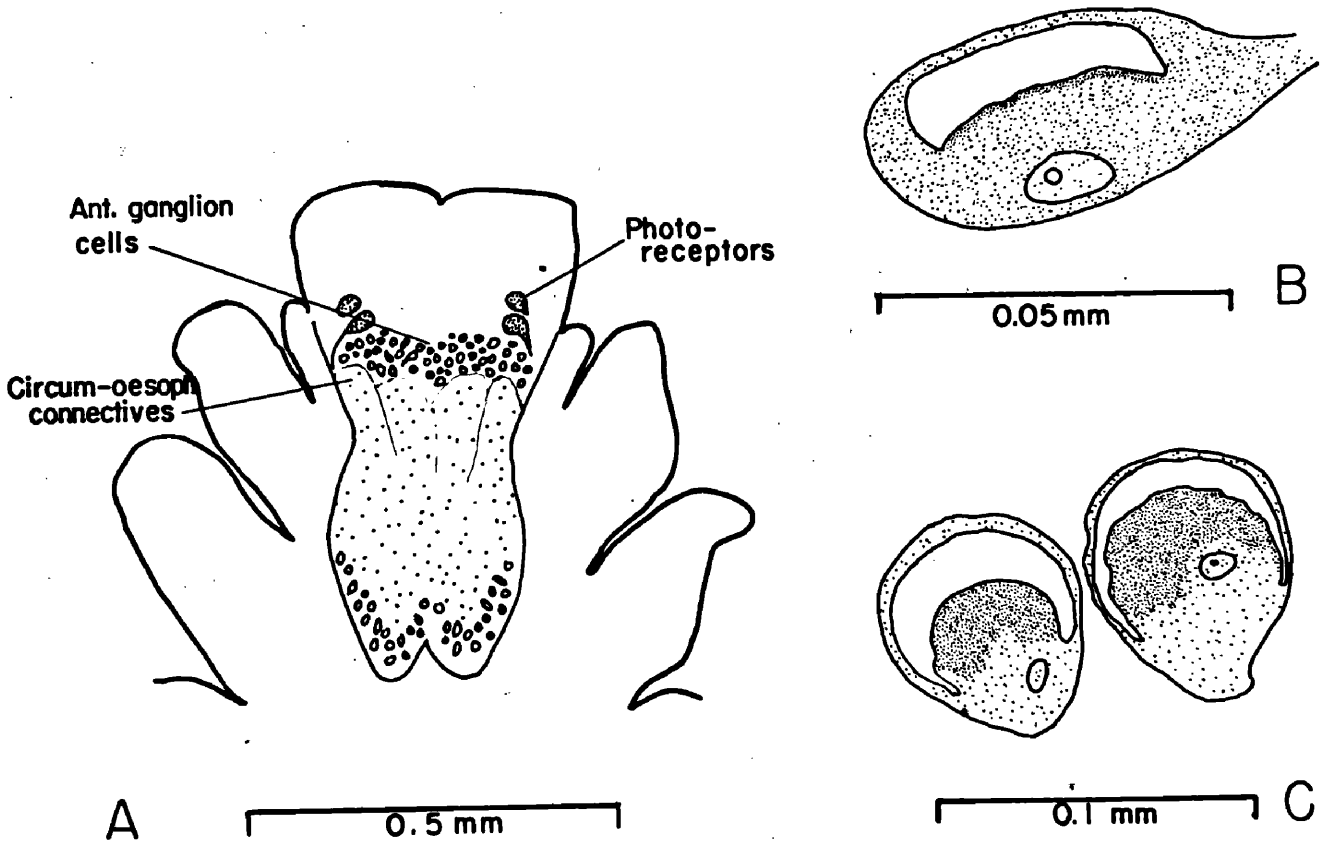
C. Transverse section through the anterior eyes of *N. californiensis*.

Figure 32. Rate of movement of *N. cirrosa* from the light compartment to the dark in a light-dark choice experiment. Upper line, single worms; lower line, groups of worms in each experiment.

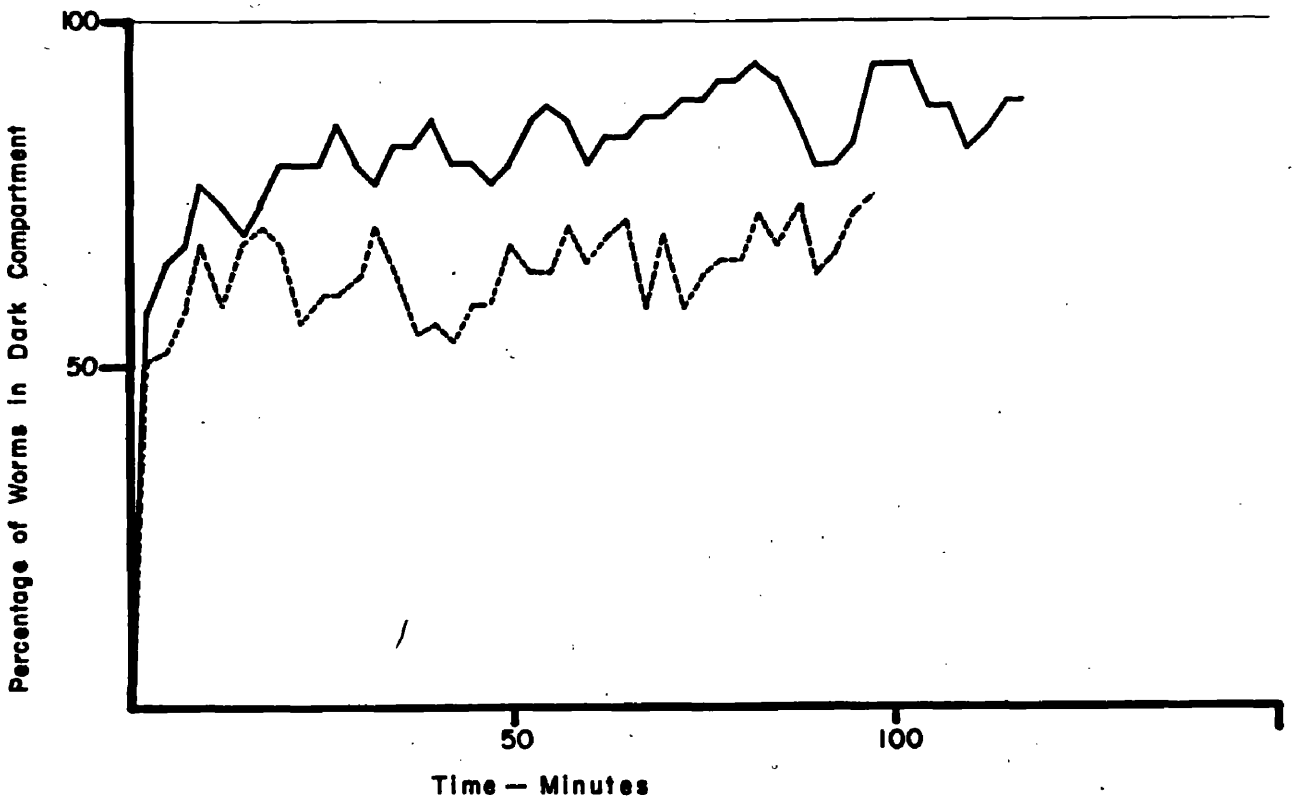
Figure 33. Number of swimming excursions of *N. cirrosa* in two hours at various light intensities, following two hours' dark adaptation.


Post. group of ganglion cells
Neuroglia
Eye
Sensory axon
Neuropile
A
0.5 mm
0.05 mm
B
0.05 mm
C

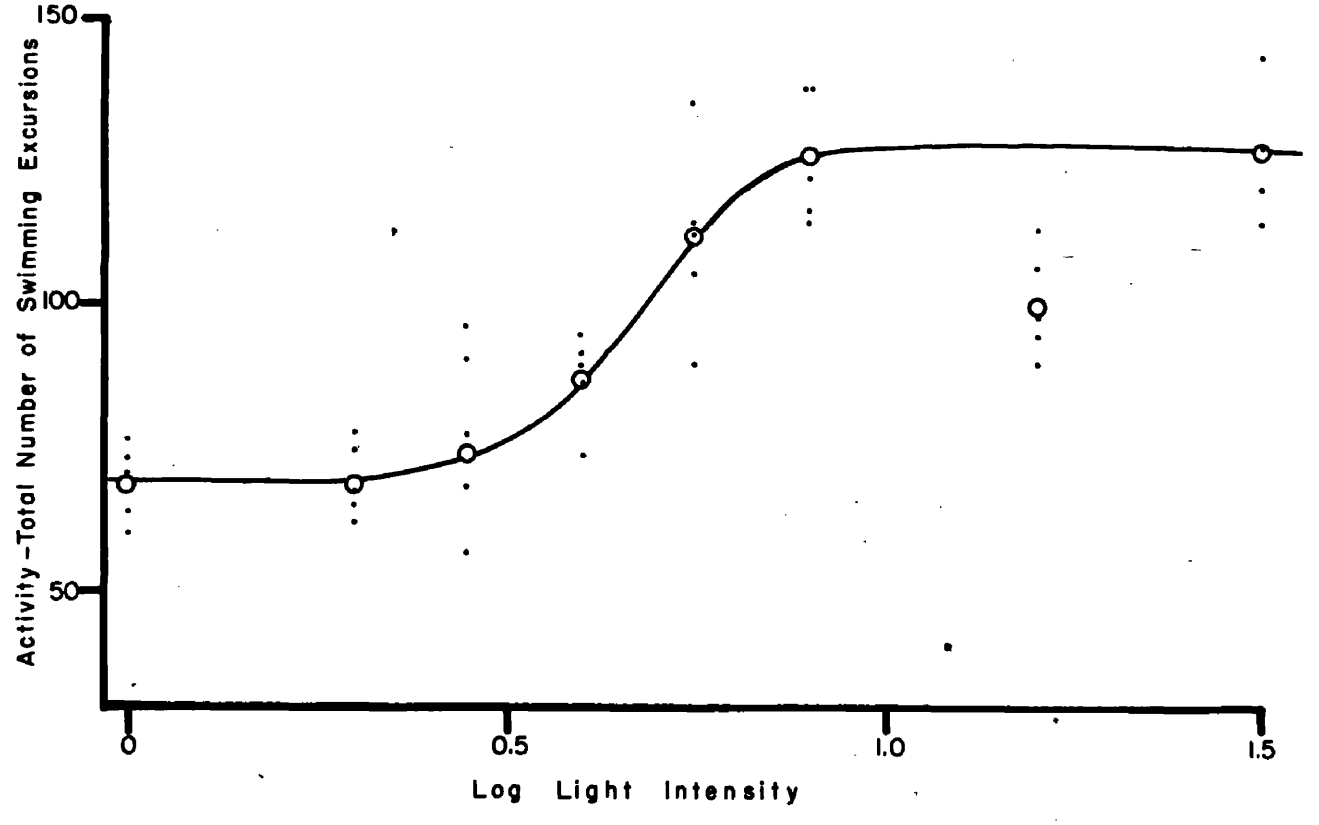
30


Ant. ganglion cells
Photo-receptors
Circum-oesoph connectives
A
0.5 mm
0.05 mm
B
0.1 mm
C

31


100
50
Percentage of Worms in Dark Compartment
50
100
Time — Minutes

32


150
100
50
Activity-Total Number of Swimming Excursions
0
0.5
1.0
1.5
Log Light Intensity

33

THE NEUROSECRETORY SYSTEM

THE NEUROSECRETORY SYSTEM OF THE SUPRA-OESOPHAGEAL GANGLION

Neurosecretory cells have been discovered in the central nervous system of most major groups of triploblastic animals and in many cases their endocrine function has been demonstrated. Among the worms, neurosecretory cells have been found in the polyclad turbellarians (Turner, 1946), sipunculids (Gabe, 1953a) and the three main classes of annelids (Scharrer, 1936, 1937; Scharrer & Scharrer, 1937, 1945; Harms, 1948; Hubl, 1953; Herlant-Meewis, 1955). It is likely that most if not all polychaetes have neurosecretory cells in the central nervous system, but so far they have been described only in the following: Aphrodite aculeata, Lepidonotus squamatus (Scharrer & Scharrer, 1937), several nereids (Scharrer, 1937; Schaefer, 1939; Bobin & Durchon, 1953; Defretin, 1955) and in three sabellids, the serpulid Apomatos similis, the terebellid Lanice conchilega, and in Arenicola marina (Arvy, 1954). Most of the detailed morphological, cytochemical and physiological studies have centred on the nereids; in the remaining polychaetes little more is known than that neurosecretory cells exist in the central nervous system. However, from the studies that have been made on other organisms, it is becoming increasingly apparent that neurosecretory cells play an important role as incretory organs

(see recent reviews by Gabe, 1954; Scharrer & Scharrer, 1954a, 1954b; Scharrer, 1955) and it is evident from the work of Durchon (1952) that this is also the case in polychaetes.

Durchon (op.cit.) has produced evidence that a humoural factor originating in the supra-oesophageal ganglion of nereids inhibits epitoky and the maturation of the male gametes. While conclusive evidence is lacking, it is likely that this hormone is produced by neurosecretory cells in the ganglion and is released into the blood vessels on the ventral surface of the brain (Bobin & Durchon, 1952). Defretin (1955) has recently offered an alternative interpretation of the blood plexus beneath the ganglion and so the method by which the hormones are released into the body remains a matter for discussion. A second group of neurosecretory cells exists in the epidermal nucleus of the brain of Nereis (Bobin & Durchon, 1953) and the secretion is transported along the axons forming the epidermal nerve to a site in the epidermis of the posterior and dorsolateral walls of the prostomium. It is not known if this secretion has any biological activity, but it is evidently related to reproduction because the cells of this group produce a detectable secretion only when the worm is mature and in the process of becoming epitokous. Other hormones may be produced in the supra-oesophageal ganglion of nereids

and Harms (1948) has suggested that a 'growth and differentiation' hormone is secreted by certain neurosecretory cells in the brain of the fresh-water nereids of the genus Lycastis.

The earlier authors (Scharrer, 1936, 1937; Schaefer, 1939) described four types of neurosecretory cells in the brain of Nereis. These are: a) cells with a homogeneous, strongly acidophilic cytoplasm, b) fusiform cells near the posterior optic nerves which have a reticulate cytoplasm containing fuchsinophilic droplets, c) large, round cells containing a fine, granular secretion in vacuoles, and, d) large cells in which the cytoplasm is reduced to fine strands crossing an enormous vacuole which contains drops or else a mass of fuchsinophilic, colloidal material. It is now generally accepted that cell types c and d represent different stages in the secretory cycle of the same type of cell (Gabe, 1954). The secretion of all these types of cell is PAS-positive and can be stained with paraldehyde fuchsin, but c and d cells are not stained by acid chrome-haematoxylin, though the others are (Gabe, 1954). Defretin (1953, 1955) has studied the histochemistry of these cells, and in particular the distribution of polysaccharides in them, and the relation between the polysaccharides and the neurosecretory material. He has produced evidence that there is a considerable quantity of PAS-positive material in neurosecretory cells in the epidermal nucleus of the brain

of Nereis during the period when no neurosecretory activity can be detected in them, and that this is gradually depleted and finally disappears as the neurosecretory granules are formed and leave the cell body. It may well be that the polysaccharides are precursors or constituents of some fraction of the neurosecretory substance, though Defretin did not study the composition of the secreted granules and could offer no more than suggestions on this point.

From this work on the nereids, we have some knowledge of the endocrine function of the brain, descriptions of the various types of neurosecretory cell in it, and a certain amount of information about the histochemistry of the cells. Unfortunately, we have no knowledge of the function of the one group of neurosecretory cells, i.e. those of the epidermal nucleus, in which the fate of the secretion is known, and it is not known which neurosecretory cells produce the hormones responsible for the control of sexual maturity and epitoky. In spite of these lacunae, comparable studies have been carried out on no other polychaete.

The Types of Neurosecretory Cell in the Brain of Nephtys

Three types of neurosecretory cell have been distinguished in the supra-oesophageal ganglion of Nephtys.

Their secretory cycles which are described below are deduced from the different appearances of cells of the same type in various parts of the brain or in different specimens. There is naturally no proof that the cycle follows the same sequence as that proposed.

Type A cells. In N. californiensis there are generally four large neurosecretory cells in the posterior part of the brain (fig.34), though they have not been identified in all specimens. In one specimen of this species, one of the cells had been replaced by two smaller ones, making five in all. The other species possess only one pair of these cells, but they occur in the same position and have the same staining properties as well as having the same appearance. The cells are oval, slightly irregular in outline, about 25-30 μ long and 15 μ in diameter*, with a nearly spherical nucleus 7-10 μ across which contains a conspicuous nucleolus at all stages in the secretory cycle. The nucleus has a few coarse granulations and is chromophobic. The cytoplasm of inactive cells is also unstained and this fact, rather than the actual absence of the cell, may account for their not having been detected in some specimens. Some cells of this type show no signs of secretory activity but have a few small vacuola-

* The dimensions of these and other cells given in this section refer to full-grown N. californiensis, one of the largest species. In small species the dimensions may be halved.

tions near the axon hillock; these appear to bear no relation to the neurosecretory material itself. In some examples of these cells there are small patches of acidophilic material in the cytoplasm around the periphery of the cell. This appears to spread until the entire cytoplasm is acidophilic and contains a large, spherical mass of very finely granular material at the proximal end of the cell (figs. 35 and 37B).

In all but a very few of the specimens examined, the cells of this type contain a great mass of secretion. The secretion is stained by neither paraldehyde fuchsin nor acid chrome-haematoxylin and contains no detectable quantity of lipids or glucids; evidently it consists mainly of proteins. The axons of these cells probably run directly into the neuropile since the cell-bodies lie immediately posterior to it and the axons run in an anterior direction from them. The secretion has never been seen in the axons, though unless it was in the immediate vicinity of the cell body it would be extremely difficult to detect. Since virtually all the worms contain these cells fully charged with secretion, there is no clue as to their function.

Type B cells. In all species of Nephtys, the great majority of neurosecretory cells in the supra-oesophageal ganglion are pyriform, about 25 μ long and 15 μ wide, with a nucleus

about 10 μ in diameter. They occur principally at the posterior corners of the neuropile where they form two conspicuous groups (fig.34). They are also found along the sides of the brain and in the dorsal anterior region immediately above the origin of the fibres which emerge from the neuropile to form the circum-oesophageal connectives. The axons of most of these cells run directly into the neuropile where they can be traced no further, but it is possible that some of those in the anterior dorsal group run directly into the connectives. The secretory cycle appears to be as follows: in some cells, granules staining with paraldehyde fuchsin can be seen in the cytoplasm of the cell, which at this stage is not vacuolated (fig.37C). In other cells there are more granules, and vacuoles in the cytoplasm in which the granules accumulate (fig.37D). Finally, in other cells, the nucleus is extremely irregular in outline and the cytoplasm is reduced to a small patch around it and connecting it to the cell walls. Most of the cell then consists of an enormous vacuole containing fuchsinophil granules 1-2 μ in diameter. These granules can be seen in the axons in the immediate vicinity of the cell bodies (fig.36).

The secretion is PAS-positive and figure 40 shows the distribution of glucids, other than glycogen, in type B cells in early and late stages of the proposed secretory cycle.

It will be observed that they correspond exactly with the distribution of fuchsinophil granules (cf. fig.36). There is also a lipoidal component of the secretion (fig.38). The distribution of osmiophil lipids is the same as that of fuchsinophil granules in the early stages of the secretory cycle, but they can hardly be detected in cells presenting a highly vacuolated appearance characteristic of late stages in the secretory cycle. Ciaccio-positive lipids, on the other hand, correspond with the distribution of the fuchsinophil granules only in late stages of the secretory cycle and are not to be seen in cells at the beginning of it. However, although the distribution of Ciaccio-positive lipids corresponds with the distribution of fuchsinophil granules towards the end of the secretory cycle (fig.42C), they by no means fill the vacuoles which must also contain a further substance that is lost during the fixation or embedding of the material.

Type C cells. The cell bodies of the third type of neurosecretory cells are among the largest of any neurones in the brain. They are ovoid or nearly spherical and may be 50 μ in diameter. The nuclei are chromophobic, ellipsoidal and about 12 μ long, and they always contain a prominent nucleolus. Cells of this type are found in the ventral part of the brain flanking the cone of fibres running to the cerebrovascular complex (see below), in the sides of the brain, and

in the antero-dorsal region where they occur with the type B cells that are also there (fig.34). It is difficult to trace the paths of the axons of these cells in all cases, but those along the sides of the brain, and probably most of the others, run into the central neuropile. The axons of the antero-dorsal cells may run into the connectives, while those in the ventral part of the brain may run into the cone of fibres and not dorsally into the neuropile.

Fuchsinophil granules can be seen in an area of basophilic cytoplasm in some cells (fig.37E), in others they collect in numerous small vacuoles that appear around the periphery of the cell (fig.36). At this stage the outline of the cell is highly irregular and it presents a blistered appearance. These vacuoles appear to coalesce to form a single large one, often, though not invariably, lying in the axon hillock (fig.37G). As in type B cells, osmiophil lipids, the distribution of which corresponds exactly with the distribution of the fuchsinophil granules, are demonstrable in cells with no, or very few, vacuolations, but not in those at later stages in the secretory cycle. Ciaccio-positive lipids, on the other hand, occur only towards the end of the secretory cycle. They can be demonstrated around the periphery of cells showing the characteristic blistered outline, and in a few large masses in cells which are apparently approaching the end of their secretory

cycle (figs.39, 42A and B). The vacuoles that appear in these cells in ordinary paraffin sections are accounted for by these lipids. They have never been demonstrated in cells which are supposed to be at the beginning of their cycle. Of course, cells in which there are no detectable Ciaccio-positive lipids may be completely inactive, but the fact that a correspondence between these lipids and fuchsinophil granules at the start of the cycle has never been seen, argues strongly that the lipid component of the secretion undergoes modification as the cycle proceeds.

The distribution of glucids in type C cells, as in type B cells, corresponds with the distribution of fuchsinophil granules (figs.36 and 40). Defretin (1955) made the interesting discovery that glucids present in neurosecretory cells of the epidermal nucleus of Nereis were depleted as the cell began active secretion, suggesting that the glucids were precursors of the neurosecretory material. No such inverse correlation between the glucids and the secretion has been found in Nephtys. Defretin unfortunately did not distinguish glycogen from other 1.2.glycols. Glycogen is present in practically all neurones and cannot be regarded as a neurosecretory product, nor as a substance peculiarly related to the neurosecretory material. Its distribution in type B and C cells of Nephtys is much the same as the distribution of glucids as shown by Defretin (cf. fig.41

with Defretin's figures) but the neurosecretory products of B and C cells in Nephtys include other 1.2.glycols. It is therefore impossible to evaluate Defretin's observations on this point.

The cell types B and C obviously have much in common, and both differ markedly from cell type A. Their staining reactions are the same and no great differences between them have been detected by the few histochemical tests that have been applied (see Table VIII). Both types of cell, when stained by conventional techniques, show numerous vacuolations. This is a common feature of neurosecretory cells. The entire content of these vacuoles in type C cells can be conserved and stained by Ciaccio's method, but not in type B cells (cf. figs. 38 and 39). In the latter, either there is some fraction of the neurosecretory product which has not been conserved, or else the droplets are lying in an unstainable material in the vacuoles, and the former conclusion is more likely to be true. Apart from the differences in general appearance, this represents the sole difference between B and C cells to be detected.

TABLE VIII

Staining reactions of the three types of neurosecretory cell.

	A	B	C
Paraldehyde fuchsin	-	+	+
Acid chrome-haematoxylin	-	+	+
Phloxin	+	-	-
Azan	red	mauve	mauve
PAS	-	+	+
Osmiophil lipids	-	+	+
Ciaccio-positive lipids	-	+	+

The Cerebro-Vascular Complex

At the base of the supra-oesophageal ganglion, there is a modified axonal tract, specialised areas of the membrane investing the brain, and a close association between the brain and the blood vascular system (fig.43). These structures correspond closely with those in nereids, for which Bobin & Durchon (1952) coined the name 'cerebro-vascular complex'. The same structure is found in all species of Nephtys, though with some variations in the details of its morphology.

In N. californiensis, a species in which the structure of the cerebro-vascular complex can be seen to best

advantage, a tract of axons emerges from the base of the neuropile and runs, diverging to form a cone-shaped tract, to the base of the ganglion (fig.45A). It has a single, if somewhat diffuse, origin in the neuropile. The axons run in a tangled mass to the connective sheath and are flanked by dense neuroglial fibres which penetrate into the cone of axons.

The ganglion is invested, at least laterally and ventrally, by a double sheath, an inner laminated connective tissue, which is continuous with the basement membrane of the epidermis, and an outer, extremely thin cellular layer. Around the sides of the brain the latter can be detected by occasional nuclei flattened against the connective tissue sheath and elsewhere only under optimal optical conditions, and not always even then. Both the connective tissue sheath and its investing membrane are modified over the region where the axon tract meets the base of the ganglion. The sheath is in most places no more than 2 μ thick, but over the mid-ventral part of the brain its thickness increases to 8 or 10 μ. It is similarly thickened in the mid-ventral line anterior to the cerebro-vascular complex, but this seems to be a separate, special adaptation for the attachment of the diagonal muscles of the prostomium. In the region of the cerebro-vascular complex, the connective tissue sheath is usually drawn up to a variable extent into the brain,

leaving a 'v' shaped depression running along the base of the ganglion in which the dorsal blood vessel lies. The cellular pericapsular membrane is also modified in this area. It is thickened and thrown into folds and ridges which may be 8 or 10 μ in height. The membrane does not show the papilla-like formations such as Bobin & Durchon (1952) described in Perinereis, nor does the membrane become thickened in regions other than the cerebro-vascular complex.

The dorsal longitudinal blood vessel runs along the ventral surface of the supra-oesophageal ganglion and is suspended from it by two fine extensions of the ganglionic membranes. The outer and middle layers of the blood vessel walls are continuous with the two membranes investing the brain. At the posterior margin of the ganglion the dorsal vessel bifurcates and the two branches run parallel courses along the base of the brain to its anterior margin where they separate and follow the circum-oesophageal connectives to the sub-oesophageal ganglion to form the paired neural vessels (see Appendix II). There are frequent anastomoses between the two branches of the dorsal blood vessel where they cross the cerebro-vascular complex, and they form a blood plexus immediately beneath the modified ganglionic membranes and the ends of the axons.

The essential features of the cerebro-vascular complex are to be found in the supra-oesophageal ganglion of all the

species of Nephtys that I have examined, but there are some variations in details of the structure (fig.44). The axon tract emerges from the central neuropile in the mid-brain of N. californiensis, but in N. ferruginea, N. incisa and N. punctata it is much more restricted in area and emerges from the extreme posterior part of the neuropile from among the axons of the postero-lateral groups of neurosecretory cells (composed principally of type B). In all the species except N. cornuta, the tract of axons is flanked by neuroglial fibres running parallel to the axons. In most species there is a certain amount of neuroglia which penetrates into the axon tract as well. Thus, in N. californiensis, N. picta and N. punctata, the degree of penetration is slight, but in N. caeca and N. cirrosa there is more neuroglia than axons, while in N. incisa the neuroglial fibres are so numerous that it is difficult to detect axons in the tract. The brain of N. cornuta is very small (the adult worm is only 5 mm long) and most of it is taken up with neuropile and neurones; there is very little neuroglia at all. The neuropile occupies a disproportionately large part of the brain and is close to the ventral surface of the ganglion. Consequently the axon tract is very short and rather indistinct, and it is not flanked by neuroglial fibres as it is in other species.

The modification of the ganglionic membranes is also variable. The connective tissue sheath is always thickened in the region of the cerebro-vascular complex, though in most species not to as great an extent as in N. californiensis. The thickening of the pericapsular membrane is also conspicuous in N. caeca and N. picta, but it is slight and difficult to detect in N. cirrosa.

The bifurcation of the dorsal longitudinal blood vessel may take place anywhere along the length of the ganglion. In N. californiensis the bifurcation is at the posterior end of the brain and the two branches run parallel courses along the base of the brain and a plexus is formed between them. This is not typical of Nephtys, for in most species the bifurcation is anterior to the cerebro-vascular complex, and there are no anastomoses between the blood vessels. In this case the single blood vessel may be flattened against the modified pericapsular membrane, as in N. picta, or to a lesser extent, in N. incisa. More commonly the longitudinal groove in the base of the brain is very deep in the region of the cerebro-vascular complex and the blood vessel is drawn into it. The result is always the same, a considerable area of the dorsal longitudinal blood vessel is apposed to the modified ganglionic membranes, by the formation of a plexus, by the flattening of the blood vessel, or by deepending the ventral groove on the brain and drawing the

blood vessel into it (fig.44). The situation in N. incisa is a little different. The dorsal vessel is neither markedly enlarged nor flattened, nor is there a pronounced ventral groove. However, the modified pericapsular membrane completely fills the space between the base of the brain and the blood vessel.

The Fate of the Secretion

The axons of the neurosecretory cells in the brain of Nephtys run into the central neuropile, into the circum-oesophageal connectives, and possibly also directly into the ventral cone of axons which forms part of the cerebro-vascular complex. Granules of secreted material have been observed in the axons near the cell bodies, on rare occasions in axons running to the neuropile, and frequently odd granules of material which stains in the same way as the neurosecretory product can be seen in the neuropile itself. The transport of neurosecretory material in the axoplasm has been observed in several animals other than polychaetes, both directly (Carlisle, 1953; Passano, 1953) and indirectly (e.g. Scharrer & Scharrer, 1954a). The granules have been traced from the cell body to their probable release site in only one case in a polychaete, and that is in the epidermal nerve of Perinereis cultrifera (Bobin & Durchon, 1953). It is not possible to do this in the brain of Nephtys. There

are signs that the secretion is transmitted in the axons in the case of type B and C cells, though not of type A cells, but the neural pathways of the secretion can only be inferred. There are no highly distinctive axonal tracts from the neurosecretory cells to the release site, as there are in more highly organised and specialised brains and nearly all axons run directly into the neuropile where they cannot be traced, and only a few cells in any brain are actively secreting at the same time so that the quantity of secretion in axons can never be great.

If the transport of neurosecretory material in Nephtys is axonal, and the axons of nearly all neurosecretory cells run into the neuropile, the eventual pathway of the secretion must be sought in the bundles of axons which emerge from it. Several nerves run between the neuropile and the anterior part of the prostomium and the antennae. They are most probably sensory nerves for the most part; they are all inconsiderable, and neurosecretory material has never been detected in them. (The fact that they are probably sensory nerves does not preclude their containing neurosecretory axons. Bobin & Durchon (1953) demonstrated that the epidermal nerve of Perinereis cultrifera, which was considered to be primarily sensory, serves as a pathway for neurosecretory material.) By far the greater number of axons emerging from the neuropile leave the brain by way of the

circum-oesophageal connectives and occasional masses of fuchsinophil material, similar to that found in type B and C cells, have been observed in them. Arvy (1954) has described neurosecretory material which originates in the supra-oesophageal ganglion of Lanice conchilega in the circum-oesophageal connectives. In neither Nephtys nor Lanice is it known what subsequently happens to this material. The third group of axons emerging from the neuropile are those in the ventral cone which run to the ventral surface of the brain where they are in intimate contact with the blood vessels.

Neurosecretory material is not usually demonstrable in the axons of the ventral cone, just as it is difficult to demonstrate it in other parts of the brain. However, granules strung out along the axons have been seen in one specimen of N. incisa (fig.45D). But it can almost always be demonstrated at the end of the axon tract, immediately above the thickened pericapsular sheath (figs.45A and C). The material accumulated in this position can be stained with paraldehyde fuchsin, acid chrome-haematoxylin; it is PAS-positive and contains Ciaccio-positive lipids. Evidently it is the same as the material which originates in B and C cells. The same material can be seen in the modified pericapsular membrane and around the blood vessels immediately beneath the axon cone (fig.45B). Granules of the secretion

can be seen in the lumen of the blood vessels and also in amoebocytes in the blood. Granules in the blood vessels have been seen only rarely, and so far only in preparations stained with paraldehyde fuchsin. Numerous substances can be stained thus, so that a positive staining reaction cannot be regarded as conclusive proof that these granules are neurosecretory. However, since they have been seen in blood vessels immediately opposite the cone of axons and the modified membranes, all of which contain masses of the neurosecretory material, it is reasonable to assume, for the present, that the granules in the blood stream are also of neurosecretory origin.

The Function of the Secretion

Experimental studies of the function of the neurosecretory cells in the supra-oesophageal ganglion of Nephtys are still in their early stages, so that no more than very tentative conclusions can be drawn from them. As far as they go, the results of them suggest that Durchon's (1952) analysis of the role of cerebral hormones in the reproduction of nereids will be found to apply equally to Nephtys. Removal of the brain of Perinereis results in the precocious epitoky of the worm, and Durchon was able to prevent this by implanting two brains of immature worms into the coelom of decapitated specimens. He also discovered that neuro-

secretory material which could be detected in the terminations of the axons in the cerebro-vascular complex of immature worms disappeared in mature ones (Bobin & Durchon, 1952). From these observations he reasonably concluded that a hormone, or hormones, originating in the neurosecretory cells in the supra-oesophageal ganglion inhibited epitoky and the maturation of the male gametes.

Attempts to repeat Durchon's experiments on Nephtys cirrosa and N. hombergi have not been very successful. Nephtys is a much more difficult animal to keep in the laboratory than Nereis and of some 350 worms from which the supra-oesophageal ganglion was removed, all but five died before the conclusion of the experiment. This is probably because Nephtys, unlike Nereis, must be able to burrow into the sand if it is to be kept in a healthy state, and it is unable to do this when the prostomium is removed. Control worms, which have been able to bury themselves, have always lived in aquaria far beyond the conclusion of the experiments. Another difficulty is that while in Nereis the external morphological changes accompanying epitoky are gross and conspicuous and can be followed during the course of the experiment, in Nephtys they are not.

The coelomic contents were examined before the experiment by drawing off a small quantity of the fluid in a capillary and examining it microscopically. The experiments

were carried out between June and January, when only a small proportion of the worms (less than 5% of them) contained eggs, sperm, or detached muscle fibres in the coelomic fluid. Those which had were discarded. The supra-oesophageal ganglion was removed by cutting off the prostomium and squeezing the dorsum just behind the ganglion so that it is pressed forwards and can then be cut out. At the end of six weeks the five survivors, which were all males, were full of sperm and the body-wall musculature was undergoing extensive phagocytosis. No change has been detected in the shape of the parapodial lobes or the number and length of the chaetae. 200 control worms were examined in the same way at the beginning and end of the experiment. In the June-July experiments two of the control animals became mature during the course of the experiment, but on this occasion, none of the experimental animals survived. Apart from this, all the controls remained immature.

Most of the specimens examined histologically have been immature. In these type B and C cells always show signs of secretory activity and neurosecretory material can be demonstrated in the cerebro-vascular complex. In the few mature worms that have been examined B cells are completely inactive, the C cells are nearly all inactive, though a few show slight signs of secretion, and there are no secretory granules in the cerebro-vascular complex. These results are summarised in Table IX. It was difficult to

TABLE IX

Species	Sex	Maturity	B1	B2	C1	C2	C3	C4
N. cornuta		++	-	-	+	+	+	
N. cornuta		++	-	-	+	+	+	
N. ferruginea		++	-	-	+	+	+	+
N. picta		++	-	-	+	+	+	+
N. hombergi		++	-		+	+		
N. cirrosa		+	+	+	+	+	+	+
N. cirrosa		+	+	+	+	+	+	-
N. californiensis		+	-	-	-	+	+	
N. californiensis		+	-	-	+	+	+	+
N. californiensis		+	+	+	+	+	+	+
N. californiensis		+	+	+	-	-	-	+
N. californiensis		-	++	++	++	++	++	++
N. californiensis		-	++	+	++		+	
N. californiensis		-	++	++	++	++	++	+
N. californiensis		-	++	++	++	++	++	++
N. californiensis		-	++	++	++	++	++	++
N. caeca		-	++	++	++	++	++	++
N. ferruginea		-	++	++	++	++	++	++
N. ferruginea		-	++	++	++	++	++	++
N. cirrosa		-	++	++	++	++	++	++
N. cirrosa		-	++	++	++	++	++	++
N. longosetosa		-	++	++	++	++	++	++
N. incisa		-	++	++	++	++	++	++
N. hombergi		-	++	++	++	++	++	++
N. hombergi		-	++	++	++	++	++	++
N. picta		-	+	++	++	++	++	++
N. punctata		-	++	++	++	++	++	++
N. punctata		-	++	++	++	++	++	++

B1, B2, and C1, C.2, etc. refer to groups of these ganglion cells in the areas indicated in fig.34.

Maturity: ++ coelom full of eggs or sperm, phagocytosis of the musculature.
+ probably fully mature, but uncertain.
- immature.

Secretory activity of the cells:
++ a number of cells of this type with secretory granules, vacuoles, etc.
+ cells nearly all inactive, but one or more with a few granules or vacuoles. (This

TABLE IX (Contd.)

category represents almost complete inactivity.)
- no cells showing any signs of secretory activity.

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determine how close to maturity some specimens were; these have been included in the table and it will be seen that they give conflicting results, but on the whole bear out the conclusion that it is these cells which are associated with the inhibiting hormones.

Discussion

The morphological and functional similarities between the supra-oesophageal ganglia of nephtyids and nereids are evident in the neurosecretory systems of the two families of worms. Indeed, the structural features of the cerebro-vascular complex are almost identical, and there can be no doubt that it is the chief, if not the only, site at which neurosecretory material is released into general circulation in the body. In both worms the granules can be detected in the complex only in immature specimens, and it is the B and C cells which are secreting in immature worms and are inactive in mature ones. There are thus strong grounds for supposing that it is these cells which produce the hormone

or hormones which are responsible for the effects discovered by Durchon. This interpretation is supported by other considerations of a more theoretical nature. Since the hormones are inhibitory and are effective during the greater part of the life of the animal, there must be a constant release of them into the body. Under these circumstances it might be expected that a great number of secretory cells would be involved and that they would secrete asynchronously: very few secretory cells are capable of constant secretion and this is the simplest way in which the constant production of a substance can be achieved. These conditions are satisfied by the B and C cells. The type A cells are quite different. There are only two or four of them in the brain, they are usually fully charged with secretion, and it is likely that they produce a substance that is used only intermittently and then only for short periods.

The neurosecretory material accumulates at the base of the cone of axons, just above the laminated connective tissue sheath around the brain. It can also usually be found in the modified pericapsular membrane, but how it crosses the connective tissue sheath is unknown. Bobin & Durchon (1952) in their study of Perinereis cultrifera described the accumulation of fuchsinophilic granules, similar to those visible in the cone of fibres, in small

'moruliform' bodies which are found in the fibre tract and especially immediately above the connective tissue sheath of the brain. They may sometimes have the appearance of small unicellular gland cells with their necks orientated towards, and perhaps projecting through, the sheath. Defretin (1955) has come to an entirely different conclusion about the nature of these fuchsinophilic granules and the 'moruliform bodies'. He suggests that they are mitochondrial structures which have migrated inwards from the cells of the modified pericapsular membrane. I have not been able to find such 'moruliform' masses in the brain of Nephtys, even when using the mitochondrial techniques which Bobin & Durchon (1952) insist are essential if they are to be demonstrated.

Defretin (1955) is of the opinion that the neurosecretory products are carried not in the blood but in the coelomic fluid. His reasons for this are that the particular modifications of the pericapsular membrane described by Bobin & Durchon (1952) occur not only in the cerebrovascular complex, but also in the region of the posterior optic nerves and on the sides of the brain. There, some of the pedunculate formations that Bobin and Durchon have described are, according to Defretin, undoubtedly coelomic corpuscles. He therefore regards the modified epithelium around the brain as ordinary coelomic epithelium, and the

modified regions as sites of formation of coelomic corpuscles; the whole cerebro-vascular complex, with the blood plexus, is a device for supplying the brain with oxygen. The pedunculate formations on the pericapsular membrane do not occur in Nephtys, so whether or not they are amoebocytes does not affect our present argument. The presence of granules, with the same staining and histochemical properties as the neurosecretory material, in the cerebro-vascular complex, suggests that it is a release site for the secretion. From the general configuration of the blood vessels in relation to the brain, whether they form a plexus or not, it might be argued that the device was a respiratory one, but this seems unlikely in view of the great thickening of the connective tissue sheath of the brain at just the place where oxygen is supposed to be diffusing into it. This is certainly not the state of affairs in the ventral nerve cord of Nephtys, where the elaborate coiling of the neural segmental blood vessels over the ganglia, particularly the sub-oesophageal ganglion, does appear to be a respiratory adaptation, and the connective tissue sheath is somewhat thinner there than elsewhere, and certainly not thickened four- or five-fold (see Appendix II).

The possibility that the neurosecretory products are taken up by amoebocytes in the blood stream as they are released into it does not necessarily imply that the amoebo-

cytes are essential transporters of the hormone to the target organs. Wigglesworth (1955, 1956) has discovered granules staining with paraldehyde fuchsin in the haemocytes of the insect Rhodnius, which are not neurosecretory products, but appear to be 'mucopolysaccharides' that play some part in the formation of the connective tissues. This is not a function one would expect to find played by the amoebocytes in the blood vessels of Nephtys because the blood vascular system of this animal, unlike that of the arthropods, is a closed one, and there are no endothelial capillaries (see Appendix II). Furthermore, the substance ingested by the amoebocytes of Nephtys is a good deal more complex than the 'mucopolysaccharides' in the haemocytes of Rhodnius. The obvious function of these amoebocytes is the ingestion of foreign particles in the blood stream, and the chief source of foreign matter is the neurosecretory substance.

CAPTIONS TO FIGURES

Figure 34. The approximate position of the three types of neurosecretory cells in frontal sections of the supra-oesophageal ganglion of Nephtys at levels indicated in the diagram on the right.

Figure 35. Type A cells at different stages in their secretory cycle. The acidophilic secretion is indicated by dense stipple. N. californiensis, zenker, paraldehyde fuchsin, light green.

Figure 36. Type B and type C cells at different stages in their secretory cycles, showing the distribution of fuchsinophilic granules. Areas of basophilic cytoplasm are indicated by dense stipple.

Figure 37. A. Transverse section through the supra-oesophageal ganglion of N. picta, showing numerous neurosecretory cells charged with dark staining material. Bouin, paraldehyde fuchsin.

B. Type A cell containing a large mass of secreted material. N. californiensis, zenker, paraldehyde fuchsin.

C and D. Type B cells at early and late stages of their secretory cycles. N. californiensis, zenker, paraldehyde fuchsin.

E, F and G. Type C cells at early, middle and late stages in their secretory cycles. N. californiensis, zenker, paraldehyde fuchsin.

Figure 38. Type B cells at the beginning, middle and end of their secretory cycles, stained with paraldehyde fuchsin (top row), with osmic acid (middle row), and with sudan black B (bottom row).

Figure 39. Type C cells at the beginning, middle and end of their secretory cycles, stained with paraldehyde fuchsin (top row), osmic acid (middle row), and sudan black B (bottom row).

Figure 40. Type B and C cells, showing the distribution of PAS-positive material other than glycogen. N. californiensis and N. hombergi, scales various.

Figure 41. The distribution of glycogen in type B and C cells. N. californiensis and N. hombergi, scales various.

Figure 42. A. Type C cell in the middle part of its secretory cycle. N. hombergi, zenker, paraldehyde fuchsin.

B. Type C cell at the same stage in its secretory cycle showing lipids occurring in the position of the vacuoles. N. hombergi, formol, sudan black.

C. Type B cell in the later part of its secretory cycle, showing the distribution of lipids. N. hombergi, formol, sudan black.

D and E. Neurosecretory granules in axons in the posterior part of the supra-oesophageal ganglion. N. hombergi, formol, sudan black.

Figure 43. Cross-section through the cerebro-vascular complex of N. californiensis. np neuropile, at axon tract, ng neuroglia, ngn nucleus of a neuroglial cell, cts connective tissue sheath, pm pericapsular membrane, mpm modified part of the pericapsular membrane, dbv dorsal blood vessel, npm nucleus of a pericapsular membrane cell.

Figure 44. Diagrammatic representation of the variations in structure of the cerebro-vascular complex of a number of nephtyids.

Figure 45. A. The ventral part of the supra-oesophageal ganglion with the dorsal blood vessel. The axon tract emerges from the neuropile (above) and runs to the ventral surface of the ganglion where a few dark-staining granules of neurosecretory material can be seen immediately above the ganglionic membranes. N. caeca, Bouin, paraldehyde fuchsin.

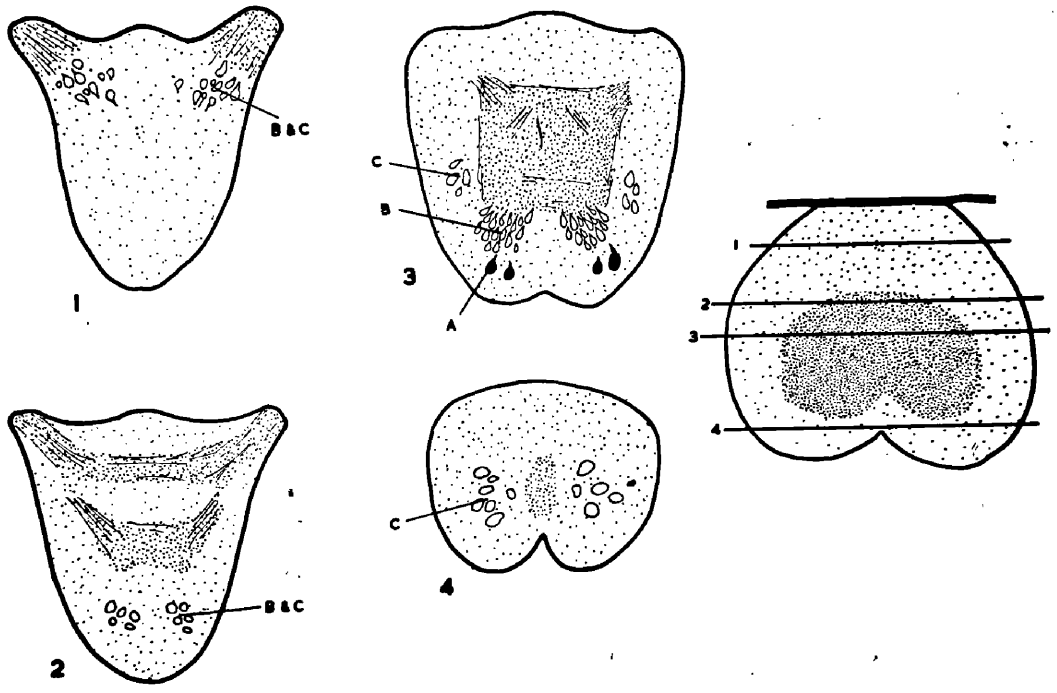
B. The base of the ganglion and the dorsal blood vessel with a large mass of neurosecretory material (darkly stained) in the space between them. N. picta, Bouin, paraldehyde fuchsin.

C. The base of the brain and the dorsal blood vessel. Some granules of secreted material

can be seen at the end of the axon tract and in an amoebocyte in the dorsal blood vessel. N. cirrosa, Bouin, paraldehyde fuchsin.

D. Granules in the axon tract of the cerebrovascular complex. The membranes and the dorsal blood vessel have become detached in this section. N. incisa, Bouin, paraldehyde fuchsin.

E. Neurosecretory material in the lumen of the dorsal blood vessel and in an amoebocyte in the vessel. N. caeca, Bouin, paraldehyde fuchsin.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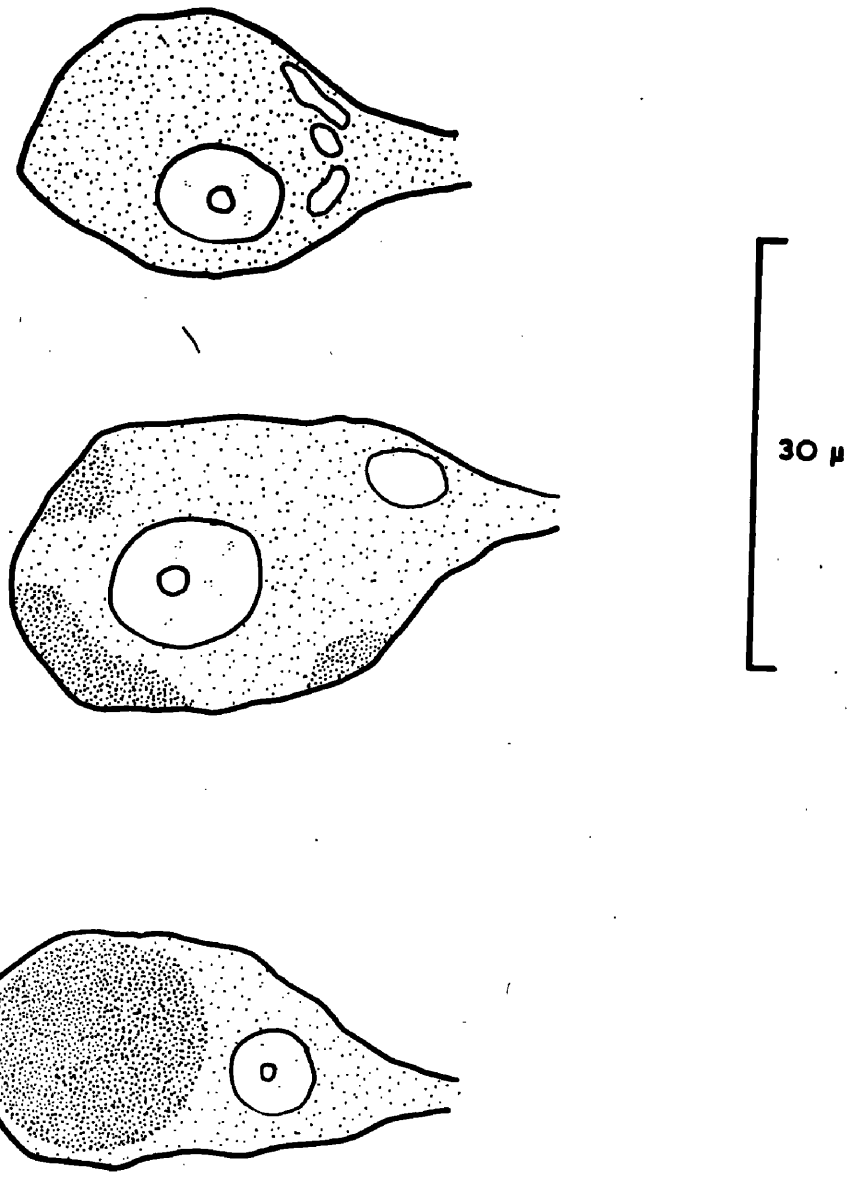
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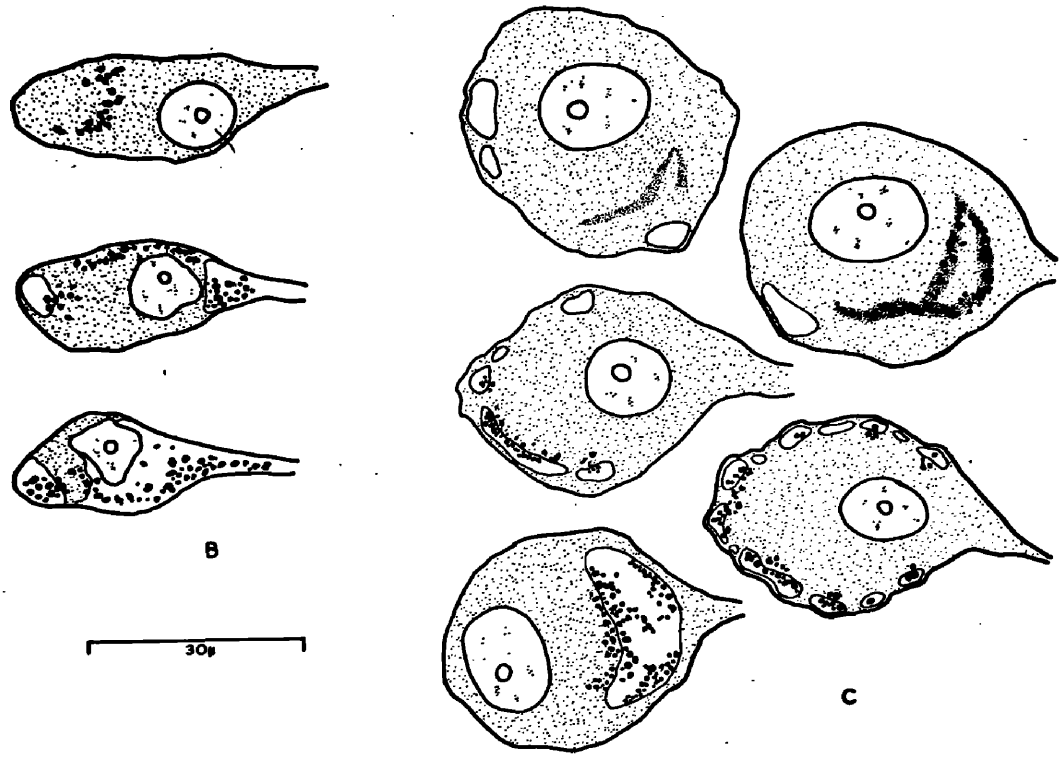
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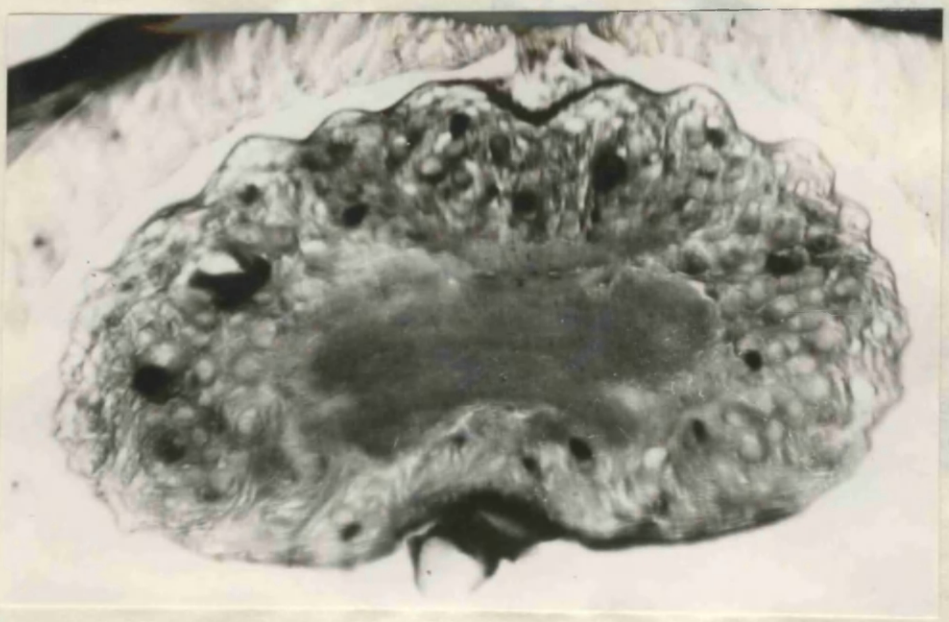

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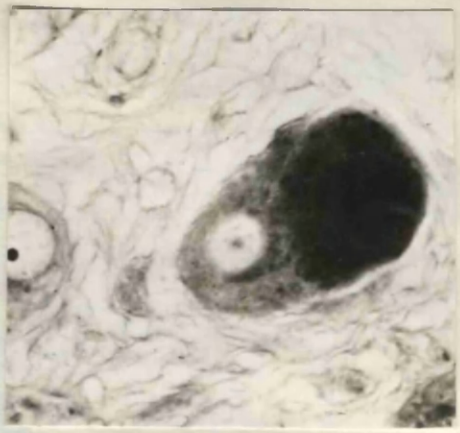

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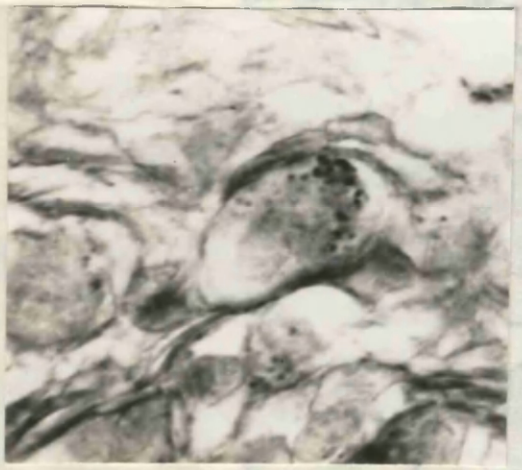

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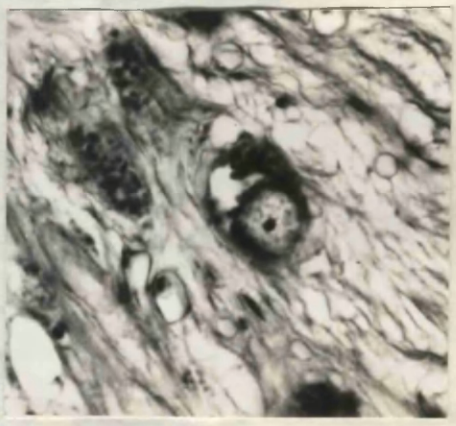

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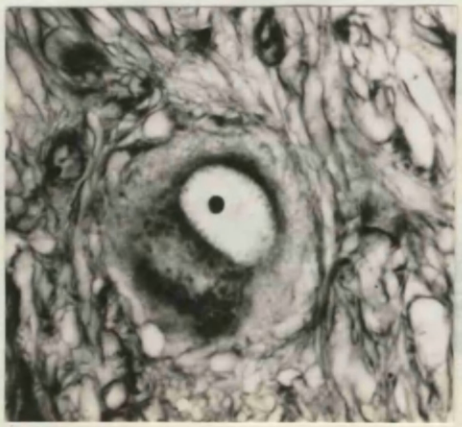

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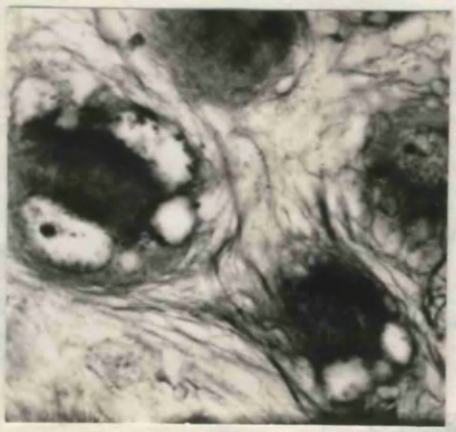

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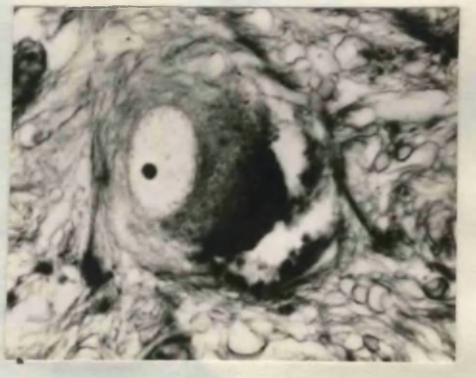

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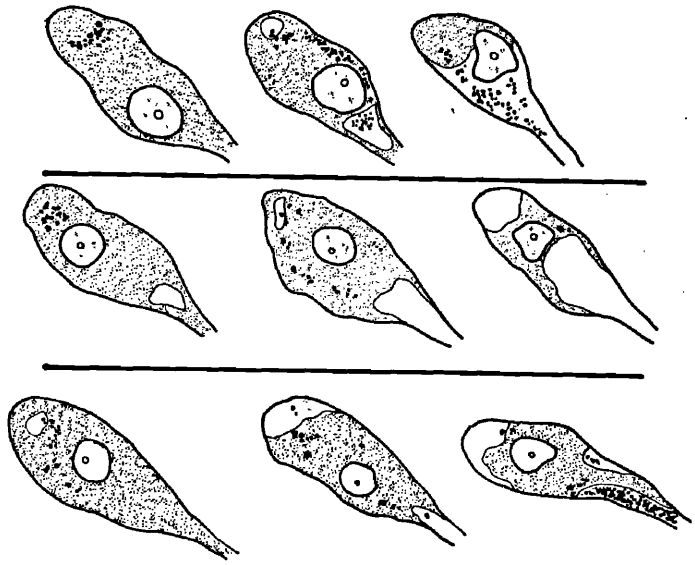

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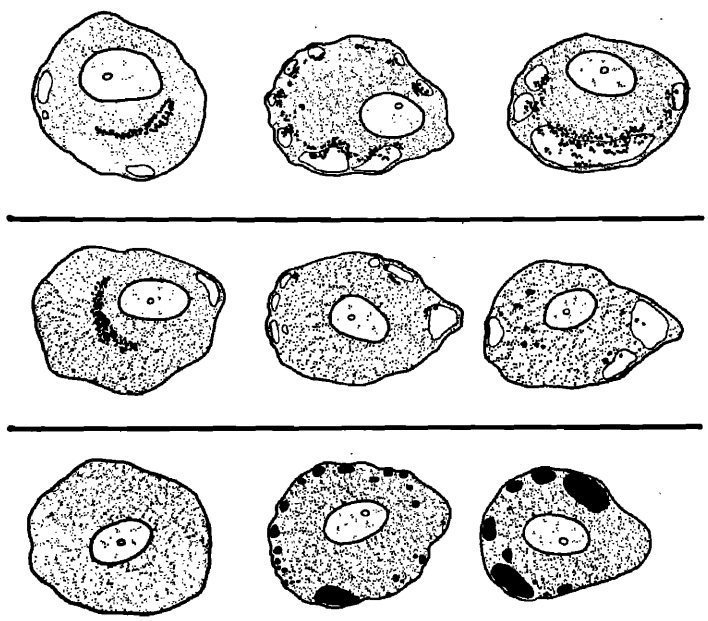

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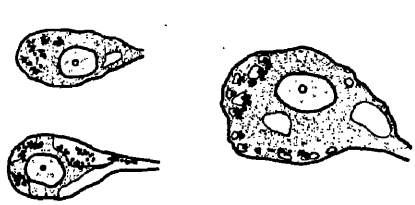

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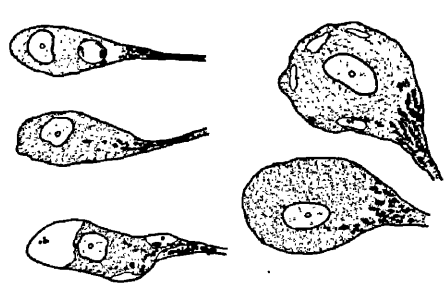

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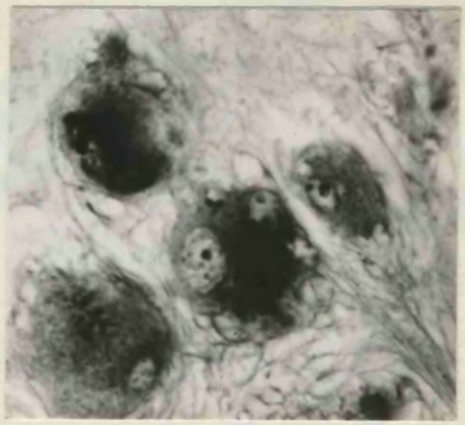

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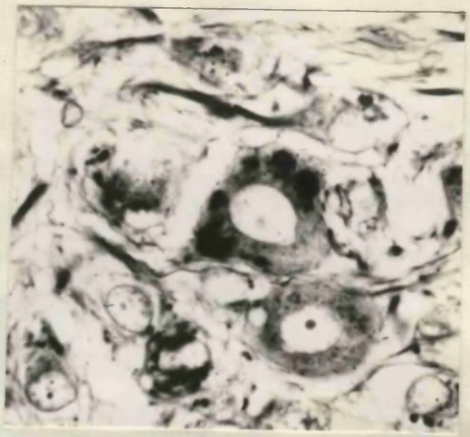

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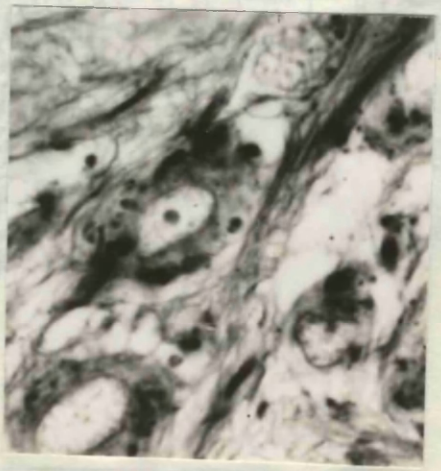

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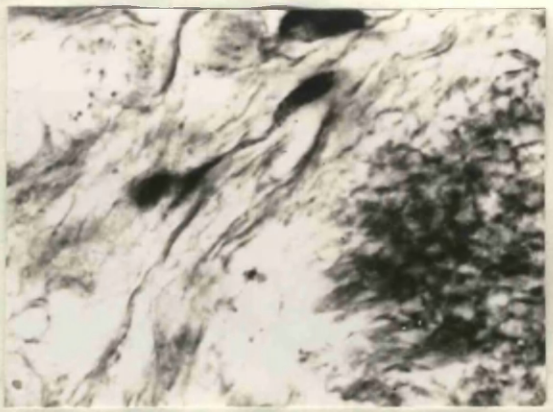

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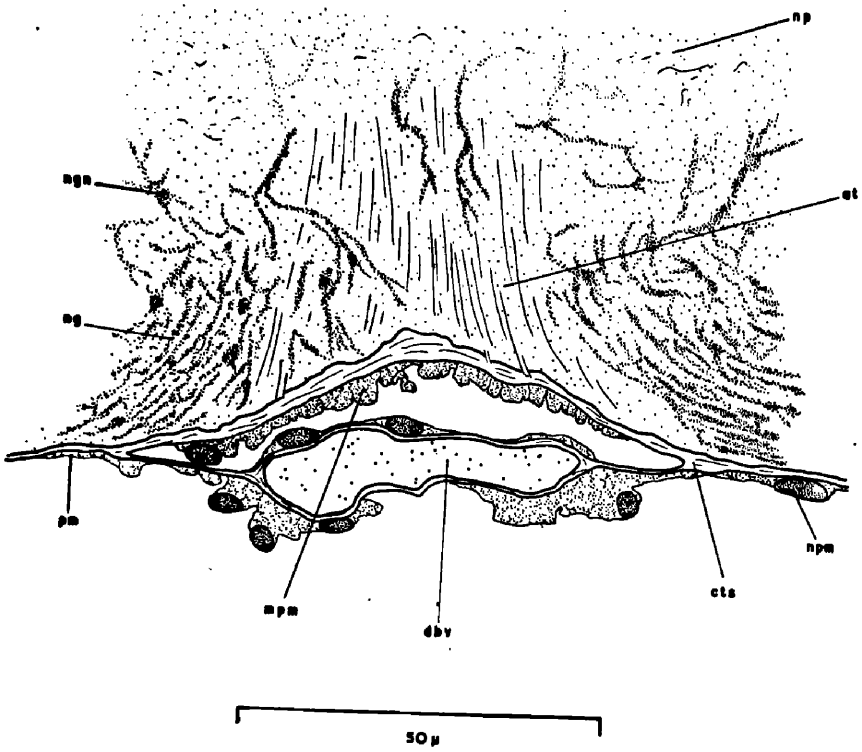
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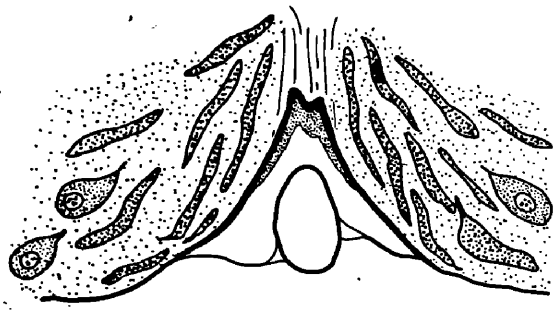
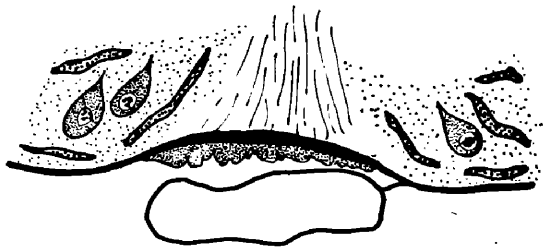
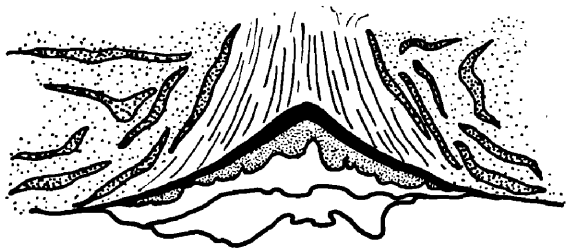


N. ferruginea

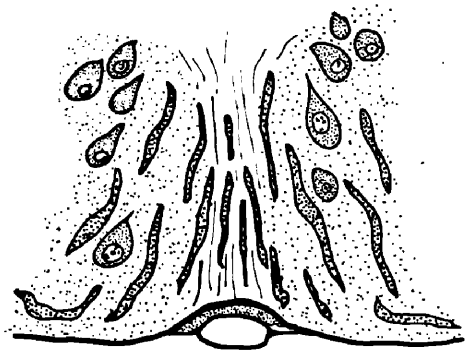


N. picta

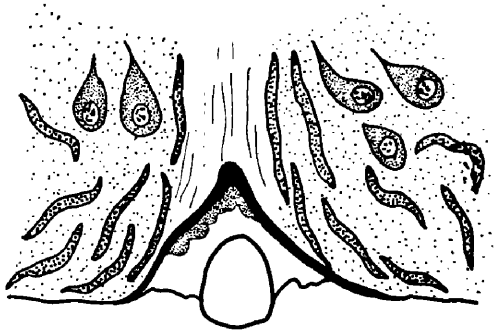


N. californiensis

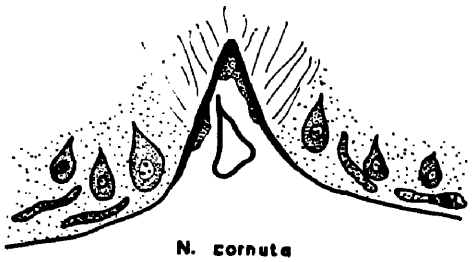
N. caeca



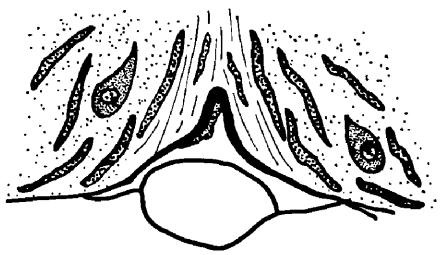
N. incisa



N. punctata

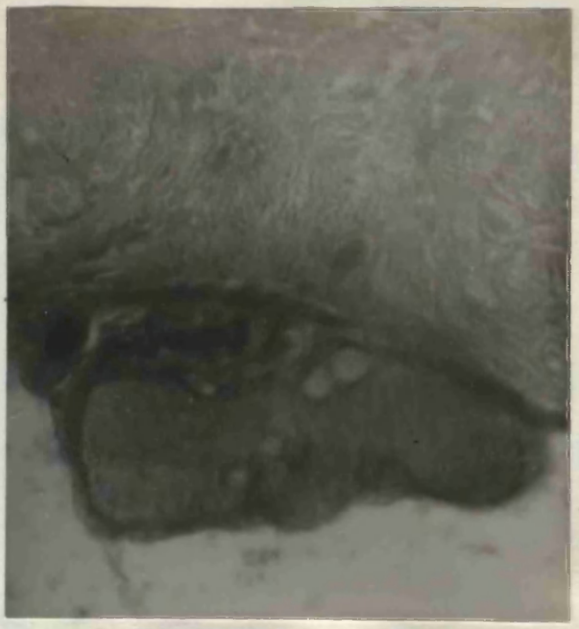


N. cornuta



N. cirrosa

A



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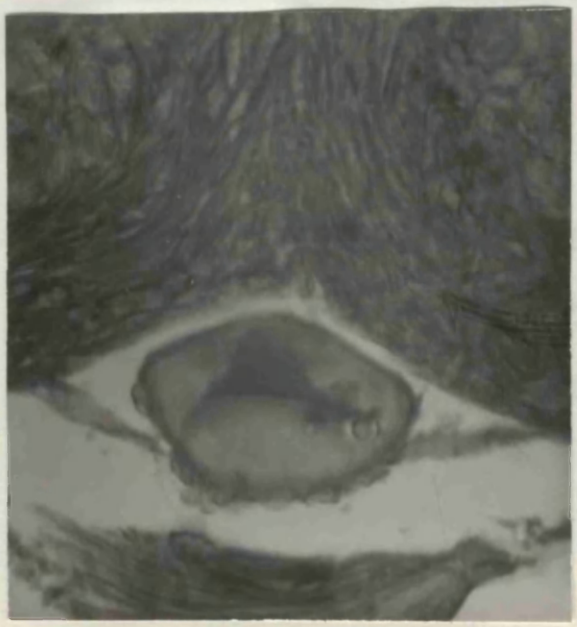

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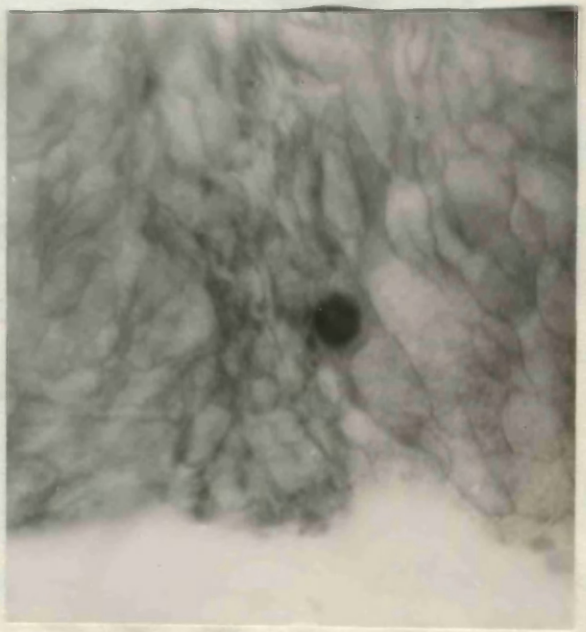

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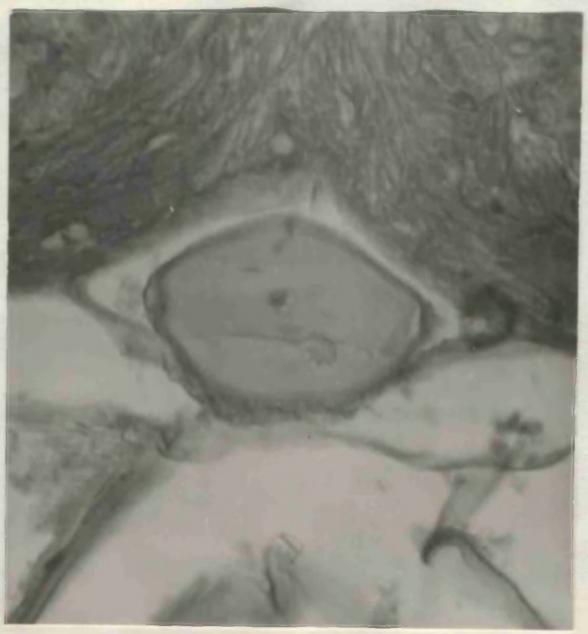

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THE HOMOLOGIES OF THE POSTERIOR LOBES OF *NEPHTYS* AND THEIR IMPLICATION IN THE THEORY OF THE NATURE OF NEUROSECRETORY CELLS

THE HOMOLOGIES OF THE POSTERIOR LOBES OF *NEPHTYS* AND THEIR IMPLICATION IN THE THEORY OF THE NATURE OF NEUROSECRETORY CELLS

There are good morphological grounds for supposing that the groups of ganglion cells in the posterior part of the supra-oesophageal ganglia of *Nephtys* and *Nereis* are homologous. In *Nereis* the ganglion cells in question include those of the epidermal, and nuchal nuclei, both of which send axons to the prostomial epidermis, and an important group of neurosecretory cells, the axons of which run into the neuropile (Retzius, 1895; Holmgren, 1916; Scharrer, 1936; Schaefer, 1939; Defretin, 1955). A little anterior to these cells, the posterior optic nerves enter the neuropile.

As we have already seen, the posterior eyes of *Nephtys*, despite their much simpler structure, are not only in the same position relative to other groups of ganglion cells, but are involved in the same type of photonegative behaviour. The behaviour is confused by the fact that the eyes of *Nephtys* are too deeply embedded in the ganglion to function properly in most species, but when they are near the surface, as in *N. cornuta*, the worms are able to execute precisely the same orientation movements as *Nereis*. The neurosecretory systems in the brains of *Nephtys* and *Nereis* are almost identical. It is most probable that the cells posterior to

the neuropile produce the maturation inhibitory hormone in Nereis which have been investigated by Durchon (Scharrer, 1936; Durchon, personal communication); the homologous cells in the brain of Nephtys are also neurosecretory and there are signs that they produce hormones having the same effect on that worm. The cerebro-vascular complexes of the two worms, which appear to be the sites at which this hormone is released into the blood stream, are identical (save for very minor differences). The nuchal nuclei are characteristic of the posterior part of the brain of Nereis and other polychaetes (Holmgren, 1916). They occur also in Nephtys, though the situation is complicated because in small species the brain tends to be elongated and to extend into the second or third segments, and in several other species the ganglion tends to shift posteriorly as the circum-oesophageal connectives are shortened. The nuchal organs are always situated at the postero-lateral corners of the prostomium, so that in some species they are actually in front of the anterior margin of the supra-oesophageal ganglion, instead of in their normal position at its posterior end. But the ganglion cells of the nuchal nucleus are always in their typical position in the posterior part of the brain.

The only structures in the posterior half of the brains between which homologies have not so far been established are

the epidermal nucleus of Nereis and the posterior lobes of Nephtys. In Nereis, the brain has sunk beneath the epidermis and the epidermal nerves run from it to the epidermis on either side of the postero-lateral part of the prostomium. In Nephtys the brain is in contact with the prostomial epidermis and there are no epidermal nerves. On the other hand, there are no posterior lobes attached to the brain of Nereis. As we have already shown, in some species of Nephtys, the mucus cells of the prostomium are found in the epidermis, principally along the sides of the prostomium (e.g. N. ~~incisa~~ picta, fig.46A). In other species, the mucus cells are located in two posterior lobes of the supra-oesophageal ganglion and open to the exterior by extremely long, fine necks which run in a tract along either side of the brain to the lateral walls of the prostomium, where, presumably, the cells were originally situated (e.g. N. ~~checa~~ californiensis, fig.46B). The mucus cells of the posterior lobes are completely enclosed within the brain capsule and are separated from the nerve cells only by neuroglial fibres which penetrate into the lobes and into the lateral tracts of cell processes running from the mucus cells to the exterior. The mucus cells, whether they are in the epidermis or in the posterior lobes, secrete an acid mucopolysaccharide. In one species, N. cirrosa (fig.46C), a different situation prevails. The posterior lobes are filled not with mucus cells, but with

neuroglial cells and fibres, large, non-secretory matrix cells, and small secretory cells which are the homologues of the posterior lobe cells of other species. Processes from these secretory cells in the posterior lobes of N. cirrosa run along the sides of the brain and end in the epidermis in the lateral walls of the prostomium. Although they are homologues of the mucus cells, these cells are morphologically similar in appearance to the neurosecretory cells in the supra-oesophageal ganglion.

The ganglion cells from which the epidermal nerves of Nereis arise lie immediately posterior to the posterior group of neurosecretory cells. The only cells lying posterior to the comparable neurosecretory cells in the brain of Nephtys are those of the posterior lobes. The posterior lobe cells lie in the same position as the epidermal nucleus, relative to other features in the brain, and processes from them run to approximately the same position in the prostomial epidermis in the two worms. The epidermal nucleus of Nereis is a neurosecretory centre (Bobin & Durchon, 1953); the posterior lobe cells of Nephtys are derived from epidermal mucus cells. In this context, the posterior lobes of Nephtys cirrosa are of special importance, for the secretory cells in them are certainly homologues of the posterior lobe cells of other species of Nephtys, but they have the appearance of neurosecretory

cells. The conclusion that the posterior lobe cells of Nephtys are homologues of the epidermal nucleus of Nereis is unavoidable.

Evidence that epidermal secretory structures may be incorporated in the brain and there become neurosecretory centres is most detailed in the Nephtys-Nereis series, but it is not unique. A strikingly similar example of epidermal secretory cells coming to have a close association with the brain has been reported in the nemerteans (Scharrer, 1941). The cerebral organ is composed of both sensory and glandular elements, and is epidermal in some nemerteans and completely incorporated into the brain of others. In this case there is no evidence that the secretory cells have the appearance of neurosecretory cells in any species, though in Malacobdella, the glandular area appears to be lacking. It would be interesting to know if the secretory component of the cerebral organ of this genus is really missing, or if the secretory cells have been modified, as they have been in Nephthys cirrosa, and have been mistaken for neurones.

The cerebral organ of nemerteans is suspected of being sensory, and a parallel has been drawn between its evolution and that of the frontal organ of crustaceans (Scharrer, 1941; Gabe, 1954). In the phyllopod crustaceans and the copepods, the frontal organ is sensory, but in the Malacostraca it has been incorporated into the central nervous system to form the X-organ (Hanström, 1949). Thus

in the Malacostraca, the principal incretory organs have been derived from epidermal sensory structures. The parallel between the evolution of the nemertean cerebral organs and the crustacean frontal organ may be even closer than this, for in some copepods the frontal organ is associated with a large secretory cell (Dahl, 1953). Because this cell is not universally present in copepods, the relationship between it and the frontal organ has been discounted, but primitive sense organs are commonly associated with secretory cells (e.g. the nemertean cerebral organ, the organs of Tomosvary of acarines, the lamellibranch osphradium and the neural gland of ascidians), and further investigation may reveal that the secretory cell of the copepod frontal organ is no mere coincidental occurrence and that it is involved in the evolution of the X-organ sinus gland system of the Malacostraca.

Since it is suggested that epidermal secretory cells have, in some cases, been incorporated into the central nervous system as neurosecretory cells, it must be established whether or not the phylogenetic transformation of a mucus cell into a neurone would be possible. All epidermal tissue has at least the potentiality of becoming nervous tissue, for neural tube can be induced to form in any part of the vertebrate ectoderm under appropriate experimental conditions. Further, nervous tissue can be regenerated

from epidermis in worms (Berrill, 1952) and insects (Wigglesworth, 1953), while the supra-oesophageal ganglion is regularly formed from epidermis in several polychaetes and oligochaetes which reproduce by stolonisation (Dawydoff, 1928; Stephenson, 1930). However, here we are suggesting that an epidermal cell, specialised for secretion, has evolved into another specialised cell, a neurone, by the sequential appearance of neuronal characteristics, rather than by dedifferentiation and reconstitution. The cells of the posterior lobes of Nephtys are unequivocally mucus-secreting, but they have some features in common with neurones. They have a cell-body containing the nucleus and a cell process which is many times longer than the greatest dimension of the cell-body; the chief difference is that they are considerably larger than most nerve cells, though not larger than the unicellular giant axons of some polychaetes. Any of the special morphological and physiological properties of nerve cells which are attributable to their peculiar shape, are likely also to be properties of any other cells which approximate to them in shape. It is likely that both the special electrical properties of neurones and the presence and configuration of the neurofibrillae fall into this category. Nissl granules are also regarded as characteristic of neurones, but they are granules of ribonucleoprotein which is present in the cytoplasm of all

cells and which tends to form aggregations resembling Nissl bodies in any active cell. Neurosecretory cells have the additional property that they secrete a substance that aggregates in droplets or granules. Epidermal mucus cells share this property, and it is significant that they and the neurosecretory cells of many animals secrete materials that contain polysaccharides.

There seems to be no over-riding objection, a priori, to the evolution of neurosecretory cells from epidermal secretory cells. In the Nephtyidae and the Nereidae this is the most reasonable explanation of the changes in distribution and morphology of the epidermal secretory cells of the prostomium. It seems that in these worms the evolutionary tendency has been first for epidermal mucus cells to migrate centripetally and to become incorporated within the supra-oesophageal ganglion, but to retain a connection to the exterior; and secondly, for the cells in the posterior lobes to undergo modification so that they resemble neurones. In the Nereidae, the incorporation of the cells into the ganglion is complete and they are indistinguishable from any other neurones. The same phenomenon may account for the evolution of the cerebral organ of nemerteans and the X-organ of crustaceans. It is not suggested that all neurosecretory cells have been evolved from epidermal mucus cells, and still less is it suggested that

all neurosecretory centres represent secondary additions to a pre-existing nervous system; it would be impossible to extend such an analysis to include the hypophyseal-hypothalamic tract of vertebrates, for example. It is suggested that neurosecretion is not a highly specialised and secondary function of nerve cells, but rather that secretion is a fundamental property of ectodermal tissue which has been retained to a marked degree by certain cells, the neurosecretory cells. In particular, it is those nerve cells in the most primitive parts of the nervous system that exhibit this primitive secretory activity to such a marked degree. It is only in a few exceptional cases that the incorporation of epidermal secretory structures into the central nervous system can be detected, but the fact that they are apparently transformed into neurosecretory cells in the process provides a clue to the fundamental nature of neurosecretory cells in other parts of the nervous system.

This view of neurosecretory cells runs completely counter to the usual interpretation of their nature. The currently accepted interpretation has recently been summarised by Hanström (1954). He emphasises that neurosecretory cells have the essential characteristics of neurones, "The fact that cells of this type originally are true nerve cells and not gland cells, which in some way or another have been incorporated into the central nervous system, is proved

by their general morphology -- like true nerve cells they possess dendrites and axons -- and their cytology -- they possess Nissl-substance and (at least sometimes in vertebrates) neurofibrils". He then goes on to arrange the various types of neurosecretory cell that are known at present into order and to suggest an evolutionary sequence of them. These are: 1. conventional neurones; 2. neurosecretory cells morphologically resembling ordinary neurones, but with granules, droplets and vacuoles of secreted substance in the cell body which does not migrate along the axon; 3. true neurosecretory cells which have retained the morphological characteristics of neurones, but in which the secretion travels along the axon to be stored in specialised terminations - the axons no longer innervate effectors; 4. neurosecretory cells which resemble those in the previous category, except that they lack dendrites.

Representing the three classes of neurosecretory cell, Hanström suggests the following: class 2. the neurosecretory cells of annelids, on the grounds that there is no sign of secretion in the axons and no storage organ has been detected; class 3. the neurosecretory cells of the pericardial and neurohaemal organs of crustaceans, some elements in the nucleus preopticus of fishes and amphibians, and the nuclei supraopticus and paraventricularis of reptiles, birds and mammals; class 4. the cells of the X-organ of crustaceans, the medial cells of the pars inter-

cerebralis in the insect brain, and some cells in the nuclei preopticus, supra-opticus and paraventricularis. Added weight is given to this interpretation of the evolutionary status of the three classes of neurosecretory cells, by the fact that no sign of neurosecretory activity has so far been detected in the nerve net of coelenterates, nor, with certainty, in the platyhelminthes. The animals lowest in the evolutionary scale to possess neurosecretory cells are the annelids, and in these the cells are of the most primitive sort. In addition, Hanström suggests that it may be possible eventually to create a fifth type of cell to accommodate neurosecretory cells without an axon which are morphological counterparts of adrenal medulla cells (he rejects the suggestion by the Scharrers (1954b) that the adrenal medulla cells represent the final stages in the transformation of conventional neurones into neurosecretory cells).

As to the second class of cells, axonal transmission of the neurosecretory substance has been demonstrated in sedentary polychaetes (Arvy, 1954), in the epidermal nerve of Perinereis (Bobin & Durchon, 1953) and in the posterior part of the brain of Nephtys. Admittedly it is unusual and difficult to show neurosecretory material in the axons of polychaetes. There seem to be two reasons for this. Much of the secretion, at least in Perinereis and Nephtys

appears to be largely in the form of alcohol soluble lipids and so, unless special precautions are taken, the chance of conserving and staining it is small. Secondly, the majority of neurosecretory cells in the brain of Nephtys secrete asynchronously, so that only a few cells are at the end of their secretory cycle in any brain that is examined. It is likely that at this stage the secretion leaves the cell body, so that the total quantity of secretion in axons in the brain is never great.

A storage and release organ of such a degree of organisation and specialisation as the sinus gland of Malacostraca or the neurohypophysis of vertebrates is not found in the annelid nervous system, but an analogous structure is found in the supra-oesophageal ganglion of Nereis and Nephtys. This is the cerebrovascular complex, and while it may be difficult to demonstrate neurosecretory material in axons in the polychaete brain, it can nearly always be found accumulated at the base of the axon tract in this complex in immature worms.

Whatever the degree of organisation of the annelid neurosecretory system, one, at least of Hanström's criteria is satisfied. There can be no doubt that the neurosecretory cells in Nephtys function as conventional nerve cells. In this worm the great majority of nerve cells in the brain show signs of secretory activity and it is inconceivable

that secretion should be their sole function. If this were true, three-quarters of the ganglion would be non-functional as a nerve centre.

But the chief reasons for including annelid neurosecretory cells in class 2, and with them the justification for designating this class of cell, has been lessened by recent discoveries. Indeed, on inspection, the whole concept of neurosecretory cells in which the secretion does not migrate along the axon, but accumulates in the cell body and later diffuses out of the cell, through the surrounding tissues and into the blood vessels, is uncertain. Attention has, on the whole, been directed towards the hormones produced by neurosecretory cells and on their biological affects, rather than on the nature of the secretion. In many cases it behaves as a colloidal material, in some animals it is very complex, and there is evidence that it is composed of a biologically inert carrier-substance together with the hormone or hormones (Scharrer & Scharrer, 1954b). As far as is known, it is constituents of the carrier-substance which are stained by conventional techniques and only in one case so far has it been possible to stain a constituent of the hormone itself (Adams & Sloper, 1955). All the information we possess about the nature of the secretion suggests that it is not a substance which diffuses readily across cell membranes, and it is this pro-

perty of forming colloidal masses which probably accounts for the material being retained in the storage and release organs, where they exist. If the carrier-substance did not serve to retain the hormone within the cells until it was required to be released into the blood stream, it would be difficult to account for its presence at all. Diffusion of the neurosecretory material from the perikaryon, which Hanström proposes in class 2 cells, therefore presents a number of difficulties and calls for some explanation.

A second difficulty arises if it is assumed that the secretion accumulates in the cell body, but does not migrate along the axon. The axoplasm is in constant proximo-distal motion, not only during the period of elongation and enlargement of the axon, but throughout the life of the mature nerve fibre. Weiss and Hiscoe (1946) conclude that this perpetual growth of neurones serves to replace catabolic protoplasmic systems, especially proteins, which cannot be synthesised in the peripheral cytoplasm. The rate of this proximo-distal convection was estimated by Weiss and Hiscoe to be of the order of 1 mm per day. This is of the same order of magnitude as the rate at which neurosecretory granules travel along the x-organ connective in the crustaceans Dromia and Lysmata (Carlisle, 1953). If the obvious conclusion that neurosecretory material is carried along the axon to its distal end by the axoplasmic currents is correct,

it is very difficult to account for the secretion accumulating in the cell body and not passing along the axon.

In view of these two fundamental difficulties, class 2 cells, instead of being simple and relatively unspecialised, must possess remarkable properties. Now that axonal transmission has been established in the majority of neurosecretory cells by both direct and indirect methods, the onus of proof rests with those who maintain that a different mechanism for the release of the secretion exists at all. Nevertheless, neurosecretory cells have been described in three groups of animals in which axonal transmission of the secretion has never been observed. These are sipunculids (Gabe, 1953a), oligochaetes (Herlant-Meewis, 1955) and in Nephtys (type A cells). An explanation of how the secretion first accumulates in the cell body and then, later, diffuses out of it (if in fact it does so) will therefore be of very great interest.

The criterion for separating the second type of cell from the others is the absence of axonal transmission of the secretion; the criterion for separating the other two types of cell is the presence or absence of dendrites. Since the four classes of neurone are proposed as an evolutionary sequence, it is implied that the differences between them are due to some change in the inherent properties of the cells. The general burden of recent research on the

growth of nerve cells in regeneration, wound-healing and tissue culture experiments, is that the development of side branches and dendrites is a function of local mechanical requirements and conditions, rather than due to directive properties of the growing cells themselves. Extreme plasticity is the chief characteristic of the nervous system during its embryonic development. If this attitude is correct, and it is supported by a great deal of evidence (Weiss, 1955), it follows that the gross morphology of an individual neurone is an unsafe guide to its evolutionary status; its form is determined by the organisation of the nervous system - a different matter altogether. The morphology of the axons depends on the arrangement of the neurosecretory system and this, in turn, depends upon the size and elaboration of the nervous system. A small and not highly differentiated nervous system like that of an annelid is unlikely to contain sufficient nerve cells for elaborate structures to be formed within it. Only in animals with large and complicated nervous systems are there highly specialised neurosecretory systems. A specialised neurosecretory system may well contain neurones of a different morphological type from those in the rest of the nervous system, but this is not to say that they have undergone any change in their inherent properties.

The alternative interpretation of the nature of neurosecretory cells that I have proposed, that they are a primitive sort of nerve cell which has retained to a marked degree the secretory properties of the epidermis from which it was derived, is attractive in that it conforms with our views of the plasticity of the nervous system and places no reliance on the morphology of the cells, but only on their physiology and function. Evidence that a chemical similarity exists between secretions produced by neurosecretory cells and epidermal cells is crucial to this hypothesis. But unfortunately, very little is known of the composition of the neurosecretory material. Often it includes PAS-positive substances; they have been demonstrated in annelids (Argy, 1954; Gabe, 1954; Defretin, 1955; Clark, above), sipunculids (Gabe, 1953a), malacostraca (Gabe, 1952a; Turchini, 1953), chilopods (Gabe, 1952b) and insects. A great many substances react positively to the PAS test, but it may be significant that the same class of substances is secreted by neurosecretory cells and epidermal cells. Of course, this similarity may mean no more than that neurosecretory cells resemble a great many secretory cells in all parts of the body. But at all events, it suggests that the secretory ability is not a specialised one, but a very general and wide-spread phenomenon. Neurosecretory cells are specialised in that they

secrete biologically active substances, whereas epidermal cells do not, but in this respect they are no more specialised than other nerve cells which secrete adrenalins and acetylcholine.

Furthermore, if neurosecretion is a primitive function of nerve cells, it would explain why a much higher proportion of neurones in the supra-oesophageal ganglion of annelids are neurosecretory than in the brains of higher animals. Scharrer and Scharrer (1945) estimated that half the brain of Aphrodite was occupied by neurosecretory cells, and in Nephtys the proportion may be even higher. But apart from Turner's (1946) report of possible neurosecretory cells in the brain of the polyclad Leptoplana, neurosecretion in turbellarians is unknown. This supports Hanström's interpretation rather than the present one, but it must be remembered that the nervous systems of turbellarians has not received much attention from investigators and that it remains to a great extent unknown.

The interpretation I have advanced is of course speculative, and much more information about the cytochemistry and ontogeny of neurosecretory cells will be needed before it can be admitted or dismissed. However, if this interpretation is validated, it might provide a basis for a less arbitrary definition of neurosecretory cells than that currently in use. They are defined as cells

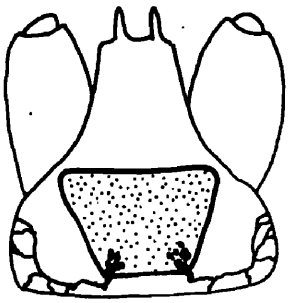
which have the morphological characteristics of neurones (axon, neurofibrillae and Nissl-bodies) and which secrete a substance that is microscopically visible in living or suitably stained material (Scharrer & Scharrer, 1954b). This definition deliberately, but arbitrarily, excludes such structures as the chromaffine cells of leeches and the epistellar bodies of octopod cephalopods, although they have some neuronal characteristics and are also secretory. The Scharrers (1954b) have distinguished them from neurosecretory cells and from ordinary neurones by giving the generic name 'neuroglandular cells'. But the chromaffine cells of leeches and the epistellar body of octopods are certainly modified neurones and they probably secrete adrenalins or substances resembling them. It may be that three types of neurone should be distinguished:

1. neurosecretory cells which are the most primitive and retain to a marked degree the secretory properties of epidermal cells, even to the extent that they secrete glycoproteins, polysaccharides, etc., which are also secreted by epidermal cells;
2. ordinary neurones in which the secretory function is much more specialised, so that they produce only adrenalins, acetylcholine, hydroxytryptamine and similar substances to facilitate synaptic transmission;
3. neuroglandular cells that are secondarily modified

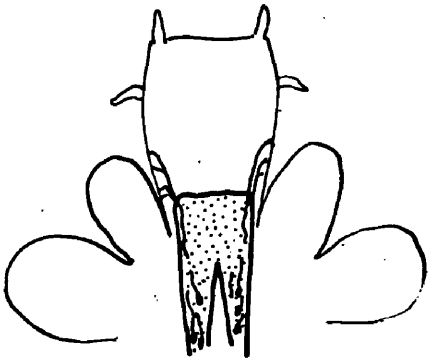
neurones in which the secretion of adrenalins (and possibly other, related substances) has become a hypertrophied activity.

CAPTION TO FIGURE

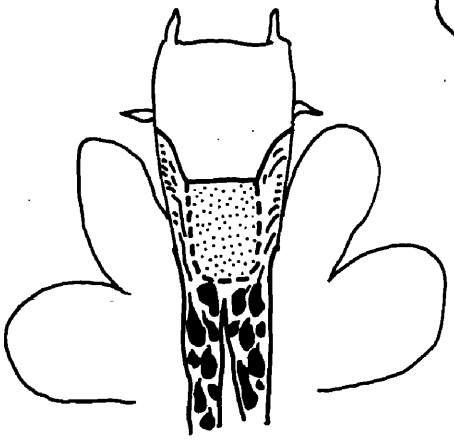
Figure 46. A summary of the evolution of the posterior lobes of *Nephtys* and the epidermal nucleus of *Nereis*. Homologous structures are shown in black. In *N. picta* these are the epidermal mucus-glands of the prostomium, in *N. californiensis* mucus glands in the posterior lobes, in *N. Cirrosa* the secretory cells of the posterior lobes, and in *Nereis* the neurosecretory cells of the epidermal nucleus.



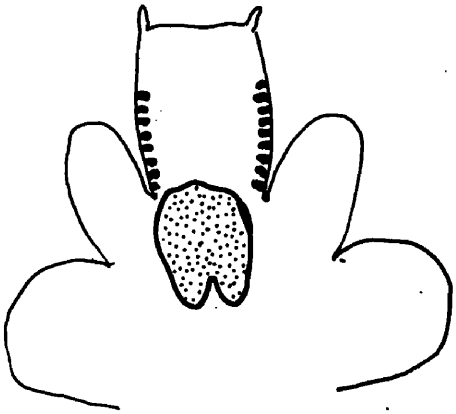
Nereidae



Nephtys cirrosa



Nephtys californiensis



Nephtys picta

APPENDIX I

PERIODIC ACTIVITY

PERIODIC ACTIVITY

Alternating periods of rest and activity play an important role in the general behaviour of a number of polychaetes. In all the worms that have been studied so far, periodic activity has been related to feeding or respiration, and the worms have been tubicolous. The most completely understood manifestation of this sort of behaviour is that of Arenicola in which feeding, defaecation and respiratory movements appear to be controlled by endogenous, pacemaker activities of the nervous system (Wells, 1937a, 1949a, 1949b, 1953). Similar behaviour has been observed in Nereis and Chaetopterus (Wells & Dales, 1951), Glycera (Wells, 1937b), Sabella (Wells, 1951) and terebellids (Dales, 1955). Since many polychaetes pump respiratory currents of water through their tubes in much the same way as Arenicola, it is likely that pacemaker activities are a common function of polychaete nervous systems.

Nephtys is not a tubicolous worm and it does not perform undulatory irrigation movements even when it can be persuaded to stay in a tube. It is a carnivore and does not show regular, periodic feeding. But it does show periodic activity in the form of repeated and fairly short swimming excursions when it is unable to bury itself in the substratum. This behaviour can be modified by applying

stresses of one sort or another to the worm.

Periodic Swimming

If the worms are placed in a rectangular aquarium without any substratum, they immediately swim and they go on swimming at intervals. This behaviour continues for a very long time, probably until the worms become moribund. For the first two or three hours after the worms are introduced into the aquarium, their behaviour is rather erratic, but after that time they settle down to swimming every one or two minutes (fig.47). The swimming excursions are brief, usually of the order of 10-20 seconds, and one lasting more than a minute is exceptional (fig.48). If the aquarium is provided with a layer of sand or mud the worms burrow into it and remain buried and inactive even if the substratum is unsuitable and within a few days the worms are all dead. Worms vary considerably in their activity and one specimen may swim only five or six times in an hour, another thirty or forty times. The pattern of periodic activity can be greatly modified and rendered more conspicuous by changing the environmental conditions.

a. Effect of lateral curvature on the worm. If Nephtys is placed in a circular vessel, it usually lies around the edge of the dish during its quiescent periods. By varying the size of the vessel relative to the worm, the curvature

of the arc into which the worm is constrained can be changed.

> Worms were placed in circular vessels of various sizes containing sea-water, but no sand. Except in some preliminary experiments, the worms were dark-adapted for two hours and then illuminated. The times at which they swam were recorded over the following two hours. At the end of the experiment the worms were relaxed in Mg-sea-water and their lengths measured. These experiments were carried out at 12°C on N. caeca and N. cirrosa.

The mere fact of keeping the worms in a circular vessel instead of a rectangular tank makes the periodic activity much more regular (figs.47 and 49). Within half an hour of placing them in the vessels, the worms settled down to frequent and regular bursts of swimming activity. Removal of the supra-oesophageal ganglion does not materially affect the behaviour. Within three hours of the operation, the worms once more settle down to a fairly regular pattern of alternating periods of rest and swimming activity (fig.50). In 31 experiments performed on 13 specimens of N. caeca and N. cirrosa, a linear relationship could be demonstrated between the frequency of swimming and the arc into which the body of the worm was constrained (radius of vessel/length of the worm). This is illustrated in fig.51. If the worm is placed in a relatively large vessel, it often does not

lie around the edge of the dish and conditions approximate to those when the worms are in a rectangular tank. The fact that the activity of the worm increases if the body is constrained into an arc of smaller radius, suggests that the stimulation of stretch receptors in the longitudinal muscles is responsible for an increased afferent flow of impulses, leading to a general increase in the level of activity of the worm.

b. The effect of light. The photonegative behaviour of a number of species of Nephtys has been examined in detail and is described in a previous section. In some of those experiments advantage was taken of the fact that the normal periodic swimming activity is enhanced when the worms are kept in circular jars.

c. Measurement of the swimming activity. In dealing with the measurement of the level of the activity of the worms, it may be appropriate to determine the frequency of the activity, its duration or its rate. The criterion and the variable factor must depend upon the biological significance of the activity. Thus in worms in which the periodic activity is related to respiration or feeding, the total length of the active period is of equal importance to its frequency. The distance, duration and speed, as well as the frequency of swimming excursions of N. cirrosa have

been measured at a variety of light intensities, but otherwise under constant conditions.

TABLE X

Average distance, duration and speed of swimming excursions of N. cirrosa at different light intensities.

log I	0.00	0.30	0.60	0.75	0.90	1.50
Worm			DISTANCE (cms)			
A	44.02	30.76	36.63	27.27	28.15	34.88
B	26.28	28.22	31.60	22.96	19.80	29.90
C	15.38	16.90	33.60	22.40	91.69	35.35
			TIME (secs)			
A	10.07	7.41	8.70	6.04	6.20	7.70
B	5.38	5.72	5.51	4.55	4.44	5.87
C	3.37	4.32	7.03	4.58	17.61	5.25
			SPEED (cm/sec)			
A	4.50	4.07	4.00	4.14	4.06	4.61
B	5.52	4.41	5.18	4.80	4.28	4.55
C	3.98	4.00	4.22	2.14	5.32	4.18

There is no obvious relation between the average distance, duration or speed of swimming excursions and light intensity, as there is with frequency of swimming (Table X). Both the distance and the duration of swimming excursions vary considerably, and even under constant conditions, there is no constancy in the length of the excursions (fig.48).

Conclusions

The almost total lack of information about the behaviour and ecology of Nephtys makes it impossible to discuss the biological significance of this periodic activity. But whatever its significance, the periodic activity and the environmental factors which influence it have some bearing on the design of experiments involving Nephtys. From the observations that have been described here, it is clear that it is important to standardise methods rigidly. The worms do not settle down to a regular pattern of activity for an hour or more after they have been placed in an aquarium, and if a circular vessel is used, the worms must all be the same length or differences in the level of activity will be caused. The light intensity also must be constant, or at any rate, fluctuating outside the range in which it affects the worms.

CAPTIONS TO FIGURES

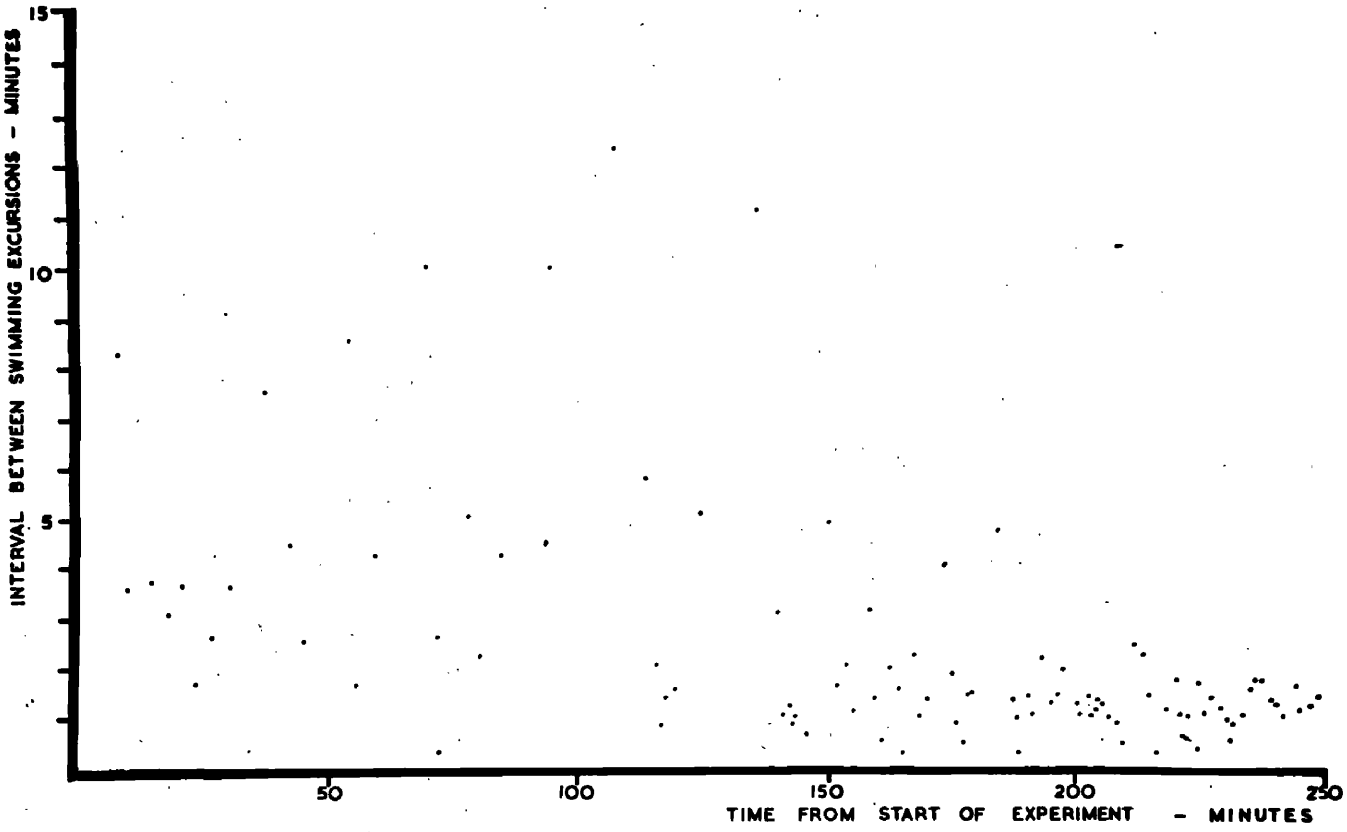
Figure 47. The activity of a single specimen of N. cirrosa during 250 minutes after placing it in a rectangular tank.

Figure 48. Frequency distribution of the duration of swimming excursions of N. cirrosa.

Figure 49. The activity of a single specimen of N. cirrosa, 90 mm long, in a circular vessel 100 mm in diameter. The record starts immediately after placing the worm in the dish.

Figure 50. Activity of the same worm as in Figure 49 after the removal of the supra-oesophageal ganglion. The record starts 15 minutes after removing the ganglion and immediately after placing the worm in the dish.

Figure 51. The effect of curvature on the swimming activity of N. cirrosa and N. caeca. Activity measured in swimming excursions per hour.


INTERVAL BETWEEN SWIMMING EXCURSIONS - MINUTES
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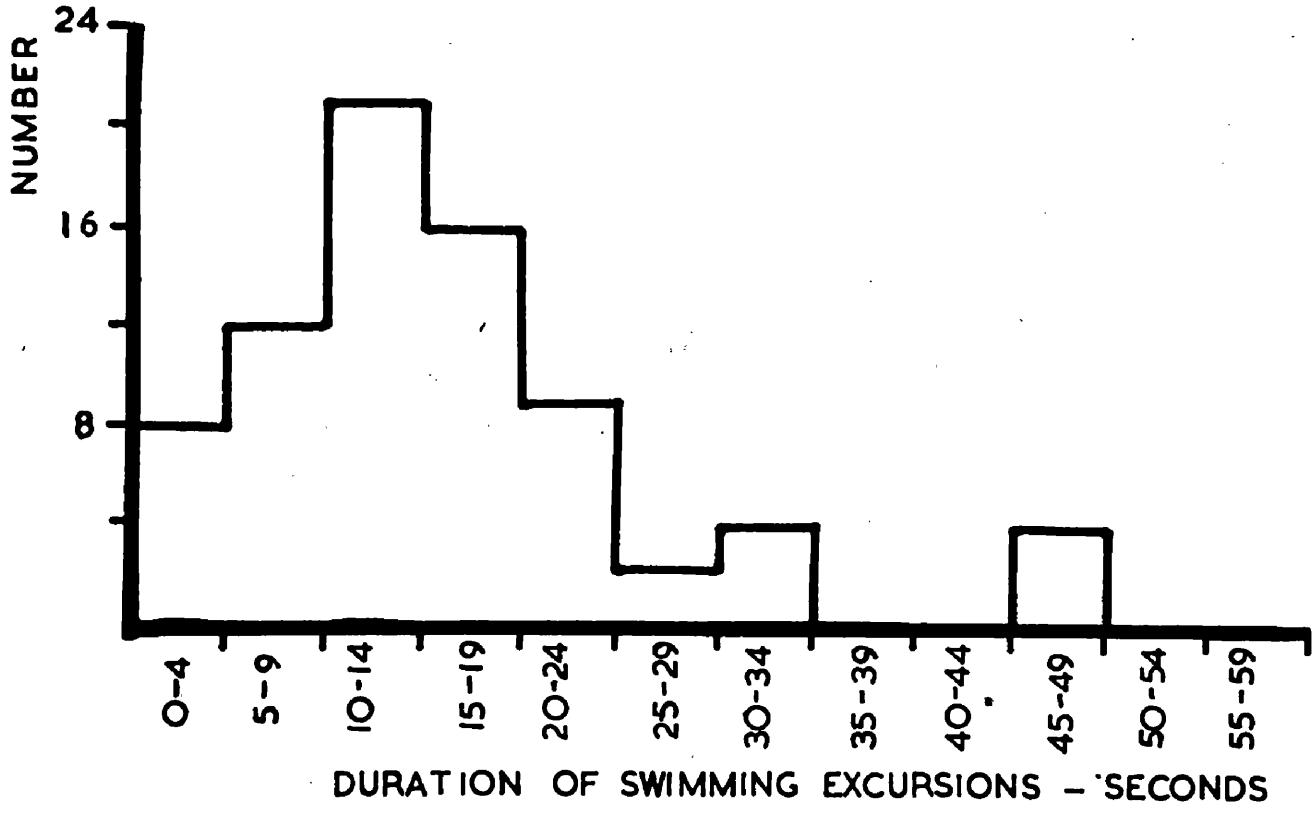
5
50
100
150
200
250
TIME FROM START OF EXPERIMENT - MINUTES

47


NUMBER
24
16
8
0-4
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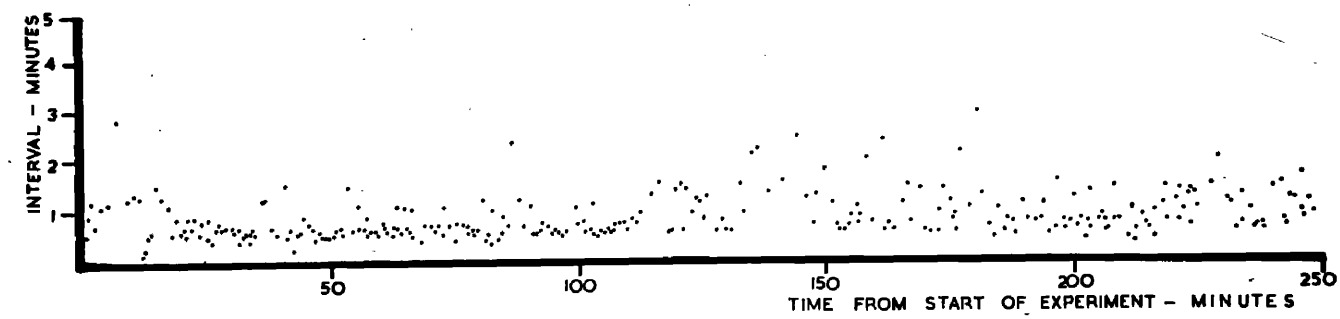
55-59
DURATION OF SWIMMING EXCURSIONS - SECONDS

48


INTERVAL - MINUTES
5
4
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2
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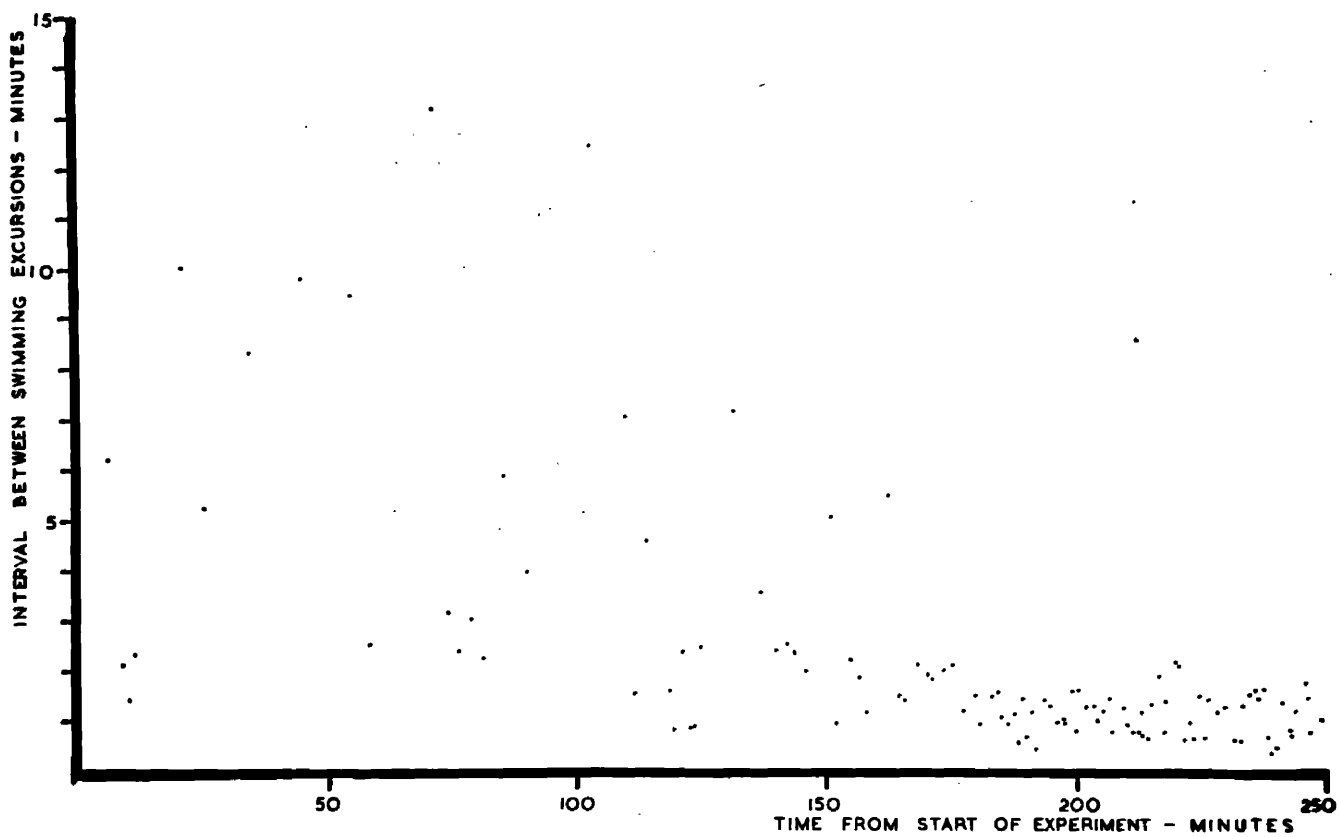
TIME FROM START OF EXPERIMENT - MINUTES

49


INTERVAL BETWEEN SWIMMING EXCURSIONS - MINUTES
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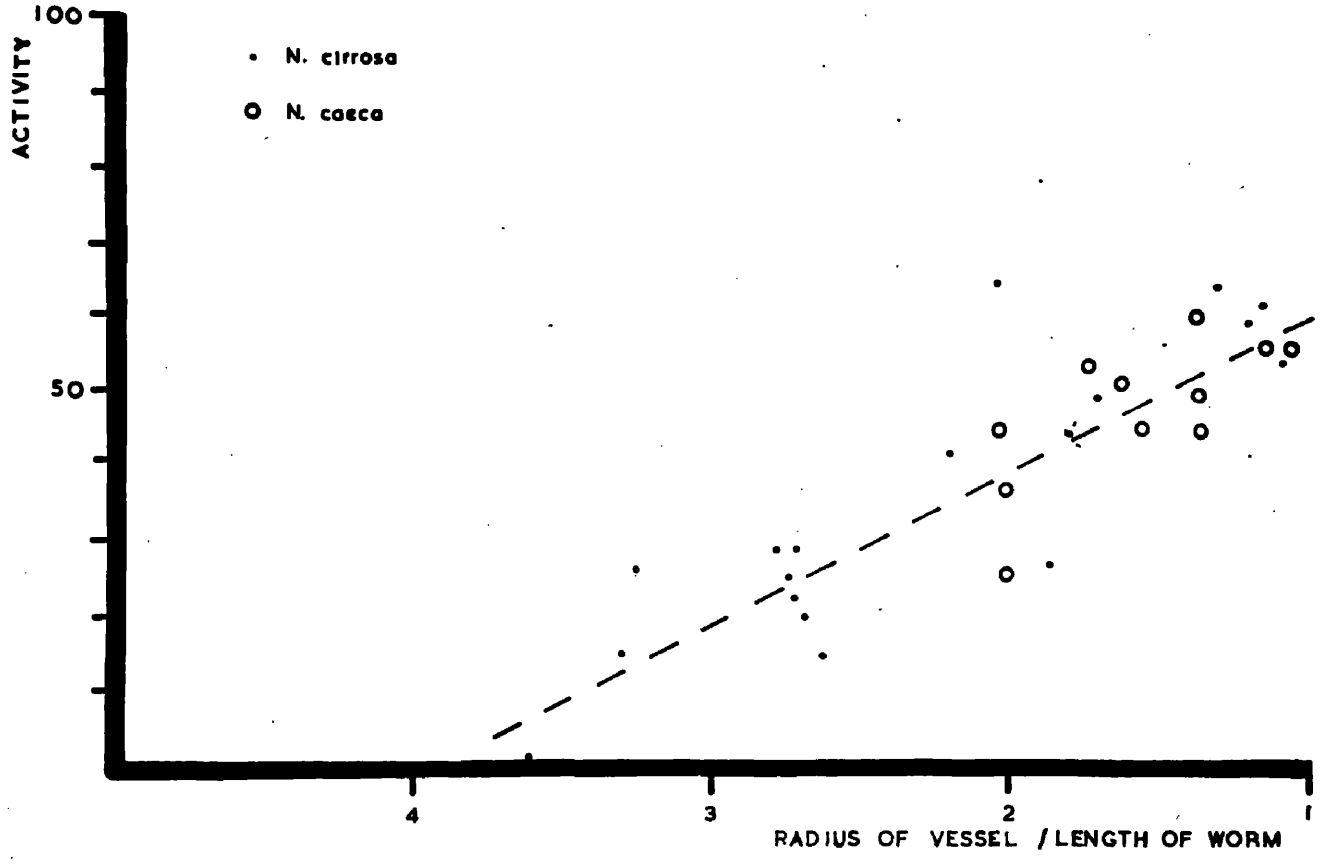
TIME FROM START OF EXPERIMENT - MINUTES

50


100
50
ACTIVITY
• N. cirrosa
O N. caeca
4
3
2
1
RADIUS OF VESSEL / LENGTH OF WORM

APPENDIX II

THE BLOOD VASCULAR SYSTEM

THE BLOOD VASCULAR SYSTEM

There has been no detailed study of the blood vascular system of Nephtys, although several nineteenth century zoologists give superficial, and mutually contradictory accounts of it. The circulatory system of N. caeca has been described by Ehlers (1864-8) and by Schack (1886) and that of N. hombergi by Milne-Edwards (1837), Jaquet (1886) and by Saint-Joseph (1894). The blood vascular system of Nephtys does not afford any anatomical surprises; it follows the general pattern found in other errant polychaetes. There are four main longitudinal vessels, these are dorsal, sub-intestinal, and paired neural vessels. Except in the anterior specialised segments, the four vessels communicate with each other directly by way of the segmental vessels, while in the anterior part of the worm, the dorsal vessel bifurcates to form the circum-oral vessels, which in turn become the neural vessels. A pair of vessels from the dorsal longitudinal one communicate with the sub-intestinal vessel by way of the proboscidial circulation. There is generally a complete segmental and a complete longitudinal circulation. In the anterior thirty or so segments, the segmental circulation is modified as an adaptation to the presence of the large muscular, eversible pharynx. The following account is based mainly on an examination of N. californiensis.

The Anatomy of the Vascular System

The whole worm, when fully grown, includes some 120-150 segments. The proboscidial apparatus, consisting of a thin-walled buccal region, a stout, muscular pharynx, and the retractor and protractor muscles, occupies the anterior 35 of them. The first ten segments are somewhat smaller than the succeeding ones, so the inverted proboscis occupies one-fifth to one-sixth of the total length of the worm. For convenience, this modified anterior part of the worm which houses the extrovert will be referred to as the proboscidial region.

The dorsal vessel for most of its course lies embedded beneath the muscle coats of the intestine. It is about 0.1 mm in diameter in a fully grown worm and does not taper appreciably except at the extreme posterior end. As it approaches the proboscidial region it is thrown into tight folds (segments XL-XXXV) and then, in segment XXXV, it dilates to form a bulb lying at the junction between the intestine and the pharynx. In the course of the next fifteen segments it crosses from the gut to the dorsal body wall and completes its anterior course suspended between the two halves of the dorsal longitudinal muscle by a fine connective tissue mesentery. In its passage from intestine to dorsal body wall, the dorsal vessel is thrown into a

generous loop which lies freely in the coelom, attached to the intestine at one end and to the mesentary at the other, but unsupported in between. Because of its disposition, the dorsal vessel is not visible from the exterior, as is that of Nereis, for example, except for a few segments anterior to segment XX, where the dorsal longitudinal muscles do not meet completely in the mid-line and the blood vessel can be seen indistinctly between them by transparency. In segment II, two branches of much the same diameter as the dorsal vessel arise from it and run, unattached, back through the coelom to the papillae at the anterior end of the pharynx (figs.52 and 53). When the proboscis is everted, these vessels run out through the mouth between the thin buccal sheath of the proboscis and the muscular pharynx, as the dorso-lateral proboscidial vessels. In segment I, at the posterior margin of the supra-oesophageal ganglion, the dorsal vessel bifurcates, though its two branches do not immediately separate. Instead, they run side by side, with frequent anastomoses between them, and form part of the cerebro-vascular complex (fig.52). This is described in greater detail in a previous section. At the anterior margin of the ganglion the two branches diverge and for a short distance follow the circum-oesophageal connectives, but in segment I they leave them to supply the body wall of segments I and II. The vessels return to

follow the connectives in segment II and converge on the sub-oesophageal ganglion, which lies in segment V. These two circum-oral blood-vessels do not meet ventrally, but pursue separate courses, one on each side of the ventral nerve cord, as the neural blood vessels.

The sub-intestinal vessel is a little narrower than the dorsal vessel and is also embedded in the muscle coats of the intestine for most of its course. In the proboscidial region this, like the dorsal vessel, is detached and it runs freely along the ventral surface of the pharynx to the terminal papillae as the ventral proboscidial vessel.

In the anterior part of the worm the dorsal and sub-intestinal vessels communicate with each other by way of the proboscidial circulatory system (fig.53). They give rise to the paired dorso-lateral and ventral probiscidial vessels respectively, and, when the probiscis is everted, they lie unattached between the buccal sheath and the muscular pharynx. At the distal end of the proboscis the ventral vessel divides and each branch runs round the side of the extrovert. There is a fine anastomosis between the two dorso-lateral vessels before they diverge to run to the sides of the proboscis. Jaquet (1886) disputed the presence of this anastomosis, which had previously been reported by Milne-Edwards (1837) in N. hombergi. It is rather difficult to see because it frequently drains of blood and

becomes invisible when a tension is applied to the vessels during dissection. Possibly this anastomosis is a regular feature of the blood vascular system of Nephtys which Jaquet overlooked. At the sides of the proboscis the vessels disappear beneath the bases of the papillae muscles to form a complicated ring with loops running into each of the eleven terminal papillae on each side of the probiscis. Nowhere does the system break up into capillaries or a fine plexus such as Nicoll (1954) described surrounding the muscular extrovert of Nereis limbata and Nereis virens. Furthermore, there is not a simple ring vessel surrounding the extrovert, connecting the dorso-lateral and ventral proboscidial vessels, as Schack (1886) described in Nephtys caeca, and the proboscidial circulation is somewhat more complicated, though essentially similar to, that described and figured by Ehlers (1864-8) in the same species.

The vascular system of an unmodified segment, that is one posterior to the proboscidial region of the worm, is illustrated in fig.54. In each segment paired vessels leave the dorsal, sub-intestinal, and ipsi-lateral neural vessels to supply the body wall and parapodia. The segmental vessels leave the dorsal longitudinal blood vessel in about the middle of the segment and run postero-laterally to the septum. In doing so they half encircle the gut, giving off an intestinal vessel which runs to the sub-

intestinal vessel, and pass under the lateral edges of the dorsal longitudinal muscles. A fine branch of the dorsal segmental vessel passes dorsally along the outer edge of the longitudinal muscle, between it and the dorsal parapodial muscles which flank it laterally, and ends where the dorsal muscle meets the dorsum of the segment. Along its course, this dorsal vessel arborises and produces a number of fine, blind-ending diverticula. These are the only type of 'capillary' found in Nephtys. Conventional endothelial capillaries have been found in a number of polychaetes (e.g. Nicoll (1954) describes them in Nereis, and Hanson (1949) reviews their occurrence in other polychaetes), but they appear to be missing from Nephtys. The blood vessels do not penetrate into any of the large muscle masses. At the insertion of the parapodium into the body wall, the dorsal segmental vessel bifurcates, the main branch running through the dorsal part of the parapodium to the branchiae and the inter-ramal area; the finer, intersegmental branch runs ventrally, following the insertion of the parapodium into the body wall just in front of the anterior face of the septum. It gives off a great many blind capillaries which lie in the posterior wall of the parapodium and in the intersegmental part of the body wall. The intersegmental vessel finally joins the sub-intestinal vessel.

The subintestinal segmental vessels, like those of the dorsal vessel, arise mid-segmentally and after partly encircling the gut, run posteriorly and laterally to the septum. They are quite long and loop down into the coelom, presumably to allow for changes in the shape of the segment during the locomotion of the worm. These vessels pass to the ventral, posterior, lateral corners of the segment and there communicate with the intersegmental branch of the dorsal vessel. They give off a small nephridial branch and also communicate with the trans-septal branch of the neural segmental vessel. The main branch runs into the neuropodium where it arborises.

The neural segmental vessels arise in the anterior part of the segment. They run up the sides of the nerve cord, are coiled into a small loop lying on top of it, and then run between the ventral longitudinal muscles and the diagonal muscles lying on top of them directly to the base of the neuropodium at its insertion into the body wall in the anterior part of the segment. After coiling into a second loop, the neural segmental vessel disappears between the parapodial muscles. At this point, at the lateral edge of the ventral longitudinal muscles, the blood vessel bifurcates. One branch runs anteriorly through the septum into the segment in front, the other runs posteriorly a short distance, gives off a small gonadial vessel, and runs

into the neuropodium.

While a significant proportion of gaseous exchange must take place across the body wall of the worm, the parapodia are the most important respiratory surfaces. The thin-walled branchiae and the inter-ramal areas are heavily ciliated (fig.55) and the entire parapodium is highly vascularised. There are, as we have seen, three main blood vessels entering or leaving the parapodium, one from each of the segmental vessels. The notopodial branch of the dorsal segmental vessel divides to form the branchial and inter-ramal vessels. A number of fine vessels from both branches supply the anterior and posterior walls of the parapodium, particularly in the dorsal half, but some extend into the neuropodium. Schack (1886) describes a small vessel in the dorsal cirrus of N. caeca and I have been able to confirm its presence in that species and in N. hombergi, but it does not occur in N. californiensis. The branchial vessel becomes very narrow and coils within the branchia; it is attached to the branchial epithelium, but apparently sufficiently loosely for the coils to slide fairly freely over each other. The return vessel from the branchia runs ventrally to the neuropodium as the deep inter-ramal branch. Both the deep and the superficial vessel give off numerous blind capillaries in the neuropodium and eventually they communicate with the two ventral vessels

those from the sub-intestinal and neural segmental vessels. Jaquin (1886) described a very fine capillary bed lying in the neuropodial post-acicular lamella of N. hombergi. He said that it was not easy to see and was frequently invisible. After careful examination, I have not been able to find any such plexus in N. californiensis. The parapodia in the middle part of the worm are highly vascularised; those at the extreme posterior and anterior ends are less so. The vascularisation is for the most part in the form of numerous blind capillaries which, in the middle segments, occupy all the space not taken up with muscles. Those capillaries in the posterior wall of the parapodium and in the intersegmental body wall are drawn from the intersegmental vessel, those in the neuropodium from the sub-intestinal and neural segmental vessels and also from the two inter-ramal vessels. There are relatively few blood-vessels in the notopodium.

In every segment, other than those in the proboscidial region, two fine vessels from the dorsal blood vessel encircle the intestine and communicate with the sub-intestinal vessel. These intestinal vessels are serpentine, presumably to permit dilation of the gut, but they do not break up into a plexus. There is no vascular supply to the pharynx except for the proboscidial circulatory system described previously.

The arrangement of the segmental blood vessels is modified in the anterior thirty-five segments which comprise the proboscidial region of the worm (figs.52 and 56). In these segments there are neither dorsal nor sub-intestinal segmental vessels, but the modifications appear even posterior to this. There are no sub-intestinal segmental vessels anterior to segment L, and between segments XL and XXXV the dorsal segmental vessels are considerably elongated, so much so that those of segment XXXV are twice as long as those of segment XLV. The intersegmental branch of the dorsal segmental vessel is a typical segment, runs into the sub-intestinal segmental vessel, but when the latter is missing (as in segments L-XXXV) the intersegmental branch communicates with the trans-septal branch of the neural segmental vessel. This does not involve the development of any new connections, since the trans-septal vessel communicates with the sub-intestinal vessel and by way of it with the intersegmental vessel, in any case. In the proboscidial segments, where there is neither dorsal nor sub-intestinal vessel, the intersegmental branch assumes a new importance. All the peripheral parts of the vascular system in these segments are the same as in more posterior, typical segments and the only way in which blood reaches the dorsal muscle and the parapodium is by way of the neural segmental and intersegmental vessels. This becomes a

large vessel in these segments, of the same diameter as the neural segmental vessel, unlike the slender intersegmental vessel in more posterior segments. The result is that in segments of the proboscidial region, the neural segmental vessel crosses the anterior part of the segment between the ventral longitudinal and diagonal muscles and passes into the segment in front (there are no septa, so it cannot be described as 'trans-septal' as in posterior segments). There it coils into a loop before sending one branch into the neuropodium. The main branch runs dorsally, in the same position as the intersegmental branch of posterior segments, to the dorsal part of the parapodium, where it sends one branch into the notopodium and another to the dorsal longitudinal muscle. The blood supply to each parapodium is therefore from the neural segmental vessel of the segment behind and there are only two vessels entering the parapodium, one dorsally and one ventrally.

The first five segments are further modified (fig.52). As in other anterior segments, blood vessels originating in segments V, IV, III, and II supply the intersegmental area, the parapodia and the latero-dorsal body wall of segments IV, III, II, and I, respectively. They all take their origin from the circum-oral vessels, which are, of course, continuations of the neural longitudinal vessels. The neural segmental vessels originating in segments V and

IV first run back along the circum-oral vessels to coil extensively over the sub-oesophageal ganglion before running back along themselves to their respective segments. The segmental vessels arising in III and II run directly to segments II and I and are connected by an intersegmental loop of considerable diameter at the level of the notopodia. Since the first and second segments are abranchiate, their parapodial circulation is correspondingly reduced and modified. Two very fine vessels leave the circum-oral vessels in segment I to supply the lateral lips.

The Adaptations of the Anterior Vascular System

The modifications of the anterior 40-50 segments can be attributed to the presence of a large muscular pharynx and to the fact that it is eversible. There is a considerable relative movement between it and the body wall of the segments through which it passes as the proboscis is everted and, in consequence, there are no dorsal or sub-intestinal vessels in the proboscidial region. In addition, those vessels which are attached to the pharynx, the dorsal vessel at its posterior end and the dorso-lateral and ventral proboscidial vessels at its anterior end, all lie freely in the coelom and are long enough to permit the complete eversion of the pharynx. The anterior part of the intestine must also be stretched as the proboscis is everted and in this region there are no sub-intestinal

segmental vessels and the dorsal segmental vessels become progressively longer and longer to allow for the displacement of the intestine relative to the body wall. The problems posed by the existence of a large, muscular, eversible pharynx are not all solved by the segmental vessels running in the body wall instead of across the coelom, however. In Nephtys the first eight or ten segments are commonly smaller in diameter than the pharynx which has to pass through them as it is everted; when retracted it lies posterior to them. The first five segments are specially modified to permit the passage of the proboscis. The ventral floor of these segments is replaced by a muscular gular membrane which is folded and normally tucked within the lateral lips formed by the edges of the lateral walls of these segments. The buccal part of the gut, which forms the thin-walled sheath of the extrovert, is attached to the anterior end of the gular membrane and to the lateral lips. When the proboscis is everted, the lateral lips are thrust aside and the gular membrane is tightly stretched. The most extreme distortion of the anterior five segments is therefore restricted to the gular membrane, and the sub-oesophageal ganglion lies at its posterior margin, in segment V. The nerves and blood vessels run in the lateral walls of segments I-V, which are displaced but not immoderately stretched when the proboscis passes

between them.

The segments immediately posterior to V have no such elastic gusset, and the body wall with its blood vessels is correspondingly distended by the passage of the pharynx. The neural segmental vessels are coiled into a loop on top of the ventral nerve cord and also at the base of the lateral body wall, proximal to the origin of the neuropodial vessel. The latter is certainly an adaptation to permit the distension of the body wall. The blood vessels are fairly strong and will resist longitudinal tension, but they cannot be stretched, at least not macroscopically. If the body wall is cut on each side, immediately above the parapodia, and the dorsum removed, these loops are clearly visible, but if the body wall is stretched in a dissection, the loops disappear. The loops over the ventral nerve cord are more doubtfully connected with permitting the stretching of the floor of the segment. The body wall cannot be stretched in such a way as to extend the loops, the diagonal muscles tear away before this stage is reached. Indeed, in one specimen of N. californiensis, an abnormal anastomosis had been formed between the two loops on each side of the nerve cord in one anterior segment, which would totally have defeated the purpose of these loops did they serve to permit the extension of the ventral body wall. It is more likely that they serve to provide a greater area

of contact between the blood vessel and the nerve cord to which they are closely apposed, though not attached, and so serve a respiratory function alone. This idea is strengthened by the elaboration and convolution of these loops in the region of the sub-oesophageal ganglion, which is a good deal thicker than succeeding ganglia.

The Cerebro-Vascular Complex

Another modified part of the vascular system is the plexus formed between the two branches of the dorsal vessel where it runs over the base of the supra-oesophageal ganglion. This has been discussed in detail in a previous section.

Circulation and Respiration

The chief contractile vessel, as in all polychaetes, is the dorsal longitudinal vessel. Peristaltic contractile waves pass along it from behind forwards as far as the bulb which lies at junction between the intestine and the pharynx. Saint-Joseph (1894) described the bulb as a 'heart', presumably in the sense that it is the chief propulsive part of the vascular system. In fact, neither it nor the dorsal vessel anterior to it show any but the slightest contractions and it seems likely that it acts as a sort of expansion chamber to equalise the flow into the the anterior vascular system. All the blood vessels, even

the fine, blind ending capillaries, appear to be contractile, though none of them show strong contractions at all comparable to those of the dorsal vessel.

The walls of polychaete and oligochaete blood vessels are commonly composed of three layers (Hanson, 1949); 1. an endothelium which may occasionally be in the form of a continuous layer, but is often reticulate and possibly sometimes composed of isolated cells; 2. a collagenous connective tissue, or skeletal layer; 3. an outer, peritoneal layer differentiated into a muscle coat, or, more often, with contractile fibres in the tails of stellate cells; no contractile fibres have been detected in the peritoneal epithelium of some of the smaller vessels of certain annelids. Retzius (1891) described stellate muscle cells on the finer vessels of Nephtys and by vital methylene blue staining, I have been able to repeat his observations. Stellate cells also occur on the walls of the segmental vessels and the blind capillaries of Nephtys californiensis; the dorsal longitudinal vessel has a complete muscular coat. Nowhere in the circulatory system are there endothelial capillaries such as are found in Nereis (Hanson, 1949; Nicoll, 1954). This is strong presumptive evidence that all vessels in the circulatory system are contractile, and while I have not been able to see contractions in the blind capillaries, I have watched the irregular, inter-

mittent contractions of the branchial vessels in parapodia removed from the body.

Nicoll (1954) in his analysis of the segmental circulation of Nereis virens and N. limbata, has demonstrated that the flow is from the sub-intestinal vessel, through the capillary beds of the parapodium, and then medially to the dorsal vessel. The return flow from the dorsal vessel to the sub-intestinal vessel is by way of an intestinal capillary plexus and a by-pass vessel which short-circuits the lower half of the plexus.

At first sight it appears from the anatomy of the vascular system of Nephtys that essentially the same segmental circulation may occur as in Nereis. However, the system in Nephtys is complicated by two factors. The circum-intestinal vessels and the inter-segmental branch of the dorsal segmental vessel may be held to be analogues of the intestinal plexus and the by-pass of the intestinal plexus, which in Nereis return blood from the dorsal to the sub-intestinal vessel. These are narrow and insignificant blood vessels in Nephtys and the quantity of blood flowing through them cannot be great, so that segmental circulation, while not interrupted, must at least be impeded. The second factor which must complicate the segmental circulation, is that while in Nereis there is no direct connection between the dorsal and sub-intestinal vessels in the

anterior part of the worm, in Nephtys there is a pair of blood vessels of considerable size connecting them by way of the proboscidial circulation. Even in Nereis, where there is what one would call, on morphological grounds, a complete and direct segmental circulation and a relatively imperfect longitudinal circulation, the segmental circulation is subordinate to the longitudinal. According to Nicoll, if both dorsal and sub-intestinal vessels are ligatured, a few segments apart, the intervening segments are quickly drained of blood. In Nephtys, where the segmental circulation appears to be relatively incomplete and the longitudinal circulation extremely well developed, segmental circulation must be even more dependent upon longitudinal.

In anterior segments, where dorsal and sub-intestinal segmental vessels are lacking, it is difficult to explain segmental circulation at all. The sub-intestinal segmental vessel is to some extent dispensable, since its function is duplicated by the neural segmental vessel, and in fact it is missing from about 15 segments (XXXV-L). However, in all the proboscial segments, the neural vessels alone exist and presumably most of the blood runs to the neuropodium and then back into the same vessel from the notopodium. Unless there is a periodic reversal of flow in the neural segmental vessel, it is difficult to imagine how parapodial

blood of these segments can be restored to general circulation, for there are no valves in the blood vessels; but this reversal has never been observed. Evidently the circulatory system of these segments is more than theoretically inefficient. The vascularisation of the anterior parapodia is much reduced and the branchiae are often small and frequently lost altogether. In N. californiensis all but the first two segments carry branchiae, but in N. punctata and some other species, they are missing from the first ten segments.

It is surprising that in Nephtys there should be such a poor blood supply to the massive dorsal and ventral longitudinal muscles. In Nereis, both sets of muscles have their own blood supply (Nicoll, 1954), and in serpulids and sabellids there are blood vessels penetrating the dorsal longitudinal muscles (Hanson, 1950). Presumably in Nephtys there is a sufficient area of blood vessels exposed to coelomic fluid for it to act as an important oxygen transport system. Direct gaseous exchange across the dorsal and ventral body walls, of which these muscles form part, is no doubt also of great importance.

The only structures in the body with a well developed vascular supply are the gonads and the nervous system. Capillaries penetrate the ovary and project from it in all directions. Thus, not only is the ovary well supplied with

blood vessels within, but the coelomic fluid immediately surrounding it is also probably kept well oxygenated. The gonadial vascular system keeps pace with the development of the ovary so that when the latter is fully developed and fills practically the whole of the ventral part of the coelom, it has a considerable blood supply, drawn not only from the gonadial vessel, but augmented by capillaries of the intersegmental branch of the dorsal segmental vessel and from the neuropodial vessels. The vascular supply to the ventral nerve cord appears to be largely incidental and it has no capillary system. In the middle and posterior segments, the neural segmental vessels run up the sides of the nerve cord from the neural longitudinal vessels, and where they are in contact with the nerve cord they are closely flattened against it. The membrane investing the cord immediately under the vessels is exceptionally thin. In the anterior segments the loops formed by the neural segmental vessels above the nerve cord become more elaborate and do not stick up into the coelom, but are flattened and coiled on top of the ganglia. It is in the most anterior segments that the loops become most elaborate and the sub-oesophageal ganglion is invested dorsally, not only with the loops of the segmental vessels of segment V, but also those of segment IV, which double back from their origin on the circum-oral blood vessels before running to their

appropriate segment. These loops do not appear to have a structural function, but seem instead to be a respiratory device.

CAPTIONS TO FIGURES

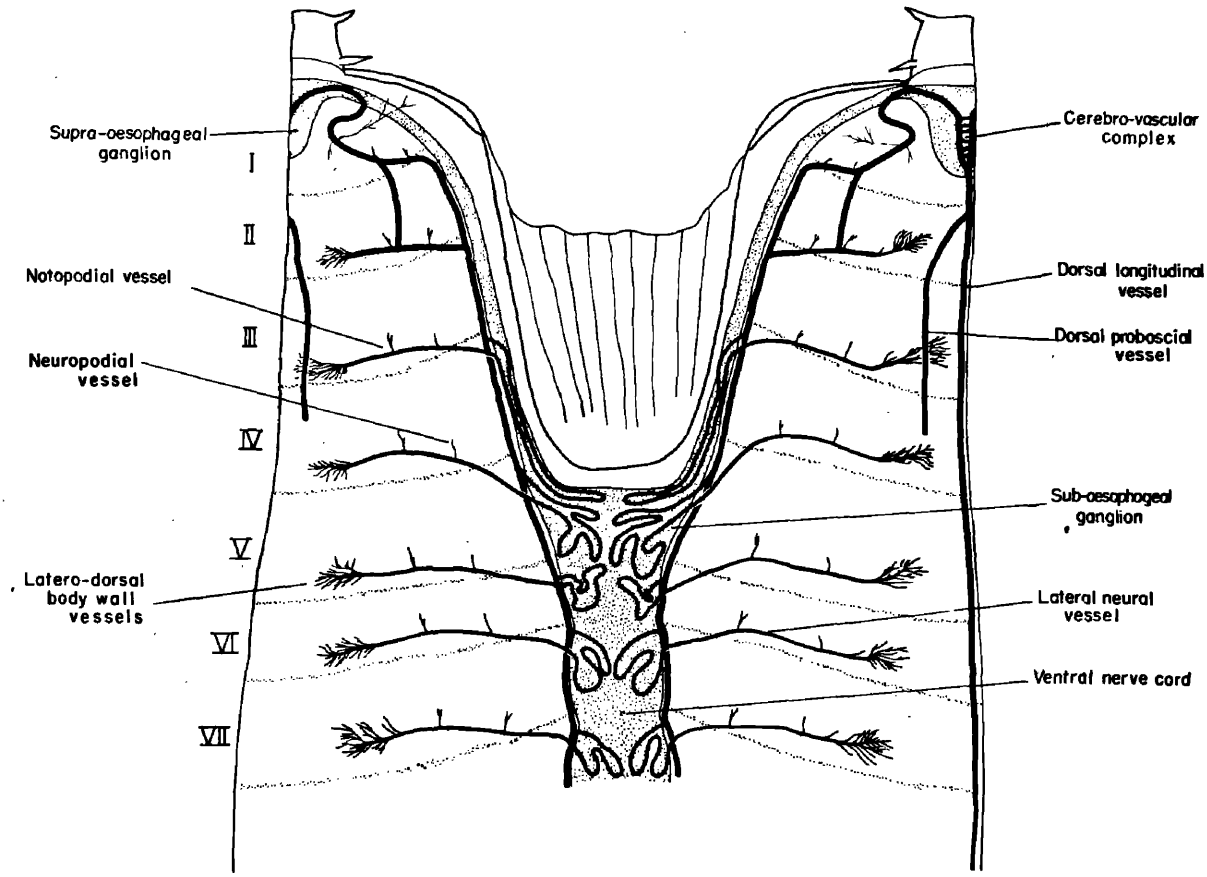
Figure 52. Dissection of the anterior segments of N. californiensis to show the blood vascular system. The worm has been opened by a mid-dorsal incision; the pharynx, and with it the sub-intestinal vessel, has been removed, and the two halves of the body-wall reflected laterally.

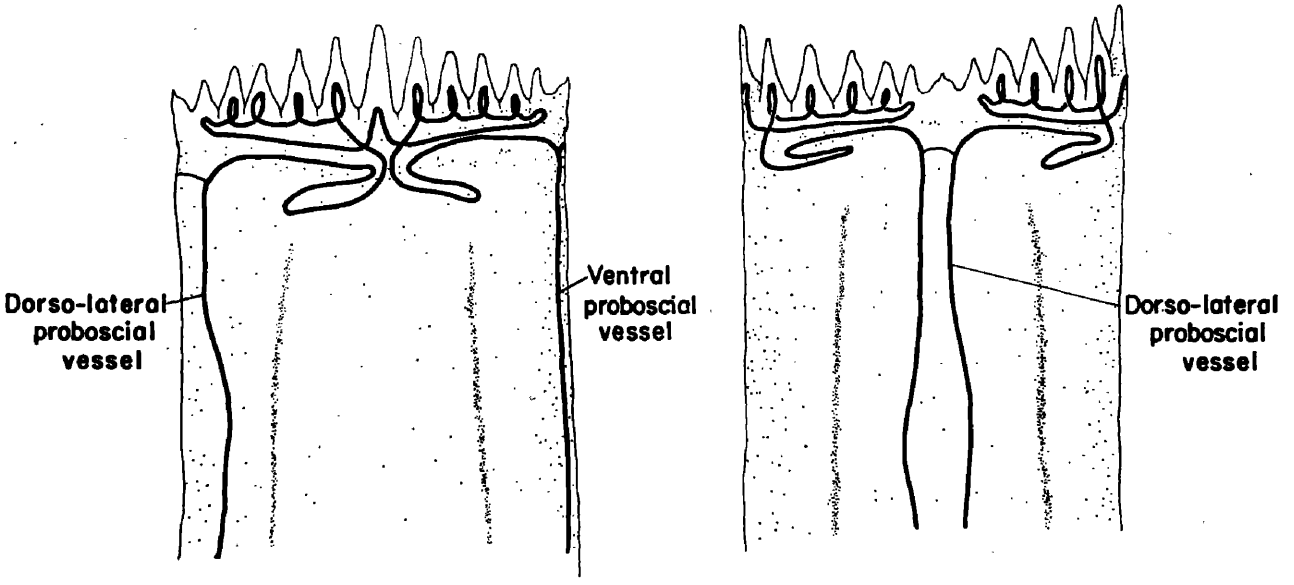
Figure 53. Proboscidial circulation of N. californiensis. A. lateral view; B. dorsal view.

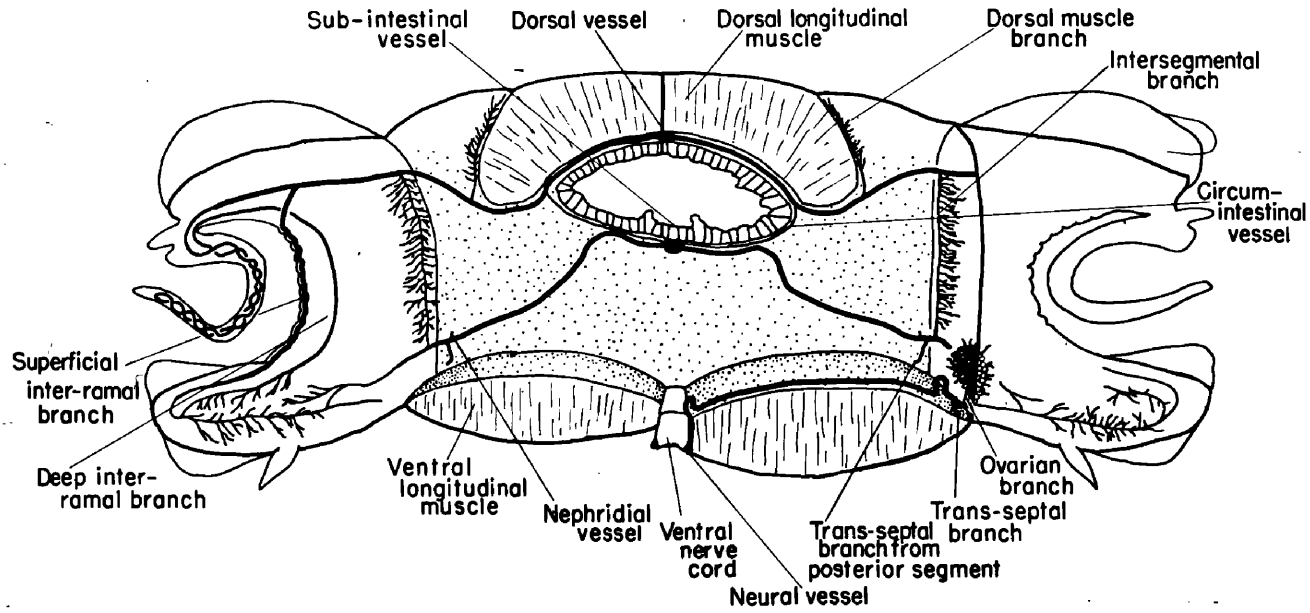
Figure 54. Schematic view of the circulatory system of a segment from the middle region of N. californiensis. The anterior septum has been removed, the posterior one is stippled. The anterior half of the segment and parapodium has been removed from the left-hand side.

Figure 55. Frontal section through the inter-ramal region of the parapodium of N. californiensis which passes through the branchia.

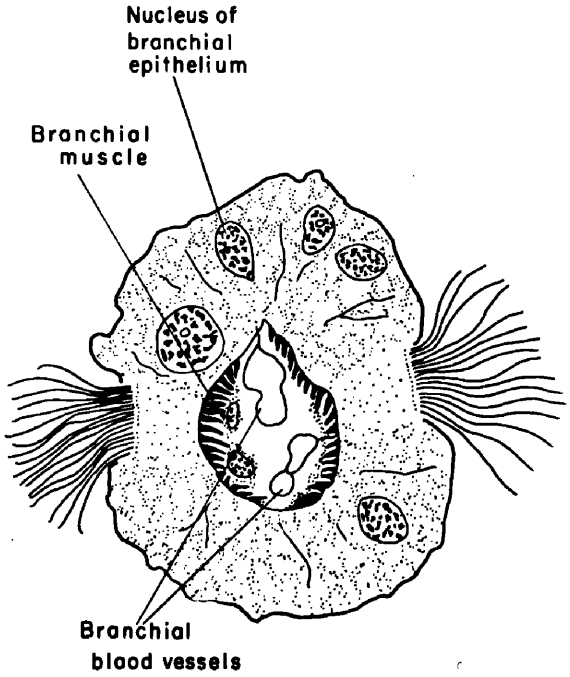
Figure 56. Schematic view of the circulatory system of a segment from the anterior region of N. californiensis. The anterior half of the segment and parapodium has been removed from the left-hand side.

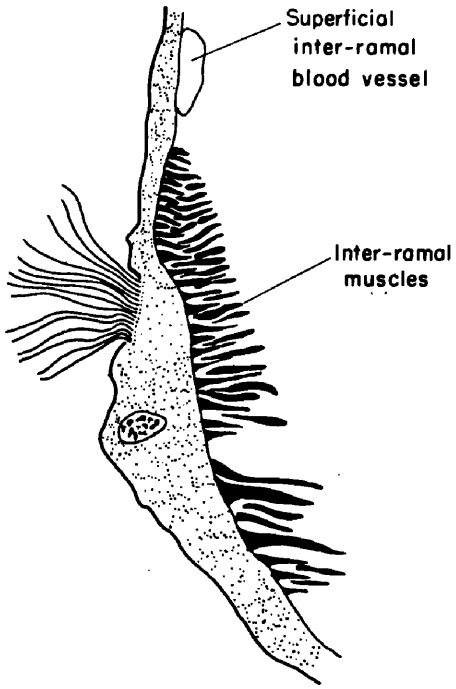

Supra-oesophageal ganglion
I
II
Notopodial vessel
III
Neuropodial vessel
IV
V
Latero-dorsal body wall vessels
VI
VII
Cerebro-vascular complex
Dorsal longitudinal vessel
Dorsal proboscial vessel
Sub-oesophageal ganglion
Lateral neural vessel
Ventral nerve cord


Dorso-lateral proboscial vessel
Ventral proboscial vessel
Dorso-lateral proboscial vessel

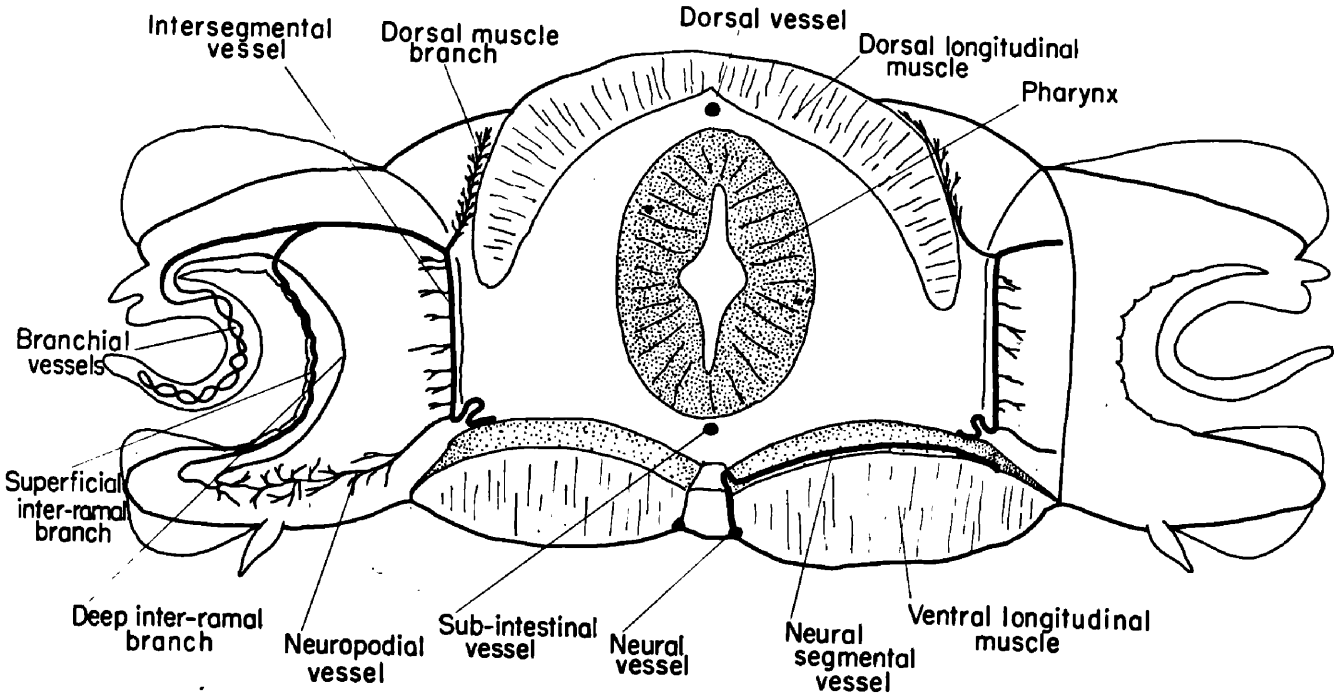

Sub-intestinal vessel
Dorsal vessel
Dorsal longitudinal muscle
Dorsal muscle branch
Intersegmental branch
Circum-intestinal vessel
Superficial inter-ramal branch
Deep inter-ramal branch
Ventral longitudinal muscle
Nephridial vessel
Ventral nerve cord
Neural vessel
Trans-septal branch from posterior segment
Trans-septal branch
Ovarian branch

54


Nucleus of branchial epithelium
Branchial muscle
Branchial blood vessels


Superficial inter-ramal blood vessel
Inter-ramal muscles

55


Intersegmental vessel
Dorsal muscle branch
Dorsal vessel
Dorsal longitudinal muscle
Pharynx
Branchial vessels
Superficial inter-ramal branch
Deep inter-ramal branch
Neuropodial vessel
Sub-intestinal vessel
Neural vessel
Neural segmental vessel
Ventral longitudinal muscle

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