The Posterior Lobes of the Brain of *Nephtys* and the Mucus-Glands of the Prostomium

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With one plate (fig. 4)

SUMMARY

A pair of lobes is attached to the posterior margin of the supra-oesophageal ganglion of some species of *Nephtys*. They are filled with large, vacuolated mucus-cells similar in appearance and histochemical properties to those found in the anterior part of the prostomium, in the lateral walls of it, and in the parapodia. The mucus-cells of the posterior lobes, and sometimes those of the anterior prostomial group also, have long necks which run in a tract to the epidermis, where they open to the exterior. When this is so, they replace the epidermal mucus-cells found in the lateral walls of the prostomium in species lacking posterior lobes. It is suggested that there has been a centripetal migration of epidermal mucus-cells into the posterior lobes and to the anterior prostomial group, a phenomenon closely paralleling that found in the evolution of the nermertean cerebral organ. In one species, *Nephtys cirrosa*, the posterior lobe cells appear to have undergone a further modification, for they are much more closely integrated with the nervous system and differ in appearance from those of other species, coming to look, at least superficially, like the majority of neurosecretory cells in the brain.

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 properly 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 crawls around in the substratum which it may leave for spawning and 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

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in detailed structure the brains of all the 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 many obvious differences in the minute structure of the ganglion in different species. In this paper attention will be directed towards a pair of

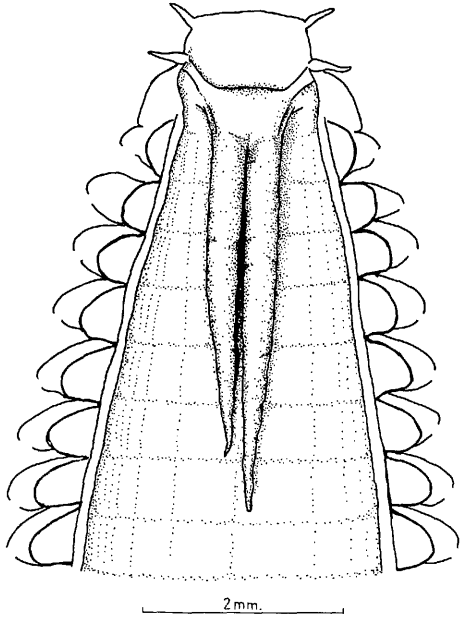

FIG. 1. Supra-oesophageal ganglion and posterior lobes of *N. californiensis*. Dissection from the ventral side.

posterior lobes of the supra-oesophageal ganglion which are present in some species but not in others.

There has been a handful of papers describing the central nervous system of *Nephtys* (Delle Chaije, 1825; Quatrefages, 1850; Claparède, 1868; Ehlers, 1864–8; Pruvot, 1885; Schack, 1886; de Saint-Joseph, 1894), and these are full of contradictions and discrepancies. The contradictions spring from the fact that the authors expected that the nervous system would be identical in all nephtyids and engaged in controversy with others who had examined different species of *Nephtys* with different results. The situation has not been improved by an apparent confusion or misidentification of species (Rullier, 1947; Clark, 1956*c*). Ehlers (1864–8), and Schack (1886) described a pair of cylindrical lobes extending from the posterior margin of the supra-oesophageal ganglion

of *N. caeca* to the fifth segment. Quatrefages (1850) and de Saint-Joseph (1894), who also examined this species, denied their existence. There was general agreement that there were no such lobes in *N. hombergi* and since it was assumed that the brain of a polychaete would not vary so greatly within a single genus, Ehlers and Schack were presumed to have been mistaken in their observations. In fact, they were right and Quatrefages and de Saint-Joseph were wrong. Apart from the (incorrect) observation by Schack that the posterior lobes are filled with exceptionally large ganglion cells, the lobes have not been investigated histologically and the purpose of the present paper is to discuss the nature and homologies of the lobes and their distribution in the Nephtyidae, and to suggest how they may have evolved. This has demanded a comparative study of all the available species of *Nephtys*. There are some 70 species in the family and it has not been possible to collect more than a small number of them. I have been able to supplement these collections with specimens from museums, although these are not ideally fixed for histological work and the examination of them has been comparatively superficial.

Materials and Methods

This study has been based on an examination of the species of *Nephtys* and *Aglaophamus* listed below. The two genera are regarded as sub-genera of *Nephtys* by some systematists. 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; the rest have been collected and fixed in Bouin's fluid made up in sea-water, as a general rule. *N. californiensis* is readily available on the central California coast and has accordingly been used for experimental purposes. It has been fixed in a variety of ways: Bouin's fluid, picroformol, Heidenhain's 'Susa', Zenker-formaldehyde, Zenker-acetic, formalin, and absolute methyl alcohol. All but the last two fixatives have been made up in sea-water mixed with distilled water so as to make the fixative approximately isotonic with sea-water, as a means of reducing shrinkage.

Species examined:

N. caeca (Fabricius)
N. caecoides Hartman
N. californiensis Hartman
N. cirrosa Ehlers
**N. cornuta* Berkeley and Berkeley
N. cornuta franciscana Clark and Jones
N. ferruginea Hartman
**N. glabra* Hartman
N. hombergi Aud. and Edw.
N. incisa Malmgren
N. longosetosa Oersted
**N. magellanica* Hartman
N. parva Clark and 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 usually 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.

Transverse and frontal serial paraffin sections have been cut at 7 or 10μ and 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 neurosecretory products. I have used Gabe's (1953) method of preparing the paraldehyde fuchsin. The final method is thus:

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

Potassium permanganate	3 g
Conc. sulphuric acid	3 ml
Distilled water	1,000 ml

8. Rinse in water.
9. Decolorize 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 (1953). 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 for 10 minutes.

Phosphotungstic acid	4 g
Phosphomolybdic acid	1 g
Distilled water	100 ml

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

Light green (fast green)	0·4 g

Orange G	1·0 g
Chromotrope 2R . . .	0·5 g
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.

Bests results have been obtained with material fixed in Bouin's fluid, but mercuric fixatives are also suitable, though they usually require a stronger solution of paraldehyde fuchsin (dilute the stock solution only 4–5 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. Although lengthy, this technique has the advantage of being a quite delicate stain for nervous tissue and also of giving excellent colour differentiation of various tissues.

Other staining techniques have been used, including Mallory triple stain and a series of histochemical techniques, which will be referred to in the text where appropriate.

Anatomy and Histology of the Posterior Lobes of *Nephtys Californiensis*

The supra-oesophageal ganglion of *N. californiensis* is a trapezoidal structure lying in the posterior part of the prostomium and the anterior part of the first segment (fig. 1). 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. 2). 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. 3). These cells vary in size, but are usually about 100μ long and 40μ wide. The vacuoles may be small and numerous, as in fig. 3, 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 of the cell 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. 4, A). Because of their high degree of vacuolation, these cells in the posterior lobes are difficult to fix satisfactorily. After fixation by mercuric chloride the material in the vacuoles has a reticulate

appearance, but after Bouin's fixation the vacuoles can be seen to be filled with numerous granules or globules of secreted material (fig. 4, B).

The nuclei of posterior lobe cells are roughly oval, approximately $10 \times 15\mu$, and are frequently irregular in outline. Often the nuclear surface has a number

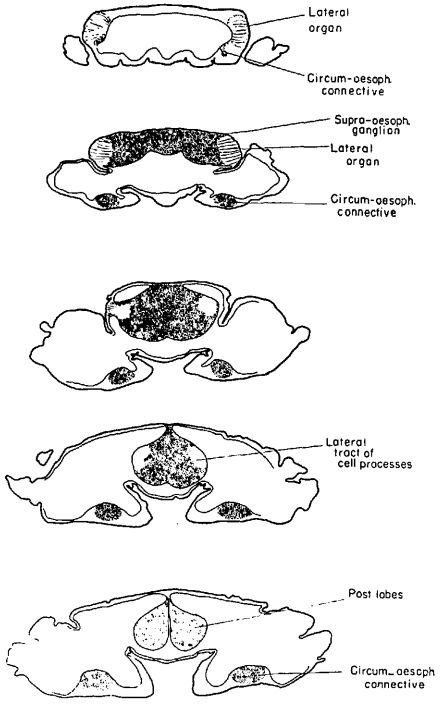

FIG. 2. Series of transverse sections through the prostomium and first segment of *N. californiensis*, to show the relation between the nervous system, the posterior lobes, and the epidermal basement membrane.

of projections and bumps on it. This does not appear to be a fixation artifact 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 and Scharrer, 1954). There is usually a single large nucleolus which is very conspicuous, but in a minority of the 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

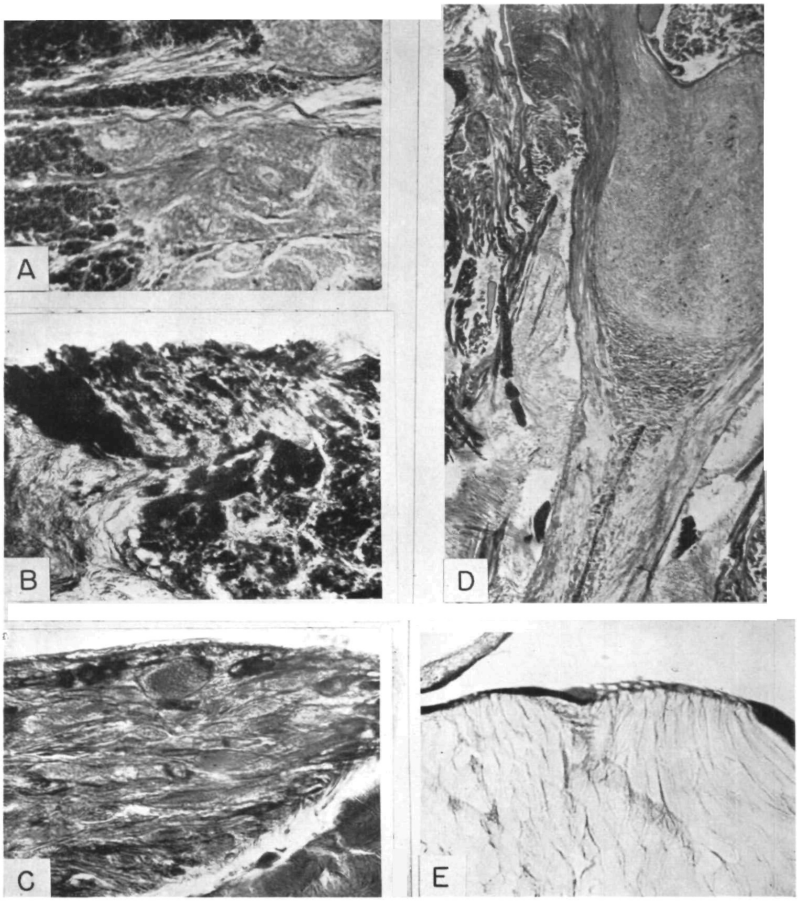

FIG. 4
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processes collect together and run in a tract along each side of the supra-oesophageal ganglion (figs. 2 and 4, D) to the anterior part of it where the circum-oesophageal connectives originate. There they turn sharply through a right angle and run perpendicularly to the lateral walls of the prostomium (fig. 5). The cell processes running to the prostomial walls are very distinctive

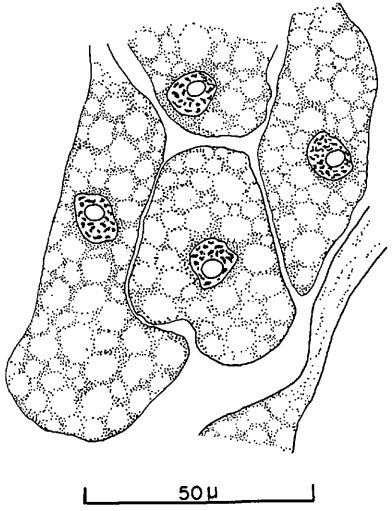

FIG. 3. Cells from the posterior lobes of *N. californiensis*. Camera lucida drawing.

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 eye-stalk. The ends of the

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FIG. 4 (plate). A, mucus-cells of the anterior prostomial group of *N. caeca*. The long necks of the cells are filled with fuchsinophil granules. Bouin; paraldehyde fuchsin; 7μ paraffin sections. No filter.

B, posterior lobe cells of *N. longosetosa* filled with strongly fuchsinophil granules. Granules can be seen filling the cell processes which run towards the upper left-hand corner to the lateral tract. Bouin; paraldehyde fuchsin; 7μ 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; 7μ 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 sides of the ganglion to the lateral organ in the upper left centre of the figure. Zenker-formol; paraldehyde fuchsin; 7μ frontal sections. Wratten 58 filter.

E, lateral organs 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; 10μ paraffin sections. Wratten 25 filter.

processes penetrate into and possibly through the cuticle overlying them. The prostomial cuticle is fairly thick (16μ) in most places, but over the lateral organs it is reduced to a thickness of 2·5μ and over the terminations of the cell processes it is either absent altogether, or else is so thin that it cannot be detected by ordinary histological methods. When the cuticle is stripped from

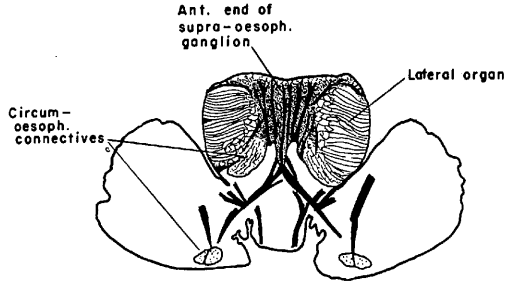

FIG. 5. Transverse section through the prostomium of *N. ferruginea*, showing the lateral organs at their greatest development.

the sides of the prostomium it is seen to be peppered with perforations marking the ends of the cell processes of the lateral organs (fig. 4, E). The lateral organs extend along the sides of the prostomium in front of the supra-oeso-

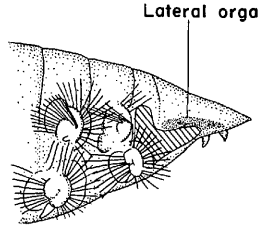

FIG. 6. Lateral view of the prostomium and anterior segments of *N. californiensis*. Cross-hatched area indicates the attachment of the first parapodium which has been removed to expose the area occupied by the lateral organ.

phageal ganglion, almost to the anterior antennae at the antero-lateral corners of the prostomium (fig. 6).

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. 7 and 4, A). 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 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

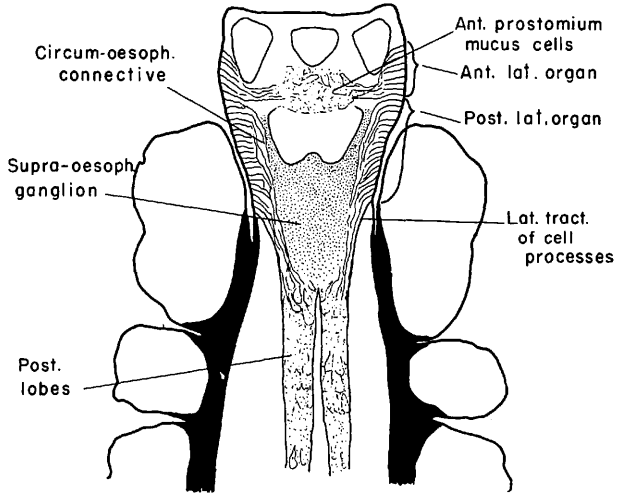

FIG. 7. Composite frontal section through the anterior end of *N. californiensis* to show the relation between the posterior lobes, the lateral tracts, the lateral organs, and the prostomial mucus-cells. Based on camera lucida drawings.

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 its outer surface (Clark, 1956*a*). 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 of neuroglial fibres separates the lateral tracts from the ganglion, although they are all within the same connective tissue sheath (fig. 4, D). 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 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 lateral tracts from the ganglionic material 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 of the ganglion proper takes up light green in the counter-stain, 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 of 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 narcotized and most of the body-wall was trimmed away in the hope of improving 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, muco-proteins, 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 (Southgate, 1927), dialysed iron (Hale, 1946), and alcian blue 8GS (Steedman, 1950), and show metachromasia with toluidine 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, while not conclusive, 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 specialized, 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 specialized mucus-cells in various parts of the body of an animal frequently produce different sorts of mucus (see, for example, 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 3 or 4 cells can be seen 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 of *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 that of 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

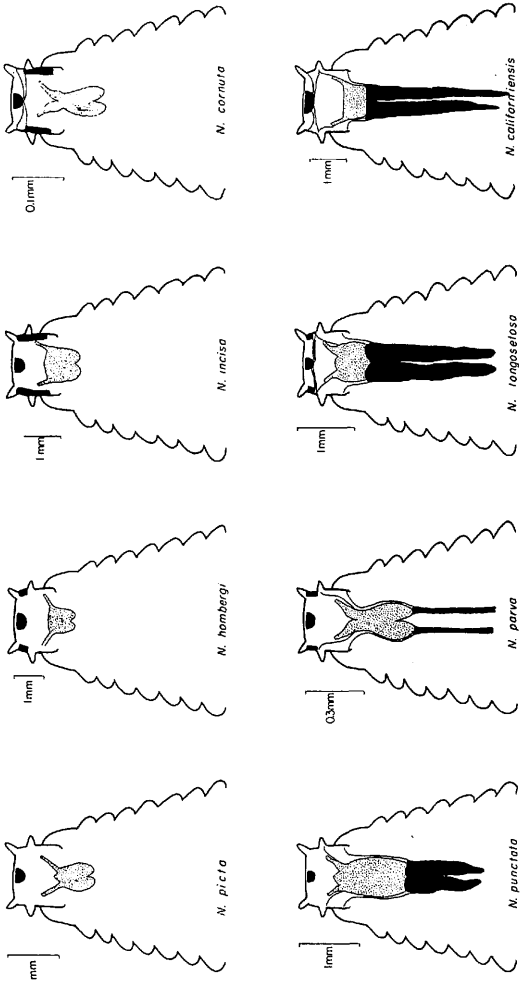

FIG. 8. The various arrangements of the prostomial mucus-gland system found in the Nephtyidae. The first three species in the upper row show an increasing development of the prostomial epidermal mucus-glands; those in the lower row indicate stages in the centripetal migration of epidermal mucus-cells into the posterior lobes and into the anterior prostomial group.

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 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 recognizable group of them is in the median anterior area. These open directly to the exterior.

The anterior and posterior parts of the prostomial 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 summarized in the following table and in fig. 8.

	Post. lobes	*Post. lat. organs*	*Post. lat. mucus-cells*	*Ant. median prostomial group*	*Ant. lat. organs*	*Ant. lat. mucus-cells*
N. californiensis	×	×	—	×	×	—
N. caecoides	×	×	—	×	×	—
N. longosetosa	×	×	—	×	×	—
N. caeca	×	×	—	×	—	—
N. ferruginea	×	×	—	×	—	—
N. glabra	×	×	—	×	—	—
N. magellanica	×	×	—	×	—	—
N. parva	×	×	—	×	—	—
N. rickettsi	×	×	—	×	—	—
N. punctata	×	×	—	×	—	—
N. cornuta	—	—	×	×	×	—
N. cornuta franciscana	—	—	×	×	×	—
N. incisa	—	—	×	×	—	—
N. squamosa	—	—	×	×	—	—
N. hombergi	—	—	—	×	—	—
N. picta	—	—	—	×	—	—
A. dicirris	—	—	—	×	—	—
A. erectans	—	—	—	×	—	—
A. virginis	—	—	—	×	—	—
N. cirrosa	*	*	—	×	×	—

* 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. 9). 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 also 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. 4, c). The cell-bodies of the secretory

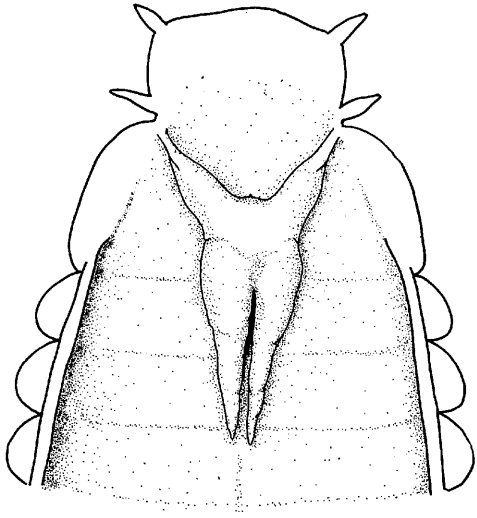

FIG. 9. Supra-oesophageal ganglion and posterior lobes of *N. cirrosa*. Dissection from the ventral side.

cells are about 15μ long by 8μ wide and are packed with strongly 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 forward 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 are positive to paraldehyde fuchsin is essentially the same in *N. cirrosa* as in *N. californiensis* (fig. 10). 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 processes from posterior lobe cells run through the epidermis in the position where lateral organs would be

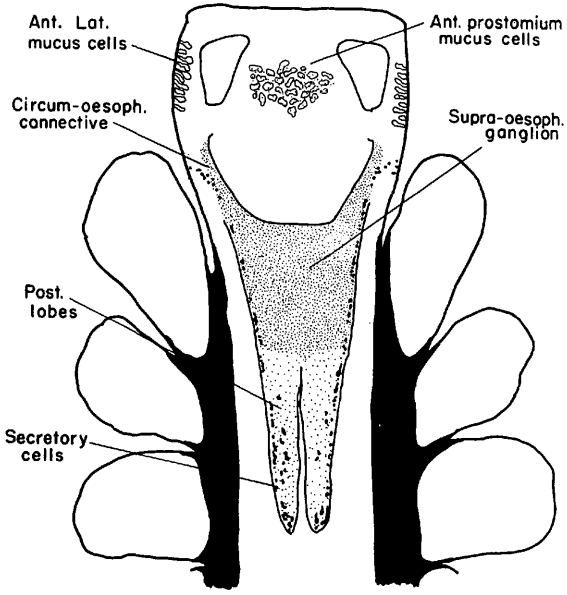

FIG. 10. Composite frontal section through the anterior end of *N. cirrosa* to show the epidermal mucus-glands of the prostomium and the path taken by granules from secretory cells in the posterior lobes. Based on camera lucida drawings.

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 opposite 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 the anterior prostomial group and 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 epidermal 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 in 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 if they have migrated centrally. To judge from the disposition of the mucus-cells in the species illustrated in fig. 8, 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 to such a condition 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 of 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.

Although the posterior part of the prostomial mucus-gland system comes to have an intimate connexion with the supra-oesophageal ganglion, the anterior part has no connexion 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. incisa*, it is impossible to distinguish between the 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 ganglionic and partly glandular. 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 *Derpanopus albolineatus* the cerebral organ is internal to the muscle layers but connected to the exterior by a cerebral 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 out. 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 the 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 possibly 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. However, the histological appearance of the lobes of this species differs so markedly from that of the posterior lobes of any other, that any discussion of them must be tentative for the present. Perhaps after the investigation of the histology of the lobes has been extended to a greater number of species of *Nephtys* and the nature of the secretion produced by the cells in the posterior lobes of *N. cirrosa* is known, it will be possible to be more positive about them. Only one of the three types of cell in the posterior lobes of *N. cirrosa* secretes fuchsinophil granules and they are much smaller than the secretory 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 homologized 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 homologized 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 this 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 probably homologous with a similar group in the posterior part of the brain of Nereids 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 of the lobes by a barrier of neuroglial fibres. The neurones of these two groups are neurosecretory in all species of *Nephtys* examined (Clark, unpublished data) and also in *Nereis* (Scharrer, 1936). These are represented in the posterior part of the ganglion, and not in the posterior lobes, of *N. cirrosa*. In addition there are four large neurosecretory cells of a different type, lateral to the two posterior groups of cells (Clark, unpublished data). These, too, are present in the ganglion of *N. cirrosa*. Finally, the eyes of *Nephtys*, which are located in this region of the ganglion and bear a constant relation to the neurosecretory cells of both types, occur in their usual position in *N. cirrosa* (Clark, 1956*b*). Thus all the recognizably constant features of the posterior part of the supra-oesophageal ganglion of other species of *Nephtys* occur also in *N. cirrosa* in their typical positions 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 *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*. They look remarkably like the majority of the neurosecretory cells of the supra-oesophageal ganglion. Whether or not there are neurosecretory cells in the posterior lobes of *N. cirrosa* can only be determined after detailed study of the histology of these cells and of the histochemistry of the secretion produced by these and the neurosecretory cells of the supra-oesophageal ganglion. An investigation of this sort is now in progress.

If this analysis is correct, 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 organs is recognizably 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 of 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-glands in polychaetes is the secretion of a tube in which the worm lives. In most species of *Nereis* this is done by the parapodial mucus-glands, but in *Nephyts* the epidermal mucus-glands in the segmental part of the body are comparatively 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 there is every indication that the worms swarm in the water for spawning and have 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 mucous net for just this purpose (Harley, 1950).

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