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NAME OF AUTHOR ..... DANIEL PETRYA .....

TITLE OF THESIS ..... AN ANATOMICAL STUDY OF THE CARDIOVASCULAR SYSTEM AND SOME ASSOCIATED TISSUES OF THE ANIMALS STERNAL GLANDS (MOLLUSCA) .....

UNIVERSITY ..... U. OF ALBERTA .....

DEGREE FOR WHICH THESIS WAS PRESENTED ..... PH.D .....

YEAR THIS DEGREE GRANTED ..... 1976 .....

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NL-91 (10-68)

THE UNIVERSITY OF ALBERTA

AN ANATOMICAL STUDY OF THE NERVOUS SYSTEM AND SOME ASSOCIATED TISSUES OF THE ANEMONE *STOMPHIA COCCINEA* (MUELLER)

by

DANIEL J. PETEYA

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOOLOGY

EDMONTON, ALBERTA

SPRING, 1976

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "An anatomical study of the nervous system and associated tissues of the anemone *Stomphia coccinea* (Mueller)," submitted by Daniel J. Peteya in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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Date... December 18th 1975.....

ABBREVIATIONS

The following abbreviations have been used throughout the text. Although some are standard, all abbreviations used are listed here:

min(s)	=	minute(s)
sec(s)	=	second(s)
hr(s)	=	hour(s)
g(s)	=	gram(s)
ml(s)	=	milliliter(s)
μ	=	micron
Å	=	angstrom
LM	=	light microscopy
EM	=	electron microscopy
ZIO	=	zinc-iodide-osmium tetroxide
CA	=	catecholamine
SMC	=	small mesogleal cell type

ABSTRACT

Stomphia coccinea has been examined in an attempt to provide a comprehensive light (LM) and electron (EM) microscopic picture of an anemone nervous system. Emphasis has been placed upon the nature of the nonnervous components of the subepithelial "nerve plexus," the question of mesogleal neurons, and the effect of the technique of study on the nature of observations made.

Both the ectodermal and endodermal nerve nets are surrounded by the processes of up to ten types of cells. These nonnervous fibres may be at least nine times more numerous than the nerve fibres, and with them form a dense subepithelial "fibre plexus" erroneously interpreted as a nerve plexus in previous studies. In an attempt to discover the structural and functional relation of these cells with the nerve net a detailed study of the histology of the endoderm has been made; however, no indication of the function of any of the nonnervous fibres has been achieved.

Although the ectodermal plexus is easily seen on a LM level it is known only from the studies of von Heider (1877, 1879) and from EM study. In place of the plexus most LM anatomists have described the "reticulum" as being the nervous system. Evidence is presented that most of the fixatives used in earlier LM studies of anemones cause a severe swelling damage to the fibres of the plexus. In many cases this results in the rupture of the fibres and in the scattering of their organelles, making the plexus impossible to identify. Also, the swelling of the fibres places a mechanical stress upon the interspersed supporting-cell peduncles, distorting them into the network known as

the reticulum. It is not known whether such a fixation artifact occurs in the endodermal plexus.

The endodermal plexus contains five neural elements, an epithelial receptor, and multipolar, tripolar, small bipolar, and large bipolar neurons. All five elements had been described originally as separate cell types, but recent studies have unified the latter three into a single type (Pantin, 1952; Batham et al., 1960). The present study supports their separation into distinct cell types. In EM study only the sensory-cell axon and the axon of one of the neurons have been defined by their synaptic morphology. Neuromuscular synapses have not been observed in the endoderm.

The ectodermal plexus is subdivided into three zones: one lies in the tentacles, oral disc and pharynx, a second in the column that overlies the sphincter, and a third in the pedal disc. The column below the level of the sphincter also contains a sparse plexus, but it is not known whether this belongs to the second or third plexus.

Seven neural elements have been identified in EM study of the ectoderm; no LM observations have been obtained. Nerve-fibre types 1, 3, and 4 are found only in the tentacle and oral disc, and type 2 in these areas and in the second plexus. Type 1 is the axon of the ciliary-cone receptor, 2 and 3 are axons of neurons, and type 4 is a fibre of uncertain nature which innervates the sunken ectodermal musculature. Types 5 and 6 are found only in the columnar plexus; both appear to be neuronal fibres. The seventh neural element is an epithelial receptor found in the pedal disc. Neither its axon nor that of any neuron has been found in the sparse plexus of the disc.

The mesoglea contains five cell and fibre types: the only nervous element is ectodermal type 4 (although it is found only in the mesoglea it is thought to be ectodermal since it innervates ectodermal muscles). The "small mesogleal cell" (Leghissa's neurocyte and fibrocyte) is described as being nonnervous because it does not bear synapses; however, histochemical study indicates that the cell may secrete catecholamines [redacted]n associated with the ectodermal muscles.

Discussion i[redacted] of the morphological criteria necessary for the identification of a neuron in study of primitive nervous systems.

ACKNOWLEDGEMENTS

I want to thank Dr. D.M. Ross for his encouragement and support throughout this study. Thanks are also due to Drs. A.N. Spencer, S.K. Malhotra, and D.M. Ross for reviewing the thesis.

The investigation written up in Chapter 5 of this thesis was carried out in large part during a visit to the Friday Harbor Laboratories, Washington, U.S.A.

This study was supported entirely by Canadian National Research Council grant A-1445 to D.M. Ross.

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Chapter 1

INTRODUCTION

The objective of the study which led to this thesis was to describe the anatomy and organization of the nervous system of an anemone. Before beginning this report it can be useful to quickly describe some aspects of the history of the study of actinian nervous systems which have influenced both the formulation and the interpretation of this study.

It is well known that the Cnidaria constitute a significant group for the study of nervous systems. With the periodic exception of the Porifera they are thought to be the most primitive phylum to have a nervous system that is composed of recognizable nerve cells, and the presence, in some taxa, of systems which contain both neuronal and neuroidal elements possibly provides our clearest glimpses at the evolutionary inception of a nervous system. Among the Cnidaria there are two groups, the medusae and the anemones, which have been of particular interest. Although they were initially chosen for study primarily because they were large enough to be studied by the techniques available to the pioneer cnidarian neurophysiologists, both present characteristics which have insured their continuing importance in the study of primitive nervous systems. While the medusae are the most primitive animals to have a centralized nervous system and to have sensory organs, with the possible exception of the Actinulida (Swedmark and Tessier, 1966), the anemones have until recently appeared to have a simple, stereotyped behaviour and to have a diffuse nervous system consisting of only a few types of neurons. Indeed, the apparent

simplicity of the anemones was so great that their leading contemporary student once proposed that if it were possible to understand the mechanism of function of any animal's nervous system then it was possible to understand that of the anemones (Pantin, 1952).

This image of simplicity of the anemones has, however, been eroded by recent behavioural studies, particularly those of *Calliactis* and *Stomphia* (Ross, 1965, 1973), which indicate that the anemones can have a surprisingly complex behaviour which exceeds the apparent capacities of contemporary models of the actinian nervous system. Although some significant advances in our understanding of the actinian nervous system have recently been made our picture is still incomplete. Batham, Pantin and Robson have demonstrated that much of the problems and confusion in the earlier neuroanatomical studies of anemones were the result of staining artifacts (Batham et al., 1961), and with techniques which minimize these effects have developed our most complete and accurate picture of the actinian nervous system (Pantin, 1952; Batham et al., 1960; Robson, 1961, 1963, 1965; Batham, 1965). It would appear, however, that their picture is incomplete as they have not duplicated some observations made in earlier and apparently accurate studies (von Heider, 1879; Hertwig and Hertwig, 1879; Leghissa, 1949). Also, neurophysiologists have recently developed techniques for recording from actinians bringing us knowledge of new conducting systems and modes of organization. Unfortunately their techniques are limited to recording from through conducting systems and thus provide no information of much of the nervous system.

This is the first comprehensive electron microscope (EM) study of

an actinian nervous system to be attempted. As such it can provide not only a new level of knowledge but, by avoiding the technical problems which have limited the light microscopists, may provide observations of components of the nervous system that have not previously been observed. The study was initiated to provide a picture of the neuroanatomy of the swimming anemone *Stomphia coccinea*, through a correlated light (LM) and EM study, in which: 1) the components of the nervous system are identified and characterized; 2) their distribution is determined; and 3) observations are made of the pattern of synaptic interaction between the types of receptors, neurons, and effectors. It is hoped that the study will ultimately provide a complete picture of the nervous system and a preliminary model of its synaptic organization as had been proven possible in an earlier EM study of the nervous system of a cerianthid (Peteya, 1973). However, the nervous system of *S. coccinea* has proven to be considerably more complex and difficult to study than was anticipated and the present report can give only a preliminary picture of parts of the nervous system and almost no observations concerning its synaptic organization.

In addition to a description of personal observations on the morphology of the nervous system of *S. coccinea* this thesis includes a report of some observations which the author believes to have a peripheral but important bearing on our concepts of the actinian nervous system. These relate to: 1) the predominance of nonnervous fibres in the so-called nerve plexuses that have been described in earlier EM studies, 2) the discovery of a fixative-induced destruction of nerve fibres which invalidates all reports of the ectodermal nerve net

which describe the 'reticulum' as neuronal, 3) observations of a fixation-dependent morphology of synapses which can have a strong influence on the apparent synaptic complexity of an actinian nervous system, and 4) some ideas concerning criteria for a morphological recognition of nerve cells and of synapses in studies of primitive nervous systems.

Chapter 2

MATERIAL AND METHODS

Species Used

The detailed study has been made of *Stomphia coccinea* (hereinafter referred to simply as *Stomphia*); for its identification I have used the studies of Carlgren (1949) and Siebert (1973). In the case of Chapter 5, study has been extended to use of *Ceriantheopsis americanus*, *Anthopleura elegantissima*, *Metridium senile*, *Pachycerianthus fimbriatus*, and *Stomphia didemon*. *Ceriantheopsis* were collected from St. Tereasa, Florida and the others from San Juan Island, Washington.

Stomphia were collected throughout the year by dredging in the San Juan Channel. They were maintained in running water or in closed-system tanks until needed. In the case of those in closed tanks no animal was used after four months. All animals used were sexually mature and had a basal-disc diameter of 2 cm or more.

As an introduction to the morphology of *Stomphia* a brief systematic description, taken in main from Carlgren (1949), is presented:

Stomphia coccinea (Mueller, 1776)

Tribe	Theneria	Carlgren, 1899
Subtribe	Mesomyaria	Stephenson, 1921
Family	Actinostolidae	Carlgren, 1932.

The basal disc of *Stomphia* is flat, adherent, and clearly distinct from the column. It has well developed circular and basilar muscles, both of which decrease in size toward the center of the disc. The column is cylindrical, smooth and is not divided into regions. The

sphincter is mesogleal, strong, and covers the tentacles. The circular muscle layer of the column is endo-mesogleal and in the basal 2/3 of the column it is well developed. The oral disc is large, circular, and bears 4—5 cycles of tentacles. The longitudinal ectodermal muscles of the tentacles and disc are formed into tubes that are sunken into the mesoglea. There are usually four cycles of mesenteries (6+10, 16, 32) of which 16 are perfect and sterile. All but the youngest imperfects are fertile and bear gastric filaments. *Stomphia* has no acontia. The mesenteries bear three muscle fields: a diffuse retractor, an exceptionally well developed parietobasilar, and a weak radial field. All three are endodermal muscles; although it has been reported that the retractor is mesogleal (Siebert, 1973) I find that only about 10% of the muscle fibres are mesogleal. A report of the cnidom has been given by Siebert (1973).

Techniques of Anesthesia

Because of the longstanding preference given to narcotization of anemones by $MgCl_2$ (or $MgSO_4$) by histologists, ethologists, and by physiologists I had unquestioningly accepted this narcotic for use in my study. However, late in the study I discovered that magnesium narcotization for 16 hrs, which is necessary before a *Stomphia* can be dissected, occasionally produces an extreme vesiculation of some of the ectodermal neurons, the axon of the ciliary-cone receptor (Fig. 77), and of some mesodermal cells (Fig. 41). Limited tests with chilling, $MnCl_2$, Ms 222, and with urethane have provided no suitable alternative to $MgCl_2$. All figures and descriptions in this report are taken from animals that

were narcotized in a mixture of equal parts of sea water and 6.7% $MgCl_2 \cdot 6H_2O$.

It is usually quite difficult to narcotize an anemone in total expansion. Mg^{++} is a slow-acting narcotic and histologists usually increase its concentration in a tank slowly to avoid the animal contracting in response to its presence (e.g. Leghissa, 1949; Batham et al., 1960). These difficulties can be avoided in *Stomphia* by introducing the animal to full-strength narcotic while it is swimming. At the end of a swimming bout there is a refractory period of 15—60 mins during which the animal is fully expanded and highly insensitive to stimuli; by the end of this period the animal is sufficiently narcotized that it cannot initiate any behaviour. It requires considerably longer narcotization, however, before an animal can be fixed or dissected without gross tissue contraction.

Narcotization for 16 hrs is necessary before an animal can be dissected into individual mesenteries. Although dissection is desirable for fixative penetration, the long narcotization necessary may introduce artifacts; thus prefixation dissections were performed only where necessary, in the case of fixation by OsO_4 and $KMnO_4$. In the case of fixation by aldehydes and most fixatives for LM usage it was possible to fix the undissected animal by perfusion. This allowed narcotization to be limited to 8—10 hrs and provided tissue in a more complete expansion that could be had if the animals were dissected prior to fixation. To perfuse an animal the tips of the primary and secondary tentacles were removed and the animal placed into fixative. Fixative was then pipetted down the mouth for a period of several minutes, after

which the animal was transferred to fresh fixative and perfused again. The animal could then be dissected during fixation with much less difficulty than if it were dissected alive.

Histological Methods

More than 100 histological techniques have been employed in this study. This section is therefore limited to a report of only those few fixation, staining, and histochemical procedures that proved to be the most useful.

Light Microscopy

Classical histology

Most of the fixatives used for LM histology cause damage to, or destruction of, the ectodermal subepithelial plexus. As a result they have been used in this study only in my investigation of this artifact. The technical section of Chapter 5 provides details of these fixatives.

Description of only one technique will be given in this section: it is one of only two techniques found that gives a good visualization of the mesogleal cell plexus. It has been found that fixation for 5 hrs in 3—4% formic acid and 0.1% OsO_4 in water at 20°C creates a metachromatic reaction to toluidine-blue staining of araldite sections. The result is an intense staining of all cell types in the mesoglea and, with 3% formic acid, of a small number of random cell bodies in the endoderm. The technique could not be used for whole mounts as osmium darkens the tissue too greatly to be seen through.

With these exceptions most of the LM study has come from 1—2 μ-thick sections of araldite-embedded material that was prepared by the

fixatives used for EM study (see below). Wax-embedded material proved useless because of the poor resolution afforded by such thick sections.

Macerations

A modified Hertwig procedure has been used for preparations of macerated tissue. The original technique (Hertwig and Hertwig, 1879, p. 457), using 0.02% OsO_4 in 0.1% acetic acid in sea water as a fixative, did not preserve all components of the ectodermal fibre plexus (see Chapter 5) and is recommended against. The author's modification uses a longer fixation in a higher concentration of OsO_4 which provides a superior fixation but which requires a longer maceration and does not provide as good a dissociation of the tissue:

1) fix for 12—24 hrs in 0.05% OsO_4 and 0.2% acetic acid in sea water at 20°C
2) macerate for 3—4 days in 0.2% acetic acid in sea water at 20°C
3) dissociate and mount in 'Aquamount' (from E. Gurr).

Whole mounts

Most parts of the body of actinians are thin enough that they can be examined in whole mounts. Specific staining of such preparations has provided the majority of our knowledge of the actinian nervous system.

Whole mounts from all parts of the body (other than the sphincter) have been prepared for study by a variety of procedures, including all of the histochemical techniques listed below. Neuronal staining, however, has been achieved only in preparations of mesenteries that were stained with the Batham-silver impregnation (Batham et al., 1960), the Wood alcian-blue-eosine technique (Wood, 1963), or by a modified zinc-iodide-osmium technique (Martin et al., 1969).

The Batham-silver impregnation:

1) fix for 24—48 hrs in 3 parts saturated aqueous picric acid and 1 part stock commercial formaldehyde at 20°C
2) rinse in several changes of 70% ethanol until the leaching of picric acid is minimal. During this rinse the animal is dissected into individual pieces and tied to slides with cotton thread.
3) wash for 10 min in distilled water
4) 60 min in 20% $AgNO_3$ in the dark
5) wash in several changes of distilled water
6) impregnate for 24—48 hrs in freshly made impregnating solution at 35°C:

2 ml	1% $AgNO_3$ in distilled water
2 ml	10% pyridine in distilled water
14 ml	0.2M H_3BO_3
6 ml	0.05M $Na_2B_4O \cdot 10H_2O$
180 ml	distilled water

7) rinse in distilled water
8) 2—3 min in 1% hydroquinone and 5% sodium sulphite in distilled water
9) rinse for 3 min in running water
10) 3—10 min in 0.2% $AuCl_3$ with a trace of acetic acid
11) rinse 10 secs in distilled water
12) place in 2% oxalic acid until neurons can be seen
13) rinse for 10 secs in distilled water
14) 5 mins in freshly made 5% sodium thiosulfate
15) wash 10 mins in running water
16) at this time it helps to wrap the slide in cigarette paper to

keep the tissue flat

17) dehydrate slowly, clear in methyl benzoate, and mount under pressure.

Wood's aniline blue-eosine technique (Wood, 1963):

This technique is believed to be a histochemical procedure for the localization of adrenaline and noradrenaline (Wood, 1963; Wood and Lentz, 1964). In the investigator's hands, however, it appeared to be a general stain, staining every type of cell that he had observed on a LM level. The technique has been used without modification except for the concentration of formaldehyde. Wood recommended using 4% formaldehyde rather than 40%; however, it was found that the latter improves staining intensity. Also, changes have been made in the rinse to accommodate the dissection.

1) fix for 24 hrs in freshly mixed fixative made from:

10 parts 2.5% $K_2Cr_2O_7$ and 1% sodium sulfate in 0.2M acetate buffer at pH 4.1
1 part stock commercial formaldehyde
adjust to pH 4.0—4.2 at 20°C

2) wash in several changes of distilled water for 4—8 hrs during which the animal is dissected and tied to slides

3) stain for 15—20 mins in freshly-mixed stain:

1 part 1% eosine Y in distilled water
1 part 0.5% aniline blue in distilled water
1 part 0.2M acetate buffer at pH 4.0
adjust to pH 4.0 at 20°C

4) the tissue must be dehydrated rapidly to avoid a complete extraction of eosin. Best results were obtained with the following schedule:

1 dip in distilled water
30 secs in 95% ethanol
30 secs in 100% ethanol
3 mins in a change of 100% ethanol

5) clear in xylene, and mount under pressure.

Zinc-iodide-osmium (ZIO) impregnation:

Although ZIO has been used to stain organelles in *Hydra* (Elias et al., 1972), it has not previously been used to provide specific staining of neurons in a cnidarian. Development of such a technique is important for cnidarian neuroanatomists not only because it will provide a new technique for visualizing neurons, but it is one of the very few techniques which provide the possibility of sequential LM and EM study of a neuron. The technique used in this study is a slight modification of one developed for cephalopods (Martin et al., 1969):

1) fix 3—4 hrs in 4% glutaraldehyde in equal parts of sea water and 6.7% $MgCl_2 \cdot 6H_2O$ at 4°C
2) wash 5 mins each in 3 changes of sea water-$MgCl_2$
3) postfix 18 hrs in ZIO at 4°C
4) after washing, the tissue can be mounted for LM or dehydrated and araldite-embedded for EM.

Preparation of the ZIO solution:

Shortly before its use prepare a solution of 7.5% zinc powder and 2.5% iodine. Stir well and filter; chill to 4°C. At the time of fixation this is mixed with 4% OsO_4 in distilled water at a ratio that varies from 1:1 to 2:3 plus 3 parts of water. Little variation in frequency of stained cells or of staining intensity has been seen with these changes in reagent concentration.

In the tentacle, this procedure gives adequate fixation quality of the mesoglea and endoderm to allow EM study, but the ectoderm is too damaged to be studied. Tests with better aldehyde prefixations and with the buffer system recommended by Martin for the ZIO medium have not significantly improved the fixation quality. Therefore this technique has had little value here for EM study. Of the variations examined the procedure described here is the one which has provided the best LM-level impregnations.

Photography for light microscopy

Figures 49, 50, 55, 56, 57, and 58 were made on a Zeiss 'Ultraphot' with Polaroid type 55 P/N film at a negative magnification of X 1250 to X 1700. All other light micrographs used here were made with a Reichert 'Photoautomat' on a Reichert 'Zetopan' with Plus X film at a negative magnification of X 500.

Electron Microscopy

Fixation

From an initial survey of fixatives two were chosen for general use: Cavey's (1973) modification of Luft's (1971) glutaraldehyde-ruthenium red fixative and saturated $KMnO_4$ in sea water-$MgCl_2$. Limited use was also made of Westfall's (1965) modified Dalton and of Peteya's (1973) cerianthid fixative.

Cavey's fixative:

Of the fixatives examined this has given the best general fixation. However, it is not optimal for the fibres of the ectodermal fibre plexuses; their morphology is fixative-dependent, and Cavey's does not

allow recognition of all types of fibres nor identification of the synapse of the ciliary-cone receptor.

1) fix 4 hrs in 2% glutaraldehyde in 0.2M sodium cacodylate with 0.275M sucrose and 0.05% ruthenium red at pH 7.3 at 20°C

2) wash 15 mins in 3 changes of 0.1M cacodylate at pH 7.3

3) postfix 1—2 hrs in 1—2% OsO_4 in phosphate.

Permanganate in sea water-$MgCl_2$:

Permanganate is one of the best fixatives for vesicle-bound proteins that are associated with catecholamine storage and thus provides a differentiation of neuronal vesicle types that is superior to more conventional fixatives. This particular fixative was developed for use as an alternate to the Richardson (1969) fixative. Whereas Richardson's gives excellent fixation of some parts of the body (e.g. the column ectoderm), other parts (e.g. the tentacle and oral disc ectoderm) are very poorly fixed. The technique described here affords a relatively poor, but adequate, fixation of all parts of the body:

1) fix 1 hr in saturated (about 0.8%) $KMnO_4$ in equal parts of sea water and 6.7% $MgCl_2 \cdot 6H_2O$ at 4°C

2) wash 15 mins in 3 changes of sea water-$MgCl_2$ at 4°C

3) postfix 1 hr in 1% uranyl acetate in sea water-$MgCl_2$ at 4°C.

Westfall's fixative:

This fixative has no clear value over other osmium fixatives, but is a popular fixative in EM studies of Cnidaria.

1) fix 1—2 hrs in 2% OsO_4 and 1% $K_2Cr_2O_7$ in 78% sea water at pH 7.2 at 4°C.

Peteya's fixative:

This fixative was developed in an EM study of the nervous system of *Cerianthеopsis americanus*; of over 20 aldehyde fixatives examined this one was the best fixative for synapse and nerve morphology.

1) fix 2—4 hrs in 2% glutaraldehyde and 2% paraformaldehyde in 70% sea water at pH 7.6 at 4°C
2) wash 15 mins in sea water at 4°C
3) postfix 1 hr in 1—2% OsO_4 in 0.2M Sorrenson phosphate at pH 7.4. During fixation the solution is warmed from 4° to 20°C.

Dehydration, embedding, and specimen preparation

All tissue used here was dehydrated in ethanol and embedded in Araldite. Sections were mounted on Formvar-coated grids and stained with uranyl acetate and lead citrate, except for some of the histochemical preparations which are specified in the following section. Grids were examined in either a Philips EM 200 or EM 300 electron microscope.

Histochemistry

Biogenic amines are widely distributed among the Cnidaria, and have been histochemically localized in presumptive neurons in two species of anemones (Dahl et al., 1963; Wood and Lentz, 1964). Early in this study it was found that catecholamines could be localized in several cell types, including nerve fibres, by EM-level chromaffin techniques and by incubating animals in exogenous amines. Subsequently a number of LM-level amine-histochemical techniques were examined in the hope of finding techniques which could give a LM-level visualization

of neurons. Although all of these techniques demonstrate amines in gland cells and some other nonnervous cells none have proven sufficiently sensitive to illustrate neurons except for the Wood and Woods techniques. They are listed here only as a record of what has not had any value for staining neurons in this study:

1) Chromaffin reactions
   A) Fixation in 2.5% $K_2Cr_2O_7$ in acetate buffer at pH 5.6 (Hillard and Hokfelt, 1953); used only on a LM level.
   B) The Jones (1967) chromargentic; on a LM level.
   C) The Leghissa (1949) chromargentic; on a LM level.
   D) The Wood (1963) aniline blue-eosine technique (see above for a description); on a LM level.
   E) The Wood chromaffin technique for catecholamines (Wood, 1966); used on both a LM and EM level.
   F) The xanthydrol technique of Lillie (1957); on a LM level.
   G) The Woods (1969) technique for catecholamines; on both a LM and EM level.
   H) Ninhydration sublimation (Holtzenberg and Benditt, 1961); on a LM level.
   I) Jaim-Etcheverry and Zieher's (1968) chromaffin; on a LM level.
2) The iodaffin reaction of Hillard and Hokfelt (1953)
3) Argentaffin reactions
   A) Fontana (1912) (as cited in Gray, 1954); on a LM level.
   B) Hakanson (1971); on a LM level.

4) Tetrazolium coupling of monoamine oxidase, activated by noradrenaline or by 5-hydroxytryptamine (Chayer, et al., 1969); on a LM level.

5) Drug histochemistry

A) Prefixation incubation with noradrenaline has been used in an attempt to increase the effectiveness of several of these techniques (1B, 1E, 1F, 1G, 1H, 1I, and 4).

B) Incubation with α-noradrenaline or with metaraminol to modify vesicle morphology on an EM level.

Of these techniques only the Wood technique (1963), described above, gives a LM-level visualization of neurons, and only the techniques of Wood (1966), Woods (1969), and drug histochemistry have provided an EM-level localization of amines in sensory and nervous fibres. They are described in detail here:

Wood (1966), for catecholamines and indolamines:

1) fix 2—4 hrs in 4% glutaraldehyde in 0.1M sodium cacodylate at pH 7.2 at 4°C

2) wash overnight in 4% sucrose in 0.1M cacodylate at pH 7.2 at 4°C

3) incubate 24 hrs in 2.5% $K_2Cr_2O_7$ and 1% sodium sulphate in 0.2M acetate at 4°C

a) if pH is 4.1 all catecholamines except adrenaline are stained

b) if pH is 7.0 all catecholamines are misplaced (Wood, 1963)

4) sections of this tissue may be stained only with lead citrate.

Woods (1969), for catecholamines:

1) fix 1 hr in 2.5% acrolein (added at use) in 0.1M $Na_2Cr_2O_7$ at pH 7.0 at 0°C
2) incubate 1—2 hrs in 0.1M $Na_2Cr_2O_7$ unbuffered at pH 3.5—4.0 at 0°C
3) the sections may be stained only with lead citrate.

Incubation in metaraminol (modified from Hokfelt, 1969):

1) narcotize for 12 hrs
2) remove animal to narcotic containing 0.02 g metaraminol bitartrate/100 cc (100 µg/ml free base). The medium is replaced with freshly made medium every 2 hrs to insure against decomposition of the metaraminol.
3) After 9 or more hours' incubation the animal is dissected and fixed in permanganate in sea water-$MgCl_2$ (see above for description).

Incubation in α-m-noradrenaline (modified from Hokfelt, 1969):

1) narcotize for 12 hrs
2) remove animal to narcotic containing, per 100 mls, 0.005 g α-m-noradrenaline, 0.200 g anhydrous glucose, 0.020 g ascorbic acid, and 0.005 g EDTA.
3) After 3 or more hours' incubation the animal is dissected and fixed in permanganate in sea water-$MgCl_2$ (see above for description).

Chapter 3

THE ENDODERM

Introduction

The most clearly understood component of the actinian nervous system is that part which lies in the endoderm. This is in part because of the greater interest that the endoderm holds, since it contains the muscles that mediate the most obvious behaviours, and in part because of the unusual technical difficulties inherent in the study of the ectodermal neural elements (see Chapter 5). Although much is known of the endodermal nerve net from the LM studies of Batham (1956, 1965; Batham et al., 1960, 1961), Pantin (1952) and Robson (1961, 1963, 1965), the picture is still incomplete, and there has never been an EM study of the endodermal net.

In addition to reporting some new observations on the nervous system this chapter reports the discovery of a complex subepithelial plexus of fibres, on an EM level, in place of the sparse nerve net expected from LM studies. Such plexuses are found in the ectoderm of every anthozoan examined by EM and are now known in the endoderm of *Dendrophyllia* (Kawaguti and Yokoyama, 1966), *Aiptasia* (Amerongen, personal communication), and of *Stomphia* (this thesis). There is, however, no LM literature of these plexuses other than the reports of von Heider (1877, 1879). Evidence will be presented below (see Chapter 5) that the ectodermal plexuses were not rediscovered until the advent of EM studies because they are destroyed during fixation by most of the fixatives used in most LM studies of anemones. It is

generally believed that the plexuses are composed primarily or entirely of nerve fibres (for review see Chapter 6); however, analysis of the ectodermal plexuses of *C. americanus* (Peteya, 1973) and of *Stomphia* (see Chapter 6) and of the endodermal plexus of *Stomphia* (this chapter) indicates that they contain large numbers of nonnervous fibres. If these findings are representative, a reinvestigation of these 'nerve plexuses' will be necessary. Moreover, this is believed to be the first report of an aggregation of nonnervous tissue that is specifically associated with a cnidarian nervous system. As a start toward the analysis of the plexus this chapter includes a report of the histology of the entire endoderm, including a characterization of most of the cell and fibre types which form the plexus. A similar attempt to characterize all components of the ectodermal plexuses of *Stomphia* (see Chapter 6) has failed because of the greater complexity of these systems; thus for the present the endodermal plexus must serve as a model system.

Material and Methods

For LM study all parts of the body have been examined, although observation of neurons has, because of technical limitations, come exclusively from stretch preparations of the mesenteries. Staining of nerve cells has been achieved with the Batham silver technique, Wood's alcian blue, and with a zinc-iodide-osmium (ZIO) impregnation. Most of the nonnervous cells described below from LM study could best be illustrated by the [illegible] technique, but could also be stained by almost any silver or [illegible]amine technique employed in this study.

For EM observations the study of the endoderm is limited to those areas indicated in Figure 1. It will be described elsewhere (Chapter 6)

that there are considerable problems in fixation of the ectodermal neurons for EM study; such problems have not been experienced in study of the endodermal plexus, and the only consideration made here in judging fixatives is quality of fixation. Therefore most EM observations have come from tissue fixed in Cavey's or in permanganate in sea water.

Results

General Histology

Throughout most of the body both the endoderm and ectoderm of anemones are organized as 'epitheliomuscular epithelia.' Such a tissue is composed of three layers: a superficial epithelium, an intermediate nerve net (or nerve plexus), and a basal muscle layer (Fig. 2). In *Stomphia* there is considerable digression from this plan of organization in that epitheliomuscular cells lack muscle fibres in some areas (the ectoderm of the pharynx, column and pedal disc, and the endoderm in the gastric filaments) and in others (the sphincter, and circulars of the column and pedal disc, and the ectodermal muscles of the tentacle and oral disc) many or all of the muscle cells and their fibres form into tubes which are sunken into the mesoglea. Nonetheless, the histology of the endoderm is relatively simple; as can be determined by LM the endoderm throughout the body (with the exception of the gonads and gastric filaments) is composed of only 11 cell types: the epitheliomuscular cell, a gland cell, the amoebocyte, a cnidoblast, two types of previously undescribed nonnervous cells, a receptor, and four types of neurons.

The endodermal epithelium is composed of the cell bodies of the epitheliomuscular cell and of a small number of gland cells, receptors and of new-cell type 1. Although there is a considerable variation in regional histology, each of these cells is found throughout the body. The cells are organized into a columnar epithelium, and have an apical junctional complex consisting of a band of septate desmosomes 1—3 μ in depth. Little has been done in this study to investigate these junctions but they are not believed to be a diffusion barrier since they are permeable to phosphotungstic acid, ruthenium red and bismuth iodide (Fig. 3).

The fibre plexus is a layer of fibres, lying between the epithelium and the muscle layer, that varies in size from 20 fibres in thickness at the insertion of mesenteries to the column to a layer of scattered, solitary fibres in the tentacles. It is composed of the nerve net and the processes of five nonnervous types of cells which do not appear to have been described previously. The amoebocytes lie in the plexus, but do not contribute fibres to it. There are few records of the existence of endodermal plexuses, and there is some question of their general occurrence in the Anthozoa since they appear to be lacking in *Metridium* (Grimstone et al., 1958; Batham, 1960).

The muscle layer is primarily composed of the contractile processes of the muscle cells; it also contains the cell bodies of some muscle cells, the axons of the receptor and of some types of neurons (Robson, 1963), and of the nonnervous fibre types 2, 3, 4, and 5. The muscle fibres are apposed to the mesoglea, and thus form a simple sheet in which the fibres are oriented parallel to the endodermal surface. In most

parts of the body the density of fibres is increased by pleating the sheet of fibres upon lamellar extensions of mesoglea (Fig. 2) or by forming the sheet into cylinders that are sunken into the mesoglea (Fig. 2). The endodermal muscle fibres are organized into about 10 functional fields and are responsible for all behavioural activity in *Stomphia* other than some aspects of tentacle and oral disc movement.

Endodermal Cell Types

The epitheliomuscular cell

This is the most common type of cell in the endoderm; its presence is integral to the organization of the endoderm into an epithelio-muscular epithelium. Its morphology is reasonably well known (Robson, 1957; Grimstone et al., 1958) so its description here is limited to its relation to the nervous system.

Those cells whose cell bodies lie in the epithelium are polygonal columnar cells (Figs. 4, 5) whose size depends on location and state of contraction of the tissue. Their surface is irregular, with numerous indentations that appear to be sites of phagocytosis (Fig. 5); their only projections are a number of microvilli and cilia. These cells are the major site of food adsorption and digestion, and are characterized by numerous lysosomes and electron-dense vesicles (1600—3200 Å in diameter) (Figs. 4, 5). In the endoderm of the sphincter, column, pedal disc, and mesenteries many of the cell bodies of this cell type are sunken into the muscle layer and are in direct contact with their muscle fibres (Fig. 6). This population of cells does not appear to be associated with food metabolism as they only rarely contain lysosomes.

Each cell that lies in the epithelium bears one or more peduncles each of which terminates in a 'T' junction to form a single muscle fibre. Depending on the degree of tissue contraction the peduncles are 1—5 μ in length and 0.3—1 μ in diameter. Unlike any of the fibres of the plexus through which they pass, the peduncles have a granular cytoplasm containing numerous mitochondria, vesicles, endoplasmic reticulum, and small bundles of fibrils (Fig. 7). This and their orientation perpendicular to the tissue surface distinguishes the peduncles from the fibres of the plexus.

Because of the importance of the muscle system in a discussion of the nervous system the muscle fibres will be described in an expanded section below.

The gland cell

The endoderm of *Stomphia* contains several classes of gland cells, but only one is found outside of the gastric filaments. This is a large epithelial cell, averaging 10 μ in diameter, that is identifiable by its 0.7—1.5 μ diameter vesicles (Fig. 8). This type of gland cell does not bear a basal process, and thus does not contribute to the fibre plexus.

They are found throughout the body, but are most common on the mesenteries in the area between the retractor and the free edge. As seen before in *Calliactis* (Vialli and Casati, 1958) the vesicles of this cell stain intensely in aminergic histochemical procedures and the cytoplasm shows a monoamine oxidase activity. On this basis the cell has been termed a chromaffin cell and is presumed to be homologous to the enterochromaffin cell system of higher phyla (Vialli, 1966).

In this study it has been found that the frequency of this cell varies among animals and appears to vary through the year; however, the seasonal data necessary to verify this latter possibility have not been kept.

Cnidoblasts

Endodermal cnidoblasts are known in *Stomphia* only in the filaments (Siebert, 1973). In the present study a single observation has been made of a cnidoblast in the endoderm of the pedal disc. From this observation, however, it is not possible to give an identification of the type of nematocyst or to describe the cell.

Amoebocytes

This is the only other endodermal cell type to have been described in the recent LM literature except for presumptive neurons. The amoebocytes are wandering phagocytes that are found in all three tissue layers. In the endoderm they are always located in the plexus and, contrary to previous reports (Batham et al., 1961), never give rise to processes. Thus they do not contribute to the structure of the plexus. The cells are usually elongated and flattened in shape, averaging 15 μ in length, 6 μ in width, and 2.5 μ in thickness; thus in sections the cells always appear to be bipolar in shape (Fig. 9). The cells are characterized by vesicles that are 2000—3600 Å in diameter and which stain in silver impregnations (Fig. 11); on an EM-level the vesicles are spherical to rod shaped, electron-dense, and either dense-cored or solid (Fig. 10) depending upon the fixative. The amoebocyte can also be identified by the presence of large lysosomes that contain eosinophilic crystals; it is the only cell in the plexus that contains lysosomes.

New cell type 1

In the whole mounts of mesenteries in this study two types of cells were observed which do not correlate to any known cell type, yet they are common cells which stain intensely with most of the techniques used here. While it is possible that some earlier description has been overlooked it is certain, however, that neither cell has been described in any of the papers of Leghissa, Batham, Pantin, or Robson.

The first is an epithelial cell that is found in all parts of the body, but which is most frequent on the mesenteries. Its cell body lies at the base of the epithelium and gives rise to a single narrow process to the tissue surface and one or more basal processes into the fibre plexus (Fig. 12). The cell and its fibres are packed with spherical vesicles (1000—5000 Å) that stain intensely with every silver and LM-level amine technique used, making this cell type a prominent feature of the endoderm. On an EM level the vesicles may appear either as a uniform class of solid vesicles (Fig. 12) or else as a collection of small dense-core vesicles and large granular and solid vesicles, depending on the type of fixative.

New cell type 2

The second new cell is a subepithelial cell found in the plexus throughout the body. It is characterized by a population of small vesicles (600—780 Å as measured by EM) that stain by both silver and amine techniques. The cells are highly variable in appearance (Fig. 13), ranging in shape from spherical to fibrous, and although they may give rise to an anastomosing web of fibres many form no fibres. Many of the cells are bi- or tripolar and thus can be confused with the small

bi- and tripolar neurons described below. They are distinct, however, in that the techniques used in this study stain the vesicles of this cell and the cytoplasm of the neurons. Also, the fibres of this cell are usually irregular in diameter while those of the neurons have a regular diameter (except when prepared by the Batham technique). This variation in size has allowed the fibres of this cell, but not its cell body, to be identified on an EM level; the fibre will be described below in the section concerning components of the plexus.

Receptor

Previous studies (Hertwig and Hertwig, 1879; Leghissa, 1949; Pantin, 1952; Batham et al., 1960; Robson, 1961, 1963) have demonstrated a small bipolar epithelial receptor that has one to three basal axons that lie in the plexus and the muscle layer. They are found scattered throughout most of the body and are particularly numerous at the junction of the mesenteries to the oral disc, column, and pedal disc. As illustrated by the plates of these papers there is considerable variation in this cell's morphology creating some difficulty in identifying it here. It has been possible, however, to identify a receptor-like cell in macerations and in ZIO preparations (Fig. 14).

As seen here, the receptor is 10—30 μ in length and about 1—2 μ in diameter in the dendrite and 4—10 μ in diameter at the nucleus. The dendrite bears a single cilium surrounded by an irregular ring of microvilli (Figs. 15, 16, 17); occasionally it also bears a small projection which does not reach the tissue surface (Figs. 14C and 17). All cells seen here have either one or two axons. The distribution of the receptor differs from that reported previously in *Stomphia* (Robson,

1963) in that: 1) they were found in the tentacles, and 2) the reported aggregation of the receptors at the junction of mesenteries to the oral disc is almost negligible.

Although the cell can easily be identified on an EM level by its shape and distribution, it is difficult to identify it as a receptor because of the simplicity of its sensory apparatus. The cilium is a 9 + 2 whose basal body has a basal foot and an accessory centriole (Fig. 15); it is unmodified from the cilia on epitheliomuscular cells except that in some receptors microtubules are found in the dendrite which attach to the basal foot. The microvilli are less than 1000 Å in diameter and less than 1 μ long; they do not contain bundles of filaments (Fig. 16) as are found in the stereocilia of some other receptors of *Stomphia* (Chapter 6), and are indistinguishable from the microvilli on epitheliomuscular cells (Fig. 17A). The receptor contains two classes of vesicles. One, which is found in large numbers throughout the cell and axon, is a solid or dense-cored vesicle (700—1800 Å) (Fig. 17C) whose morphology is dependent upon the fixative. The other class consists of a small number of agranular vesicles (500—1100 Å) that are found in the axons and which includes the synaptic vesicles (500—900 Å). The axon and its synapse are described in the following section concerning the components of the fibre plexus.

The large and small bipolar neurons

These are large cells, up to 4—8 mm in length and with axons up to 1.0 μ in diameter, that are believed to constitute the through-conducting system that mediates protective withdrawal. They form a network throughout the mesenteries (for technical reasons it is not known if they or

any other neuron lies over the parietobasilars) (Pantin, 1952; Batham et al., 1960) which extend onto the column over the sphincter and descend into it (Robson, 1965). Robson (1963) has noted but not illustrated or described a smaller bipolar neuron, also known from the studies of the Hertwigs (Hertwig and Hertwig, 1879) and Leghissa (1949) which is found on the pedal disc, column and mesenteries, and presumes it to be part of the same class of neurons as the large bipolars. Also, the small tripolar neuron described below has been described as a variant of this class (Pantin, 1952, fig. 6). The presence of the bipolar neuron in the endoderm of the oral disc and tentacles is uncertain; Robson (1963) presumed its presence but has never seen it. Batham (1965) on the other hand reported that they extend from the mesenteries onto the disc where they cross the mesoglea to run in the ectoderm of the disc and tentacles. Although the latter idea fits with physiological knowledge, the validity of the report is questioned and needs verification.

The endodermal nerve net is a remarkably diffuse system (see Pantin, 1952, fig. 13) making EM study of it through random sectioning almost impossible; nerve fibres and interneural synapses have been seen too rarely to allow a characterization of any of the nerve fibre types, and possible nerve somata have been seen only three times in this study. Although for this reason the study has been limited largely to LM observations, one important discovery has been made: that the bipolar neuron described by Batham, Pantin and Robson is actually a collection of three types of neuron that are distinct both by their size and shape (compare Figs. 18, 19, 20, 21) and by their staining

affinities (Table 1). Both the small bipolar and the tripolar cells that they had described as variants of the large bipolar neurons are distinct classes of neurons. Their identification as separate types of cells was not possible in the studies of Batham, Pantin and Robson because the techniques which they had used only rarely stain either the small bipolar or the tripolar neurons (Table 1).

In this study LM staining of the large and small bipolars has been obtained only on the mesenteries, and, like Robson (1963), this investigator has been unable to find any neurons overlying the darkly staining parietobasilar muscle field. Both the large and small bipolars are spindle-shaped and invariably bear two opposing axons which rarely branch (Figs. 18, 19). The large cells are 3—12 μ in diameter and 14—70 μ long (Fig. 18), and the smaller ones are 2—3 μ in diameter and 5—12 μ long (Fig. 19). The axons of even the largest bipolar neurons are less than 1 μ in diameter. In tissue prepared by the Batham silver procedure the axons are 2—4 μ in diameter, but this and the unusual varicosities of the axon (Batham et al., 1960) appear to be a fixation artifact. Although the large and small cells approach each other in size, their separation into two classes is clearly demonstrated by comparing the size ranges of observable bipolar neurons in ZIO preparations (which stain only the large class) against the range of neurons seen in Wood's preparations (which stain both classes) (Fig. 20).

Multipolar neuron

This cell is known only from methylene-blue preparations of the column endoderm of *Calliactis* and *Stomphia* (Robson, 1963, 1965). In the Batham-silver preparations of mesenteries in this study this cell

was found on the retractor face in the area between the parietal and retractor muscles; but staining was achieved too rarely to define the limit of its extension onto the mesentery. As seen here it is a stellate cell, about 15 μ in diameter, which bears five to eight unbranching axons (Fig. 21A). Neither the cell nor its axon have been identified on an EM level.

The tripolar neuron

This cell has been described previously as a rare variant of the bipolar neuron of Batham, Pantin, and Robson (see above). The tripolar cell is, however, distinct from both the large and small bipolar neurons described here in its staining affinities (Table 1). It is found throughout both faces of the mesenteries (except in the area of the parietobasilars) and in tissue prepared by the Wood's alcian blue or by the ZIO procedures it is relatively common, one tripolar cell being found for every five to six large bipolar neurons.

The tripolar neurons are small, 3 x 11 μ to 7 x 17 μ, triangular cells which vary in shape from equilateral triangles (Fig. 21C) to cells that have two axons displaced onto a single end of the cell (Fig. 21D). Many of them have an aggregation of silver-staining material near the origin of their axons (Fig. 21B).

The Fibre Plexus

The plexus lies in the zone between the epithelium and the muscle layer. It is composed of the axons of the four types of neuron and of the receptor, the processes of new-cell types 1 and 2 (fibre types 1 and 2), and of three fibre types (3, 4, and 5) which have been

identified only on an EM level. For convenience, Table 2 provides a summary of the characteristics of the fibres identified on an EM level.

The degree of development of the plexus varies through the body, and closely parallels the development of the nervous system even though it appears that less than one in ten fibres in the plexus are neuronal. Thus it varies from a layer of scattered, solitary fibres in the tentacles and oral disc to a layer over 20 fibres thick at the junction of the mesenteries to the column and pedal disc. The plexus is found throughout the endoderm with the exception of the gastric filaments. The medial lobe of the filaments has a small plexus, composed of fibres that are not found elsewhere in the endoderm, which does not extend into the intermediate tract or the lateral lobes. In this work a study was not made of this second plexus and it is not known whether it has communication with the general plexus or whether it contains neural elements.

With the exception of the plexus of the gastric filaments the endodermal plexus shows little regional variation in composition or of organization. All but one of the six types of fibres whose distribution has been determined (types 1, 2, 3, 4, 5, and the receptor axon) have been found in all parts of the body examined. In the column and pedal disc, where the muscle layer is partly displaced into the mesoglea, the plexus shows some evidence of being organized into two layers (a superficial and an intramuscular layer) which differ in composition, in orientation of the fibres, and in their spatial contact with the muscle layer.

Fibre type 1 belongs to the cell described above as new-cell type 1. It is found throughout the body but is most frequent on the mesenteries. Each cell bears one to three processes (Fig. 11) that lie exclusively in the superficial layer of the plexus. The processes are no more than 40 μ in length and are between 0.2 and 2 μ in diameter. They are characterized by the presence of large numbers of the solid vesicles (1000—5000 Å) (Fig. 22) that are found in the cell body. These vesicles stain for catecholamines by LM-histochemical techniques and show a weak response to prefixation incubation in metaraminol. The only other contents are a small number of 700 Å agranular vesicles, reticulum, mitochondria, and glycogen. The fibres occasionally come into close contact with muscle fibres, but there is no evidence for a specialized contact or of vesicle aggregations in these areas. Also, no evidence was found that these fibres bear junctions with other cell types or are engaged in secretion of their vesicles.

Fibre type 2 is the process of new-cell type 2. Although their cell body has not been observed on an EM level, their processes are easily identified by their large and irregular size, ranging from 0.3—7 μ in diameter. The fibres, which are found throughout the body, lie only in the superficial layer of the plexus and may cause a displacement of the epithelium overlying larger varicosities (Fig. 23A). They are characterized by the presence of two types of vesicles: the more numerous type is a spherical vesicle (600—800 Å) that is dense cored in Cavey-fixed tissue and solid with Petęya fixation (Fig. 23, B and C); the other is a small number of agranular vesicles averaging 540 Å in diameter, found primarily in the varicosities (Fig. 23C).

The electron density of the dense-cored vesicles is sensitive to metaraminol incubation indicating that these vesicles contain catecholamines; they also stain by the Wood alcian blue and by the Batham techniques.

Fibre type 3 is found in both layers of the plexus throughout the body, although it is most frequent in the column and mesentery. It is a small fibre, 0.2 μ in diameter, with periodic varicosities up to 0.8 μ in diameter. It contains two classes of vesicles; one is a dense-core vesicle (600—800 Å) that is found throughout the fibre and the second is a solid vesicle (1200—2000 Å) that is rarely found in the varicosities (Fig. 24). This fibre is known only from EM study; its vesicles apparently do not stain by any of the LM procedures employed.

In the column and pedal disc the majority of these fibres are found in the intramuscular layer of the plexus where they are in close association with the type B form of muscle fibres. Although the fibres, particularly their varicosities, are often closely apposed to the muscle fibres there is little evidence of vesicle aggregations at these sites and there is no sign of membrane specializations.

Fibre type 4 is known only from EM sections of the column where it is rarely found in the intramuscular layer of the plexus. It is a small fibre, 0.4—0.8 μ in diameter, whose 1.3 μ varicosities are filled with opaque vesicles (650—1500 Å) and a small number of 1100 Å dense-cored vesicles (Fig. 25).

Fibre type 5 is also known only from EM study. It is found in the

intramuscular layer of the plexus of the column and pedal disc and in the superficial layer in the rest of the body. It is 0.2 μ in diameter with 0.4—1.0 μ periodic varicosities. The fibre is characterized by large numbers of solid vesicles (1000—1500 Å) that are concentrated in the varicosities (Fig. 26). It is the only endodermal fibre type, other than recognized receptor and nerve-cell axons, that has been seen to contain microtubules.

The axon of the receptor is easily recognized on an EM level by its content of the same dense-core vesicles (700—1500 Å) that characterize the cell body (Fig. 17). The fibres may be either uniform in diameter at about 0.15 μ or they may have varicosities of 0.2—0.7 μ in diameter every 2—4 μ along their length. If there are varicosities there is a strong tendency for the vesicles and other organelles to be concentrated within them. In addition to the dense-core vesicles the axons contain microtubules, mitochondria, glycogen, swollen membrane sacs, and a small number of agranular vesicles of varying sizes.

These fibres have been observed throughout the body, and are concentrated at the junction of mesenteries to the column and pedal disc. They lie in both layers of the plexus, and show a strong tendency to run with the muscle fibres, even running with the muscle bundles that run under the insertion of mesenteries to the column.

The sensory synapse has been identified (Fig. 27). It appears to be poorly fixed in Cavey-fixed material; however, interneural synapses in the same tissue show relatively good preservation (Figs. 28, 29). The area of apposition is distinguished by an increased electron density of the pre- and postsynaptic membranes and of the interposed extracellular space, and by an increased separation of the two fibres from

150—200 Å in the surrounding areas to 200—250 Å in the area of the junction. In the adjacent cytoplasm there is an aggregation of agranular, irregularly spherical vesicles. These are 500—900 Å in diameter and are identifiable as the vesicles found scattered through the axon in nonsynaptic sites. The postsynaptic fibres appear to all be of a single type, but they have only partly been characterized (see below).

Neurons. As seen by LM (Pantin, 1952, fig. 13) the frequency of neurons in the endoderm of anemones is much lower than that of the ectodermal neurons in both anemones (this thesis) and cerianthids (Peteya, 1973). Thus, although it has been possible to learn something of the ectodermal nervous systems through observation of random sections, the same technique of study has provided almost nothing of the endodermal system.

Two or possibly three types of interneural nerve fibres have been observed. Most of the nerve fibres have been recognized because they are postsynaptic fibres at receptor synapses (Fig. 27). All such fibres appear to be a single class containing microtubules and small agranular vesicles, but the organelle content is too low to permit a characterization of a fibre type upon the limited observations here. A second possible type of nerve fibre is seen in the unique observation of the postsynaptic fibre in Figure 28; its irregular agranular vesicles are larger than those seen in any other fibre identified. It has been possible to characterize only the third type of nerve fibre (Figs. 28, 29); although observed in only five cases its large aggregations of synaptic vesicles, both at synapses (Fig. 28) and at

nonsynaptic areas, permits its characterization. The synaptic vesicles are between 600 and 900 Å in diameter, spherical, and agranular in aldehyde-fixed tissue. They, some larger agranular vesicles, microtubules, and glycogen are the only organelles observed in these fibres. The gap is 200 Å wide, and no gap or cytoplasmic specializations are evident. In two of the interneural synapses seen there is a sac of agranular reticulum closely apposed to the postsynaptic membrane (Fig. 29); because it is absent in other synapses of this type (Fig. 28) its significance is uncertain.

No neuromuscular synapses in the endoderm of *Stomphia* have been found in this study even though all major muscle fields have been studied and a variety of fixatives used.

The Muscle System

The muscle system of actinians is surprisingly complex in comparison to the organizational complexity of the rest of the body. It forms about ten functional fields, some of which are capable of both fast and slow contractions. Although physiologists have built an extensive knowledge of the physiology of 'neuromuscular preparations', anatomists have done little to provide the basis needed for such studies. Histological studies have been limited to the distribution of muscle fields and to basic aspects of the organization of the epitheliomuscular cell (see above for description) (Batham and Pantin, 1951; Robson, 1957). It is currently believed that there is only one functional class of muscle fibre in anemones (Batham and Pantin, 1951; Robson, 1957), and therefore that it receives a double innervation (Horridge, 1956). However this idea is based only on the erroneous idea that the muscle

fibres all look alike in LM study. There have been no EM (except for Grimstone et al., 1958) or histochemical studies of an actinian muscle system.

The present report is limited to two observations: 1) that two morphologically distinct types of muscle fibre are found in the endoderm and ectoderm of *Stomphia*; and 2) that their distribution indicates that some muscles, such as the column circulars, are organized into two functionally discrete zones. A more complete study is in progress by Amerongen.

As described above the cell bodies of some muscle cells are epithelial in location and are joined to the fibres through a peduncle, while others are fusiform cells sunken into the muscle layer which are directly attached to their muscle fibres. Although fibres that attach directly to their cell bodies contain more organelles in the area near their attachment than do those fibres which attach to peduncles there is no other morphological difference between the muscle fibres borne by cells in these two positions. Both types of muscle cell may bear either of the types of muscle fibre described below (types A and B). The association, seen in the circulars of the column and pedal disc, of type A fibres with sunken cell bodies and type B fibres with epithelial cells is probably due to the organization of those tissues rather than to the existence of different cell types.

The type A fibres are 0.75—1.75 μ in minimum diameter, averaging 1.3 μ, and the type B are 0.5—1.5 μ in minimum diameter, averaging 0.6 μ; neither type appear to be striated. The contractile fibrils fill the fibre with only a small number of mitochondria and glycogen

particles interposed within the fibril-free areas within the mass of fibrils. There are other areas of sarcoplasm, located adjacent to the periphery of the fibre, which are devoid of fibrils and have an accumulation of mitochondria and glycogen. No evidence of a sarcoplasmic reticulum, as is found in the ctenophores (Hernandez-Nicaise, 1974) and higher phyla, is found.

Muscle-fibre type A has two types of fibril that are disposed parallel to each other. The thick fibrils are 90—170 Å in diameter, and appear as hollow tubes in cross section (Fig. 30). These fibrils appear regularly spaced (about 350 Å apart); although a hexagonal array is sometimes apparent, the fibrils are not disposed in a precise geometric order. In longitudinal sections the thick fibrils do not show the banding characteristic of paramyosin. In the same sections, the diameter of the thin fibrils is approximately 60 Å. They have no association with the thick fibrils (Fig. 30), but are found scattered throughout the fibre including those areas which do not contain thick fibrils. Limited counts indicate a ratio of about 10 thin fibrils to 1 thick fibril. Dense bodies, found in smooth muscles of some invertebrates, are not found in either type of muscle fibre.

The type B muscle fibres differ from A fibres primarily by the presence of paramyosin-like fibrils, 70—400 Å in diameter, in place of the thick fibrils found in the A fibres. In longitudinal sections the paramyosin-like fibrils are spindle-shaped and show the periodic banding (Fig. 31 inset) that distinguishes paramyosin in muscle fibres in other invertebrates. Their tapered shape accounts for much of the size range as measured in cross-sectioned fibres. They are surrounded

by a circle of thin fibrils identical to those seen in the A fibrils (Figs. 31B, 32, 33).

The type B fibres are highly variable in appearance and many appear to be intermediate between types A and B (Figs. 32, 33), indicating that the two types may represent different states of contraction or extension of a single type of muscle fibre rather than two distinct morphological classes of fibre. Indeed, it seems possible to distinguish the degree of conversion of an A fibre into a B fibre by the relative frequency of thick to paramyosin-like fibrils and by the diameter of these fibrils. In fibres that have only a few paramyosin-like fibrils they are only 70—180 Å in diameter (Fig. 32) whereas in fibres that have only a few thick fibrils the paramyosin-like fibrils approach 400 Å in maximum diameter (Fig. 33).

There is, however, some evidence that the A and B fibres may represent functionally distinct classes of muscle fibres. In most of the muscle fields the A fibres are either the only type found (as in the transverse muscle) or else comprise 97—99% of those found (as in the circular muscles of the tentacle and oral disc, the sphincter and retractor). In contrast, the type B fibre is the only type found in the parietals and comprises over 70% of the muscle fibres in the circular muscle of the column and pedal disc (Fig. 2); thus there is a clear association of B fibres with muscles involved in swimming. Moreover, there is evidence that in the circulars of the column and pedal disc the A and B fibres may have different mechanisms for innervation. Unlike the circulars of *Metridium* (Batham and Pantin, 1951), the circulars of *Stomphia* are organized like a mesogleal sphincter (Fig. 2).

All of the type B fibres of the circulars are found in a band at the endodermal face of the mesoglea, while all of the type A fibres are found in tubes that are sunken into the mesoglea. Unlike the sunken muscle tubes of the tentacle ectoderm (see Chapter 6) these tubes have never been seen to fuse with the overlying endoderm; and they only rarely contain a nonnervous element of the plexus. The A fibres therefore appear to be largely sealed off from the endodermal nervous system whereas the layer of B fibres is in direct contact with the fibre plexus. However, since it has not been possible to identify neuromuscular synapses in the endoderm any idea of the interaction between the nervous system and muscle system is premature.

Discussion

Most of our histological knowledge of anemones has come from neuroanatomical studies which have devoted little attention to non-nervous histology; as a result the Hertwigs (Hertwig and Hertwig, 1879) remain our major source of anatomical knowledge. There are three observations from this study which have some bearing upon our basic concepts of the actinian endodermal nervous system: 1) that it is composed of twice the number of nerve-cell types that have been described previously; 2) that the nervous system lies within a complex plexus of fibres of unknown nature; and 3) that some of the muscle fields contain what appear to be two physiologically and functionally distinct classes of muscle fibre.

The Nervous System

The histological study of actinian nervous systems is subject to

several technical and conceptual difficulties (see Chapters 4, 5, and 7) which have led to a literature which is largely a collection of contradictory accounts. Only the endodermal neural elements have lent themselves to analysis; some of the elements can be traced back as far as the Hertwigs (Hertwig and Hertwig, 1879) and a detailed model of the endodermal nerve net has recently been worked out by Batham, Pantin and Robson (Pantin, 1952; Batham et al., 1960; Robson, 1963). According to their studies the endodermal nerve net consists of three elements: 1) a bipolar, epithelial receptor which is found concentrated at the insertion of the mesenteries to the oral disc, column, and pedal disc and scattered on the oral disc, mesenteries, column and pedal disc; 2) a large bipolar neuron (believed to represent the "through-conducting-system") which is found in the pedal disc, mesenteries, column, and oral disc; and 3) a large multipolar neuron found only in the column.

The present LM study is based on two techniques, ZIO impregnation and the Wood's alcian blue technique, not previously utilized in the study of cnidarian nervous systems (although it should be noted that the applicability of the Wood technique has already been noted by Wood and Lentz, 1964). Through the use of these techniques two new types of neuron have been demonstrated. These are a small bipolar neuron, first described by Leghissa (1949), and a tripolar neuron, which was probably first described by the Hertwigs (Hertwig and Hertwig, 1879). Both cell types were seen in the studies of Batham, Pantin and Robson (Pantin, 1952; Batham et al., 1960; Robson, 1963) but were interpreted as being rare variants of the large bipolar neuron which they had discovered. However, counts here of neurons in Wood's stained mesenteries give a

ratio of 5 to 6:1:1 for the large bipolar : small bipolar : tripolar cells. Each is a separate cell type distinguished by its size, shape, and staining affinities (Figs. 18, 19, 20, 21; Table 1). On the basis of the present LM study it is proposed that the endodermal nerve net of *Stomphia* is composed of five elements: 1 and 2) the receptor and large multipolar neuron as described by Robson (1963), 3) a large bipolar neuron which is 3—12 μ in diameter and 14—70 μ in length, 4) a small bipolar neuron which is 2—3 μ in diameter and 5—12 μ long, and 5) a small tripolar neuron which is about 10 μ in average diameter.

Even with the addition of these new nerve-cell types the endodermal nerve net is still highly diffuse, making its EM analysis through study of random sections impractical. This study has been limited to the EM observation of only a small number of possible nerve fibres, five interneuronal junctional complexes, and t[illegible] possible nerve somata. Therefore almost nothing has been lea[illegible] the EM morphology of the interneurons. It has been possible, bec[illegible] of their concentration at the junction of mesenteries to the column, to recognize the receptor and its axon and synapse, but it appears that this is the only aspect of the endodermal nerve net that can be easily studied without the development of a technique which will permit the sequential LM and EM examination of identified neurons. In the study of vertebrate nervous systems Golgi impregnations have been successfully used for sequential studies (Blackstad, 1970). Although Golgi impregnations are believed to be worthless in the study of anemones (Batham et al., 1961) it should be possible to develop a comparable technique suitable for anemone research since both ZIO (Martin et al., 1969) and methylene blue (Richardson,

1969) impregnated neurons can be studied on an EM level. With the development of such a technique it would be possible to locate a desired type of nerve soma or interneural junction, cut it free from the tissue, and resection it for EM; its potential value in the study of actinian nervous systems is immeasurable.

The Fibre Plexus

Little is known of the nature of the fibre plexus; the only LM record of a plexus is that of von Heider (1877, 1879) who was discredited by others who failed to see a plexus in their own studies (Hertwig and Hertwig, 1879). However, a fibre plexus, currently thought to be a nerve plexus, has been found in the ectoderm of every actinian, cerianthid, and madreporarian examined by EM (Kawaguti, 1964; Leghissa and Quaglia, 1966; Kawaguti and Ogasawara, 1967; Westfall et al., 1970; Peteya, 1973, unpublished) and in the endoderm of the coral *Dendrophyllia* (Kawaguti and Yokoyama, 1966), *Aiptasia* (Amerongen, personal communication) and *Stomphia* (this thesis). The rediscovery of a plexus was delayed until the first EM studies in large part because the fixatives used by most anemone light microscopists destroy or severely damage the components of the ectodermal plexus thus making them difficult or impossible to see (see Chapter 5). When first seen in EM study the plexus was thought to be an extraordinarily complex nerve plexus which for some reason had been missed in earlier LM studies (Kawaguti, 1964); most subsequent workers have not questioned either idea. However, detailed study of the components of the plexuses of *Ceriantheopsis* (Peteya, 1973), the tentacle ectoderm of *Stomphia*, and the mesentery

endoderm of *Stomphia* shows that only 50% to less than 10% of the fibres in these plexuses are neuronal.

Thus we are presented with a complex of cells, about which almost nothing is known, which is in intimate association with both the nervous system and the muscle system. Such an association of cells with the nervous system is unprecedented in the Cnidaria, and it is tempting to postulate that these nonnervous cells could have a trophic function on the muscles or neurons, or serve as a glia, or represent the neuroidal conducting system that is believed to exist in anemones (for review see Shelton, 1975).

Although analysis of the function of the nonnervous elements of the plexus will likely require extensive experimental and histochemical work, the first step must be to select a model system for analysis and to build a catalogue of the cell types that contribute processes to a plexus. From my experience, in *Stomphia* the endodermal plexus is preferred over that of the ectoderm for such an analysis. The ectodermal plexus can be highly complex and its composition varies in different parts of the body and between species. It is difficult to study, and because of the fixation artifacts discussed in Chapter 5 little is known of its histology. In contrast, the endodermal plexus of *Stomphia* contains only 5 types of nonnervous fibres (some of which are also found in *Metridium* and *Aiptasia*); all but one of the nonnervous fibre types is found throughout the body, and we now have a clear picture of most of the endodermal neural elements.

This chapter is largely a report of the efforts in this study to catalogue the cells whose processes form the endodermal fibre plexus.

The report is incomplete; some of the cells described are known only on either a LM or EM level, and some others have been seen too rarely to be described here. Despite this it is apparent that there are no obvious anatomical clues as to the function of any of the nonnervous cells or fibres of the plexus. Only two observations of possible value to this question have been made in this study. Firstly, that none of the nonnervous fibres form recognizable junctions with either the nerve fibres or the muscle fibres. However, several of them run for long distances along muscle fibres with only a 200 Å gap separating them; such appositions between putative nerve fibres and muscles have often been described as functional junctions in other invertebrate phyla (Rosenbluth, 1972; McKenna and Rosenbluth, 1973) and in the vertebrate autonomic nervous system (see Bennett, 1972 for review). Secondly, that several of the fibres of the plexus (types 1, 2, 3) contain catecholamines and show an uptake of exogenous catecholamines. No evidence, however, has been found that any of the fibres are secretory.

Mention must also be made that an argument can be put forward against the idea that the nonnervous components of the fibre plexus have any particular and special function. The ectodermal fibre plexus of *Cerianthéopsis* contains only neural elements and a "type B fibre" of unknown nature (Peteya, 1973). Subsequent study has shown that the B fibre is a process from muscle-cell peduncles whose function is to increase the area of contact between the muscles and the nervous system (Peteya, unpublished observation). Thus the nervous system of this cerianthid does not have any associated nonnervous cells to which this special function could be ascribed.

The Muscle System

In contrast to the findings of previous studies of actinian muscle fibres (Batham and Pantin, 1951; Robson, 1957; Grimstone et al., 1958), two morphologically distinct types of muscle fibres have been observed in *Stomphia*. The two types, A and B, merge into each other indicating that they may represent different states of contraction of a single type of muscle fibre rather than being two distinct morphological classes. In a study of contraction states of the tentacular muscle of *Chrysaora* fibres similar to type A in this study were described as being fully extended or else as contracted, whereas fibres like my type B were described as being partly extended (Perkins et al., 1971). Since a comparable experiment has not been performed here it cannot be determined whether the A and B fibres represent two states of expansion of a single class or whether they are two morphologically distinct classes. Nonetheless, the more important question of whether they represent two physiologically distinct classes can be answered. If they are distinct morphological types their differing morphology is probably the expression of some physiological distinction. On the other hand, if they are simply two states of a single type of muscle, the fact that they are found in different states of extension in a single tissue implies either that they have different resting lengths or that one is being held in a state of contraction by some Mg^{++}-insensitive mechanism. In either case both a physiological distinction and the possibility of separate innervations of the type A and B muscle fibres is implied.

If it is true that the two types of muscle fibres identified here represent two physiologically or functionally distinct classes of muscle

cell, then this is the first such record in the Actiniaria and directly contradicts an assumption that is basic to the interpretations of physiological studies of actinian 'neuromuscular preparations'.

Chapter 4

THE MESOGLEA

Introduction

The mesoglea is a connective tissue, analogous to mesoderm, that lies between the ectoderm and endoderm in all parts of the body and between the two layers of endoderm that form the mesenteries. It is composed of fibrous protein embedded in a mucopolysaccharide matrix and a variable number of one or more cell types, depending upon the taxa. The mesoglea of anemones is particularly well developed and contains a large variety of an unknown number of cell types. Although the actinian mesoglea is of special interest since its cellular complexity matches that of the mesoderm of some higher phyla little is known of it other than its biochemical and mechanical properties (for reviews see G. Chapman, 1966, 1974); its cellular components are almost unknown.

The major problem limiting our knowledge of the mesogleal cells is conceptual. The question of the presence or absence of mesogleal neurons is the greatest controversy arising from studies of the actinian nervous system. Transmesogleal nerve fibres and endogenous mesogleal neurons have been described in most studies of anemone mesoglea, and a clear picture exists of three mesogleal cell types (the amoebocytes, fibrocytes and neurocytes) from the LM studies of Leghissa (Leghissa, 1949, 1965; Leghissa and Mazzi, 1959), although he has not been able to identify the neurocyte in EM study (Leghissa and Quaglia, 1965). The major argument against the existence of mesogleal neural elements is the claim of the Hertwigs that such elements do not exist in the

anemones (Hertwig and Hertwig, 1879). Although it might be expected that the Hertwigs' report would have been replaced by the findings of subsequent studies, particularly so in light of the long-standing LM and EM knowledge of a mesogleal nerve net in the related Alcyonaria (Hickson, 1895; Ashworth, 1899; Kassianow, 1908; Titschack, 1968, 1970; Buisson and Franc, 1969; Buisson, 1970), the reverse has occurred. Out of an exaggerated respect for these famous histologists contemporary workers have discredited every study that has reported the presence of mesogleal neurons or transmesogleal nerve fibres (Pantin, 1952; Batham et al., 1961). Even though these same workers have subsequently reported the existence of transmesogleal nerve fibres (Batham, 1965; Robson, 1965), reviewers continue to follow their initial papers and ignore the findings of the detailed mesogleal studies of Havet (1901, 1922) and Leghissa, acknowledging the existence of only the amoebocyte (Bullock and Horridge, 1965; Josephson, 1974; G. Chapman, 1974).

This chapter reports the presence of three endogenous mesogleal cell types: the amoebocyte, the 'small mesogleal cell' which is comparable to Leghissa's fibrocyte and neurocyte, and a new cell type found only in the tentacles, oral disc and pharynx. The only neural element found in the mesoglea is a presumptive ectodermal nerve fibre which innervates the sunken ectodermal muscles of the tentacles and oral disc.

Materials and Methods

In contrast to the experiences of previous studies it was found difficult in this one to stain mesogleal cells for LM study. Of all

the LM techniques employed (see Chapter 2 for a list) only ZIO impregnations and a technique developed here have provided a consistent and adequate LM visualization of mesogleal cells. The ZIO technique gives an intense nonspecific staining of mesogleal cells in the mesenteries in areas where the tissue is thin and contains few overlying muscles (e.g. the free edge of the mesentery) or is relatively thick and contains few muscles (e.g. the mesentery near the sphincter). If the tissue is thick and contains a thick muscle layer only the muscles and a few endodermal cells will stain. Also, Araldite sections of tissue fixed in 3—4% formic acid and 0.1% OsO_4 show an intense staining of all cells in the mesoglea when stained with toluidine blue. Unlike the ZIO technique this one is applicable to all areas of the body. Since both techniques stain all types of mesogleal cells the shape of the cells is the only character by which the types described below have been distinguished.

For EM study the Cavey and Peteya fixatives were found to be the most suitable for general histology. The Wood and Woods chromaffin techniques and prefixation-incubation with metaraminol have been used for the EM-level localization of catecholamines in the 'small mesogleal cell' (SMC). Although limited examination has been made of the mesoglea throughout the body, this report is limited to LM study of mesentery whole mounts and to EM study of mesenteries, column, tentacle and oral disc.

Results

In LM examination of mesenteries two populations of mesogleal cells

are evident. One is the amoebocyte, which through its large size, lysosomes, and highly variable shape is easily recognized and has usually been identified as an amoebocyte in previous studies (Havet, 1901; Leghissa, 1949; Batham et al., 1960). The other, which I will call the small mesogleal cell (SMC), is a small bi-, tri-, or multipolar cell which has fibres that may or may not branch. This cell has either been identified as a neuron (Havet, 1901, 1921; Leghissa, 1949), separated into fibrocytes and neurons (Leghissa and Mazzi, 1959; Leghissa, 1965), or else has been overlooked or confused with amoebocytes in most other studies.

The mesogleal amoebocyte has the most variable shape and appearance of any cell type in an anemone. In the mesenteries they vary from spheres to long filiform cells, but are usually elongated and only rarely bear processes. In the tentacles and oral disc the amoebocyte is usually elongated and may bear up to eight processes that are usually only a few microns in length (Fig. 34) but which may be as large as the rest of the cell (Fig. 35). In ZIO impregnations the amoebocytes stain intensely, more so than the SMC; only the cytoplasm and some vesicles stain leaving the lysosomes and other vesicles clear (Fig. 37).

On an EM level the amoebocytes are characterized by their lysosomes and by large numbers of dense-core or solid vesicles (1500—3800 Å and 1300—2000 Å) and by a small number of agranular vesicles (650—800 Å) (Fig. 56). The appearance of the electron-dense vesicles is dependent on the fixative; in Peteya-fixed tissue the smaller class is more electron-dense and is compressed into rod-shaped vesicles,

while with Cavey fixation the smaller class is only slightly more dense and both sizes are spherical (Fig. 36).

It is difficult to confidently identify the other mesogleal cells as a single cell type as is done here. As Leghissa has shown (Leghissa and Mazzi, 1959; Leghissa, 1965) their appearance is highly variable. After initially describing the SMC as a single class of neuron (Leghissa, 1949) he separated them into the neurocytes and fibrocytes. He reported that the neurocyte is distinguished from the fibrocyte by its larger volume, its different staining affinities, larger nucleus to cytoplasm ratio, and by the tendency to be bipolar rather than multipolar, agranular rather than granular, and to have fibres that branch only rarely. The ZIO observations in this study closely follow those of Leghissa in that nearly all of the bipolar SMC observed can be identified as 'neurocytes' or 'fibrocytes'; however, no clear subdivision can be made of the tri- and multipolar SMC of *Stomphia*. Moreover, EM examination of these cells indicates that all of the SMC are of a single class despite their variation in shape; Leghissa also has not found EM evidence for a subdivision of the SMC (Leghissa and Quaglia, 1965). Nonetheless the presence of areas of mesentery which contain only one form of SMC argues that there may be some significant variation within this group. The present study cannot fully answer the question; further study of the mesogleal cells is badly needed.

The small mesogleal cell (SMC) is either bipolar (Figs. 38; 39, A,B,C,D,E), 3 x 15 μ, tripolar (Figs. 39, F,G,H.; 40, A,B), 3 x 10 μ, or multipolar (Fig. 40, C,D,E,F,G), 4 x 12 μ, in shape. The nuclei of the bipolar cells are ovoid and fill much of the cell

bodies while those of the tri- and multipolar cells are circular and proportionally smaller. They are easily distinguished from amoebocytes by their smaller size and small uniform-diameter fibres. The description of amoebocytes that have long branching fibres (Batham et al., 1961) is probably the result of confusing SMC with amoebocytes.

The clearest evidence that the SMC is composed of two cell types comparable to the fibrocyte and neurocyte of Leghissa is found in the morphology of the bipolar cells. As Leghissa has shown, a small number of the bipolar cells are characterized by a cytoplasm which contains no visible vesicles and by a relatively large (0.3—1 μ) fibre which infrequently shows first-order branching (Fig. 38, A,B,C). Most of the remaining bipolar SMC resemble the fibrocyte in that their cytoplasm is granular and their fibres branch frequently (Fig. 38, D,F,G). It is rare to find a bipolar cell which appears to be intermediate between these states in that it has granular cytoplasm and unbranching fibres or is agranular and branching. In contrast, numerous bipolar cells are seen which appear to grade into tripolar cells in that they have a third fibre which arises from either one end or from the middle of the spindle-shaped cell body (Figs. 38, E,H; 39, A,B). Thus the bipolar SMC of *Stomphia* closely resemble those of *Actinia* (Leghissa and Mazzi, 1959) and appear to be composed of a discrete class of 'neurocytes' and a class of 'fibrocytes' which grade into the tripolar fibrocytes.

The tri- and multipolar cells are more variable in shape than the bipolar SMC, but are usually elongated, 3—4 x 10—12 μ, and usually give rise to three to five processes. Their nuclei are spherical and proportionally smaller than those of the bipolar cells. Their fibres

and cell bodies may contain small stained or unstained vesicles as in the bipolar cells, and the cell bodies also may contain large unstained vesicles (Fig. 40A) which are not seen in the bipolars. The presence of neither type of vesicle can be associated with the occurrence of branching of the fibres. The fibres of the tri- and multipolar SMC are estimated to be 0.2—0.5 μ in diameter, with rare varicosities that are up to 2 μ in diameter. Although the fibres of some cells do not branch those of most cells show first, second, and third order dichotomous branching (Fig. 40, A,B,E). In almost all cases of branching there is a large aggregation of cytoplasm, 0.5—3 μ in diameter, at the junction of the branch; in extreme cases therefore the junction may resemble a tiny tripolar cell (Fig. 40A) and give the impression that the network formed by these cells is a syncytium.

Despite the great variability in shape and appearance of the SMC on a LM level it appears from EM study that all of them belong to a single morphological type. In EM study only three types of cells and two types of fibres can be found in the mesoglea of *Stomphia*; they are the amoebocyte, the SMC, a cell that is found only in the tentacles, oral disc, and pharynx, the axon of an ectodermal nerve which innervates the sunken ectodermal muscles (see Chapter 6), and one fibre seen too rarely to have been identified in earlier LM studies.

As seen by EM the SMC is an irregularly elongated cell (Figs. 41, 42) that has a proportionally smaller nucleus than the amoebocyte and which contains more mitochondria and reticulum than does the amoebocyte. The SMC is characterized by large numbers of spherical to elongated vesicles (1200—3700 Å) that, in aldehyde-fixed tissue,

are solid (Fig. 43) but which in permanganate-fixed tissue are solid- or dense-cored.

There is no direct evidence, from this EM study, to support Leghissa's identification of some of these cells as being a separate class of neuron. The SMC only rarely come into contact with each other or with amoebocytes, and have never been seen to form intercellular junctions. However, in the tentacles and oral disc the fibres of the SMC, but not the cell bodies, regularly invade the tubes of sunken ectodermal muscle fibres (Fig. 44), so that at any point along the tube one to four SMC fibres can be found. Although they do not appear to form junctions with the nerve fibres or with the sarcoplasmic extensions of the muscle fibres they are in close proximity to both. There is some evidence that the fibres that are within the tubes are histologically different from those that lie in the mesoglea providing preliminary evidence that the SMC may have a secretory function:

1. Although a large number of cell types of *Stomphia* contain catecholamine (CA)-containing vesicles, as evidenced by their ability to take up exogenous CA, only a few contain sufficiently high endogenous concentrations to show a positive reaction to the EM-level chromaffin techniques of Wood (1963, 1966) and Woods (1969). These are the lysosomes of ectodermal amoebocytes, the secretory vesicles of some ectodermal and endodermal gland cells, the large vesicle found in ectodermal fibre type 2, and the vesicles of the SMC (Fig. 45). Thus the vesicles of the SMC have not only one of the highest endogenous concentrations of CA among the cells of *Stomphia*, but a concentration higher than that of the vesicles of some ectodermal neurons known to

contain CA (see Chapter 6). Only those cells and fibres that lie free in the mesoglea have chromaffin-positive vesicles; those within the muscle tubes are always negative. This apparent decrease in CA content of the vesicles can be explained in only two ways: 1) that the muscle fibres constitute a penetration barrier to the fixative which allows for a greater prefixation loss of CA from the processes within the tubes than from those within the mesoglea; and 2) that the SMC is secretory, and that its mechanism of CA release does not involve the loss of the vesicle or its matrix. From present knowledge neither possibility is acceptable; the question of a secretory function of the SMC will require further study before it is resolved.

2. Prefixation incubation of an animal in metaraminol shows an uptake of the exogenous CA into two types of ectodermal nerve fibres, into several of the nonnervous endodermal fibre types, and into the vesicles of the SMC (Fig. 44). Although uptake of metaraminol into the ectodermal and endodermal fibres was regularly seen, the results with the SMC were often negative or questionable. In some cases one tentacle from an animal would be negative while the next showed an incorporation into the SMC, implying the presence of an unknown variable in the tissue which affects observations of the SMC or of a higher sensitivity of the SMC to Mg^{++}-induced inactivation of CA uptake than in the other cells studied.

In addition to the amoebocyte and the SMC there are only three other cell or fibre types found in the mesoglea. One is the ectodermal nerve fibre which innervates the sunken ectodermal muscle fibres. While there is a strong tendency for this fibre to run within the tubes

of muscle fibres it is frequently found crossing the mesoglea between adjacent tubes (see Fig. 92) and neuromuscular junctions are often found on sarcoplasmic extensions of the fibres which have extended out from the tube rather than into its core. This fibre will be described in the following chapter; it is mentioned here only to record the presence of a known nerve fibre within the mesoglea. The second is a fibre characterized by solid vesicles (1000—3500 Å) (Fig. 46, A,B). It is the process of a large, 5 x 25 μ, mesogleal cell of unknown nature; the cell body has been observed only once, on an EM level, and its shape is therefore unknown. It is found in low numbers only in the mesoglea of the tentacle, oral disc and pharynx. The third is known from a unique observation of a fibre, in the oral-disc mesoglea (Fig. 47, A,B), which contains agranular vesicles (500—1300 Å). Its vesicles are similar in appearance to those found in ectodermal nerve fibre type 4 (Chapter 6; Fig. 48) and to those found at endodermal interneural synapses (Chapter 3; Figs. 28 and 29) but are distinct from each in their size range.

Discussion

In this study, as in any neuroanatomical study of the mesoglea of an anemone, there is only one question to be investigated: whether there are neural elements in the mesoglea. Although it is a question that is basic to our understanding of the anemone nervous system, it is a controversial subject in which speculation has effectively been discouraged.

The Hertwigs believed that there were no neural elements in the

mesoglea, and that the only site of functional interaction between the ectoderm and endoderm was at the aboral edge of the pharynx where the two tissue layers are in direct contact (Hertwig and Hertwig, 1879). Although several subsequent studies have indicated the presence of a mesogleal nervous system in anemones (Havet, 1901, 1922; Parker and Titus, 1916; Parker, 1919; Leghissa, 1949, 1965; Leghissa and Mazzi, 1959; Leghissa and Quaglia, 1965) all reports of mesogleal neural elements have been rejected (Pantin, 1952; Batham et al., 1961; for reviews see Bullock and Horridge, 1965 and D. Chapman, 1974). This is a particularly questionable action in light of our detailed LM and EM knowledge of a similar mesogleal nerve net in the Alcyonaria (Hickson, 1895; Ashworth, 1899; Kasianow, 1908; Titschack, 1968, 1969; Buisson and Franc, 1969; Buisson, 1970). As a result of this adherence to the Hertwigs' claim that the anemone mesoglea does not contain nerves there is little known, from accepted studies, of the mesogleal cells other than that amoebocytes similar to those in the ectoderm and endoderm are also found in the mesoglea.

Since the time of the Hertwigs there has been no generally accepted modifications in our understanding of how the ectoderm and endoderm interact other than: 1) to postulate interaction at other known sites of direct contact between the two tissue layers (Pantin, 1952; Batham et al., 1961, Robson, 1965); 2) to suggest that endodermal receptors can be directly stimulated by mechanical stimulation to the column ectoderm (Passano and Pantin, 1955); and most significant, 3) to claim that the large bipolar endodermal neuron crosses the mesoglea to innervate the sunken sphincter muscle fibres (Robson, 1965) and to

enter the oral disc ectoderm (Batham, 1965). Despite these new reports of transmesogleal nerve fibres there is little more known of the mesogleal histology than the Hertwigs had shown; the reports of the fibres are poorly documented and need verification, and the only recent histological study of the mesoglea, other than Leghissa's EM studies, has described only the amoebocyte (Young, 1971; cited by G. Chapman, 1974). However, there now exists preliminary physiological evidence for a transmesogleal pathway in the column of *Stomphia* (Lawn, personal communication). With the coming of such knowledge a closer look will have to be taken at the mesoglea and at Leghissa's studies.

The present study has illustrated the presence of three endogenous mesogleal cell types and of two other cells, known only from their fibres, which may belong to the mesoglea or to one of the other cell layers. Observations allow limited discussion of the possibility of the occurrence of nerve fibres and of neurons in the mesoglea.

Many anemones have sunken muscle fields; *Stomphia*, for example, has sunken ectodermal longitudinal muscles in the tentacles and oral disc, circulars in the column and pedal disc, and a sunken sphincter. If such muscle fields are neuronally innervated we should expect to find transmesogleal nerve fibres running from the muscle tubes to the nerve net in the overlying epithelium. Robson's (1965) claim that the large endodermal bipolar crosses the mesoglea to innervate the sunken sphincter of *Calliactis* is the only credited report of this type of invasion of neurons into an anemone mesoglea; unfortunately, however, she did not substantiate her claim with any micrographs. The report in this study of an ectodermal nerve fibre in the tentacle and oral

disc of *Stomphia* (see Chapter 6 for description) which runs through the mesoglea separating adjacent muscle tubes is therefore the first documentation of a nerve fibre which lies in an anemone mesoglea specifically for the innervation of sunken musculature.

The question of whether elements of the ectodermal and endodermal nerve nets cross the mesoglea to enter the other layer is more difficult to explore. Such fibres have been identified in only three studies (Havet, 1901; Leghissa, 1949; Batham, 1965) and, as already stated, Havet and Leghissa have been discredited for their descriptions of endogenous mesogleal neurons and the report of Batham is too poorly documented to be readily acceptable. The present study can provide little relevant information on the question. Large areas of the mesoglea of the oral disc have been examined on an EM level without observing any structure which could be interpreted as a transmesogleal nerve fibre; but such fibres could easily be missed if their density is low.

According to Leghissa (Leghissa and Mazzi, 1959; Leghissa, 1965) there are three types of mesogleal cell, one of which he thought to be a neuron; however, in EM study he has been able to identify only the amoebocyte and fibrocyte. From the present EM study it appears clear that the fibrocyte and neuron described by Leghissa are a single cell type, described here as the 'small mesogleal cell', but the results of this LM study are not as clear. In LM study the majority of the bipolar SMC conform closely to Leghissa's description of neurons and fibrocytes but the frequency of intermediate cells among the tri- and multipolar SMC is so high that their subdivision cannot be accepted here. No[illegible] the occurrence of zones of mesenteries which

contain only neuron-like or fibrocyte-like cells indicates that there may be some validity to Leghissa's classification of these cells.

This small mesogleal cell is known on an EM level only from the studies of Leghissa and myself. Neither of us have found it to form synapses or other specialized junctions with other types of cells; thus the clearest evidence of its neuronal nature is lacking. Circumstantial evidence has been presented here that may indicate a secretory nature of the SMC. The vesicles in its processes contain relatively high levels of a CA as evidenced by a positive reaction to the Woods chromaffin reaction and they take up exogenous CA, and in the tentacle and oral disc, where the cell's processes are associated with the sunken ectodermal muscle fibres, the vesicles have a measurable lower concentration of CA as evidenced by a negative reaction to the Woods reaction. If this apparent drop in the concentration is related to a secretory activity of this cell, then the SMC may be seen to be secretory or hormonal in nature. Clearly it is doubtful that a secretory mechanism exists in which there is no loss or change in morphology of the vesicle such as appears to be the case here. Nonetheless, it is hoped that these histochemical data will provide a basis for further study of this almost unknown class of cell.

It is clear that we know very little of the degree to which the anemone nervous system invades the mesoglea. Although it appears that some sunken muscle fields are innervated by fibres of neurons that extend through the mesoglea from the overlying nerve net, there is no clearly demonstrated case of nerve fibres crossing the mesoglea to interconnect the ectoderm and endoderm or of endogenous mesogleal neurons.

Our knowledge of the histology and function of the nonnervous mesogleal cells is also inadequate. What little is known of their function from the few recent studies of Polteva (1970) and Young (1971) (both as cited in G. Chapman, 1974) has been ascribed to 'amoebocytes' in the erroneous belief that that was the only mesogleal cell type. Of particular importance for future studies is our need for a detailed knowledge of the physiology and the histochemistry of the small mesogleal cell.

Chapter 5

A STUDY OF THE FIXATIVE-INDUCED DESTRUCTION OF COMPONENTS OF THE ECTODERMAL FIBRE PLEXUS OF ACTINIANS AND CERIANTHIDS

Introduction

> Everyone who has endeavoured to anatomize one of the Actiniae must acknowledge the excessive difficulties which accompany the attempt. The irritability of the muscular tissues, their persistent contraction during manipulation, the confusion caused by the abundance of different cellular histological elements, and the general sliminess of the whole, render the minute examination very troublesome and usually very unsatisfactory.
>
> Duncan (1874)

From the earliest studies neuroanatomists working on Cnidaria have felt a need to include with their data an explanation of why their studies have achieved so little. Even today the nervous system of Cnidaria, particularly that of the anemones, is reputed to be among the most difficult for the LM histologist to study. Partly as a result of this our literature is a collection of contradictory reports. It has been only since 1952, when Batham, Pantin, and Robson introduced new and standardized techniques of staining neurons, that a reproducible picture has been obtained of some aspects of the nervous system (Pantin, 1952; Batham et al., 1960, Batham, 1956, 1965; Robson, 1961, 1963, 1965). Considerable problems remain however. There have been only two generally accepted additions to our knowledge of the ectodermal nerve net since the Hertwigs' study (Batham, 1965; Robson, 1965). Similarly, almost nothing is known of the mesoglea (for review see Chapter 4).

Of greater importance than the incompleteness of our knowledge is that there is evidence that our entire concept of the ectodermal nerve net is incorrect. Recent EM studies of the anemone nervous system (Leghissa and Quaglia, 1966; Kawaguti and Ogasawara, 1967; Westfall, 1970, Peteya, 1973) have revealed a dense ectodermal plexus of fibres that has never been seen in LM studies except for the long-forgotten studies of von Heider (1877, 1879). The only explanation suggested for this is that the majority of the presumptive nerve fibres are below the level of LM resolution (Kawaguti, 1964). However, von Heider's accurate description of this fibre plexus disproves this possibility and also provides an alternative explanation for the inaccurate picture seen in all subsequent LM studies of sectioned tissue. Von Heider described as being nervous a dense plexus of fine-diameter clear fibres that lie in the lattices of the "Interbasalnetz" (= reticulum), a network of darkly staining fibres which he described as the peduncles of epithelial supporting cells. He had used 1% OsO_4 in sea water as a fixative although fixatives that contained only OsO_4 had fallen into disrepute by the 1870's; thus his nerve plexus was discounted as a fixation artifact when it was not found in studies based on tissue fixed by other fixatives (Hertwig and Hertwig, 1879, p. 582). As a consequence the Hertwigs, and most subsequent workers, have interpreted the reticulum, which is the only remaining subepithelial tissue, as being composed of the nervous system and of peduncles. Torelli's (1952) theory that cerianthids have no nervous system and that the reticulum is an anastomosing web of peduncles and branching receptor axons has been generally discounted, and is disproven by the recent EM-level demonstration of a nerve net in a cerianthid (Peteya, 1973).

While recent EM studies appear to have confirmed von Heider's observations on the nervous system there is still confusion over the nature of the reticulum. It would appear that the reticulum is composed of the supporting-cell peduncles, as von Heider had said; however, in every species that this writer has examined the peduncles do not lie in the conformation described by von Heider or by any other worker.

Moreover, some question must be raised as to how the ectodermal nerve net and fibre plexus have largely escaped recognition for almost a century. Since accurate observations of the plexus have not been made until the reintroduction of OsO_4 as a fixative in the EM studies cited above, it would appear that the morphology of the plexus and possibly of the reticulum is influenced by an unknown fixation artifact. This chapter is a report of a LM and EM study of effects of histological fixatives on the gross morphology of the ectodermal plexus of the tentacles of several cerianthids and actinians. A newly discovered fixation artifact, causing the apparent destruction of the fibres of some types of cells, including neurons, is described which precludes the possibility of an accurate and complete observation of the nervous system in studies that use many of the most commonly used histological fixatives.

Materials and Methods

Two cerianthid species, *Ceriantheopsis americanus* and *Pachycerianthus fimbriatus*, and three anemone species, *Stomphia coccinea*, *Metridium senile*, and *Anthopleura elegantissima*, have been used for study. *Ceriantheopsis* was collected from St. Tereasa, Florida and the others from San Juan Island, Washington.

Prior to fixation the animals were narcotized and dissected in equal parts of sea water and 6% $MgCl_2 \cdot 6H_2O$. Approximately 50 histological fixatives have been examined, including most of those used previously in studies of actinian and cerianthid nervous systems. Tables 3 and 4 list the fixatives, their components, the fixation times, and which species were fixed in each. The procedures for the preparation and usage of a fixative have been taken from the sources cited in Table 3. All fixatives were prepared immediately before their use. Isolated tentacles were fixed in the light (except for the Kenyon fixative) and at 20°C (except for the two Alexandrowicz fixatives which were at 4°C) in about 50 times their volume of fixative. After fixation the tissue was dehydrated in ethanol (except for those fixed in the Alexandrowicz fixatives, which required butanol-ethanol dehydration) and was embedded in Araldite. Preliminary work using wax embedding proved useless because of the relatively poor resolution of details.

With the exception of Figure 49, which is from a 5-μ wax section, all light micrographs were made from 1—2 μ Araldite sections that were stained in toluidine blue.

Results

As indicated in Table 4, the fixatives examined may be separated into three groups on the basis of the degree of damage that they cause to the fibres and peduncles: 1) those that produce a reticulum that has the configuration reported in most studies, and which do not preserve the fibre plexus; 2) those which produce little observable damage to either the plexus or to the peduncles, and thus do not present a reticulum;

and 3) those fixatives that preserve the fibres of the plexus but which cause their swelling, a swelling which causes a mechanical distortion of the peduncles.

The Reticulum

A reticulum is formed by 12 of the fixatives tested, including many of the most popular: Bouin, Brasil, Bathan, Fol, VomRath b, Susa, Orth, Zenker, Helly, Zenker's with formic acid, formalin-acetic-alcohol, and DeCastro. When examined in wax sections such tissue reveals a reticulum identical to that described in most of the earlier studies of a reticulum (Fig. 49). In such thick sections the reticulum appears as a dense network of interconnected fibres that lies between the epithelium and the muscle layer. The fibres either lie in the plane of the reticulum or else run through it from the epithelium to the mesoglea, but it is not possible to differentiate the two groups of fibre by their size or staining characteristics. The fibres of the reticulum freely interconnect and appear, as Torelli described (Torelli, 1952), to be a network of branching fibres.

In thin sections the separation of the fibres into two classes is obvious (Fig. 50). The radial elements, interpreted as peduncles, are thicker and do not branch or interconnect with each other or with the fibres lying in the plane of the reticulum; the second, possibly nervous, fibre appears to form a branching network and usually cannot be traced into the epithelium or onto the mesoglea. While it is too fine a detail to reproduce in photographs, it can sometimes be seen on the microscope that both types of fibre are aggregates of a finer fibre, and that at

junctions in the reticulum these fine fibres may cross over from one bundle to another. This was seen by Torelli (1952) who interpreted some of the fine fibres as being branching receptor axons that run along anastomosing peduncles. EM examination, however, indicates that all of the small fibres that compose the large fibres of the reticulum are of a single type. The two types of bundle seen in LM appear to differ only in the number of fibres that they contain (Fig. 51). It is possible to identify these fibres as the peduncles since they can be traced into the epithelium where some join with supporting-cell cell bodies; also, in EM study of osmium-fixed tissue the only comparable structures within the zone of the reticulum are bundles of microfilaments that run within the peduncles (Fig. 52) (Peteya, 1973, Chapter 6 of this thesis, and unpublished).

In tissue preserved by any of these fixatives which produce a reticulum the only histological elements that can be identified by LM study, including dark-field phase, in the zone of the reticulum are the peduncles of supporting cells and of muscle cells, and the cell bodies of muscle cells, amoebocytes and neurons. In such tissue the fibres which comprise the subepithelial plexus cannot be seen, and EM examination of Bouin-fixed tissue indicates that the plexus is not present in that tissue (Fig. 51). Since there is evidence that the fibre plexus is composed of as many as ten different types of fibres (Peteya, 1973, Chapter 6 of this thesis), it would appear that the mechanism whereby the fibres are destroyed is nonspecific; indeed, of all the fixatives which destroy fibres only the Hertwig macerating fixative distinguishes among the fibres, of *C. americanus* but not of actinians, destroying some

types only.

The Fibre Plexus

Most of the fixatives listed in Table 3 preserve the fibre plexus intact, but with considerable variation in the average diameter of the fibres (Figs. 52, 53, 55, 56, 57, 58, 59). Such swelling is also seen in some fixation procedures for EM study. Out of over 40 fixatives for EM that this writer used in the study of *Stomphia* and *Ceriantheopsis* only aldehyde-dichromate fixatives (Wood and Barrnett, 1963; Woods, 1969) caused a noticeable swelling of the fibres in *Stomphia*, but in *Ceriantheopsis* each of more than 20 aldehyde fixatives examined caused a swelling of the fibres (Fig. 53) that resulted, in some cases, in the rupturing of fibres, including those of neurons, and of nerve somata (Fig. 54) and in the deformation of the interspersed supporting-cell peduncles. From observation of varying degrees of fibre swelling and of the concomitant peduncle deformation a model has been developed relating the destruction of the fibre plexus to reticulum formation.

There is little evidence of fibre swelling in osmium- and permanganate-fixed tissue, and it is believed that these fixatives present a 'normal' picture of the plexus and of the supporting-cell peduncles (Figs. 52, 55, 56). In such tissue the peduncles run in a direct course from their cell bodies in the epithelium to the mesoglea; they do not cluster, branch, or anastomose (except in *Metridium*, where the peduncles are clustered at their insertion to the mesoglea). Thus nothing resembling the reticulum, as described above or in the literature, is present in well-fixed tissue (Figs. 55, 56). In addition, a fibre plexus identical to that described by von Heider (1879) is apparent.

The plexus consists of a layer, up to 20 fibres in thickness, of tightly-packed fibres that are oriented parallel to the tissue surface and which fill the space between the epithelium and the muscle layer. Although quite variable between species in its constituents (Westfall et al., 1970) the plexus appears similar on a LM level in all the species examined here. The average diameter of the fibres is less than 0.3 μ making them just visible in light micrographs (Figs. 55, 56). They are well characterized from the peduncles by their lighter staining, but individual types cannot be distinguished on a LM level.

Among the fixatives that preserve the plexus there are a few that did little or no visible damage to the fibres: Champy, Johnson, Flemming 1882 and 1884, Flemming without acetic acid, formic acid with osmium (Fig. 56), and Herman. But most caused a swelling of the fibres to two or more times their normal size. Beyond a certain degree of swelling the peduncles become distorted, apparently by the pressure that the surrounding fibres put upon them (compare Figs. 56 and 58).

Some insight into the mechanism of the formation of the reticulum and the disappearance of the plexus can be had from EM examination of tissue fixed in 10% commercial formaldehyde (in sea water, adjusted to pH 7.3) (Figs. 59, 60, 61). In previous studies formaldehyde-fixed tissue has been reported to have a reticulum identical to that described above, and no fibre plexus was seen (Torelli, 1938). However, in LM dark-field phase study of 1—2 μ Araldite sections the plexus is evident and a reticulum, in the sense of a network-like configuration of the peduncles, is no longer seen. Thus formaldehyde is unique among the fixatives listed in Table 4 in presenting an intermediate step between

those fixatives that produce a reticulum and those which preserve the plexus.

Unlike the supporting-cell peduncles in tissue which show a true reticulum, those in formaldehyde-fixed tissue are all attached to the mesoglea. Most of the peduncles are found lying in bundles of five to ten which run irregularly through the fibre plexus to the mesoglea (Fig. 59); seen in thick sections the bundles overlap giving the false impression of a reticulum. Their aggregation into bundles supports the theory that the large radial elements of a reticulum are bundles of peduncles, and by inference that the similar thin fibres are solitary peduncles. Also, since the peduncles are normally separated by fibres, their aggregation during fixation in which this degree of fibre swelling is seen indicates that the resulting pressures between the peduncles and fibres causes a rupturing of the fibres. It is possible that with greater swelling of the fibres some of the peduncles that are not bunched into these low-pressure channels, created by the rupturing of fibres, are dislodged from the mesoglea to form the smaller unoriented elements of the reticulum.

The inability to resolve the fibre plexus in formaldehyde-fixed tissue except by dark-field phase light microscopy appears to result from a poorer fixation of the fibres than of the surrounding tissue (Figs. 59, 60, 61). While the cell membranes and most of the organelles are preserved in both the fibres (Fig. 60) and in the peduncles and muscle fibres (Fig. 61), the cytoplasm of the fibres is washed out and contains unusually low numbers of organelles. Also, the membranes of both the fibres (Fig. 60) and of the cytoplasmic ridges of the muscles

(Figs. 59, 61) have been broken by the swelling of the fibres and muscles. This damage is limited in the case of the muscles and it is easy to trace their limits (Fig. 61); in the case of the fibres, however, it is difficult to find enough membrane to distinguish one fibre from another (Fig. 60). If this damage, seen with fixation by formaldehyde, occurs also in fixation procedures that do not preserve membranes and microtubes it would appear that nothing would be left of the neurons and associated cells of the plexus but their nuclei, mitochondria, and vesicles.

Discussion

The Reticulum

The purpose of this study has been to define the reticulum and to determine its relation to the nervous system and to the fibre plexus. There have been three theories of the nature and composition of the reticulum: 1) that it is composed of the peduncles of the supporting cells and that the fibres of the plexus lie between them (von Heider, 1879; Peteya, 1973); 2) that it is made up of both peduncles and neurons and that there is no fibre plexus (Hertwig and Hertwig, 1879; McMurrich, 1890; Cerfontaine, 1909; Torelli, 1938; Leghissa, 1949; Arai, 1965); and 3) that the reticulum is composed of peduncles and of receptor axons, and that there is no nervous system (Torelli, 1952). Further, there is considerable variation in the detailed description of the appearance of the reticulum; although all studies, except Peteya (1973), have agreed that its constituents are interconnected to form a network. It has been found here that the reticulum is composed solely of the peduncles of supporting cells, and that their morphology is dependent on a previously

undescribed fixation artifact. In well-fixed tissue the peduncles run radially from their cell bodies in the epithelium to the underlying mesoglea; in this conformation they do not constitute a reticulum, in the sense of the network that has been described in all previous studies. Many fixatives, however, cause a severe swelling of the fibres of the plexus which in turn mechanically distorts the peduncles into a network; it is in this distorted conformation that the peduncles comprise what has been described as the reticulum.

In an earlier study it was suggested that the reticulum be defined by its components (Peteya, 1973). However, after seeing the great variation in the appearance of peduncles it is now clear that the reticulum must be defined by its morphology: that the reticulum is a network-like configuration of the supporting-cell peduncles. Defined in this way the term not only encompasses all previous structures described under this term (except for the 'reticulum' of formaldehyde-fixed tissue which has been shown here not to be a reticulum), but may continue to be used to describe this particular type of tissue formation.

The presence or absence of a reticulum, in tissue being used for study of the nervous system, is of some diagnostic value since the fixatives that produce one do so by causing a severe swelling damage to the fibre plexus which makes the plexus difficult or impossible to see in LM study. EM study of tissue fixed in formaldehyde indicates that some fixatives preserve the plexus, but modify the staining and optical properties of the fibres so that they can be seen, in this writer's experience, only with phase microscopy. Therefore, in the case of fixation by formaldehyde and perhaps by Golgi-Cox, Cajal 2a A, and by

Miller (see Table 4), the possibility exists that the staining procedure determines whether or not the plexus can be visualized. Of greater importance, however, is that many techniques, including most of those used in previous studies of anemone nervous systems, apparently destroy the fibre plexus, which includes the nerve net, during fixation. This conclusion is based on three observations: 1) that during the last century anemone histologists have put in over 100 work years studying a number of species of anemones with a large number of staining and fixation procedures, but have never (except for von Heider) observed the fibre plexus; 2) in study here the plexus cannot be resolved in tissue having a reticulum even with optical enhancement of thin sections; 3) in the case of tissue fixed in Batham's the fibre plexus cannot be identified on an EM level.

Technical Considerations

Regardless of the effects stains might have on the visualization of poorly fixed fibres the effect which the destruction of the plexus by fixatives such as Batham's, Susa, Zenker, Helly, Bouin, and FAA has on our knowledge of the ectodermal nerve net is obvious. In future studies of actinians, cerianthids, and possibly other anthozoans care must be taken to employ histological techniques that do not significantly damage the fibre plexus.

Previous study of the procedures that have been used in studies of anemone nervous systems indicates that most are without value because of staining artifacts (Batham et al., 1961). As a result, most current work utilizes two techniques, both developed by Batham, Pantin and Robson (1960), that are relatively free of stain artifacts and which provide

a good and consistent demonstration of part of the nervous system. These are a modified Holmes silver impregnation which requires fixation in Batham's and a methylene-blue stain for which Susa's or Alexandrowicz's 1960 fixative have been used as fixatives. Of these only the Alexandrowicz preserves the plexus, and it does considerable damage to the fibres.

The present study provides little direction to a search for new techniques of study as no LM techniques used here gave staining of ectodermal neurons. But it is possible to make a few statements and recommendation which may prove helpful:

1. From the fixatives listed in Table 4 a generalization can be made that fixatives containing either picric acid or mercuric chloride are almost worthless as fixatives for the ectodermal nerve net.
2. There is evidence that the postfixation treatment of a tissue can selectively destroy elements of the plexus; in glutaraldehyde-fixed tentacles of *Stomphia* that are prepared by the bismuth-iodide technique for synapses (Pfenninger, 1971) the majority of the fibres in the ectodermal plexus are destroyed even though the remaining fibres are well preserved.
3. Finally, two unexplored techniques, Leghissa's (1949) chromaffin and the Champy-Maillet ZIO impregnation, should be pointed out. These, in the writer's experience, give an adequate preservation of the plexus and have some promise for staining its components.

Leghissa has been discredited for his description of mesogleal neurons (Pantin, 1952; Batham et al., 1960; for discussion see Chapter 3); but has presented a detailed picture of the ectodermal nerve net from

studies based on his chromaffin technique which has largely been verified by the less complete studies of those who discredited him (Batham, 1965; Robson, 1965). Thus, when we disregarded his observations, we also lost what appears to be the most successful known technique for the LM study of ectodermal neurons. A ZIO impregnation has been used here (see Chapter 3) to stain some types of endodermal neurons and receptors. Although staining of ectodermal neurons was not achieved, it should be possible to apply this relatively sensitive technique for amines to the ectodermal neurons since histochemical study (see Chapter 5) indicates that several types contain amines.

Chapter 6

THE ECTODERM

Introduction

The ectoderm is the outermost tissue layer of an anemone and is in direct contact with the milieu. As might therefore be expected, both its nonnervous and nervous histology are considerably more complex than those of the endoderm and mesoglea. The physiological and anatomical complexity of the ectoderm has long been recognized, and the great majority of our efforts in the study of the anemone nervous system has been directed toward its ectodermal components. Indeed, it is only through the recent studies of Batham, Pantin and Robson (Pantin, 1952; Batham et al., 1960, 1961; Batham, 1965; Robson, 1961, 1963, 1965) that we know anything of the histology of the endodermal components other than from the classic Hertwig study (Hertwig and Hertwig, 1879).

Despite our interest in the ectoderm there is little known about its nerve net. With the exception of von Heider (1877, 1879) all neuroanatomists who have worked with sectioned tissue described a structure known as the 'reticulum' as being the ectodermal nervous system. As shown in Chapter 5, however, the reticulum is composed solely of the peduncles of epithelial supporting cells, and their distortion into the network-like conformation that characterizes the reticulum is the result of a fixation damage which also, in most cases, results in the destruction of the fibre plexus. As a result, the majority of the literature on the ectodermal nerve net is invalid or at least questionable; only the observations of Leghissa and of Batham and Robson appear to have a

clear validity.

From her methylene blue study of *Stomphia*, Robson (1963) has described a complex of bi- and multipolar neurons in the oral disc. The multipolar cells usually have three or four axons, appear to be 10—15 μ in diameter (measured from her plates), and are aligned along the insertion of mesenteries. The bipolar neurons are larger, and although it is stated that they "run obliquely to the radii or along them" the plates show that they have a clear orientation in a circle around the mouth. Both cells extend throughout the disc and are most frequent in the outer oral disc, but neither were seen in the tentacles. These bipolar neurons are presumably the same as those described by Batham (1965) in *Mimetridium*. Batham used her silver technique rather than methylene blue and, although she did not see the multipolar cells, apparently achieved a more complete visualization of the bipolar neurons than did Robson. Batham described two size classes of ectodermal bipolar neurons. One is a small cell that lies without clear orientation in the tentacles and oral disc. The second, probably the bipolar neuron of Robson, is a large cell, 4—7 μ in diameter (presumably measured in the axon), which is oriented in circles around the mouth and which is concentrated in the outer disc. In addition, the axons of the large bipolar extend into the tentacles (only four to six enter each tentacle, and have a diameter of only 2—3 μ), and extend through the disc mesoglea to connect with the large endodermal bipolar neurons. Batham believed both sizes of ectodermal bipolar neurons to be identical to the large endodermal bipolar neurons; however her demonstration of cells passing through the mesoglea is not clear and needs verification.

Also, if it is true that the same class of bipolar neuron is seen in the disc ectoderm, disc endoderm, and mesentery endoderm then there is some difficulty in understanding why Robson's methylene-blue study illustrated the bipolar neurons in the disc ectoderm and mesentery endoderm but not in the disc endoderm.

Leghissa has also described a complex of small and large bipolar and larger multipolar ectodermal neurons that are concentrated in the oral disc (Leghissa, 1949, 1950, 1965; Leghissa and Quaglia, 1966). Although his data are discounted because of his description of mesogleal neural components (see Chapter 5 for discussion), his observations of the ectodermal nerve cells closely match those of Batham and Robson except that Leghissa did not find bipolar cells that cross the mesoglea. In addition to those observations which Batham and Robson have duplicated Leghissa has claimed that a bipolar neuron (he does not state what size) extends into the pharynx and that the multipolar extends throughout the tentacles. In EM study Leghissa has identified only a bipolar (apparently the small type) and the multipolar neuron which are 1.2 x 2.8 μ and 2.3 x 3.5 μ, respectively (Leghissa and Quaglia, 1966). Although these measurements are considerably less than any of those from LM study they are taken from osmium-fixed tissue and are probably the most accurate measurements of these cells. It has already been demonstrated (see Chapter 5) that the ectodermal nerve fibres are subject to a fixative-induced swelling. It appears that this has severely affected the apparent LM morphology of the multipolar and bipolar neurons described by Leghissa, Batham and Robson. For example, Robson's plates of the multipolar neuron in the oral disc of *Stomphia* indicate that they are

about 10—15 μ in diameter, whereas in this EM study of permanganate-fixed discs of *Stomphia* these same cells are only 5 μ in diameter.

Although there have been a few EM studies since Leghissa's (Kawaguti and Ogasawara, 1967; Westfall, 1970, 1973; Westfall et al., 1970) little has been added to our knowledge. In all species examined, a subepithelial plexus of fibres comparable to that first described by von Heider (1877, 1879) has been found; such a 'nerve plexus' is also known in the ectoderm of madreporia (Kawaguti, 1964) and ceriantharia (Peteya, 1973). In the species examined by this writer these plexuses vary from 5—20 fibres in thickness (measured in cross sections at the base of a tentacle). Thus a section through the base of a tentacle would show several thousand presumptive nerve fibres, a higher number than found in the marginal ganglion of a medusa (Mackie, 1969). Despite this implication the possibility that the nerve plexus contains a significant proportion of nonnervous elements has not been explored. Recently however (Peteya, 1973), it has been shown that the nerve plexus of *Ceriantheopsis* contains about 50% of apparently nonnervous fibres, and that the endodermal plexus of *Stomphia* contains less than 10% nervous fibres (see Chapter 3). For this reason it has been suggested that the misleading term 'nerve plexus' be abandoned for 'fibre plexus' (see Chapter 3).

This is the first attempt made at a comprehensive EM study of an actinian ectodermal fibre plexus; previous studies have been limited to the plexus of the tentacles and have described only recognizable nerve fibres. Three anatomically distinct ectodermal plexuses have been found in *Stomphia*; one in the pharynx, oral disc, and tentacles, a second in the column ectoderm that overlies the sphincter, and a third in the pedal

disc. In addition, a sparse plexus is found in the column that contains fibre types found in the second and third plexuses and which possibly connect the two. The definition of the plexuses is preliminary; there is considerable variation in the distribution of the fibre types within the first plexus and several types are found in both plexuses 1 and 2 and in 2 and 3. However, each plexus as defined here can be characterized by several predominant fibre types which are unique to that plexus.

Although it has not been possible in this study to characterize all of their components, a tentative identification of over 20 types of fibres in the ectodermal plexuses has been made. The distinction between types is often small and the morphology of many is influenced by the type of fixation creating considerable problems in the accurate characterization of types of fibres. Because of this, it has proven too difficult to undertake a complete study of the ectodermal plexuses and my report of them in this chapter is limited to recognizable receptors and neurons.

Observations indicate that fixatives also have an unusually great influence on the morphology of synapses in *Stomphia* and in some cases can determine their recognition. Thus, depending on the technique of fixation the tentacular nerve net can vary in apparent complexity from four types of nerve fibres, each defined by a morphologically distinct synapse type, to a plexus such as Leghissa saw in *Actinia* (Leghissa and Quaglia, 1966) which contains no recognizable interneural synapses and whose interpretation is therefore more difficult.

A description of the ectodermal amoebocyte will also be given; because of its morphology it may have been described previously as a neuron.

Materials and Methods

All of the LM techniques listed in Chapter 2 under sections 'whole mounts' and 'histochemistry' have been used, without success, to stain neurons in the ectoderm. LM visualization of neuron-like cells has been achieved only with the modified Hertwig maceration procedure, but it has not been possible to determine their nature and the technique has been little used.

Great difficulty has been experienced in the EM study of the ectodermal fibre plexuses of *Stomphia* since the morphology of the vesicles used to identify fibre types is dependent on the type of fixative used. Of the fixatives used in this study Cavey's gave the best preservation of the fibres, but only permanganate in sea water provided for a clear identification of all of the nerve-fibre types identified in the plexus of the tentacles and oral disc.

Catecholamine localization has been achieved by the Wood (1963) and Woods (1969) chromaffin techniques, and by metaraminol incubation. Some experimentation has been made with the Pfenninger (1971) synapse stain and with ZIO impregnations (Martin et al., 1969), but neither has given positive results.

Results

General Histology

The histology of the ectoderm of an actinian is complex and little known; some description of it must be given as a background. Although each region of the body (pedal disc, column, tentacle, oral disc, pharynx, and siphonoglyph) is histologically distinct, the ectoderm of *Stomphia*

can be divided into two major zones on the basis of general similarities in their organization.

The first zone is composed of the tentacles, oral disc and pharynx. In the tentacles the ectoderm is composed of the three distinct layers that are described in textbook accounts of Cnidaria; they are a superficial epithelium, a basiepithelial fibre plexus and a muscle layer, which in *Stomphia* is formed into tubes that are sunken into the mesoglea (Figs. 62, 63, 73). The oral disc is identical except that the frequency of muscle fibres and cells decreases to near zero in the inner oral disc; and muscle fibres are rarely found even in the pharynx establishing a continuity of organization between these three regions of the body. In these areas, the epithelium is composed of tall columnar cells with supporting cells and gland cells predominating (also receptors and cnidoblasts in the tentacles and disc). The nature of the fibre plexus is largely unknown; it contains a nerve net in the tentacle and disc, but no neural elements have been found here in the pharynx. The muscle system consists of the same two types of fibres described in the endoderm (see Chapter 3); the majority are type A fibres with a small number of type B randomly distributed. They are innervated in at least two ways: 1) the sunken tubes periodically fuse with the fibre plexus where they receive a direct innervation, and 2) nerve fibres have been found lying in the mesoglea between the plexus and the muscle tubes and have been seen running between adjacent tubes.

The second zone of the ectoderm is the column and pedal disc; it is distinguished from the first zone by the absence of a muscle layer and by a change in the major cell types found in the epithelium and

plexus. Three histologically distinct regions can be distinguished in the column and disc: 1) the column ectoderm which overlies the sphincter, 2) the column below the level of the sphincter, and 3) the pedal disc.

Ross (Ross and Sutton, 1966) has found that a *S. didemon* will swim in response to a contact between its tentacles and the column of a *S. coccinea*. No other part of the body of *S. coccinea* is effective in evoking this response, and the column which overlies the sphincter is nine times more effective than the column below the level of the sphincter. There are several histological differences between these two areas of column. While the epithelium of both is composed primarily of supporting cells and gland cells the epithelium over the sphincter contains fewer gland cells and a different type (Fig. 64) than is found in the column (Fig. 66). Also, nematocysts are found only in the ectoderm overlying the sphincter. The ectoderm over the sphincter contains a possible receptor and a well developed basiepithelial fibre plexus (Fig. 65) in which three apparent nerve fibre types have been identified. Below the level of the sphincter the plexus breaks down into a number of isolated bundles of fibres (Figs. 66, 67) which may provide an interconnection with the plexus of the pedal disc; no nerve fibres have been observed in this area of the column.

The epithelium of the ectoderm of the pedal disc is composed of supporting cells, cnidocytes, and a variety of gland cells (Fig. 68). A superficial receptor has been found which is unique to the disc. The basiepithelial fibre plexus is sparse (Fig. 69) and no nerve fibres have been identified within the disc.

The Neural Elements of the Tentacles, Oral Disc, and Pharynx

Receptors

Only one type of receptor, the ciliary-cone sensory cell, has been identified in these regions of the body. In *Stomphia* it is found throughout the tentacles and the outer oral disc. It is a bipolar epithelial cell whose sensory apparatus, the cone, is well known but which has often been confused with cnidocils. Similar but distinct types of receptors are found in the column and pedal disc of *Stomphia*. It has been found here that the cone is not the apparatus of a single cell, as has been reported in all earlier studies, but is the combined sensory apparatuses of a cluster of receptors. This is the first demonstration of a multicellular sensory structure in the Anthozoa.

The cone is formed of the combined sensory apparatuses of a cluster of five to seven sensory cells (Figs. 70, 71), rather than just one cell as has been reported previously (von Heider, 1877; Hertwig and Hertwig, 1879; Groselj, 1909; Pantin, 1942; Leghissa, 1949; Mariscal, 1974). Each is a primary, bipolar sensory cell lying in the ectodermal epithelium, and each bears a single axon that extends into the basiepithelial fibre plexus (Figs. 72, 73). The cells never occur in isolation and within each cluster two types of receptor can be distinguished (Figs. 72, 74). There is one central cell that has a short, narrow dendrite surrounded by four to six morphologically distinct peripheral cells whose dendrites are expanded to several microns in diameter at their surface.

The dendrite of the central cell bears a cilium within a circle of five to ten stereocilia. These lie at the center of the cone and are the tallest structures in it. Most of the peripheral cells bear only

stereocilia, but occasionally cells were found that had a single cilium (Fig. 74) and many were seen that had a basal body but no cilium. Each peripheral cell contributes between 30 and 100 stereocilia to the formation of a cone, and some cells were seen that gave stereocilia to two separate cones. These stereocilia are directed toward the tip of the central-cell cilium; thus the combined sensory apparatuses of the cells of a cluster form a discrete cone-shaped mass that is between 2 and 9 μ in height.

The 9+2 cilium is unmodified from those of adjacent nonsensory cells. It is 0.3 μ in diameter and between 2 and 15 μ in length; it is usually the height of the cone, but may extend beyond it. The basal body has an accessory centriole and a narrow striated rootlet that extends up to 4 μ into the dendrite.

The stereocilia of the central cell are between 2 and 9 μ in length and 0.3—0.5 μ in diameter at their base, tapering to 0.2 μ at the tip. They form a circle around the cilium keeping a distance of 0.3—0.6 μ from it in their basal two-thirds (Fig. 75) but approach and touch the cilium near the apex of the cone. The regularity of this arrangement may be related to the numerous cross bridges found between the stereocilia (Figs. 75, 76). The stereocilia of the peripheral cells are between 0.4 and 9 μ in length and are only 0.05—0.2 μ in diameter. They are clustered onto the side of the cell nearest the central cell and are arranged so that their length and diameter decrease with distance from the center of the cone (Figs. 70, 71). The stereocilia contain fine fibrils about 40 Å in diameter that are more electron-dense in the stereocilia of the peripheral cells than in those of the central cell

(Fig. 76). In central cells these fibrils extend as bundles for about 1 μ into the dendrite whereas those of the stereocilia of peripheral cells quickly disperse to form a small web.

The sensory-cell dendrite contains the fibrils of the stereocilia, microtubules, mitochondria, endoplasmic reticulum, and several types of vesicles. The surface of the peripheral-cell dendrite contains large numbers of 2200 Å electron-dense vesicles that are characteristic of it (Fig. 72). The cell body contains an ovoid nucleus, well-developed Golgi complexes, mitochondria, endoplasmic reticulum and lysosomes. The axon may contain some reticulum near its origin, but otherwise contains only filaments and 600—1500 Å vesicles. It has been possible to identify the axon in the fibre plexus; it will be described in the following section.

The nerve fibre of the plexus

The fibre plexus of the tentacles, oral disc and pharynx is composed of about ten types of fibres (as with the endodermal fibres types are defined by variations in their vesicle population). Many of the fibres contain CA-containing vesicles which show a fixation-dependent morphology comparable in form to that experienced in EM study of vertebrate aminergic nerve fibres (Thureson-Klein et al., 1973). Although some attempt has been made to determine the correct definition of the various fibres through comparison of apparent types produced by a variety of fixatives, in this study no characterization of all components of the plexus has been reached. Rather than describe a dubious catalogue of fibres, this report and those of the other two plexuses are limited to only those fibre types clearly identifiable as nervous by the presence of synapses.

For ease of comparisons, the characteristics of the ectodermal nerve-fibre types are summarized in Table 5.

Nerve-fibre type 1 is believed to be the axon of the ciliary-cone receptor. It is numerous throughout the tentacle and outer oral disc but is not found in the inner disc or pharynx. It is identified as the axon of the receptor because of a similarity in their frequency and distribution and in one case a fibre of this type was traced to a cell body in the epithelium believed to be that of a ciliary-cone receptor (Fig. 77). The fibre is 0.3—1.0 μ in diameter and contains two populations of vesicles; the major class is 600—900 Å in diameter and is found at the synapse (Figs. 78, 79, 80, 81) and in large numbers throughout the axon and occasionally in the soma; the second consists of a small number of vesicles, 800—1600 Å in diameter found scattered through the soma and axon. The morphology of both types is dependent upon the type of fixative; in osmium-fixed tissue both are agranular, in aldehyde-fixed tissue the synaptic vesicles are agranular and the large are dense-cored, and in permanganate-fixed tissue both are dense-cored or solid. The synaptic vesicles show an uptake of exogenously applied metaraminol indicating that they are CA-containing vesicles (compare Figs. 79 and 80).

The synapses are about 0.25 μ in diameter and are characterized by a small aggregate, one or two rows deep, of vesicles and by a slightly increased electron density of the 200 Å-wide synaptic gap (Figs. 79, 80, 81). For reasons that are unclear this synapse is extremely difficult to recognize. It is best observed in permanganate-fixed tissue taken from an animal that was incubated in a CA just prior to fixation; in

such tissue this synapse has an observed frequency as high as 50 synapses per 1000 μ^2 of section area of fibre plexus at the base of a tentacle. In contrast, in permanganate-fixed tissue of an animal that was not incubated in CA the synapse is rare and in osmium- or in aldehyde-fixed tissue only the most doubtful identification of this synapse has been made. In these latter tissues apparent synapses can be identified by the apposition of vesicles against a membrane in an area where the fibre is apposed to another (Fig. 81), but more frequently such vesicle [redacted]ns occur where there is no postsynaptic fibre.

Nerve-fibre type 2 is found throughout the tentacle and oral disc, but not in the pharynx; it has also been found in the plexus of the column (see the next section). Its morphology is closely similar to that of nerve-fibre type 1 and is easily confused with it. Type 2 is a 0.4—1 μ diameter fibre characterized by the presence of microtubes and three classes of vesicles; the most numerous is a 600—900 Å vesicle found both at synapses and scattered in the cytoplasm, the second is 800—1700 Å, and the third is 2000—3000 Å in diameter (Fig. 82). The first two types of vesicles are identical in appearance and behaviour to fixatives to the two types found in nerve-fibre type 1; in addition the synaptic vesicles of type 2 also take up exogenous metaraminol (Fig. 83). The only distinction between the two types of fibres is the presence of the third class of vesicle in type 2 and that the synapse of 2 is larger than those of type 1. The third class of vesicle in this fibre is electron-dense and dense-cored or solid in all fixation procedures used in this study; it is chromaffin-positive by the Woods techniques but its morphology is not affected by incubation in metaraminol.

The synapse of nerve-fibre type 2 appears to be identical to that of type 1 except that its average diameter is about 0.5 μ, twice that of those of type 1. However, whereas the synapses of type 1 can usually be seen only in permanganate-fixed tissue, those of type 2 can be identified in tissue fixed by any of the fixatives used in this study (Figs. 83, 84, 85) except for the Peteya fixative which blocks recognition of not only this synapse but also those of fibre types 1 and 3.

Nerve-fibre type 3 is a giant fibre that is seen only in the proximal half of the tentacles and in the outer oral disc. These fibres are either 0.3—1.2 μ or 2—6 μ in diameter; limited evidence suggests that the larger fibres branch dichotomously (Fig. 86) and that the smaller fibres are collateral branches of the large fibres (Fig. 87). Both sizes of fibre contain two size classes of vesicles; the synaptic vesicles are between 450 and 630 Å and are agranular in all fixation procedures used, and the other is a small number of vesicles (1300—2000 Å) that are dense-cored in all procedures used. The fibre does not appear to be aminergic as the morphology of the vesicles is not changed by prefixation incubation in metaraminol or in α-m-noradrenaline.

The synapses of both the giant fibre and its collateral are large, up to 1.5 μ in diameter, and are characterized by gap and postsynaptic structures (Figs. 88, 89, 90, 91) not found at other synapses. The synaptic vesicles are loosely packed into a group rarely more than one vesicle in thickness; there are no organized presynaptic projections but there is often an aggregation of electron-dense material within the cluster of vesicles. The gap is 200 Å wide and is crossed by bars

similar to those found at scalariform junctions (Fig. 88) (compare to Fain-Maurel and Cassier, 1972). The bars are 60—70 Å in width and spaced about 300 Å apart, center to center; they can be seen only in permanganate-fixed tissue, and then are not found at all synapses of this type. The postsynaptic cytoplasm usually contains a membrane sac that varies in complexity from what appears to be a sac of reticulum (Fig. 89) to large highly convoluted sacs and tubes of an unknown nature or organization (Fig. 90). Occasionally these sacs are missing in which case periodic electron-dense projections may be found attached to the postsynaptic membrane (Fig. 91). With the possible exception of the interneural synapse identified in the endoderm (Chapter 3) this is the only type of synapse found in *Stomphia* which has shown clear substructural specializations that could be used in the recognition and characterization of a synapse.

Nerve-fibre type 4 is found in the tentacles and in the oral disc, except for the area just surrounding the mouth; it lies primarily within the sunken tubes of ectodermal muscle fibres. The fibres are frequently seen crossing the mesoglea between adjacent tubes (Fig. 92), and can also be found lying in the mesoglea between the tubes and the fibre plexus indicating that they may cross from the plexus to the tubes of muscle fibres. However, this fibre has never been found in the plexus of the ectoderm; it is described as an ectodermal fibre only because it innervates the ectodermal muscles.

Type 4 is a branching fibre (0.2—1.4 μ in diameter), which contains mitochondria, reticulum, glycogen, microtubules, and vesicles that are 500—800 Å and 800—1800 Å in diameter. The larger class is dense-cored

in all fixation procedures used, and does not take up exogenous metaraminol. The smaller vesicles are found scattered in the fibre and aggregated at synapses (Figs. 93, 94, 95, 96); in tissue fixed by OsO_4 or by aldehydes they are agranular (Fig. 93), but in permanganate-fixed tissue the synaptic vesicles of a small number (less than 1%) of the nerve fibres have a dense core (Fig. 94) and in ZIO impregnations the majority of the fibres have synaptic vesicles that have a weakly stained dense core (Fig. 95). Prefixation incubation in α-m-noradrenaline does not affect the morphology of the synaptic vesicles. Although the synapse often shows an increased electron density of the membranes and gap in relation to adjacent membranes and extracellular space there are no evident substructural membrane specializations at this synapse.

Neuromuscular synapses though found on the muscle-cell cell bodies are usually on the muscle fibres or on 'sarcoplasmic extensions' from the fibres (Fig. 92, 96). Synapses on the cell bodies or the fibres usually have a mitochondrion closely apposed to the postsynaptic membrane while there is only rarely a mitochondrion associated with those onto the extension. These extensions are between 600 and 3000 Å in diameter and are at least 3 μ in maximum length; most are directed toward the core of the tubes of muscle fibres, where the nerve fibres are concentrated, but others extend out of the tube and synapses are occasionally seen between these extensions and nerve fibres that lie free in the mesoglea.

The effect of fixatives on the recognition of synapses. In several vertebrate systems the recognition of synapses has been found to be dependent upon the technique of fixation, with the apparent synaptic

frequency varying as much as 25% between two fixation procedures (Vrensen and DeGroot, 197[illegible]. There are no similar reports among the invertebrates; however, the failure of some studies (Leghissa and Quaglia, 1966; Cobb and Mullins, 1973; for a review of molluscan literature see Pentreath and Berry, 1975) to find any interneural synapses may indicate a comparable effect of fixatives on the recognition of synapses in some invertebrates.

Of the four types of synapses identified in the tentacles and oral disc only the neuromuscular type can be found in approximately the same numbers in tissue fixed by any of the procedures in this study (1, Westfall's osmium-dichromate; 2, Cavey's glutaraldehyde-ruthenium red; 3, Peteya's glutaraldehyde-formaldehyde; 4, permanganate). By comparison, the interneural-synapses of nerve-fibre types 2 and 3 appear to be two to three times more frequent in permanganate-fixed tissue than in tissue fixed by aldehyde or OsO_4; and the sensory synapse of type 1 can be confidently identified only in permanganate-fixed tissue. The potential effects of fixatives upon the apparent synaptic complexity of the nervous system is further emphasized by the effects of the Peteya fixative. This fixative was developed and used in an earlier study of the nervous system of a cerianthid (Peteya, 1973) in which it gave a fixation of synapses superior to 20 other aldehyde procedures used; however, in *Stamphia* this fixative somehow obscures recognition of each of the interneural synapse types identified in this fibre plexus.

The cell body of the multipolar neuron. In the pharynx, oral disc and tentacles the fibre plexus contains several types of cell

bodies including three types seen to bear fibres. None can be identified as neuronal by their morphology; neither the cell bodies nor the initial segments of their processes contain any of the vesicles which characterize the various types of fibres. One, however, can tentatively be identified as the multipolar neuron, described by Leghissa (1949) and Robson (1963). Like that neuron this cell is found only in the oral disc and tentacles and is found in remarkably large numbers in the inner oral disc. The cell averages 3 x 5 μ and has a small circular nucleus averaging 1.6 μ; it bears several 0.2 μ-diameter processes (Fig. 97). The cells contain two classes of vesicles: one, produced by the Golgi apparatus is a 600 Å agranular vesicle and the second is a dense-cored or solid vesicle (750—2000 Å). In addition the cell contains endoplasmic reticulum, mitochondria, and multivesicular bodies.

The Neural Elements of the Column

The ectoderm of the column has not been studied in the detail given to that of the tentacle and oral disc; in this study it is possible to give only a partial description of the three or four apparent neural elements identified in the column. Each has been found only in that part of the column that overlies the sphincter. This area of the column has a large fibre plexus (Fig. 65), composed of approximately six types of fibres, which decreases in size and complexity toward its proximal end until, below the sphincter, it consists only of scattered bundles of fibres which do not appear to contain any of the nerve fibres (Fig. 67). These fibre bundles extend throughout the column and possibly provide a connection between the plexuses of the column and pedal disc. The elements interpreted here as being neuronal are a possible epithelial

receptor, the fibre identified as nerve-fibre type 2 in the plexus of the tentacles and oral disc, and one or two other types of fibres which bear what appear to be synapses.

Receptors

Study of the column ectoderm has given only one observation of a possible receptor. Only the sensory apparatus was seen; it consists of a cilium surrounded by five 0.6 μ-diameter stereocilia (Fig. 98). This structure is distinct from the sensory apparatuses of the gland cells and cnidoblasts found in the column ectoderm, and therefore may be the sensory apparatus of a receptor.

The nerve fibres of the plexus

Nerve-fibre type 2 is the predominant type in the column; like the others in the column it is found only in the area overlying the sphincter. Unlike those seen in the tentacles and oral disc, the columnar type 2 fibres usually have a system of branching tubules, 400 Å in diameter, of an unknown nature, but are otherwise identical to those in the tentacles and disc (compare Figs. 82 and 101).

Nerve-fibre type 5 is a fibre which bears synapses, up to 1.1 μ in diameter, that have irregularly compressed dense-core vesicles (500—750 Å) (Figs. 99, 100). The gap is 200 Å wide, which in some cases is an obvious increase in separation of the two fibres from that at nonsynaptic areas. There are no other features which indicate that these areas of apposition are synapses, and their identification as such is tentative. In addition to the synaptic vesicles the fibre contains a small number of dense-core vesicles, reticulum, mitochondria, glycogen, and a network of anastomosing tubes which are 400 Å in diameter.

Nerve-fibre type 6 is a class created for a fibre seen only twice, to bear possible unpolarized synapses (Fig. 102). The synaptic vesicles are between 500 and 800 Å in diameter, are spherical to irregularly flattened in shape, and are agranular in aldehyde-fixed tissue. The gap is 200 Å wide, which is comparable to that seen at known synapses, but there are no gap nor membrane specializations which aid in the identification of these structures as synapses.

The Neural Elements of the Pedal Disc

The ectoderm of the pedal disc consists of a tall columnar epithelium (Fig. 68) and of a sparse subepithelial fibre plexus (Fig. 69). The epithelium is predominated by supporting cells and gland cells, and appears to have a density of receptors comparable to that of the tentacle ectoderm. The fibre plexus appears to be composed of numerous interconnected bundles of fibres, which vary from one to five fibres in thickness, rather than being a homogenous layer as in the tentacles. The plexus is composed of only about four types of fibres, none of which have been seen to bear synapses. The presence of a nerve net in the plexus may, however, be presumed since the epithelium contains a receptor.

The epithelial receptor is a solitary, bipolar cell whose sensory apparatus lies at the free surface of the tissue. The sensory apparatus closely resembles that of the central cell of a cluster of ciliary-cone receptors (see above); it consists of a single cilium within a circle of stereocilia (1500—3500 Å in diameter) (Figs. 103, 105) that are at least 4 μ in maximum length. Unlike those in the ciliary-cone receptors the fibre bundles of the stereocilia of this cell extend into the dendrite for at least 3 μ as discrete bundles (Fig. 103). The dendrite

may also contain numerous microtubes (Fig. 103). In sections tangential to the tissue surface it can be seen that the sensory apparatus of this cell is usually surrounded by about 50 stereocilia, or fibre-filled microvilli, that arise from adjacent cells (Fig. 105). In this way it closely resembles the organization of a ciliary cone in which the sensory apparatus of the central cell is surrounded by stereocilia which arise from peripheral receptors. However, in this case the accessory cells surrounding the receptor are supporting cells characterized by solid vesicles (7000 Å) (Fig. 103).

The dendrite is about 1.5 μ in diameter at the surface and is 5—12 μ long (Fig. 104). In addition to the fibres of the stereocilia and the microtubes the dendrite contains small numbers of mitochondria, reticulum and agranular vesicles. The cell body lies high in the epithelium and contains an ovoid nucleus and a large supranuclear Golgi complex. The axon has not been seen.

The Ectodermal Amoebocyte

A description of the amoebocyte has been included in this report of the ectodermal neural elements because of the general belief that amoebocytes have been confused with neurons in some LM studies and because of the close similarity in shape between some amoebocytes seen in *Stomphia* and a type of ectodermal neuron described in *Actinia* (Leghissa, 1949).

In *Stomphia* the amoebocyte is found throughout the ectoderm of all parts of the body, and in well-fed or freshly-collected animals is very numerous in the oral disc and lower tentacle. The cell lies in the fibre plexus and in the lower epithelium and has been seen crossing into

the mesoglea. Although the writer has not observed them on a LM level the amoebocytes are easily recognized in EM study; their cell bodies are filled with a variety of organelles, including agranular vesicles (600—1300 Å), solid CA-containing vesicles (1600—2400 Å), and lysosomes that are up to 5 μ in diameter and which often contain recognizable parts of ectodermal cells. While the lysosomes provide a morphological character for separating the amoebocytes from the neurons on both a LM and EM level, they disappear from the cells in animals which have been starved for only seven days. In their absence there is a possibility that amoebocytes can be mistaken for neurons if there is a similarity in shape since the high CA content of the amoebocytes would give them nerve-like staining properties.

The amoebocytes are large cells, 5 x 5 μ to 5 x 20 μ, which are usually spherical to elongate in shape. They usually bear one or more major processes that are 1—2 μ in diameter and of unknown length; the cells frequently have two such processes in an opositopolar position giving them the appearance of bipolar cells (Fig. 106). In addition, both the cell body and the major processes may bear a number of 0.2—0.4 μ-diameter processes that are at least 3 μ in length (Fig. 107). These small fibres give some amoebocytes the appearance of being multipolar or a combination of bipolar and multipolar in shape (Fig. 107).

Compare to this Leghissa's description of a bipolar neuron which is found in large numbers in the oral disc ectoderm of *Actinia equina*:

> Observations . . . show a bipolar element with a nucleus of large dimension considering the size of the cell body, we can also see two prolongations which emerge in diametrical opposition . . . . With the chromargentic reaction and with gold chloride it is possible to distinguish a body with only two

> prolongations, instead using the different impregnation methods it is possible to stain also thin expansions which give to this element the aspect of a peculiar multipolar cell in which two opposite prolongations are functionally and structurally prevalent.
>
> (Leghissa, 1949, p. 278)

The only difference between this neuron and the amoebocyte is the length of their fibres; in the neuron they are several hundred μ in length, whereas those of the amoebocyte are probably far shorter (unfortunately this writer has no LM observations of the amoebocyte and therefore does not know the length of their fibres). The similarity in shape of a significant number of the amoebocytes to cells identified as neurons in an anemone and to the general model of what a neuron looks like in a diffuse nerve net raises some question of the value of cell shape in the recognition of neurons in an animal such as an anemone (see Chapter 7 for discussion).

Discussion

Although the ectodermal neural elements have received special attention throughout the history of anatomical study of the anemone nervous system there is very little known about the ectodermal components of the nervous system. It has been found in this study that both the neural and nonnervous fibres of the ectodermal plexus of the tentacles, oral disc, and pharynx are subject to a fixation-induced swelling damage which can result in the destruction of, or in the inability to resolve, the plexus. At the same time the fixation damage causes a distortion of the supporting-cell peduncles into a configuration known as the 'reticulum' (see Chapter 5). It is difficult to interpret the full significance of this discovery but it can be inferred that it is an

artifact that has had serious effects on our understanding of the nervous system since all histological studies of actinians and cerianthids that are based on sectioned tissue, except for those of von Heider (1877, 1879), have described the 'reticulum' as being the nervous system.

On this basis it is suggested that only the recent EM studies and the LM studies of Leghissa (1949, 1965), Batham (1965) and Robson (1965, 1971) can be accepted with any confidence. While earlier LM studies undoubtedly contain accurate reports of some aspects of the nervous system their accuracy can be determined only by duplication, whereas the studies of Leghissa, Batham, and Robson are illustrated by micrographs and therefore are open to direct interpretation.

From the studies of Leghissa, Batham, and Robson we have a good knowledge of the tentacle, oral disc and pharynx in which is found a dense aggregate of small and large bipolar and large multipolar neurons and the ciliary-cone receptors. Recently Leghissa and Quaglia (1966) have identified the multipolar and one of the bipolar neurons on an EM level, but have not seen their synapses, believing the actinian nervous system to be a syncytium. The pedal disc and column have not been studied extensively, though Robson (1971) reports a sparse network of small multipolar neurons in the column ectoderm of the swimming anemone *Gonactinia*.

The study reported in this chapter is the first detailed EM study of the ectodermal nervous system since that of Leghissa and Quaglia (1966) and is the only EM study of the ectoderm of the pharynx, oral disc, column, and pedal disc. Although several new observations have been made few can be discussed in relation to the model of the nerve

net developed by Leghissa, Batham, and Robson since my study is limited to EM observations of the nerve fibres. Also, it is not even possible to make direct comparisons to the EM study of *Actinia* (Leghissa and Quaglia, 1966). Leghissa had characterized his nerve-fibre types by somata morphology and was unable to identify synapses, where in this study nerve-fibre types are identified by synapse morphology but the writer has been unable to identify the somata of any of the nerve fibres. Consequently discussion must be limited to some observations peripheral to the question of the organization of the nervous system.

A Fibre Plexus vs. A Nerve Plexus

The classic picture of an anemone nervous system is that of a nerve net, which is defined as "a system of neurons dispersed, generally in a plane, and so connected . . . as to permit diffuse conduction" (Bullock and Horridge, 1965, p. 1603). In practice, however, the definition is much more restrictive; from early observations of the schyphomedusan subumbrellar net and the actinian endodermal net cnidarian neuroanatomists believed the cnidarian nerve net to be a highly diffuse assemblage of neurons which are in proximity to each other only at synaptic sites; elsewhere their separation might be tens or hundreds of times greater than the diameter of the nerve fibres (for illustration of such nerve nets see Batham et al., 1960; Batham, 1965).

This image of a diffuse nerve net has been attacked by the EM observation, in every species of actinian, madrepoarian, and cerianthid examined, of an ectodermal subepithelial 'nerve plexus' in which the presumptive nerve fibres are closely apposed to each other (in *Stomphia* the separation is between 200 Å and 1 μ) and form a layer as much as

20 fibres in thickness (Kawaguti, 1964; Leghissa and Quaglia, 1966; Kawaguti and Ogasawara, 1967; Westfall, 1970; Westfall et al., 1970; Peteya, 1973). Although such a plexus of fibres had never been seen previously, except for von Heider (1877, 1879), there has not been any question to Kawaguti's initial presumptions that all of the cellular processes comprising the plexus are nerve fibres and that they had not been seen previously because most were too small to be resolved by LM (Kawaguti, 1964).

The assumption that all, or nearly all, of the fibres in the 'nerve plexus' are neuronal must be tested. The majority of EM studies of such plexuses has been limited to simple reports of the existence of a plexus or of the presence of synapses. The only studies which provide any information concerning the content of the plexuses are those on *Actinia* (Leghissa and Quaglia, 1966) and on *Ceriantheopsis* (Peteya, 1973). Leghissa's study is limited to descriptions of the two nerve fibre types he identified, but his plates indicate that the plexus includes other, presumably nonnervous, types of fibres. Peteya's (1973) study of the cerianthid is the only published attempt to characterize all types of fibres comprising an anthozoan 'nerve plexus'; the plexus consists of two types of receptor axon, three types of neuron axon, and one unknown type (called type B) which made up approximately 50% of all fibre profiles in the plexus. Fibre-type B has subsequently been identified as a sarcolemmal projection of the peduncles of the epithelio-muscular cells (Peteya, unpublished data); therefore in *Ceriantheopsis americanus* only about 50% of the fibres comprising the 'nerve plexus' are neuronal. Similarly, an analysis of the fibres comprising the

endodermal plexus of *Stomphia* (see Chapter 3) indicates that over 90% of the fibres are not neuronal. Indeed, it appears that the existing LM picture of the density of the endodermal nerve net of an anemone is quite accurate and that it is mainly the nonnervous components of the plexus which had not been resolved until EM study. It has not been possible to characterize the components of the ectodermal plexuses of *Stomphia* but evidence indicates that the nonneural elements comprise between 50% (as in the tentacles and oral disc) and 100% (as in the pharynx) of the fibres in the ectodermal plexuses.

More detailed data will come in time, but it is already clear that a reevaluation of our concepts of the gross organization of the 'nerve plexus' is necessary. There has never been, to this writer's knowledge, a report of an aggregation of nonnervous cells and their processes that is associated with the nerve net of any cnidarian. Yet in every anthozoan examined by the writer (the tentacle, oral disc and column ectoderm of *C. americanus*, tentacle ectoderm of *Pachycerianthus fimbriatus*, tentacle ectoderm of *Metridium*, tentacle and oral disc ectoderm of *Stomphia*, and endoderm of *Stomphia*) the nerve net is embedded within a tangle of cellular processes which are at least as numerous as are the nerve fibres. It is suggested that the term 'nerve plexus' is misleading and should be replaced with the less specific term 'fibre plexus'.

Because of the complexity of such plexuses and of the technical difficulties in their study it will be some time before we have an understanding of the nature and function of their nonnervous elements; however, it is imperative that an understanding be achieved. As a

possible model for analysis the endodermal plexus of *Stomphia* is suggested (see Chapter 3). It contains a relatively small number of fibre types and their morphology is not sensitive to the fixation procedure. A preliminary characterization of most of the endodermal fibre types has already been achieved in this study.

Fixative-dependent Recognition of Synapses

In the course of this study of the ectodermal plexus of the tentacles, oral disc and pharynx of *Stomphia* it was found that some aspects of the morphology of some of the synapse types identified are labile to fixation and that the observed frequency of synapses is therefore dependent upon the fixative employed. Although this phenomenon is well known in the study of the vertebrate central nervous system (Vrensen and DeGroot, 1973), it is little known in the invertebrates and the effects found here in *Stomphia* are greater than reported in any other animal.

Each of the four types of synapse identified in the ectodermal nerve net of the tentacles and disc is sensitive to this fixation artifact, and all but the neuromuscular synapse can be made completely unrecognizable by certain fixatives whose quality of fixation is otherwise adequate. While the neuromuscular synapse is found in tissue fixed by each of the procedures used (Westfall, Cavey, Peteya, permanganate in sea water) its observable frequency is higher with osmium or permanganate fixation than with aldehydes. The synapses of nerve-fibre types 2 and 3, both the axons of neurons, have a higher observed frequency in Cavey or in permanganate fixation than in Westfall, and cannot be recognized in Peteya-fixed material; and the

synapse of type 1, the axon of the ciliary-cone receptor, can only tentatively be identified in Westfall- and Cavey-fixed tissue and not at all in Peteya-fixed tissue although it is numerous in permanganate-fixed tissue.

The significance of such a severe effect of fixatives upon synapse morphology is obvious. If this study had not been based on several fixatives the synapse of the ciliary-cone receptor could not have been identified as a separate class, and had only the Peteya fixative been used, no interneural synapses would have been seen. The uniqueness of the artifact, however, is impossible to estimate. There are numerous reports of the inability to find synapses in studies of molluscan nervous systems (for review see Pentreath and Berry, 1975), but the only comparable report in other invertebrate phyla that the writer is aware of is Leghissa's report that the ectodermal nerve net of *Actinia* is a syncytium (Leghissa and Quaglia, 1966). Also, there is no way of determining from these studies whether the absence of synapses is the result of a fixation damage or because it is an asynaptic syncytium as suggested by Leghissa, or because the synapses of that animal do not fit existing concepts of a synapse and thus escape recognition (Cobb and Mullins, 1973). Some evidence exists, however, that this problem is not limited to the Cnidaria; McFarlane (personal communication) reports that there is a study in progress of some noncnidarian invertebrates whose synapses are unrecognizable when prepared by certain fixation procedures.

Chapter 7

SUMMARY AND GENERAL DISCUSSION

Although this study has not fully achieved any of the goals set out in the Introduction, it has substantiated and reinterpreted some obscure observations. In addition, several new observations have emerged from the EM work. It is now possible, therefore, to propose a model of an actinian nervous system which is more complex than any picture previously presented. Observations made also allow a discussion of some of the major questions and problems which are inherent in the morphological study not only of an actinian nervous system but of any primitive nervous system. Discussion has been presented earlier of a unique fixation artifact which has led to considerable confusion in the LM study of anemone nervous systems (see Chapter 5), of a fixation effect on the morphology of synapses which can determine their recognition (see Chapter 6), and of a new interpretation of the subepithelial plexuses found in the endoderm and ectoderm (see Chapters 3 and 5).

This chapter is limited to a review of the major findings of this study and to a discussion of some ideas concerning the identification of neurons, synapses, and of nonnervous conducting systems in morphological studies of Cnidaria.

The Nervous System of *Stomphia coccinea*

The Endoderm

1) The endodermal nerve net lies within a network of fibres which originate from a variety of previously undescribed epithelial and subepithelial cells. The five major nonnervous fibres of the plexus have

been described; one or two others possibly exist. None appear to form junctions with each other or with the nerve fibres or muscle fibres, but all are intimately associated with the nerve net and several with the muscle system.

2) LM study, utilizing two techniques that are little used in this phylum, supports two changes in the nerve-net model developed by Batham, Pantin and Robson. It is proposed that the nerve net is composed of five elements: (i) the receptor originally described by the Hertwigs (Hertwig and Hertwig, 1879); (ii) the multipolar neuron of Robson (1963); (iii) a large bipolar neuron, which is comparable to the main variant of the large bipolar described by Batham, Pantin and Robson (Pantin, 1952; Batham et al., 1960); (iv) a small bipolar, originally described as a separate cell type (Hertwig and Hertwig, 1879; Leghissa, 1949) but recently thought to be a variant of the large bipolar neuron (Robson, 1963); and (v) a tripolar neuron, possibly described before by the Hertwigs (Hertwig and Hertwig, 1879) but known mainly as a variant of the large bipolar (Pantin, 1952).

3) The endodermal nerve net is almost as sparse as indicated in earlier LM studies (Pantin, 1952) making its EM study through observation of random sections impractical. Practical EM analysis of the endodermal net will depend on the development of a suitable technique for the sequential LM and EM study of identified and isolated neurons and junctions. A detailed report of the receptor, its axon and synapse has been made. Preliminary EM descriptions have been made of two or three types of possible nerve fibres, but only one could be characterized by its vesicle population and synaptic morphology.

The Mesoglea

1) The only neural element that has been found in the mesoglea is an ectodermal nerve fibre (type 4) which lies in the sunken ectodermal muscle tubes and which occasionally passes through the mesoglea that separates adjacent tubes.

2) A cell type, the 'small mesogleal cell', is described which represents the 'mesocyte' and 'neurocyte' of Leghissa and Mazzi (1959). Despite its remarkable similarity to neurons in LM study, EM examination has not revealed synapses or other junctions on this cell and, for this reason, it is described here as a nonnervous cell type. Its fibres have an intimate association with the sunken ectodermal muscle fibres in the tentacle and oral disc. Limited histochemical study suggests that such fibres have a lower endogenous level of catecholamines than do those fibres lying in the mesoglea, possibly indicating a secretory function of this cell.

The Ectoderm

1) The subepithelial plexus of the ectoderm is not a nerve plexus as has been described in earlier EM studies; instead it is composed of the nerve net and the fibres of an unknown number of nonnervous cell types. It has not been possible to determine all of the nonnervous components of the plexus. The ectoderm contains three major subareas each of which has a separate plexus composed mainly of fibre types unique to that plexus.

2) The 'reticulum', previously thought to represent the ectodermal nerve net, is composed solely of the peduncles of supporting cells. A unique artifact is described in which certain fixatives cause a severe

swelling of the fibres of the plexus which ruptures the fibres scattering their organelles and membranes throughout the zone of the plexus. At the same time the pressure placed upon the peduncles by the swelling fibres displaces the peduncles into a network-like configuration known as the reticulum.

3) The plexus of the tentacles, oral disc and pharynx contains four types of nerve fibres. Type 1 is the axon of the ciliary-cone receptor, and innervates type 2. Types 2 and 3 appear to be axons of interneurons; their synaptic interactions are largely with themselves but synapses have been seen that are polarized from 2 to 3. Type 4 has been observed only in the mesoglea where it innervates the ectodermal musculature; it has never been seen to interact with the other types of nerve fibres. None of these fibres were found in the pharynx.

4) The morphology of the synapses of nerve fibre types 1, 2, and 3 show an exceptional sensitivity to fixation effects. One fixative, used previously in study of an anthozoan nervous system, obscures the recognition of all three types of synapse; and the synapse of the receptor can be easily recognized only if fixed by permanganate. It is possible that some of the simplicity of the synapses observed in the Cnidaria may be the result of similar fixation damage.

5) The second major zone of the ectodermal plexuses is the column that overlies the sphincter. In this area the epithelium has an infrequent receptor and the subepithelial plexus contains three types of nerve fibres. Of the fibres observed here only one is found also in the tentacle (type 2); the other two are unique to this part of the column. Below the level of the sphincter the plexus decreases in size

to a number of small bundles of fibres which may provide a connection to the third ectodermal plexus lying in the pedal disc. Synapses have not been found in the pedal disc, and therefore nerve fibres are not known in this part of the body. They are probably present, however, as the epithelium of the pedal disc contains large numbers of solitary mechanoreceptors.

The Recognition of a Neuron

The Cnidaria, more than the Porifera and other primitive phyla, are looked to for not only our concepts of the functional organization of the most primitive existing recognized nervous systems but for our concepts of the evolutionary inception of a nervous system and of its early phylogenetic development. All of these general questions plus several that are specific to the anemones, such as the possible presence of mesogleal neurons, are largely dependent upon our definition of neurons since their accurate recognition determines our picture of the nervous system that they comprise. From comparative LM and EM studies (for review see Bullock and Horridge, 1965) it appears that most of the morphological characteristics useful in the recognition of a neuron are less developed in a primitive system than in an advanced nervous system. We have only to look at the controversies surrounding the poriferan 'protonervous' system (for reviews see Pavans de Ceccatty, 1974a, 1974b) and Hydra's nervous system (for reviews see Lentz, 1968; Westfall et al., 1971) to realize the importance of, and the difficulty in, obtaining an accurate definition of a neuron. It is important, therefore, to periodically examine our concepts and definitions in order to bring attention to and to evaluate new ideas for the characterization

of neurons and to reexamine controversial cell systems in light of any new findings.

It is beyond the scope of this study to examine our concepts of what constitutes a neuron (for reviews see Pavans de Ceccatty, 1974a, 1974b). This review will be limited to a consideration of the criteria useful in the identification of neurons in morphological study. It should be noted, however, that the two problems are not as isolated as they appear. Except in obvious cases the decision as to whether a particular animal, organ, or tissue layer possesses a nervous system can be arrived at only by extensive multidisciplinary studies which may take decades, as was the case with the Porifera. In other cases where physiological and behavioural knowledge is lacking, such as is the case with the actinian mesoglea, the question of the presence of neural elements must be explored and debated solely by the anatomist. Moreover, the development of a set of valid criteria for the recognition of a neuron could provide for an alternate line of study of the distinctions between a protonervous system and a true nervous system.

Cell Shape

Until the advent of EM study the shape of a cell and its staining characteristics were the sole anatomical criteria for the recognition of a neuron. Cell shape has been particularly important in the study of cnidarian nervous systems since their neurons have a limited range of shapes. Moreover, since several contested descriptions of actinian neurons have been attacked as being artifactual staining of nonnervous structures (Batham et al., 1961), cell shape is actually the only anatomical criterion for identifying neurons upon which our current

model of the actinian nervous system is based.

It appears, however, that the only valid specifications we can make of a cnidarian neuron are that all observed have been isopolar and that unipolar neurons are unknown. While the fibres of many neurons have a uniform diameter, are unbranching, and tend to cross over other neurons or effectors, others have been seen that have varicosities, branch dichotomously, have collateral branches, are syncytial, and nearly all seem to terminate free in the extracellular space (as determined in LM study).

The possible effects of an attempt to build a concise picture of the neurons of a primitive nervous system can be seen in an examination of the only modern attempt at a rigid supraspecific definition of a cnidarian neuron (Batham et al., 1961). From study of a single class of neuron (Pantin, 1952; Batham et al., 1960), Batham, Pantin and Robson developed four criteria for the recognition of a cnidarian neuron and used them as a basis for a review of some of the literature of anemone neuroanatomy (Batham et al., 1961). This study has been widely accepted as an authoritative review (Bullock and Horridge, 1965; Josephson, 1974).

A particularly important conclusion of their review was that since the mesogleal neurons described by Havet (1901, 1922) and Leghissa (1949) did not match their criteria for neurons these cells were not really neuronal. This interpretation of Havet's and Leghissa's studies has led both to a general disregard for the findings of these workers (which in the case of Leghissa's studies of the ectodermal nerve net has set back our knowledge for 15 years until his observations

were duplicated by Batham and Robson), and to the universal belief that the Hertwigs were correct in stating that the anemone mesoglea does not contain neurons (Hertwig and Hertwig, 1879). It is apparent today, however, that none of the neuronal criteria of Batham, Pantin and Robson are valid, and it must be said that most are suspect even when compared to the literature available to them at the time of their review:

1) Their most important criterion, that the axons of cnidarian neurons do not branch, is supported only by their own studies and those on the central nervous system of medusae. Most other studies have reported that cnidarian neurons tend to branch dichotomously, and collateral branching is known in two cases (Peteya, 1973, Chapter 6).

2) Their idea that nonnervous cells can be identified by the fine diameter of their fibres is based on the study of one of the largest neurons known in the phylum and this is unacceptable without support from other studies. Neurons seen in classic studies vary from 0.3 μ (von Heider, 1879; Hertwig and Hertwig, 1879) to 12 μ (Bozler, 1927, as cited in Bullock and Horridge, 1965) in diameter, and a recent EM study has reported neurons with fibres up to 20 μ in diameter (Peteya, 1973). Thus it appears that the size of a fibre has no value as an indication of its neural nature.

3) The criterion that a cnidarian nerve net cannot be syncytial is questionable in light of the wide occurrence of neuronal syncytia in other invertebrate phyla (for review see Bullock and Horridge, 1965). Although they are probably correct in questioning much of the early literature that supports the possibility of syncytial nervous systems

in the Cnidaria (Batham et al., 1961), Mackie's study of *Velella* (1960) has provided a clear demonstration of a syncytial nerve net which cannot be dismissed.

4) Finally, it was stated that the fibres of a possible nerve cell should terminate on other neurons or on effectors and that they should exhibit the peculiar 'wetting junction' interpreted as an *en passant* synapse (see Pantin, 1952, fig. 10; Batham et al., 1960, figs. 7a, 7b). The functional interconnection of a neuron to other neurons, receptors and effectors is essential to the function of a nervous system, and the morphological observation of such junctions is an important criterion for the identification of a neuron. Their observation is, however, not usually possible by LM study. The studies of Batham, Pantin and Robson are the only reports of the endfeet that they have described as neuro-effector junctions and most other studies report that the axons terminate randomly. Similarly, while the wetting junction may represent a synapse it is unique to the actinians, its morphology appears to be fixation-dependent, and it is found on nonnervous cells. In the present study wetting junctions were often seen in Batham-fixed mesenteries but could not be identified in ZIO- or in Wood-fixed mesenteries. Also, since such junctions were rarely found on the small mesogleal cells (Fig. 39°), it appears that the junction has little if any value in the characterization of a neuron.

Although it appears that we cannot rigidly define the morphology of cnidarian neurons on a supraspecific level it has been possible to characterize them on a species level in many cases. The greatest problem in their identification has been experienced in the Actiniaria where the

presence of presumptive nonnervous cells that have the shape and staining characteristics of neurons has led to considerable debate over the accuracy of the various reports of the actinian nervous system. From my own study and literature review of anemones it appears that there are only two nonnervous cell types that can be, or have been, confused with neurons: the ectodermal amoebocyte and the 'small mesogleal cell' (SMC).

The ectodermal amoebocyte has a highly variable shape but is usually globular to elongated. A significant number, however, have the appearance of neurons because of the spatial arrangement of their two types of processes (Figs. 106, 107). This subpopulation closely resembles a type of bipolar neuron described by Leghissa (1949) not only in its morphology, but in its distribution and in its histochemical staining characteristics. It is not known whether this is a case of an erroneous description of amoebocytes as neurons or of a true similarity between the amoebocytes and a neuron. In either case the ectodermal amoebocyte of *Stomphia* appears to be distinguishable, on a LM level, from cells that would otherwise be identified as neurons only by their greater range of shape than what is expected of a neuron, and possibly by the presence of numerous lysosomes.

The SMC has variously been described as a neuron (Havet, 1901, 1922; Leghissa, 1949), as a mesocyte and a neuron (Leghissa and Mazzi, 1959; Leghissa, 1965), and as the mesogleal amoebocyte (Batham et al., 1961). The question of whether this cell is a neuron is one of the oldest and greatest controversies in the study of cnidarian nervous systems; in the absence of relevant physiological and behavioural data

the question has been explored solely by morphological criteria. The SMC are bi- to multipolar, isopolar cells whose fibres form a complex multilayered network throughout the mesoglea. Leghissa has divided them into neuronal and nonnervous elements on the basis of a number of morphological and staining differences (Leghissa and Mazzi, 1959). Although the present study could not evaluate all of these criteria those which could be observed clearly distinguish the SMC into two cell types. The supposedly neuronal SMC of *Stomphia* are a small population of bipolar cells whose fibres are at least a few hundred μ in length, have a regular diameter, and do not branch; they are almost identical in appearance to endodermal cells described as neurons (Robson, 1963; Peteya, Chapter 2 of this thesis) (compare Figs. 19 and 38 A,B,C). EM examination, however, reveals that the SMC are a single cell type, and provides no evidence that they are neuronal.

It appears that the great value which cell shape has had as a criterion for the recognition of neurons in the Cnidaria is the result of the relative absence in this phylum of nonnervous cells which might be confused with neurons because of their shape. The small mesogleal cell and the ectodermal amoebocyte of anemones appear to be the only cell types in the phylum which can be, or have been, erroneously described as neurons, excluding of course the old reports that the [illegible]orhagi are photoreceptors (Duncan, 1874).

[illegible]*ing Characteristics*

In their review of artifacts and misconceptions in the identification of actinian neurons, Batham, Pantin and Robson have justifiably given little value to the staining characteristics of a cell (Batham

et al., 1961). There appears to be no histological technique which does not fail to stain cells in the anemones which could be confused with neurons. It is possible to silver-impregnate amoebocytes (Havet, 1901; Batham et al., 1961; Peteya, Chapter 3 of this thesis), the SMC (Leghissa, 1949; Batham et al., 1961) and even collagen fibres (Batham et al., 1961); gold impregnations stain both neurons and the SMC (Leghissa, 1949). Similarly, chromaffin and chromargentic techniques both stain amoebocytes and the SMC (Leghissa, 1949; Peteya, Chapters 4 and 6 of this thesis), methylene blue stains the SMC (Batham et al., 1961), and the ZIO technique stains both endodermal neurons and the SMC (see Chapters 3 and 4).

Microanatomy

In most EM studies of Cnidaria and Porifera the identification of possible neurons has been made by correlation between LM and EM observations either by sequential LM and EM examination of silver-stained neurons (Jha and Mackie, 1967) or by establishing cell shape by EM study (Davis et al., 1968; Leghissa and Quaglia, 1966). In addition, attempts have been made to utilize vesicles (Jha and Mackie, 1967), microtubules (Mackie, personal communication; Stokes, 1974), and synapses in the recognition of neurons.

In most hydro- and scyphopolyps examined by EM, cells have been observed which contain large numbers of dense-core vesicles that are between 1000 and 2400 Å in diameter (Davis et al., 1968; Crawford and Webb, 1972; Stokes, 1974). There is evidence, in *Chrysaora* (Crawford and Webb, 1972) and in *Hydra* (Burnett et al., 1964), that these cells are neurosecretory, indicating the possible existence of a type of

neuron that is common to much of the phylum. However, there are few records of neurosecretory cells in the Cnidaria and none in the Anthozoa. Also, several nonnervous cells have been seen in this study which contain similar dense-core vesicles which, on a LM level, show a positive reaction in histological techniques for amines and neurosecretory substance. Therefore the observation of such vesicles cannot be taken as a demonstration of a nerve- or neurosecretory-cell type.

From study of a number of hydromedusae Mackie has proposed that the presence of microtubes is a valid EM-level criterion for the identification of neurons and receptors (Mackie, personal communication). This possibility has been supported by studies of neurons in all three classes of Cnidaria (Horridge and Mackay, 1962; Peteya, 1973; Stokes, 1974), and appears valid for every cnidarian receptor that has been examined. It does not, however, appear to be a foolproof technique for the identification of neurons. In the present study some identified nerve fibres, such as type 3, appear to be devoid of microtubules while other nonnervous types of fibres, such as endodermal type 5, do contain microtubes.

By definition a neuron is a component cell type of a nervous system which interconnects receptors and effectors either directly or via other neurons. It is possible, therefore, to define a neuron by its functional interconnections; an approach which is particularly favorable since only two types of transmitting junctions, the synapse and the gap junction, have been found in any of the nervous systems examined by EM. Limitations to the approach are mainly that only the synapse is unique to the nervous system, and that some cnidarian nerve nets are possibly syncytial (Mackie, 1960; Leghissa, 1965) and may

therefore be devoid of synapses other than for neuroeffector junctions (Leghissa and Quaglia, 1966).

The presence of a synapse is the only known foolproof criterion for the identification of a nerve cell, and is of growing importance in the study of cnidarian nervous systems. Its successful application in studies of primitive nervous systems, however, may be subject to both technical and conceptual problems (see below).

The Recognition of a Synapse

The synapses observed in the various invertebrates and vertebrates studied by EM show a remarkable similarity in basic morphology, and it would therefore seem that the recognition of a synapse is a straightforward matter. However, there is a growing number of studies, particularly in the Cnidaria (Jha and Mackie, 1967; Leghissa and Quaglia, 1966; Davis et al., 1968) and of molluscs (for review see Pentreath and Berry, 1975), in which synapses cannot be identified or in which they can be identified only with great difficulty (Westfall et al., 1971). Barring the possibility that the neurons of these systems interact by some unknown or unexpected mechanism (e.g. that they form a syncytium), the inability to identify synapses or gap junctions implies either a failure in our definition of a synapse as suggested by Cobb and Mullins (1973) or else that the technique of study is affecting the morphology of the synapses in such a way that they become unrecognizable.

Support for the theory that some synapses may not be recognizable by present definitions is easily found both in the vertebrate autonomic nervous system and throughout the lower invertebrates. The vesicle-filled varicosities of autonomic fibres have been identified as neuromuscular

junctions solely because they are the site of transmitter aggregation in nerve fibres that are known to innervate smooth muscle (for review see Bennett, 1972). The identification of similar varicosities as the site of transmitter release in invertebrate nervous systems is becoming popular (Rosenbluth, 1972; McKenna and Rosenbluth, 1973), but in the absence of physiological evidence this practice is questionable. More important, however, is the trend, throughout the invertebrates, toward synapses of increasing complexity in the higher phyla. Few taxa but the vertebrates and cephalopods show organized presynaptic projections, and the gap and postsynaptic projections are often missing or difficult to identify in most invertebrates. The difficulty in recognizing a synapse is directly related to its simplicity; the simplest known is that of *Hydra* which was first discovered only after over 10 years of research, in a number of labs, on the microanatomy of its nervous system (Westfall et al., 1971). Since some of the membrane specializations of a synapse are associated with attachment rather than transmission (for review see Pfenninger, 1973), Cobb and Mullins have suggested that many synapses, in which mechanical strength is unimportant, may be recognizable only by an aggregation of vesicles, and that even this criterion may not be valid. They conclude, "Only where there is physiological evidence can electron microscopy be reliably used to show synaptic contact . . . ."

An alternate explanation for the great simplicity of some invertebrate synapses can be found in observations of the effects of some fixatives on the morphology of synapses in some anemones. The interneural synapses of *Ceriantheopsis* have pre- and postsynaptic cytoplasmic

structures which are similar to, but simpler than, those known otherwise only in the synapses of cephalopods and vertebrates (Peteya, 1973). They can be seen, however, only in tissue prepared by one fixation technique out of the 20 techniques used in that study; in tissue prepared by any of the other fixatives the synapses appeared like those seen in other studies of cnidarian nervous systems and were recognizable only by the apposition of vesicles near a junction defined by dense membranes and a regular separation of the two membranes. The synapses of *Stomphia* do not appear to be as complex as those of *Ceriantheopsis* and, perhaps because of this, show a greater sensitivity to the effects of fixation. Of the four fixatives that were used extensively in this study only permanganate gave a fixation which allowed the recognition of the synapse of ectodermal nerve-fibre type 1. Similarly, in tissue fixed in the fixative which gave the best preservation of cerianthid synapses (Peteya, 1973) it was not possible to identify synapses of nerve-fibre types 1, 2 or 3.

In the absence of well-defined membrane specializations (which may itself be a fixation artifact since the synapse of ectodermal nerve-fibre type 3 has elaborate gap structures seen only in permanganate-fixed tissue), recognition of the synapses of *Stomphia* is dependent on the regularity of gap width and on the presence of an ill-defined membrane density. It is premature to suggest the mechanism whereby fixatives effect the recognition of these synapses, but there is some evidence that the synapses can be dissociated. In tissue in which synapses were difficult to recognize, such as in Westfall-fixed tissue, numerous nerve fibres were seen which had peripheral aggregations of synaptic vesicles but only rarely was there a postsynaptic fibre associated with

the aggregations. It appears that some fixation procedures do not preserve the gap material of some types of synapses allowing the gap width to become irregular or the synapse to break apart at the gap. If such features are labile, recognition of some synapses could become impossible.

It is not possible to estimate the relative importance of the theories of Peteya (1973) and of Cobb and Mullins (1973) for the simplicity and occasional absence of synapses in some invertebrate nervous systems. Such a severe fixation effect on synapse morphology as is described here showed up only in studies of *Cerianthéopsis*, *Stomphia*, and of *Metridium* conducted by this investigator. There is, however, another study in progress of the effect of fixatives on the recognition of synapses in some noncnidarian invertebrates (McFarlane, personal communication). Clearly the possibility that fixatives affect synapse morphology and may prevent their recognition must supercede Cobb and Mullins' theory in any study of an invertebrate nervous system. Exploring such a possibility may be extremely difficult. It may require the use of dozens of fixatives to find one that shows the true complexity of the synapses in a particular species, and then that fixative may prove worthless in a related taxa as the Peteya fixative was in *Stomphia*.

The Question of Neuroidal and Myoidal Conducting Systems in the Actiniaria

One of the most distinctive and remarkable histological features of the Cnidaria is the epitheliomuscular cell. Except in cases where the cell has been secondarily modified the cell bodies form a superficial epithelium and are connected to one or more of the underlying contractile elements of the muscle system. Their organization immediately suggested

the possibility that the cell bodies could constitute a nonnervous conduction pathway and that as each cell was excited it would elicit a contraction of the muscle fibres attached to it (Kleinenberg, 1872). As a consequence, the epitheliomuscular cell has played a major role in the development of several theories of the inception and early evolution of the nervous system, most notably that of Parker (1919), and has been the cell system basic to the belief that nonnervous conduction systems might exist in the Cnidaria.

The most recent revival of Kleinenberg's theory has been sparked by Mackie's (1965) demonstration that the epithelium of some hydromedusae conducts behaviourally significant events in the apparent absence of nerve fibres and, in some species, also of muscle fibres. Since then there have been few electrophysiological studies of cnidarians that have not attributed most of their findings to neuroidal or myoidal systems rather than to a nervous system. Unfortunately, few physiologists have made any attempt to relate their records to existing anatomical knowledge and instead have interpreted them on the basis of tenuous physiological criteria which may lead to contradicting findings in different taxa. Valuable auxilliary support for the claimed presence of nonnervous conducting systems in the Cnidaria could be had from the morphological demonstration of gap junctions. Although it is thought that a significant coupling can occur between cells that are separated by a gap of 200 Å or more (Bennett and Auerbach, 1969), there is no neuroidal or myoidal conducting system which has been studied by EM which does not have gap junctions. Thus while the presence of gap junctions may not imply that the tissue is excitable

(Kuffler et al., 1966), their presence does appear to be essential for the function of nonnervous conducting systems.

Among the dozens of cnidarian species examined by EM, gap junctions have been found in only two, both in tissues believed to constitute nonnervous conducting systems. These are the ectodermal and endodermal epithelium and muscle layer of *Hydra* (Hand and Gobel, 1972) and the epithelium of the exumbrellar ectoderm of *Nanomia* (Mackie, unpublished; in D. Chapman, 1974, Fig. 31). Unfortunately the few other EM studies of tissues which have now been described as possible conducting systems have not included reports of the junctions observed.

The present study is therefore in the unique position of contradicting the claims of the physiological reports. According to McFarlane and his students every actinian that they have examined, including *Stomphia* (Lawn, personal communication), has two slow conducting systems which constitute nonnervous conducting systems (for review see Shelton, 1975). One is supposed to be ectodermal and the other endodermal, and both are found throughout the extent of these tissue layers. Unless the actinians should prove to be the first known exception to the correlation between nonnervous conducting systems and gap junctions this study should have revealed a high frequency of gap junctions between either the epithelial cells or between the muscle fibres. Instead, none have been observed by this investigator in over four years of EM study. *Stomphia* has only three types of intercellular junctions, other than synapses: 1) septate desmosomes which interconnect all cell types that lie at the surface of both ectodermal and endodermal epithelia; 2) the 'dove-tail junctions' of Wood (1961) which are found at end-to-end

junctions of muscle fibres in both the endoderm and ectoderm; and 3) simple desmosomes which are found between adjacent muscle fibres in the endoderm and ectoderm. Of these three only the septate desmosome has been suggested as a coupling junction. This was done in the erroneous belief that it was the only intercellular junction in the giant salivary gland of *Drosophila* larvae (Wiener et al., 1964); the subsequent discovery of the correlation of gap junctions with conducting epithelia has led to the belief that septate desmosomes have only an adhesive function. In the absence of gap junctions in *Stomphia* the burden of proof for the claim that actinians have through-conducting nonnervous conducting systems in both the ectoderm and endoderm lies with the physiologists, a burden which some physiologists who work on nonnervous conduction in Cnidaria believe they cannot handle and instead look to a morphological study for proof' (Mackie, personal communication; Spencer, personal communication).

TABLE 1 The staining affinities of the endodermal neurons and sensory cell.

	sensory cell	large bipolar	small bipolar	tripolar	multipolar
Batham's silver*	-	+	(-)	(-)	(-)†
Methylene blue*	+	+	?	?	+
Zinc-iodide-osmium†	+	+	-	+	-
Wood's alcian blue†	-	+	+	+	-

* - Taken from the studies of Batham, Pantin and Robson (see text for references).

† - Taken from the present study.

\+ - frequent staining; - = never stained; (-) = very rare staining.

? - No record.

TABLE 2 Distinguishing characteristics of the major endodermal fibre types.*

Type	Diameter	Diameter of varicosities, if any	Microtubules	Vesicles: synaptic	Other	Other
1	0.5-2 μ		-		1000-5000 Å solid	700 (av.) agranular
2	0.3-7 μ		-		600-800 Å dense core	540 (av.) agranular
3	0.2 μ	0.8 μ	-		600-800 Å dense core	1200-2000 Å solid
4	0.4-0.8 μ	1.3 μ	-		650-1500 Å opaque	1100 Å (av.) dense core
5	0.2 μ	0.4-1.0 μ	+		1000-1500 Å solid	
SCA	0.15 μ	0.2-0.7 μ	+	irregular 500-900 Å agranular	700-1500 Å dense core	irregular 500-1100 Å agranular
NF	?	?	?	600-900 Å agranular		

*All data are taken from Cavey-fixed tissue. All vesicles are spherical unless otherwise noted. SCA = the axon of the receptor; NF = the axon of the only type of neuron characterized; ? = seen too rarely to determine characteristics.

TABLE 3 Chemical composition of the fixatives studied.* (From: Lee, 1928; Leghissa, 1949; Gray, 1954; Batham et al., 1960; Humason, 1962; Robson, 1963; Westfall, 1965; Ramón-Moliner, 1970).

Fixative:	Components:							
	OsO_4	Picric acid	$K_2Cr_2O_7$	Form.	Formic acid	$HgCl_2$	Acetic acid	Etoh Others:
Bouin, 1897		0.81	9.52				4.76	
Bouin, 1898		0.42	7.27	9.09				0.36 $PtCl_4$
Brasil, 1904		0.44	10.7				6.67	53.3
Batham et al., 1960		0.86	10.0					
Mayer, 1881		0.13						4.76 HNO_3
Rawitz, 1905	0.29	0.11						4.08 HNO_3
Fol		0.11						0.25 CrO_3
VomRath, a, 1895	0.01	1.07					0.93	
VomRath, b, 1895		1.08					0.94	0.47 $PtCl_4$
VomRath, b, (Lee)	0.21	0.96					0.84	0.42 $PtCl_4$
Susa			7.69		4.50		3.85	1.92 CCl_3COOH
								0.29 NaCl
Stievie, 1948			8.00		3.40		4.00	
Mann, 1898					2.50			2.50 CrO_3
Kenyon, 1896		3.80	8.00					2.00 $CuSO_4$
Orth, 1896		2.27	3.64					0.91 Na_2SO_4
Kolmer, 1938		1.82	1.45				9.09	0.45 CCl_3COOH
								0.09 uranyl acetate
Zenker, 1894		2.38			3.81		4.76	0.95 Na_2SO_4
Helly, 1904		2.38	1.90		3.81			0.95 Na_2SO_4
Zenker-formic acid		2.38		4.76	3.81			0.95 Na_2SO_4
Zenker-osmium	0.47	1.68		5.88	2.68			0.67 Na_2SO_4
Golgi Cox (Lee)		0.95			0.95			0.76 K_2CrO_4

*For ease of comparison, the composition of the fixatives is given in percentages; liquid stocks are expressed as v/v, solids as w/v. Values for formaldehyde assume stock is 40%. *trace* denotes small, unspecified quantities.

Table 3 (page 2)

Fixative:	Components:							
	OsO_4	Picric acid	$K_2Cr_2O_7$	Form.	Formic acid	$HgCl_2$	Acetic acid	Etoh	Others:
Leghissa, 1949			2.00	13.3					
Champy, 1913	0.45		1.17						0.39 CrO_3
Johnson, 1895	0.20		1.75		5.00				0.15 $PtCl_4$
Westfall, 1966	2.00		1.00						78 sea water
Golgi, 1880	0.20		1.60						
Golgi, 1900	0.33		2.00						
Sanfelice				12.8			4.00		0.64 CrO_3
Flemming, 1882	0.10						0.10		0.25 CrO_3
Flemming, 1884	0.40						5.00		0.75 CrO_3
Flemming, w/o AA	0.42								0.79 CrO_3
formaldehyde				4.00					90 sea water
Faa				1.82			4.54	63.6	
Carnoy, 1886							10.0	60.0	30 chloroform
Carnoy-Lebron, 1887						8.8	33.0	33.0	33 chloroform
original	0.10				3—4				
Hermann, 1889	0.40						5.00		0.75 $PtCl_4$
Frenkel, 1883	0.50						trace		0.75 $PdCl_2$
Hertwigs, 1879	0.025						0.10		in sea water
", modified	0.050						0.10		in sea water
DeCastro, 1925								46.2	2.40 chloral hydrate
									2.88 HNO_3
Alexandrowicz, 1932	0.02								10 ammonium molybdate
									35 sucrose
									0.01 $PtCl_4$
Alexandrowicz, 1960	trace								10 ammonium molybdate
Apathy, 1897									1.0 $HAuCl_4$
Cajal 2a, A, 1910								96.0	2.0 pyridine
Miller, 1923									4.0 citric acid
freezing in dry ice									
freezing in liquid N_2									

TABLE 4 The effects of fixatives on the plexus and peduncles.

Fixative	Fix time	Species used[a]	Results: plexus[b]	peduncles[c]
Bouin, 1897	3,12 hr.	all	0	0
Bouin, 1898	3,12 hr.	S	?	+
Brasil	3,12 hr.	all	0	0
Batham	1,2 day	all	0	0
Mayer	3 hr.	S	+	+++
Rawitz	3 hr.	S	+	+++
Fol	3 hr.	S	0	0
VomRath a	2 hr.	S	+	+++
VomRath b	2 hr.	S	+	0
VomRath b (Lee)	2 hr.	S	++	+++
Susa	3,12 hr.	all	0	0
Stievie	1 day	S	+	+
Mann	5 hr.	S	+	++
Kenyon	7 days	S	+	+++
Orth	12 hr.	S	0	0
Kolmer	1 day	S	+	+++
Zenker	3,12 hr.	all	0	0
Helly	3,12 hr.	all	0	0
Zenker with FA	6 hr.	all	0	0
Zenker-OsO_4	6 hr.	S	+	+++
Golgi-Cox (Lee)	30 day	S	?	0
Leghissa	1 day	M	+	+++
Champy	6 hr.	S	+++	+++
Johnson	12 hr.	S	++	+++
Westfall	2 hr.	S,M,C	+++	+++
Golgi, 1880	1/2,5 day	S,M,C	+	+++
Golgi, 1900	1/2,5 day	S,M,C	+	+++
Sanfelice	6 hr.	S	+	++
Flemming, 1882	1,12 hr.	S	++	+++
Flemming, 1884	1,12 hr.	S	++	+++
Flemming w/o AA	6,12 hr.	S	+++	+++
formaldehyde	12 hr.	S,M,C,A	+	+
Faa	3,12 hr.	all	0	0

Table 4 (page 2)

Fixative	Fix time	Species used[a]	Results: plexus[b]	peduncles[c]
Carnoy	6 hr.	S,M	+	+++
Carnoy-Lebron	8 hr.	S	+	+++
original	4 hr.	S,P	++/+++	+++
Hermann	12 hr.	S,P	++	+++
Frenkel	6 hr.	S	+	+++
Hertwigs	1 day	S,P	+, but 1/2 are missing	+++
Hertwigs; my modification	1 day	S,P	++	++
DeCastro	1,3 day	M	0	0
Alexandrowicz, 1932	1 day	S	+	+++
Alexandrowicz, 1960	1 day	S,C	+	+
Apathy	1/4 to 4 hr.	S.P	++	+++
Cajal	5 hr.	M	+/?	+++
Miller	30 min.	P	?	++
dry ice	---	S,C	+	+++
liquid N_2	---	S,C	++	+++

a. S = *Stomphia*, M = *Metridium*, C = *Ceriantheopsis*, A = *Anthopleura*, P = *Pachycerianthus*, all = all of these.

b. The fixation of the plexus is summarized: +++ = excellent fixation; ++ = good preservation; + = present, but with moderate to severe swelling damage; ? = not possible to determine whether fibres were preserved; 0 = the fibres were not preserved.

c. The fixation of the peduncles is summarized: +++ = good preservation, with little or no distortion; ++ = distorted, but none broken from the mesoglea or aggregated; + = showing some characteristics of a reticulum, either aggregated or broken; 0 = the peduncles are in a 'reticulum' conformation.

TABLE 5 Distinguishing characteristics of the ectodermal nerve fibres.*

Type	Diameter	Micro-tubules	Vesicles: synaptic	Response to drugs	Other	Response to drugs	Other	Response to drugs
1	0.3-1.0 μ	+	600-900 Å agranular (DC/MnO_4)	+/met.	800-1600 Å dense core (DC/MnO_4)	+(?)/met.		
2	0.4-1.0 μ	+	600-900 Å agranular (DC/MnO_4)	+/met.	800-1700 Å dense core (DC/MnO_4)	+(?)/met.	2000-3000 Å solid (S/MnO_4)	+/Woods -/met.
3	0.3-1.2 μ and 2-6 μ	-	450-630 Å agranular (A/MnO_4) (A/ZIO)	-/αmNA	1300-2000 Å dense core (DC/MnO_4)	-/met.		
4	0.2-1.4 μ	-	500-800 Å agranular (1:100 DC/MnO_4) (1:1 DC/ZIO)	-/αmNA -/met.	800-1800 Å dense core (DC/MnO_4) (DC/ZIO)	-/met.		
5	0.2-0.4 μ	-	irregular 500-750 Å dense core		? dense core			
6	?	?	flattened 500-800 Å agranular		?			

*All unbracketed data are taken from Cavey-fixed tissue; important changes in morphology seen with other fixatives are given in brackets. All vesicles are spherical unless otherwise noted. DC = dense core; S = solid; A = agranular; met = metaraminol; αmNA = α-m-noradrenaline; ? = seen too rarely to determine characteristics. Also see Table 6 (p. 225).

PLATE 1

Figure 1. Semidiagrammatic representation of a quadrant of a *Stomphia* illustrating some aspects of its anatomy and of the location of areas chosen for EM study.

Figure 2. A section of the column endoderm, cut parallel to the oral-aboral axis. Note that the muscle layer contains two classes of muscle fibre which lie in discrete sublayers; the type A fibres lie in tubes which are buried in the mesoglea and appear to have no contact with the fibre plexus (FP), while the type B muscle fibres lie in a sheet at the surface of the mesoglea and thus are in direct contact with the plexus. (Cavey; column). X 4,000.

Figure 3, A,B. Endodermal septate desmosomes indicating their permeability to ruthenium red (A) and to bismuth iodide (B). (A: Cavey, mesentery; B: Pfenninger, tentacle). X 120,000.

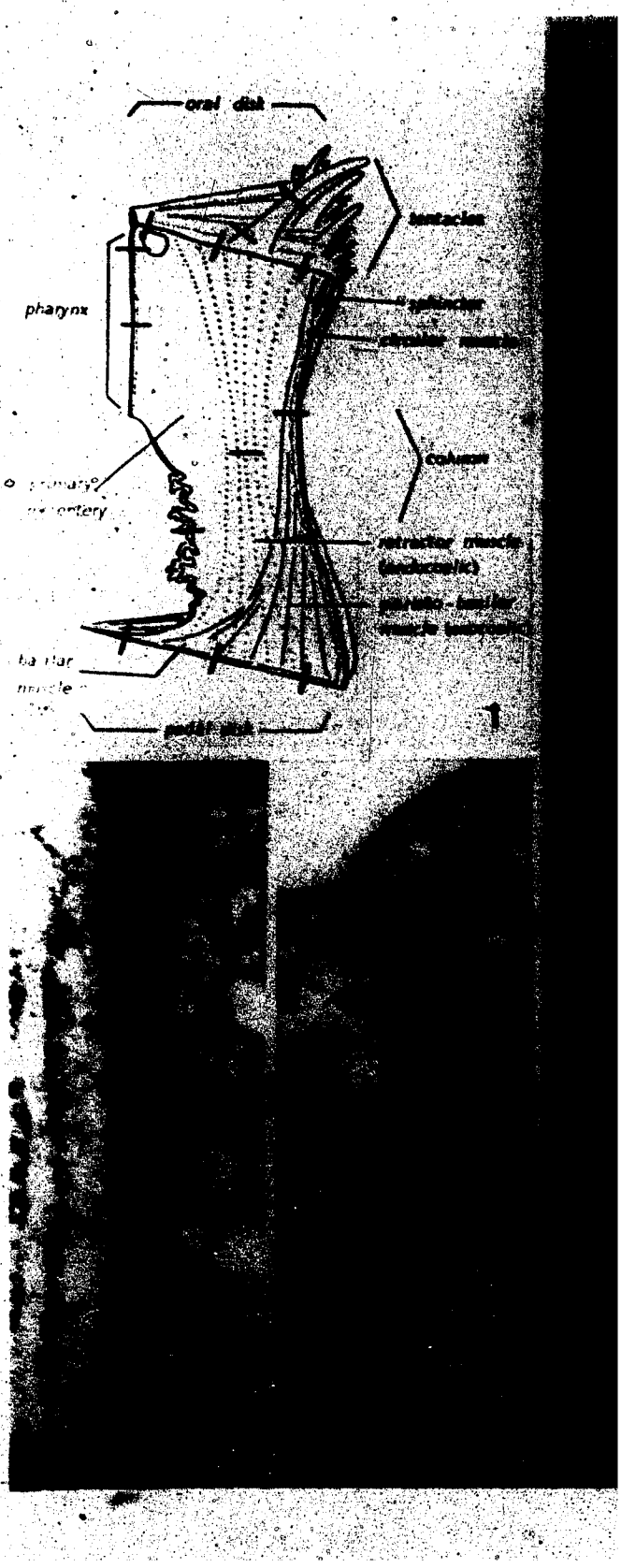
oral disk
tentacles
pharynx
sphincter
circular muscle
coelom
primary
mesentery
retractor muscle
(endocoelic)
basilar
muscle
pedal disk
1

PLATE 2

Figure 4. An epitheliomuscular cell in a whole-mounted mesentery. N, nucleus; L, lysosomes; arrows, transverse muscle fibres. (Wood's alcian blue, mesentery). X 2,000.

Figure 5. A micrograph of an epitheliomuscular cell whose cell body lies in the epithelium. P, peduncle; L, lysosomes; arrows, surface indentations with membrane-associated extracellular particles. (Cavey, mesentery). X 23,000.

Figure 6. A sunken epitheliomuscular cell, showing a continuity between the cell body and its contractile process. Note the absence of lysosomes. The cell has been reproduced in two parts; a point of overlap of the two is indicated by the arrows. (Cavey, column). X 23,000.

Figure 7. A muscle-cell peduncle. Note the bundles of fibrils which distinguish the peduncles from elements of the fibre plexus. (Cavey, column). X 28,000.

4

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PLATE 3

Figure 8. The endodermal gland cell. (8: Cavey's mesentery; inset: Wood's alcian blue, mesentery). 8: X 25,000; inset: X 2,000.

Figures 9 and 10. An elongated amoebocyte showing the lysosomes (large arrows) and large solid vesicles (small arrows) which characterize the cell. (Peteya, tentacle). 9: X 6,000; 10: X 25,000.

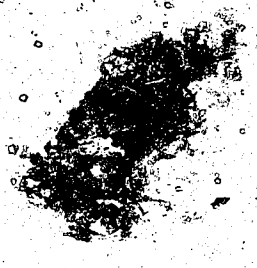
Figure 11. A spherical and an elongated amoebocyte in which both the vesicles and lysosomes have been stained with silver. (Batham, mesentery). X 2,000.

PLATE 4

Figure 12 Inset. A focus montage of a new-cell type 1. The basiepithelial cell body gives rise to a process which reaches the tissue surface, to a single fibre in the plane of the epithelium, and to two fibres which lie in the plexus. (Wood alcian blue; mesentery). X 2,000.

Figure 12. An electron micrograph of a type 1 cell. (Cavey, column). X 9,000.

Figure 13. Four examples of new-cell type 2 showing some of the variation in its appearance. (Wood's alcian blue, mesentery). X 2,000.



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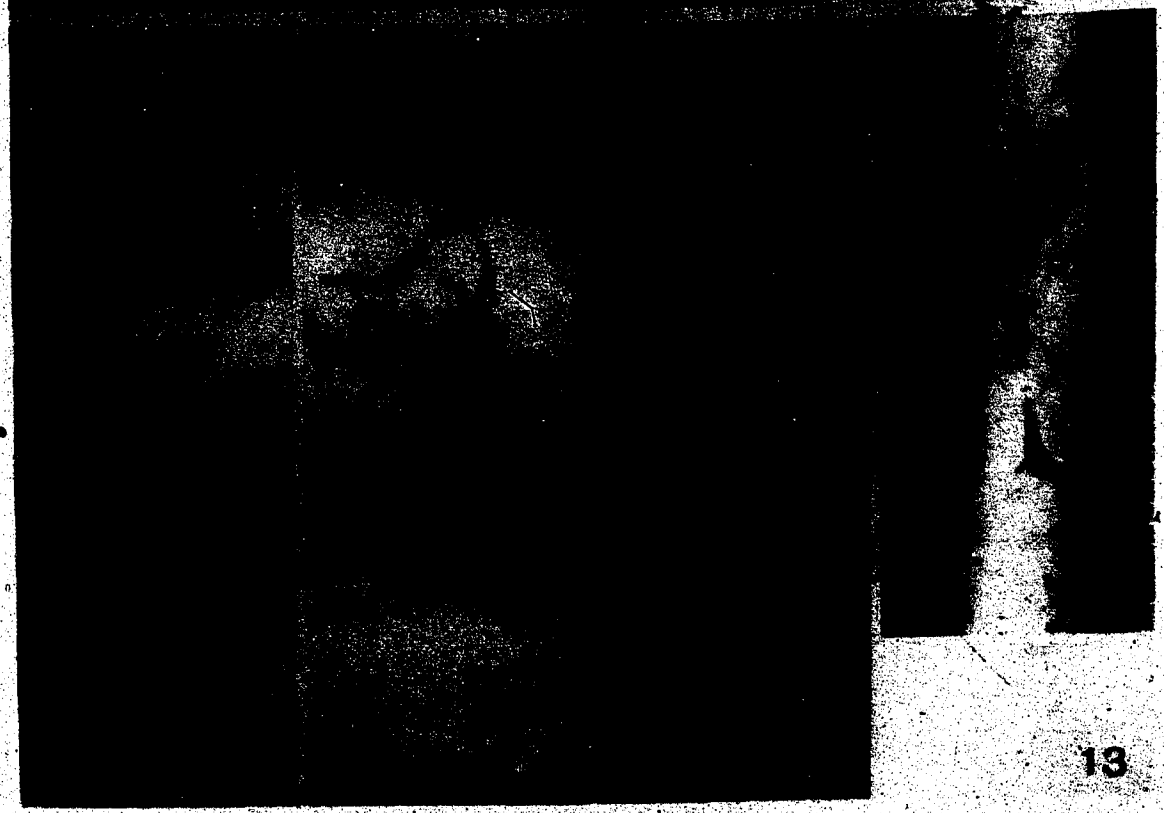

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PLATE 5

Figure 14. Endodermal receptors. Note that the dendrite of Fig. 14C has a sac (large arrow) similar to that seen in Fig. 17A. Small arrow, cilia. (A,B,C: ZIO impregnations, mesenteries; D: Modified Hertwig maceration, tentacle). X 2,000.

Figure 15. The basal apparatus of a sensory-cell cilium, consisting of a basal body with a foot and an accessory centriole. The root is not in the plane of this section. (4% glutaraldehyde in sea water, mesentery). X 55,000.

Figure 16. Dendrite of a receptor showing attachment of microvilli, the large dense-core vesicles, and an unusual array of tubules which appear to open onto the cell surface. (Cavey, column). X 55,000.

PLATE 6

Figure 17, A,B. An endodermal receptor and the initial segment of one of its axons. Note the presence of large electron-dense vesicles throughout the cell, and the similarity between the microvilli of the receptor and of the adjacent epitheliomuscular cells. The cell has been reproduced in two parts; a point of overlap of the two is indicated by the arrows. (Cavey, column). X 8,000.

Figure 17, C. A higher magnification micrograph of the axon of the same cell, showing the predominant axonal contents: large solid vesicles, various agranular vesicles, endoplasmic reticulum, and microtubes. (Cavey, column). X 45,000.

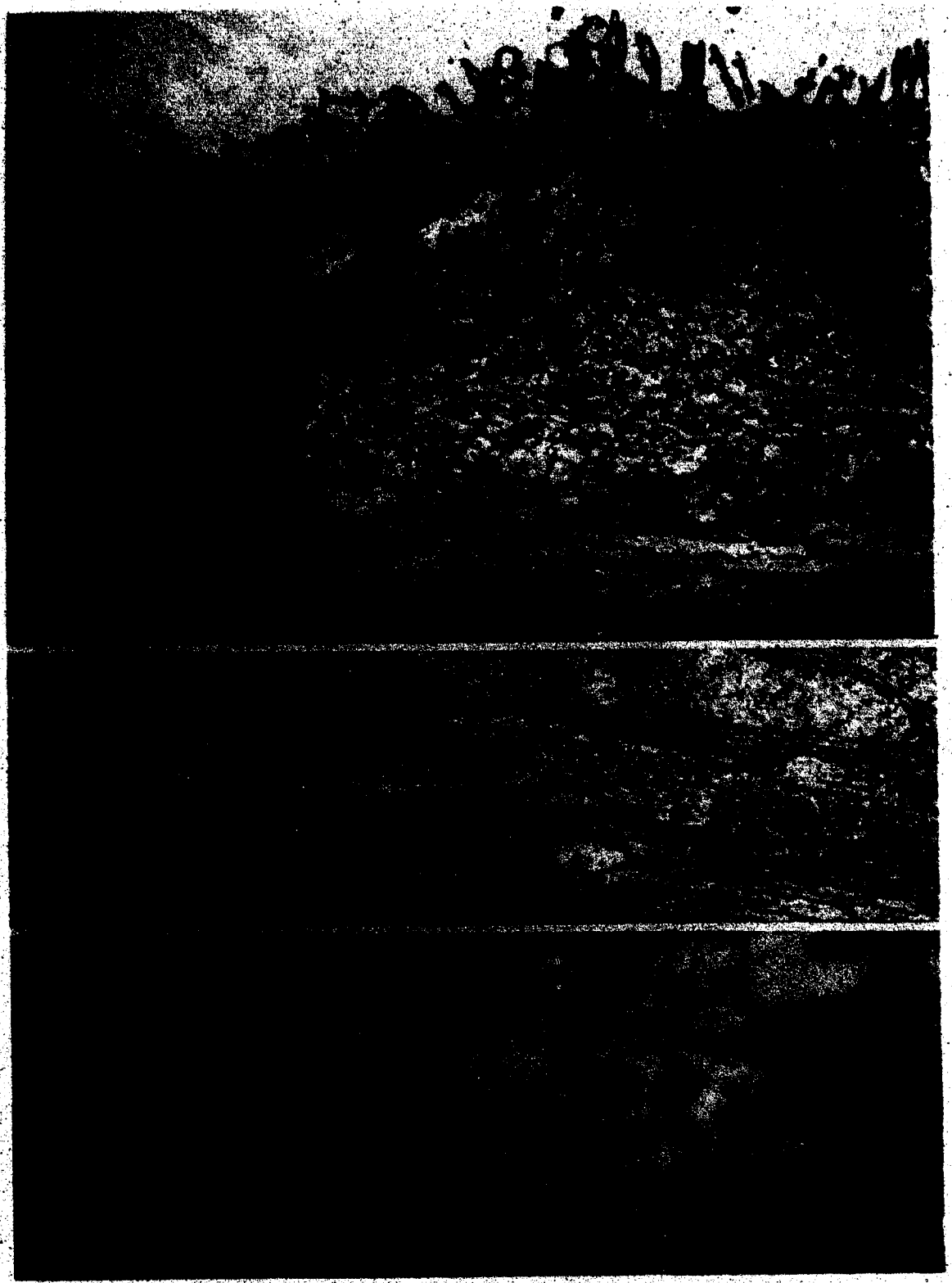
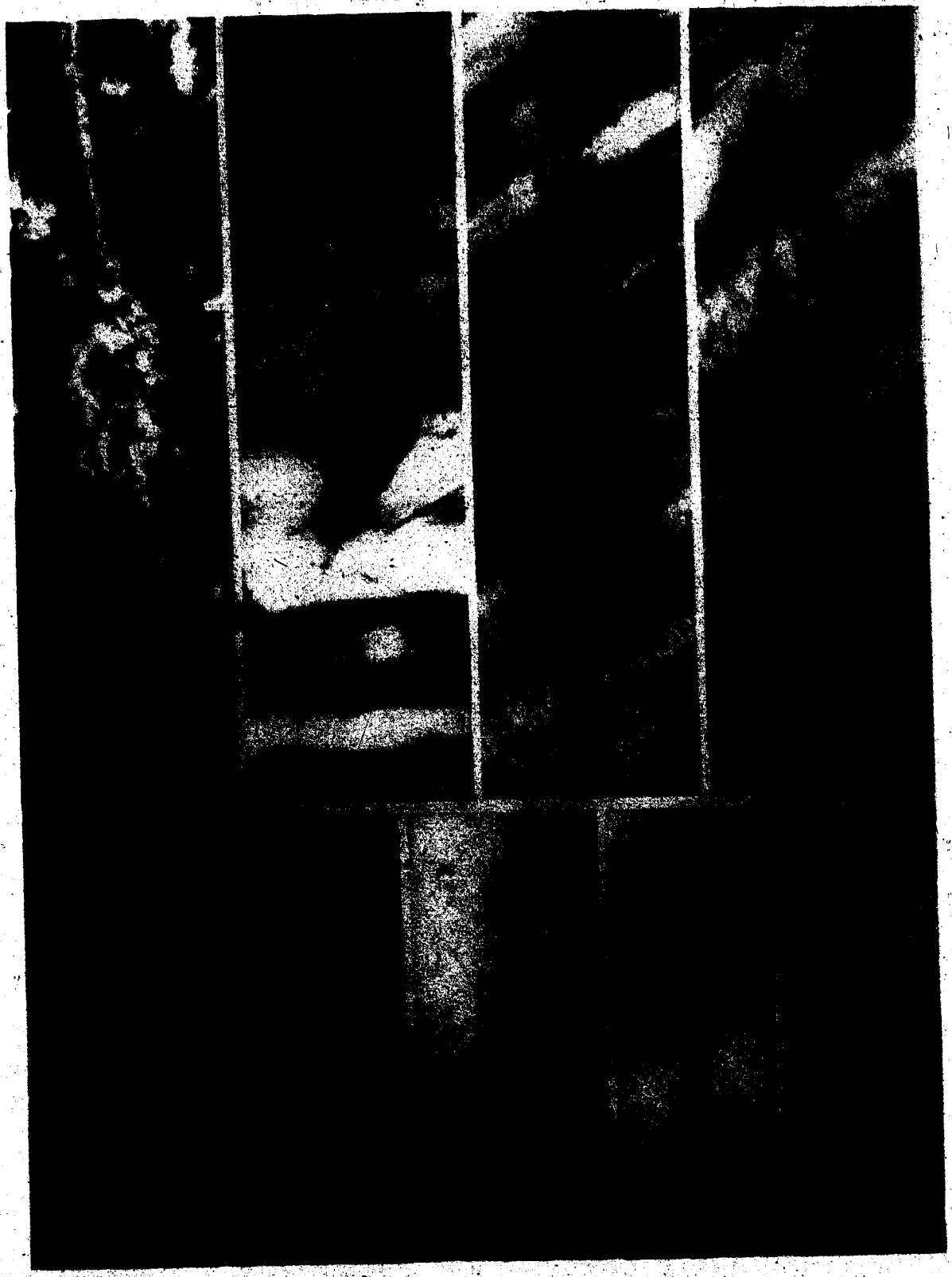


PLATE 7

Figure 18. Large bipolar endodermal neurons. (A: modified Hertwig maceration, oral-disc endoderm and ectoderm; B: Batham, mesentery; C and D: ZIO impregnation, mesentery). A: X 2,500; B, C, and D: X 2,000.

Figure 19. Small bipolar endodermal neurons. (Wood's alcian blue, mesenteries). X 2,000.



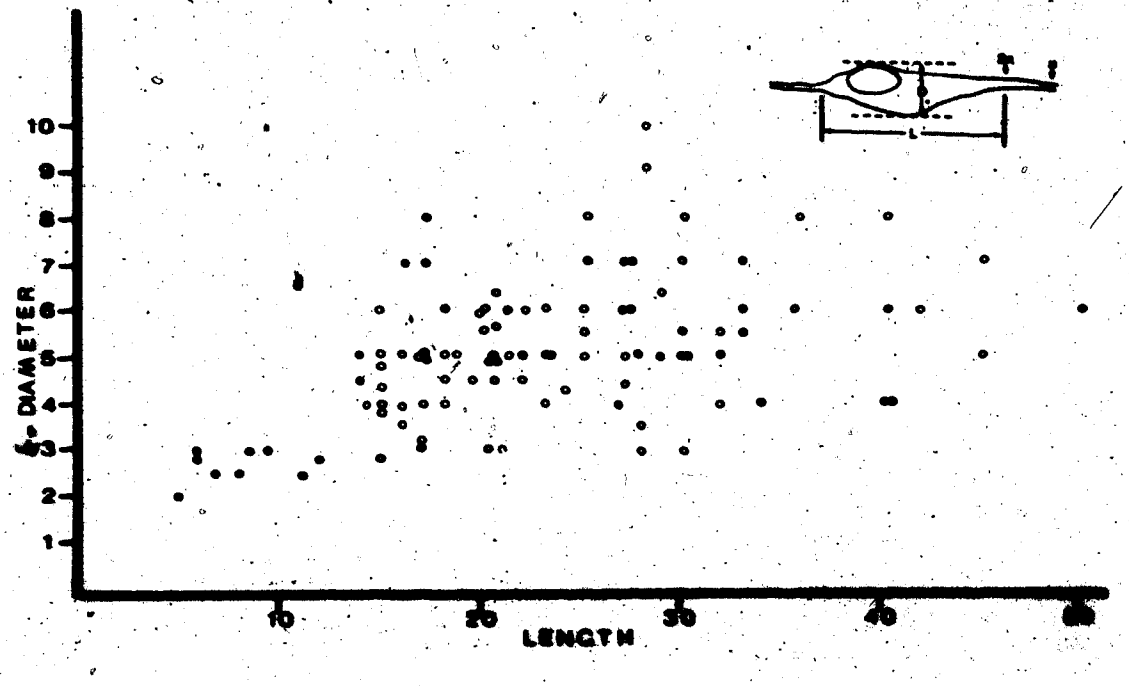


PLATE 8

Figure 20. A scattergram of endodermal bipolar neurons found on 1° mesenteries. Open circles represent 70 neurons found in ZIO-impregnated tissue, and the closed circles represent 30 neurons found in Wood's alcian-blue stained tissue. Note the cluster, around 3 x 10 μ, of Wood-stained neurons that lie outside the range of cells seen in ZIO-impregnated tissue; these represent the small bipolar neurons.

PLATE 9

Figure 21, A. A multipolar neuron bearing six fibres. (Batham, mesentery). X 1,000.

Figure 21, B,C,D,E,F. Tripolar endodermal neurons. Note the presence of silver-staining masses at the insertion of the nerve fibres to the cell in 21B, and of vesicles in 21D and 21E that are Wood-positive and ZIO-negative. (B: Batham, mesentery; C, D, and F: Wood's alcian blue, mesenteries; E: ZIO, mesentery). X 2,000.

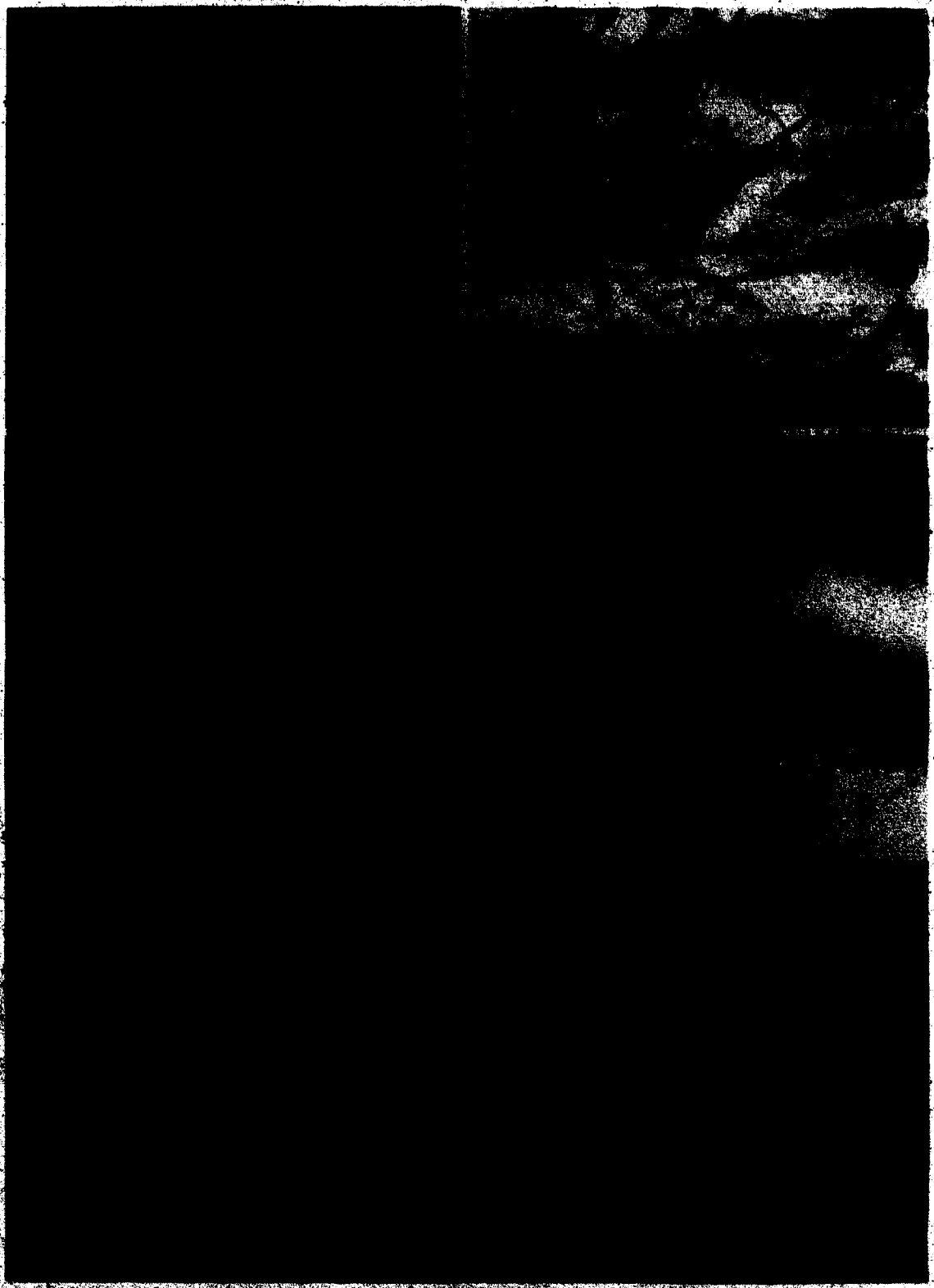


PLATE 10

Figure 22, A. A fibre of new-cell type 1. The fibres of this cell contain little but the small agranular vesicles and the large solid vesicles that characterize the cell. (Cavey, column). X 14,000.

Figure 22, B. A single vesicle printed at a higher magnification for direct comparison to plates of the vesicles characterizing the other nonnervous types of fibres (Figs. 23, B and C, 24, 25, 26). (Cavey, column). X 65,000.

Figure 23, A. A varicosity of the fibre of a new-cell type 2. Although relatively small it has caused a deformation of the overlying epithelium. (Peteya, mesentery). X 10,000.

Figure 23, B,C. The agranular vesicles, solid vesicles, and some other organelles of this type of fibre. (Peteya, mesentery). X 65,000.

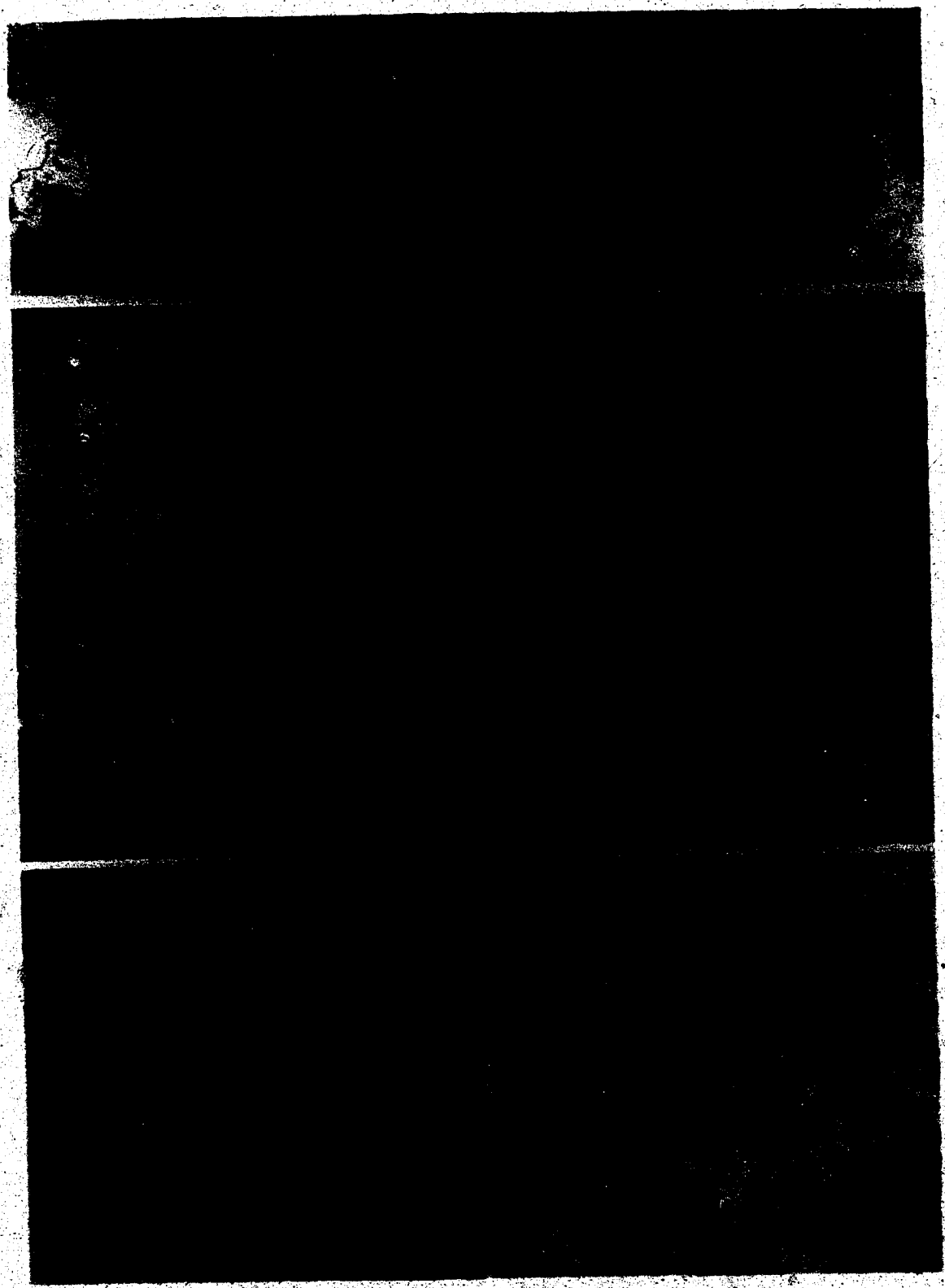


PLATE 11

Figure 24. Two varicosities of fibre type 3. They contain small dense-core vesicles, large solid vesicles, glycogen, and small membrane sacs. (Cavey, column). X 65,000.

Figure 25. A varicosity of fibre type 4, containing opaque vesicles and a small number of dense-core vesicles. (Cavey, column). X 65,000.

PLATE 12

Figure 26. A fibre type 5, containing an accumulation of the solid vesicles which characterize the type. Note the presence of microtubes; this is the only endodermal nonnervous fibre type which contains microtubes. (Cavey, pedal disc). X 65,000.

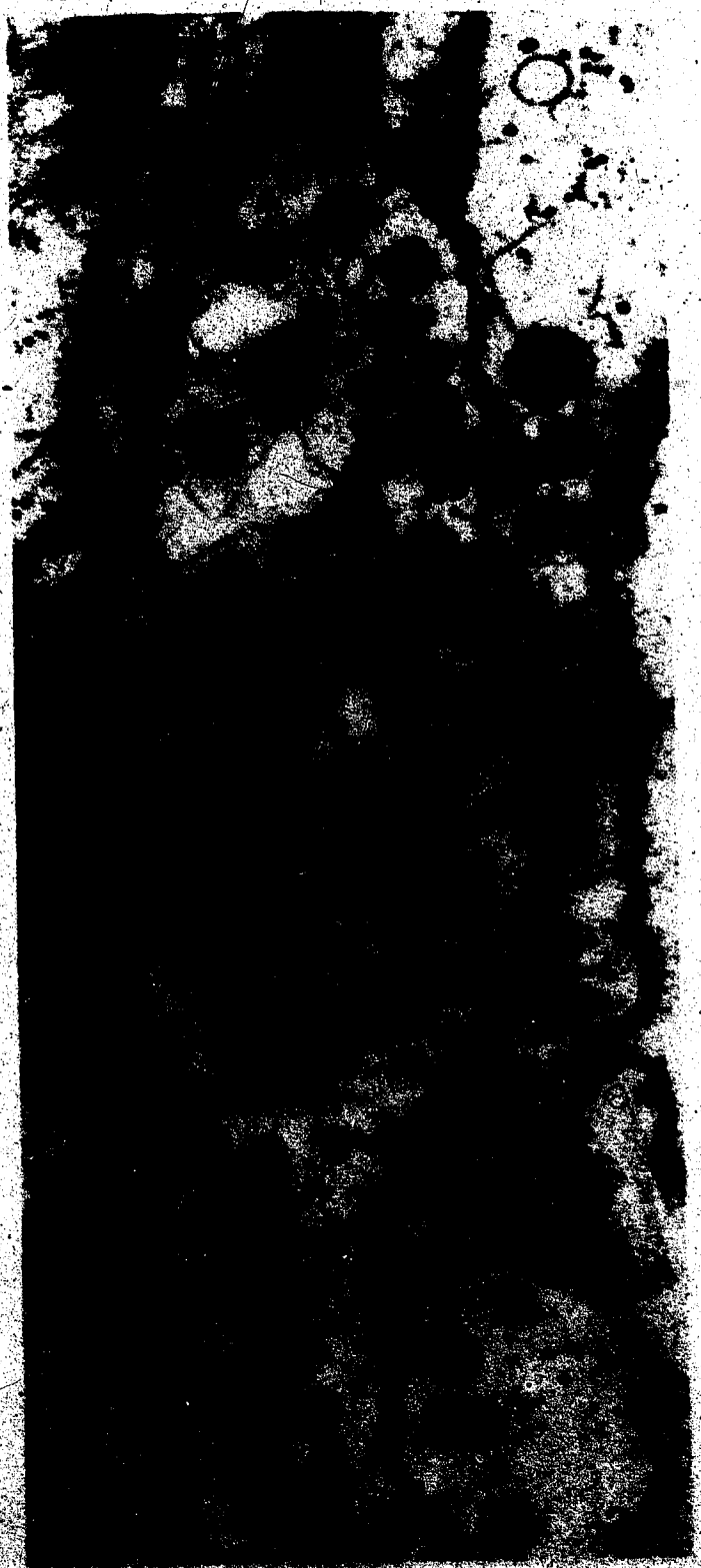


PLATE 13

Figure 27. A sensory-cell axon, identifiable by the large class of solid vesicles, synapsing on an unidentified type of fibre. The synaptic vesicles are agranular, irregularly spherical, and constitute a subclass of the agranular vesicles that are found throughout the axon and cell body. Note that the gap, which is 200 Å wide, is larger than in adjacent nonsynaptic areas. (Cavey, column). X 100,000.

Figures 28 and 29. Synapses of one of the neurons. In Fig. 28 the postsynaptic fibre is of an unidentified class; in Fig. 29 the two synapses are reciprocal between two fibres of the same class as the presynaptic fibre in Fig. 28. The synaptic vesicles are 600—900 Å, spherical, and agranular and may form large accumulations. The gap is 200 Å wide. In the synapses of Fig. 29 postsynaptic sacs of reticulum can be seen (arrows). (28: Cavey, column; 29: Cavey, mesentery). X 100,000.

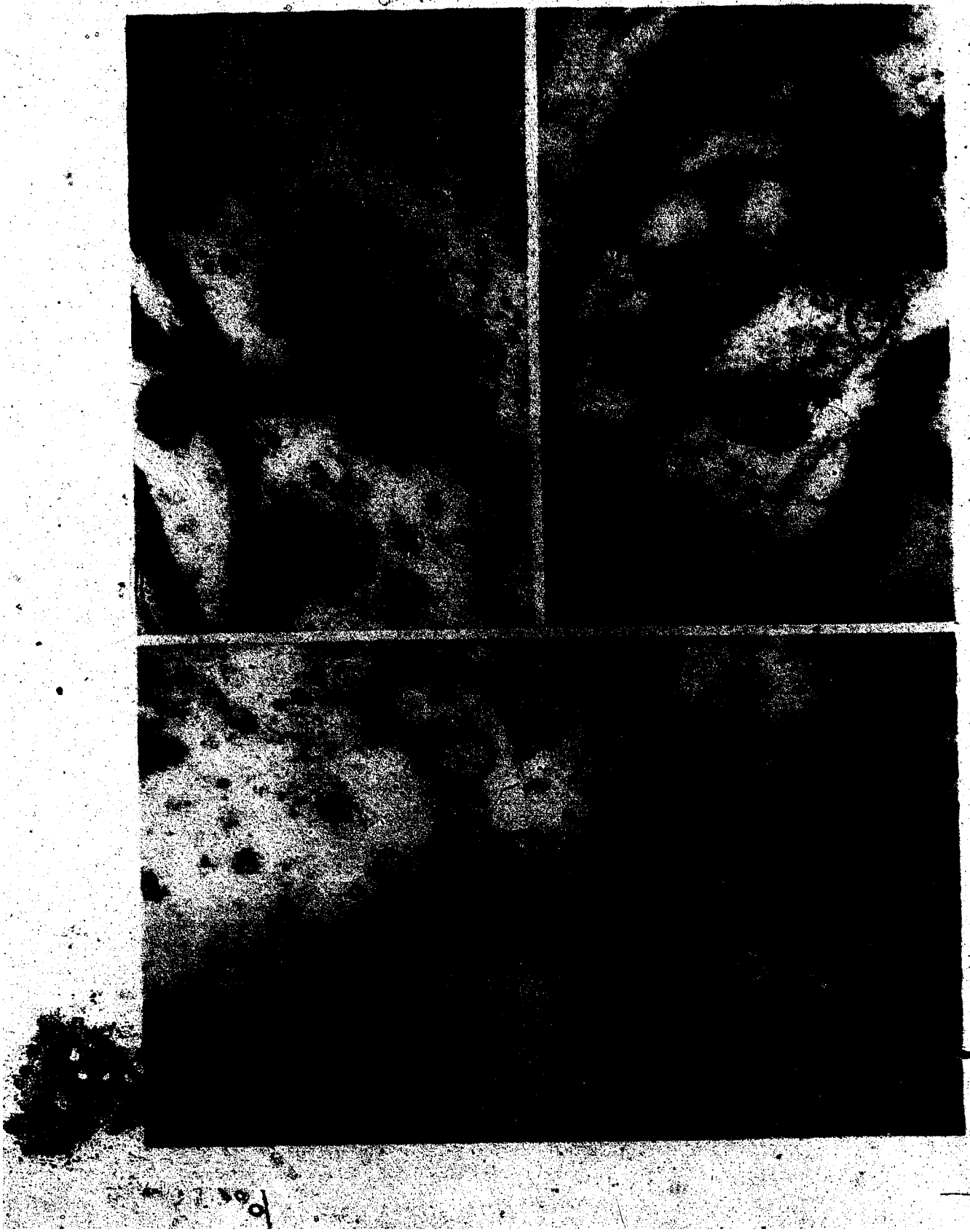


PLATE 14

Figure 30. A type A muscle fibre containing hollow thick fibrils and thin fibrils. The thin fibrils appear to be randomly distributed throughout the fibre, and show no apparent association with the thick fibrils. (Cavey, column). X 120,000.

Figure 31. A type B muscle fibre containing very large thick fibrils. In such fibres it is difficult to resolve the thin fibrils, but in some cases it can be seen that they form circles around the thick fibrils. (Cavey, column). X 120,000.

Figure 31, inset. A longitudinal section of a thick fibril of a type B fibre, showing its periodic banding. On this basis it is suggested that this may be a paramyosin fibril. (Cavey, pedal disc). X 120,000.

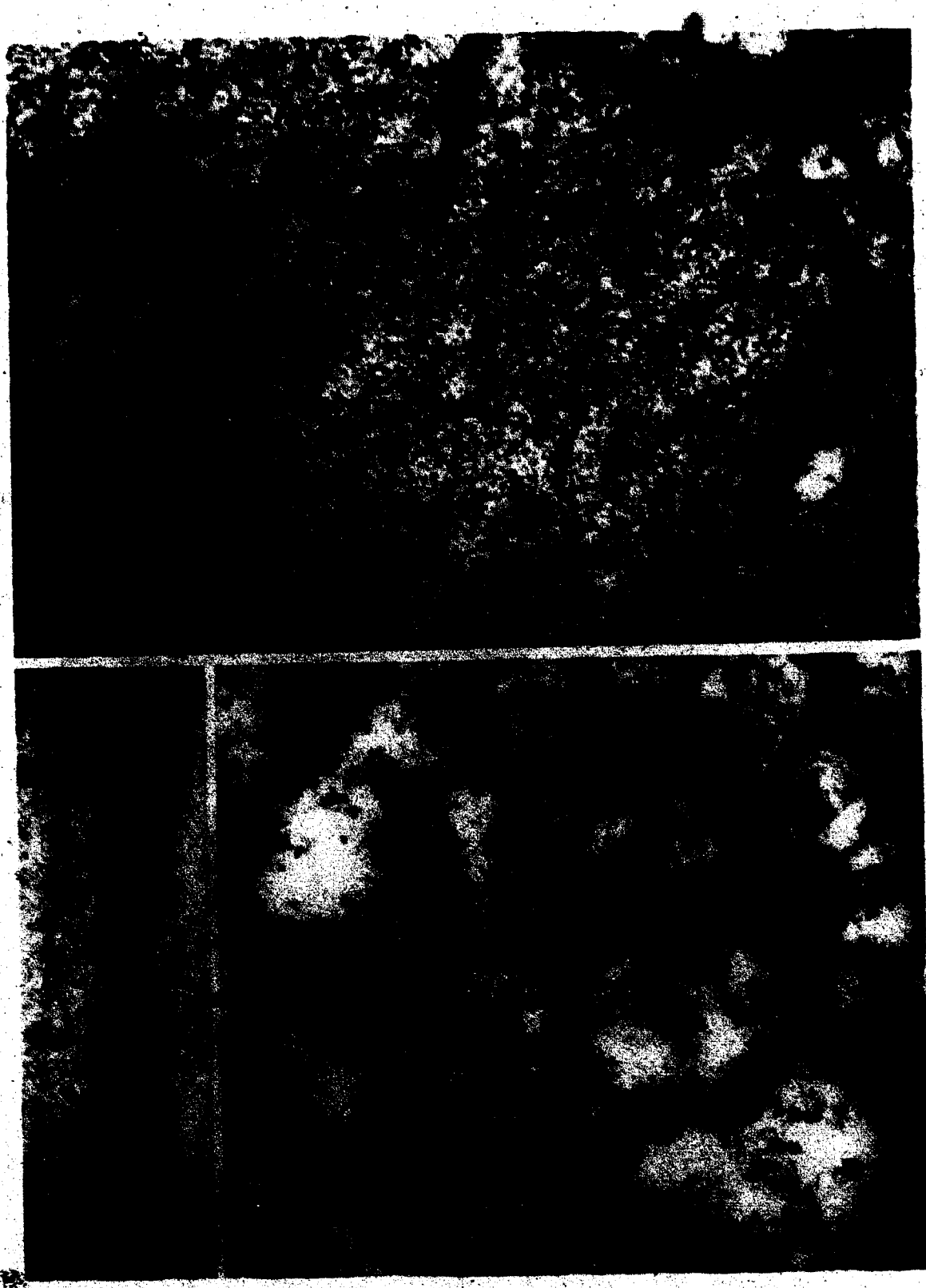


PLATE 15

Figures 32 and 33. Type B muscle fibres which appear to be intermediate between type A and the type B fibre seen in Fig. 31. In Fig. 32 the central area of the fibre contains the hollow thick fibrils which characterize a type A fibre, while the rest of the fibre contains small paramyosin fibrils. Note that the thin fibrils show an association with the paramyosin fibrils but not with the hollow fibrils. The fibre in Fig. 33 contain no hollow fibrils and the paramyosin fibrils are larger than in Fig. 32 but smaller than those in Fig. 31. Most of the thin fibrils are arranged in a circle around the paramyosin fibrils. (Cavey, column). X 120,000.

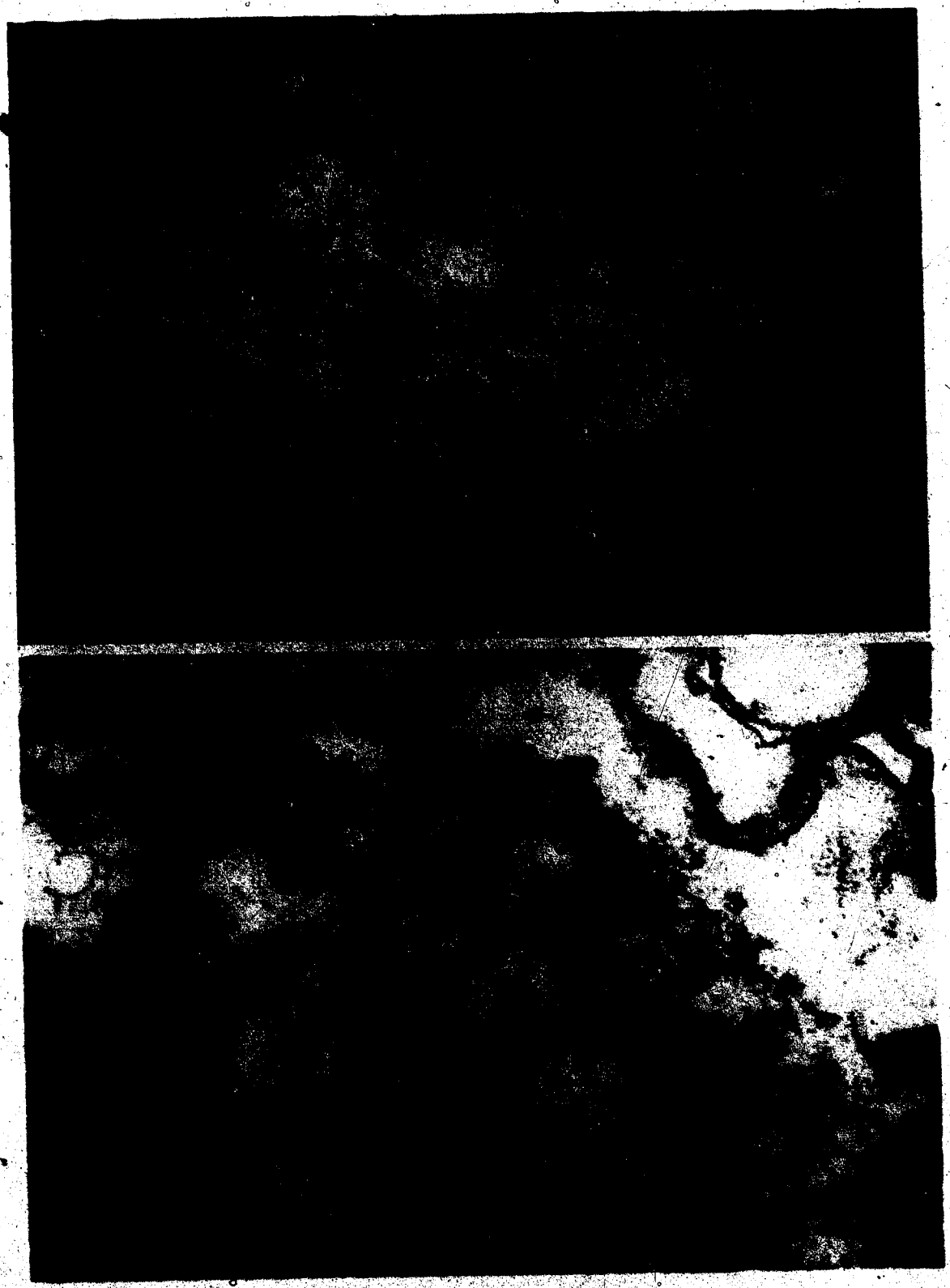


PLATE 16

Figures 34 and 35. Mesogleal amoebocytes of the form characteristic of the tentacles, oral disc and pharynx. Both are elongated and bear numerous processes of varying sizes. (Permanganate in sea water-$MgCl_2$: tentacles). X 15,000.

Figure 36. The three types of vesicles found in mesogleal amoebocytes: (A) 1500—3800 Å solid or dense-core vesicles, (B) 1300—2000 Å solid or dense-core vesicles, and (C) 650—800 Å agranular vesicles. (Cavey, column). X 85,000.

Figure 37. A typical mesogleal amoebocyte of the form characteristic of the mesenteries; it is elongated and bears no processes. (ZIO impregnation, mesentery). X 2,000.

PLATE 17

Figure 38. Small mesogleal cells.

Figure 38, A,B,C. Bipolar cells with an agranular cytoplasm and unbranching fibres. Cells like these are comparable to the mesogleal neurons described by Leghissa and are distinguishable from endodermal cells described here as neurons (compare with Fig. 19).

Figure 38, D,E,F,G,H. Bipolar cells with a granular or agranular cytoplasm and branching fibres. Note a third fibre which originates from the cell body in 38E, and a third which originates at one end of the cell body in 38H.

(All plates: ZIO impregnations, mesenteries). X 2,000.

PLATE 18

Figure 39. Small mesogleal cells.

Figure 39, A. A bipolar cell with branching fibres, and a third fibre which originates from the cell body.

Figure 39, B,C,D,E. Cells, with granular cytoplasm and branching fibres, which cannot be catalogued as either bi- or tri- or multipolar cells.

Figure 39, F,G,H. Tripolar SMC. Note in 39G two fibres of the cell 'wet' each other (arrow) in what has been described as a synaptic junction (Batham, et al., 1960).

(All plates: ZIO impregnations, mesenteries). X 2,000.

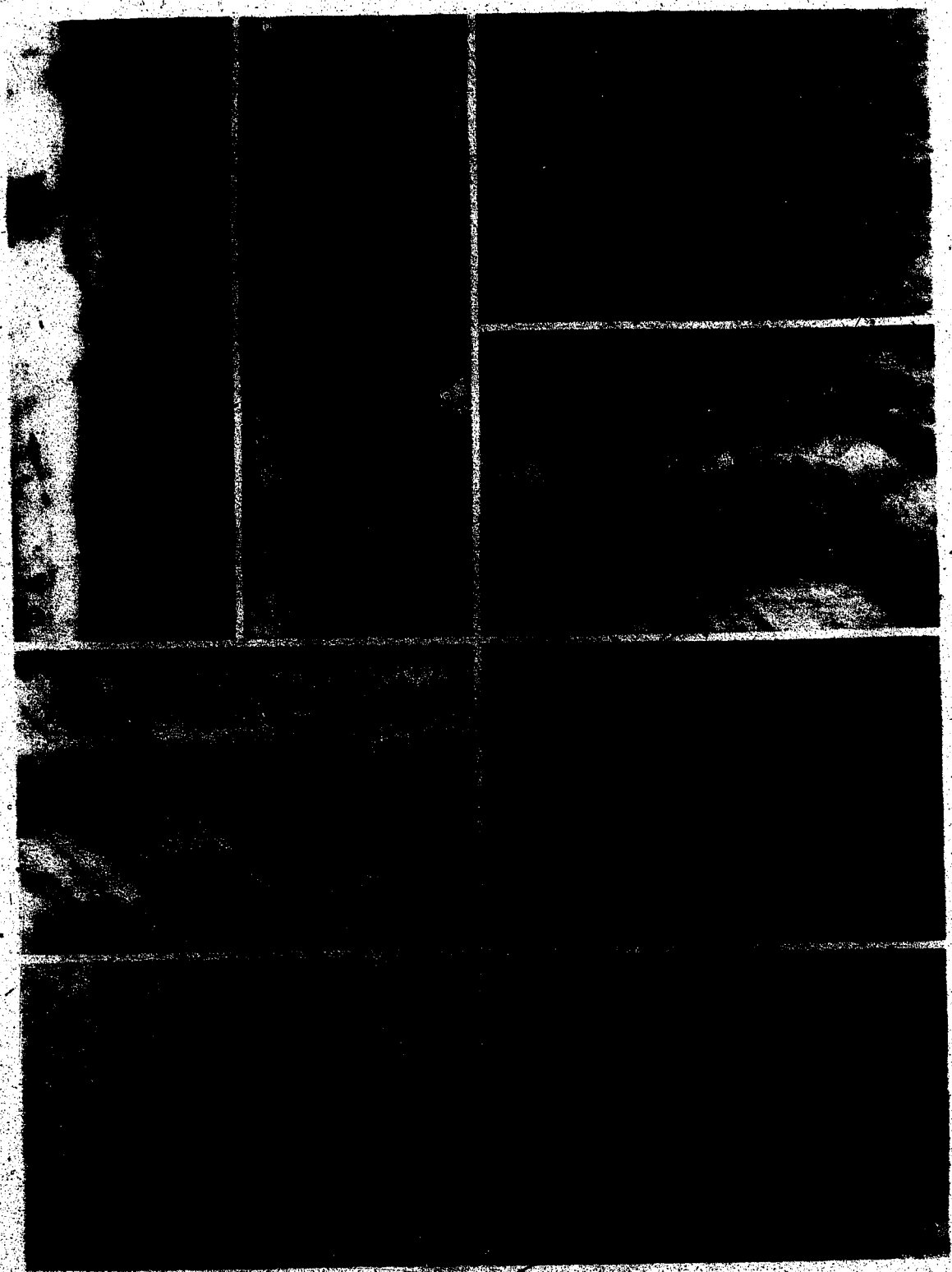


PLATE 19

Figure 40. Small mesogleal cells.

All plates are of multipolar cells with branching fibres.

(All plates: ZIO impregnations, mesenteries). X 2,000.

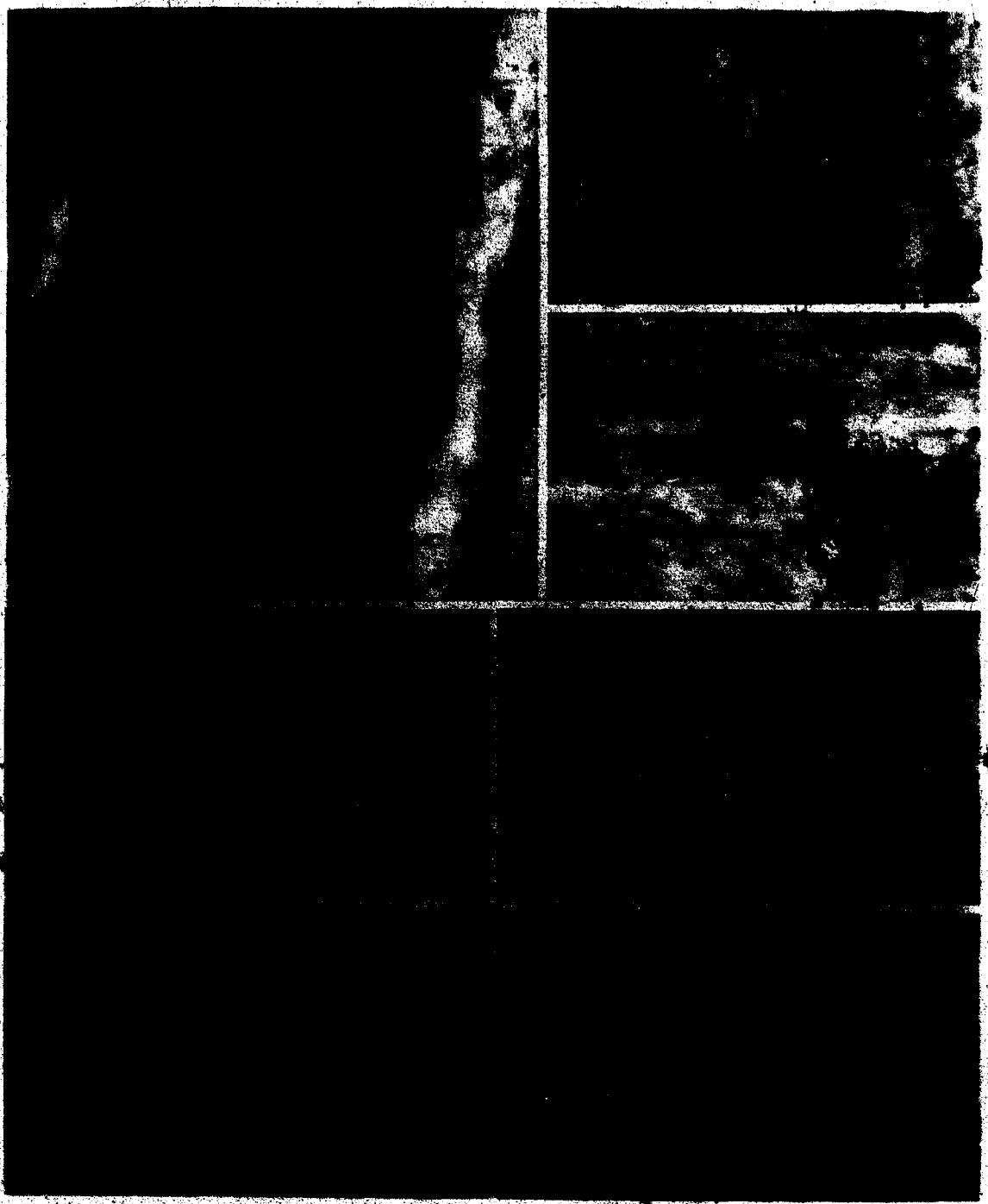
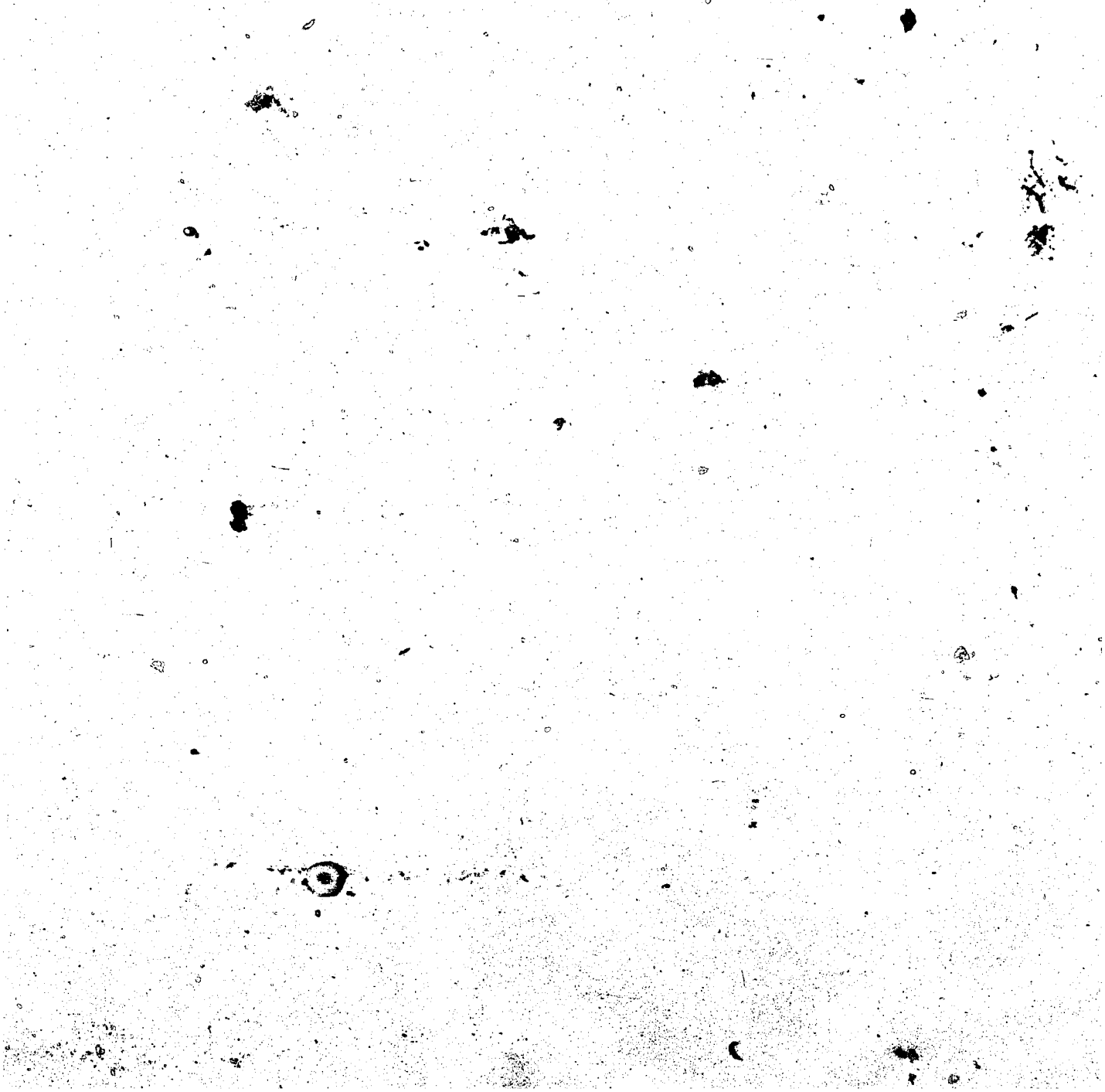


PLATE 20

Figures 41, 42 and 43. Electron micrographs of SMC. Regardless of their appearance in LM study all SMC observed in EM study contained the same class of solid vesicles seen here. (41: permanganate in sea water, tentacle; 42 and 43: Cavey, mesentery). 41: X 18,000. 42: X 18,000. 43: X 65,000.



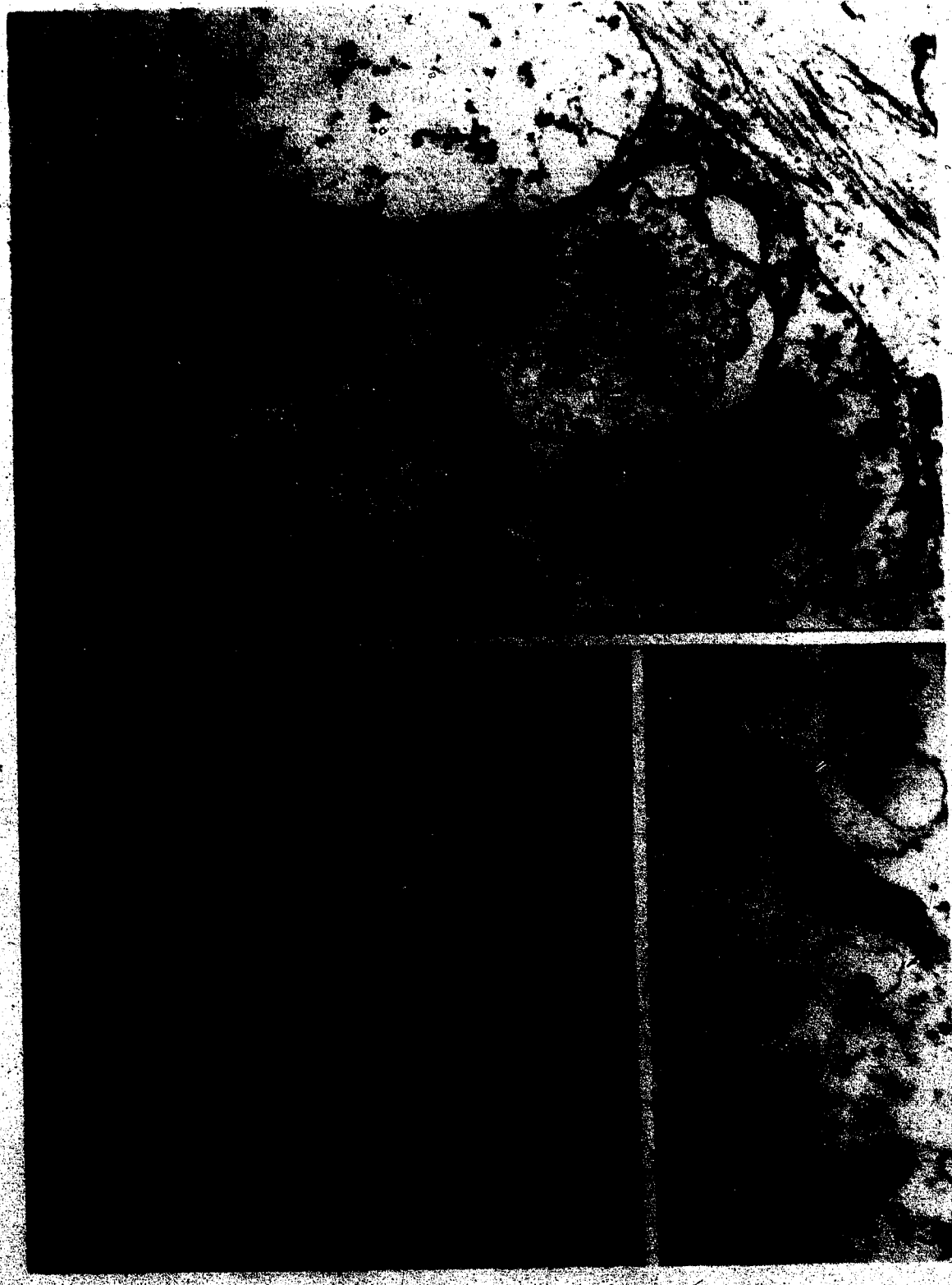


PLATE 21

Figure 44. A fibre of a SMC entering a tube of the sunken ectodermal muscles. Note that the cores of the vesicles of this and the other fibres of the SMC have fallen out leaving holes in the section, a phenomenon unique to a single experiment with metaraminol incubation. (Permanganate in sea water-$MgCl_2$, tentacle). X 25,000.

Figure 45. A fibre of a SMC showing a localization of endogenous catecholamines in its vesicles. (Hodd' chromaffin, tentacle). X 40,000.

PLATE 22

Figure 46, A. The only cell body observed of a mesogleal cell type whose fibres are found only in the tentacles, oral disc and pharynx. The cell body is 5 x 25 μ in size and contains small agranular vesicles and large solid vesicles. (Permanganate in sea water-$MgCl_2$, tentacle). X 10,000.

Figure 46, B. The spherical solid vesicles which characterize the fibres of this cell type. Note also two elongated vesicles that have a heterogenous matrix (*); this form of vesicle was found in the cell body near the origin of a fibre but has never been seen in the fibres. (Permanganate in sea water-$MgCl_2$, tentacle). X 90,000.

Figure 47, A fibre of a type observed only once. Its vesicle population consists of 500—1300 Å opaque vesicles and possibly some small solid and large agranular vesicles. (Permanganate in sea water-$MgCl_2$, oral disc). 47A: X 25,000. 47B: X 70,000.

Figure 48. An ectodermal neuromuscular synapse photographed from the same section as was Fig. 47, for comparison to Fig. 47B. (Permanganate in sea water-$MgCl_2$, oral disc). X 70,000.

PLATE 23

Figure 49. Cross section of the column showing the reticulum (R) as a network of interconnected fibres among which only one morphological type can be distinguished. (Formalin-acetic-alcohol, body wall; 5-μ wax section). (*Cerianthéopsis*). X 1,700.

Figure 50. In sections less than 2.5 μ in thickness, two types of fibre can be distinguished in the reticulum. One (large arrows) runs radially from the supporting-cell cell bodies in the epithelium (E) to the mesoglea (M) while the second (small arrows) lies irregularly in the plane of the reticulum. (Susa, marginal tentacle). (*Cerianthéopsis*). X 1,700.

Figure 51. In EM study both types of fibre seen by LM are seen as aggregates of a single type of fibre (arrows). (Batham, marginal tentacle). (*Cerianthéopsis*). X 20,000.

Figure 52. Part of the fibre plexus, showing the size of the fibres and of the extracellular space in tissue fixed in osmium. Note the bundle of filaments within the supporting-cell peduncle (arrow). (2% OsO_4 in 0.13M Millonig, tentacle). (*Cerianthéopsis*). X 13,000.

Figure 53. Part of the plexus, showing fibres swollen to the point of near exclusion of the extracellular space. This is typical of aldehyde fixation of cerianthids. (Peteya, marginal tentacle). (*Cerianthéopsis*). X 8,000.

Figure 54. A nerve soma that was exploded by osmotic damage. (4% glutaraldehyde in sea water, oral disc). (*Cerianthéopsis*). X 12,000.

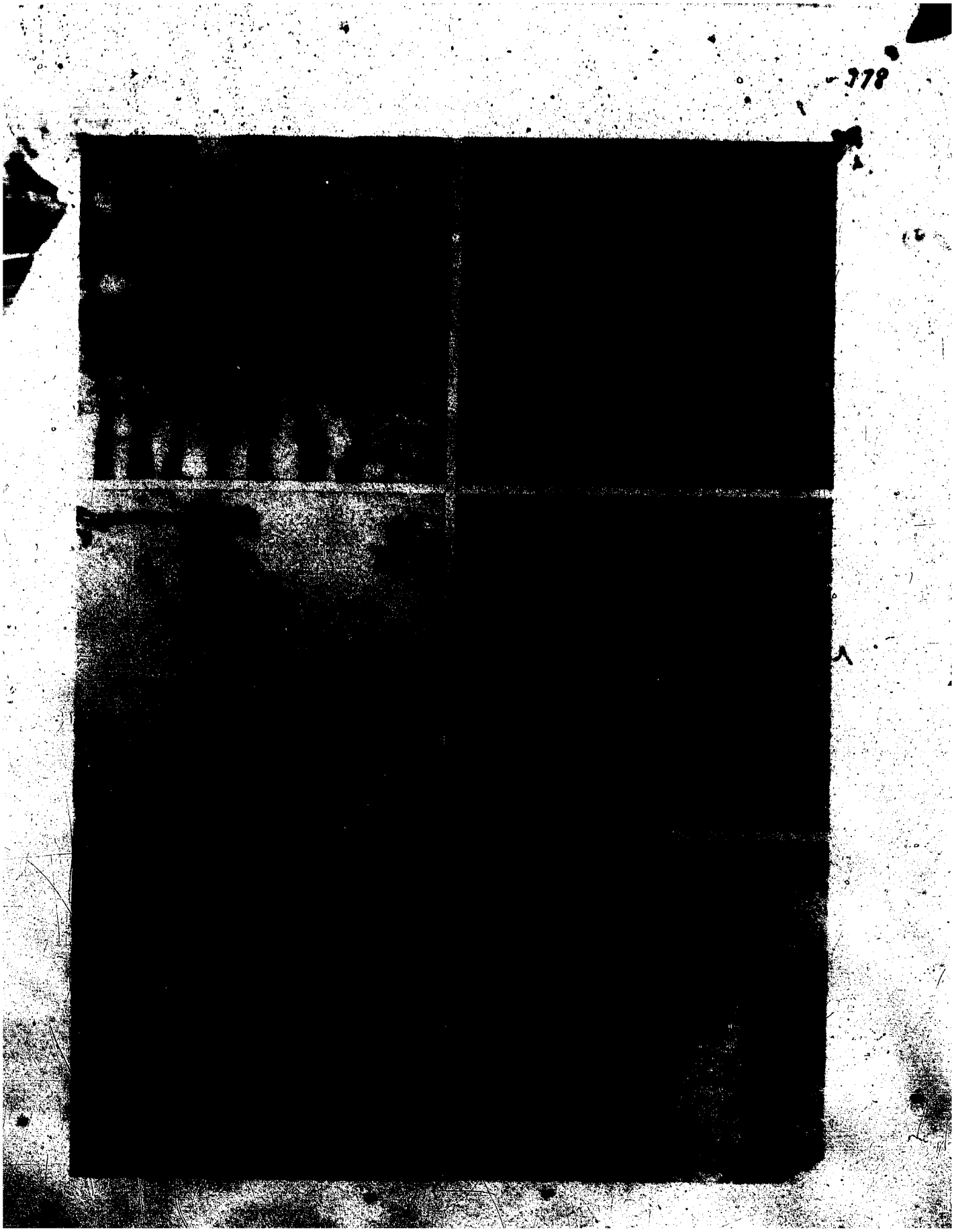


PLATE 24

Figure 55. A light micrograph of tissue fixed in osmium, illustrating the very small size of the fibres of the plexus. (2% OsO_4 in 0.13M Millonig, marginal tentacle). (*Cerianthéopsis*). X 1,700.

Figure 56. A plexus in which there is a limited swelling of both the fibres (small arrows) and of the peduncles (large arrows). (3.5% formic acid with 0.1% OsO_4, marginal tentacle). (*Pachycerianthus*). X 1,700.

Figure 57. Light micrograph of tissue in which there is severe swelling and some rupturing of the fibres (small arrows) but little distortion of the peduncles (large arrow). (Stevie, tentacle). (*Stomphia*). X 1,700.

Figure 58. Light micrograph of tissue that shows some characteristics of a reticulum. The peduncles (arrows) are highly distorted, and some are aggregated or broken from the mesoglea. Further, it is not possible to recognize the fibre plexus in this tissue. (Bouin 1898, tentacle). (*Stomphia*). X 1,700.

Figure 59. Electron micrograph of the plexus from tissue fixed in formalin. On a LM level this tissue looks like that in Fig. 58, and it is not possible to recognize the fibres although the 'cytoplasmic ribbons' of the muscle fibres are easily discerned. N, fibres; *, ribbons of muscle fibres. (10% commercial formalin in sea water adjusted to pH 7.3, tentacle). (*Cerianthéopsis*). X 13,000.

Figure 60. Three plexus fibres (1,2,3) from the tissue seen in Fig. 59 showing unusually good preservation of their microtubes and membranes (arrows). The membranes between fibres 1 and 2 are almost intact, while those between 2 and 3 are more typical. X 100,000.

Figure 61. Part of a supporting-cell peduncle (P) and of a ribbon of a muscle fibre (R) from the tissue seen in Fig. 59, showing that while the membranes (arrows) of these cells are also damaged they are as well preserved as the best seen in the fibres. X 100,000.

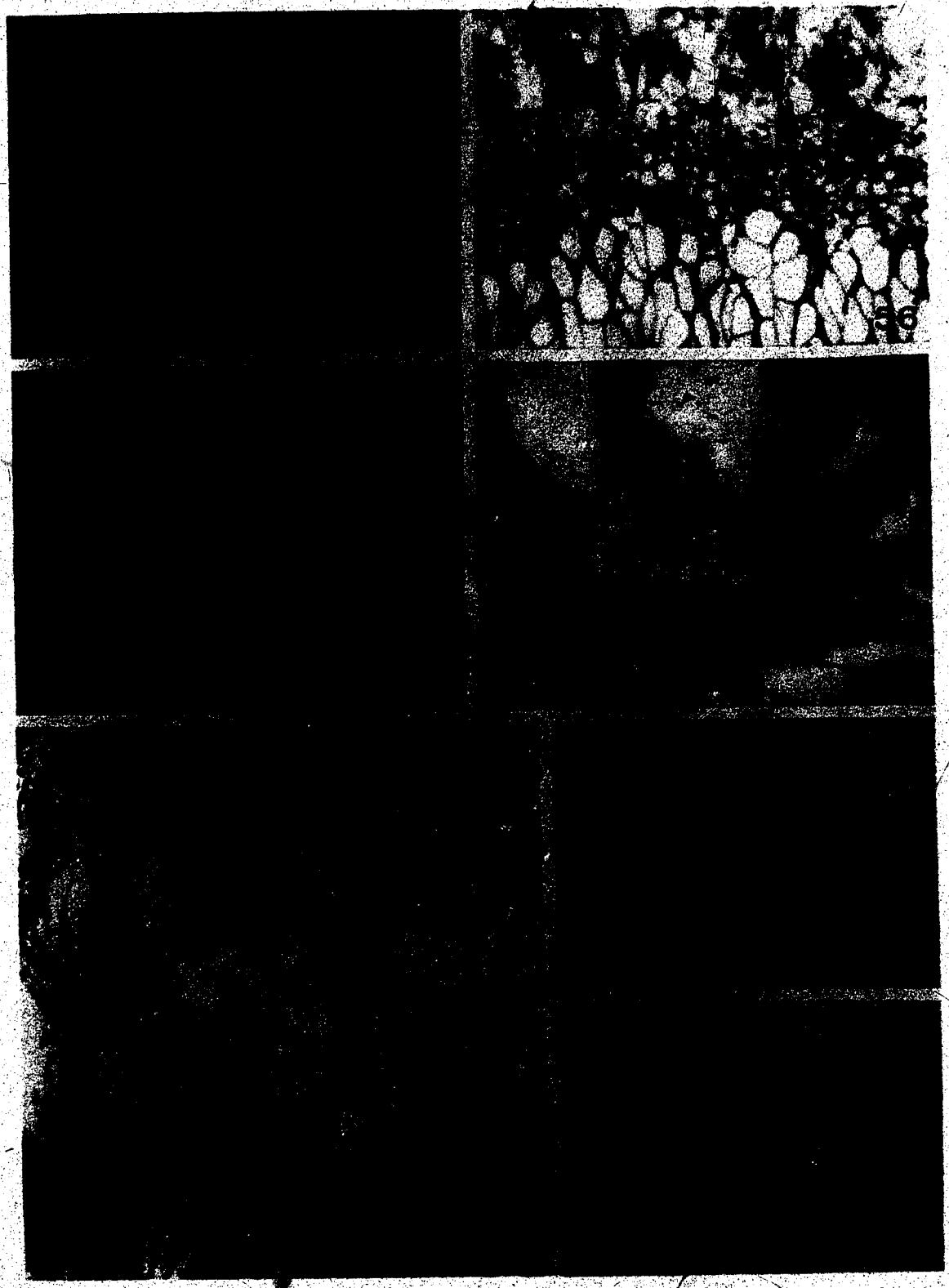


PLATE 25

Figure 62. The ectoderm at the base of a primary tentacle. The epithelium is composed mainly of supporting cells, gland cells, receptors and cnidoblasts. The fibre plexus is well developed and contains four types of nerve fibres which constitute about 50% of all fibre profiles seen. The muscle layer is formed into tubes which are sunken into the mesoglea. (Cavey, tentacle). X 2,200 (approx.).

Figure 63. The fibre plexus of the same section as Fig. 62. Note the supporting-cell peduncles (arrows). For comparisons Figs. 63, 65, 67, and 69 are at the same magnification. (Cavey, tentacle). X 10,000.

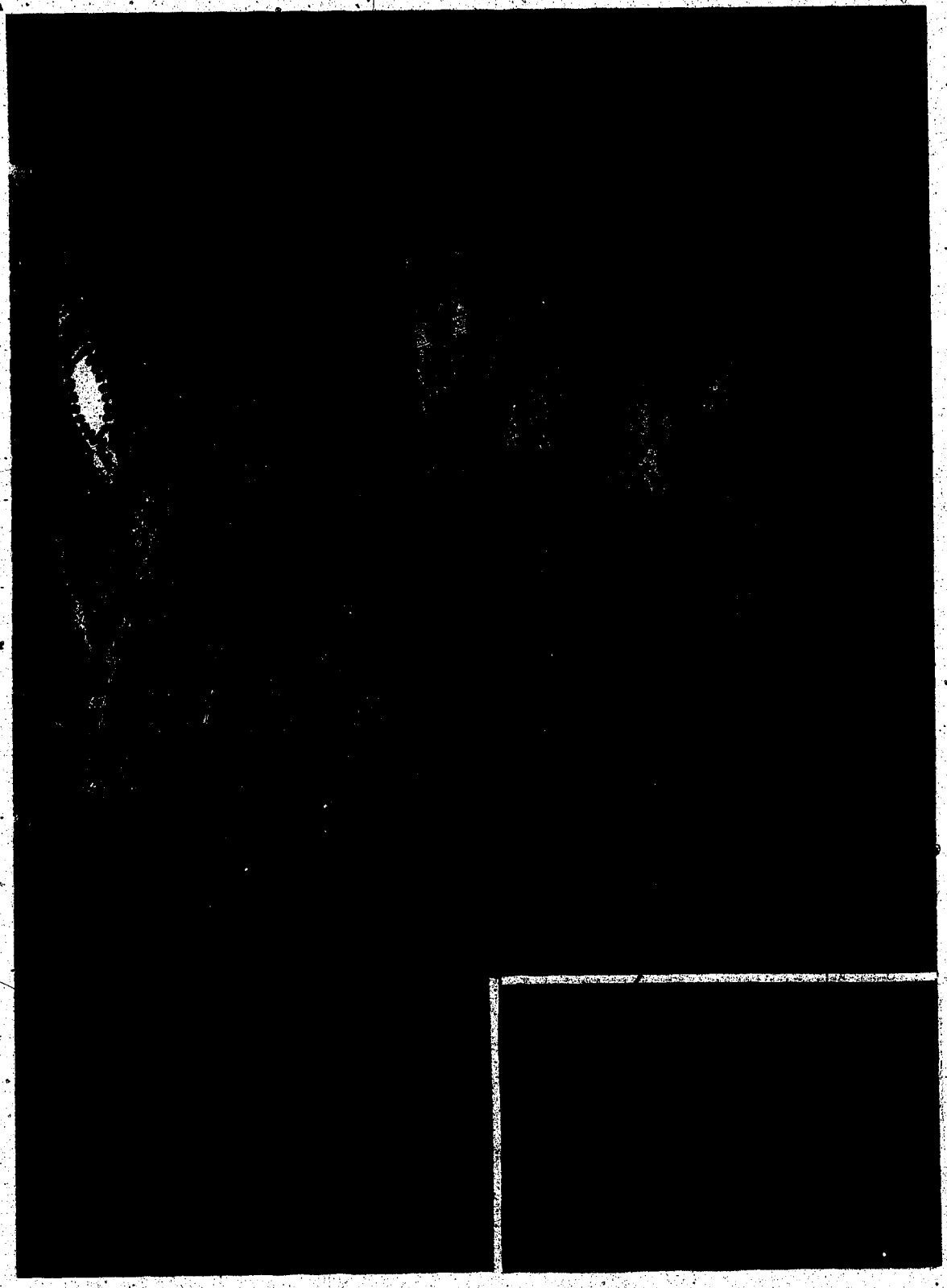


PLATE 26

Figure 64. The column ectoderm in the area overlying the sphincter. The effective stimulus which elicits swimming in *Stomphia didemon* when touched by a *S. coccinea* is localized in this epithelium. The epithelium is composed primarily of spirocysts (not recorded in the cnidome of this species) and basitrics, three types of gland cells, and supporting cells. Note the presence of amoebocytes (arrows) which have invaded the epithelium. (Cavey, column overlying the sphincter). X 2,000 (approx.)

Figure 65. The fibre plexus from the same section as Fig. 64. It appears to be a continuation of the plexus of the tentacles in that both contain many of the same elements. However, only one nerve fibre, type 2, is found in both the tentacle and column. Note the supporting cell peduncle (arrow). (Cavey, column overlying the sphincter). X 10,000.

[redacted]

PLATE 27

Figure 66. The ectoderm of the column below the level of the sphincter. The epithelium is predominated by supporting cells and gland cells. (Cavey, column below the sphincter). X 6,000.

Figure 67. The base of the epithelium seen in Fig. 66. There is no fibre plexus but isolated bundles of one to six fibre profiles can be seen at intervals of up to 50 μ along the basement membrane. (Cavey, column below the sphincter). X 10,000.

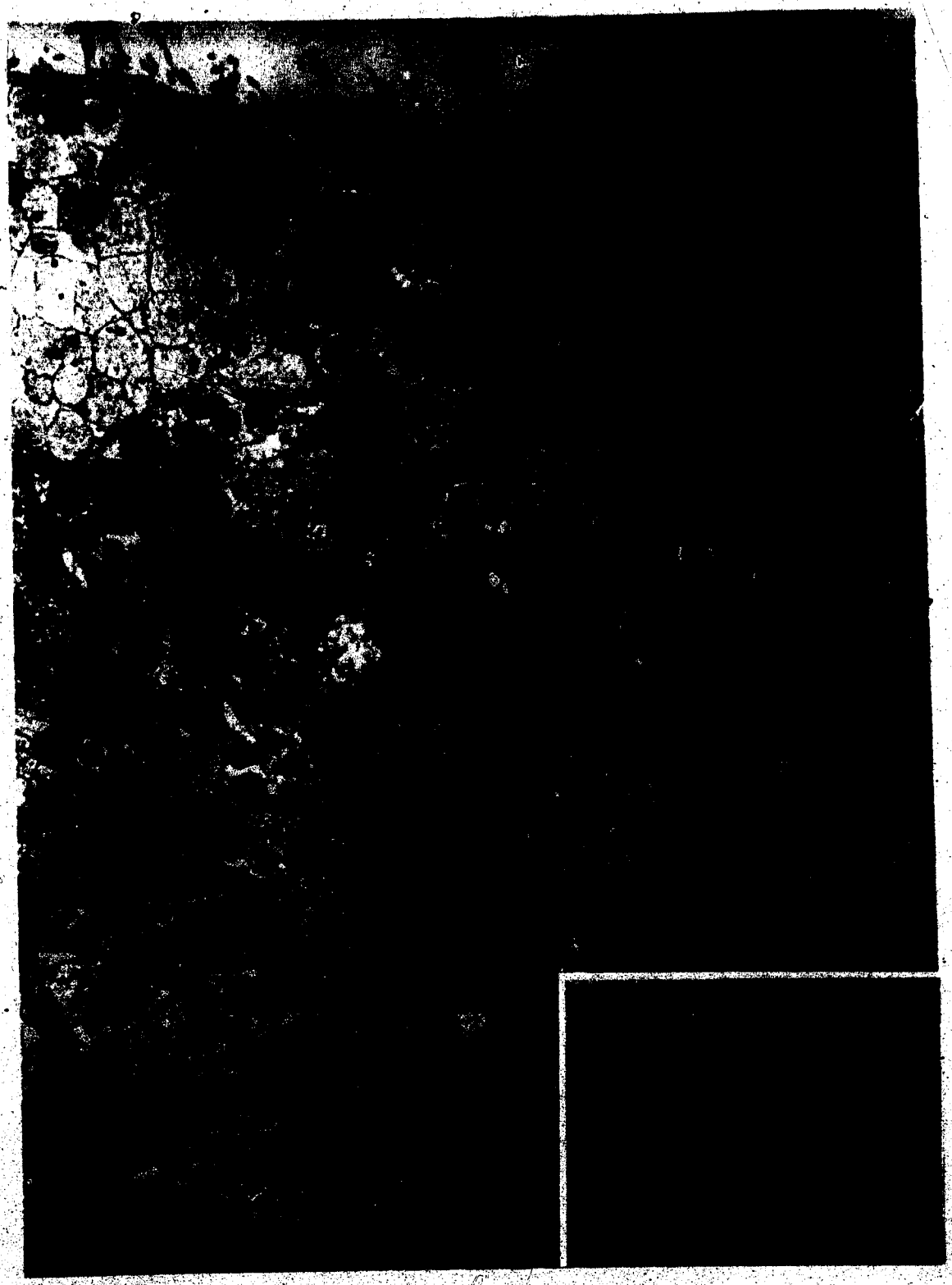


PLATE 28

Figure 68. The ectoderm of the pedal disc is predominated by gland cells, supporting cells, and solitary receptors. (Cavey, pedal disc). X 6,000.

Figure 69. The fibre plexus of the pedal disc is small and discontinuous, but may be five fibres in thickness and contains at least four types of fibres. At this time nerve fibres have not been identified in this plexus. (Cavey, pedal disc). X 10,000.

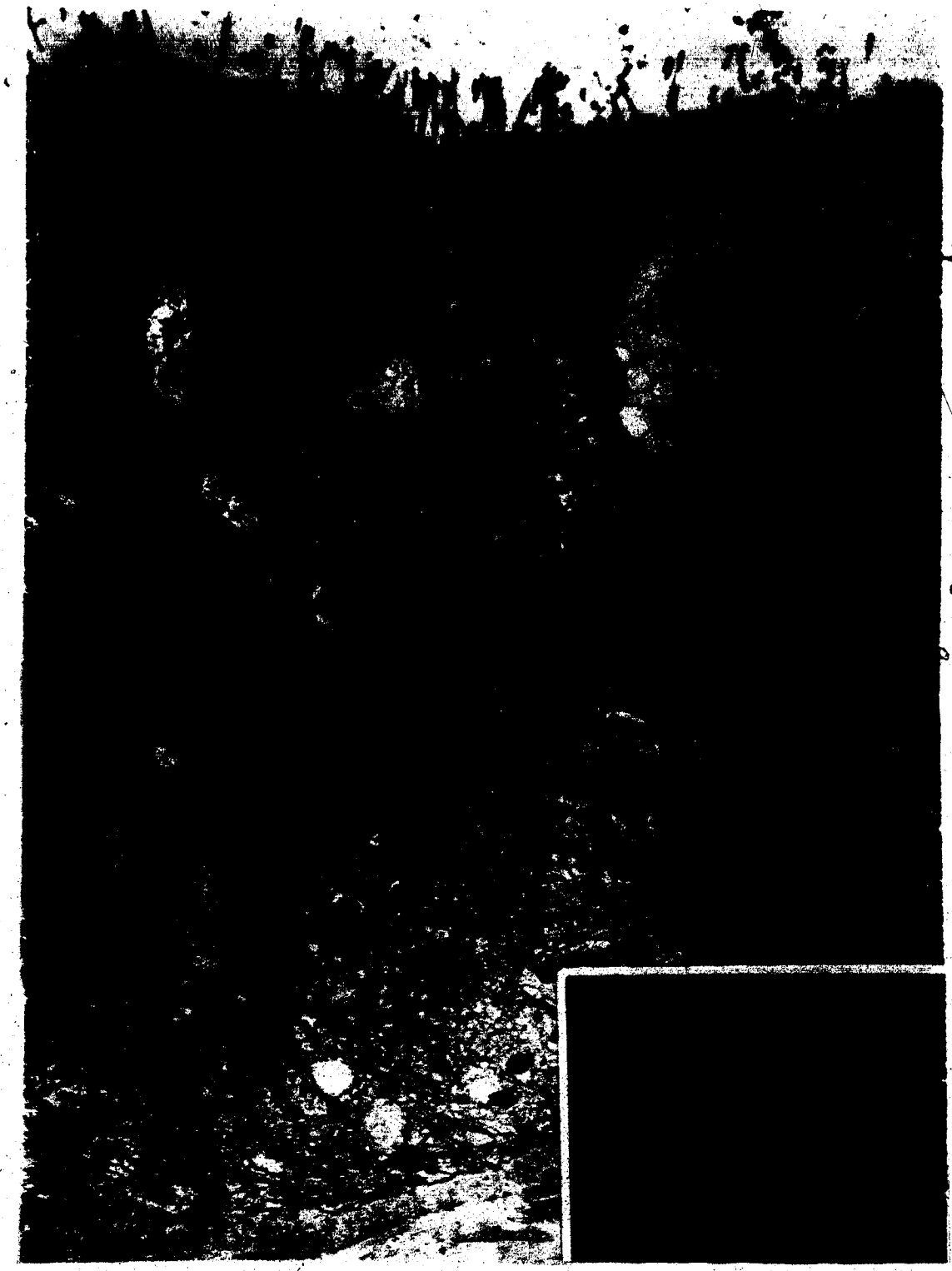


PLATE 29

Figures 70 and 71. Longitudinal sections through the cone of the ciliary-cone receptor, illustrating its conical shape and the axial location of the central cell (*) and its sensory apparatus. The cone consists of the cilium (C) and stereocilia (large arrows) of the central cell, surrounded by the stereocilia (small arrows) of the peripheral cells. (Westfall, tentacle). X 10,000.

Figure 72. A longitudinal section of a peripheral (PC) and a central (CC) cell. The central cell has a narrow dendrite that contains numerous microtubes, and whose surface is covered by its sensory apparatus. The peripheral cell is characterized by the aggregate of large dense-core vesicles at the surface of its expanded dendrite. (Cavey, tentacle). X 33,000.

Figure 73. A cross section of the tentacle ectoderm in a juvenile, showing the relation of two peripheral cells to the rest of the ectoderm. At this age these cells are proportionally longer than in an adult and extend nearly to the base of the epithelium. Thus axons (A) can often be traced to the plexus (P), although in only one case into the plexus (see Fig. 77). Note the greatly expanded dendritic surface of the one receptor (between the arrows). (MT, muscle tube; M, mesoglea. (Westfall, tentacle). X 5,000.

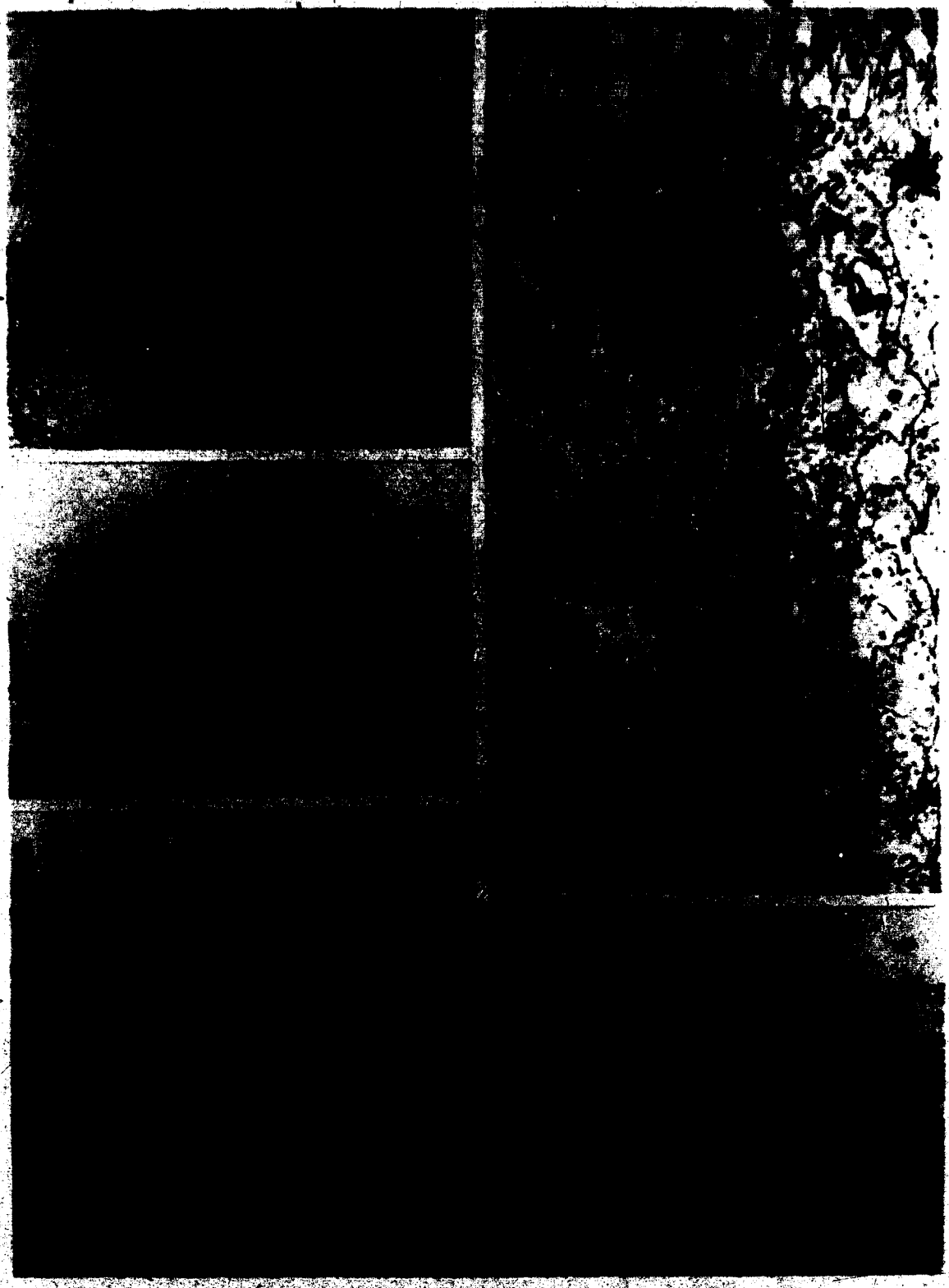


PLATE 30

Figure 74. A tangential section of the tentacle surface passing through the dendrites of two receptors. The central-cell dendrite (CC) contains only a ciliary rootlet surrounded by five bundles of fibres extending from the stereocilia. The dendrite of the peripheral cell contains large vesicles (large arrows) and a disperse web of fibres (small arrows) which are characteristic of this cell. The cilium (C) is a rare occurrence in a peripheral cell. (Westfall, tentacle). X 21,000.

Figure 75. A tangential section through the middle of the cone showing its symmetrical organization. The stereocilia of the peripheral cells (small arrows) surround a circle of central-cell stereocilia (large arrows) at the center of which is the central-cell cilium. (Westfall, tentacle). X 28,000.

Figure 76. The cilium and stereocilia (large arrows) of the central cell, and the stereocilia (small arrows) of a peripheral cell. The fibres of both types of stereocilia are of the same size, but differ in electron density. Note the numerous cross bridges between adjacent stereocilia. In most aldehyde fixatives these bridges are not preserved and in such cases the stereocilia may collapse to the tissue surface. (Westfall, tentacle). X 53,000.

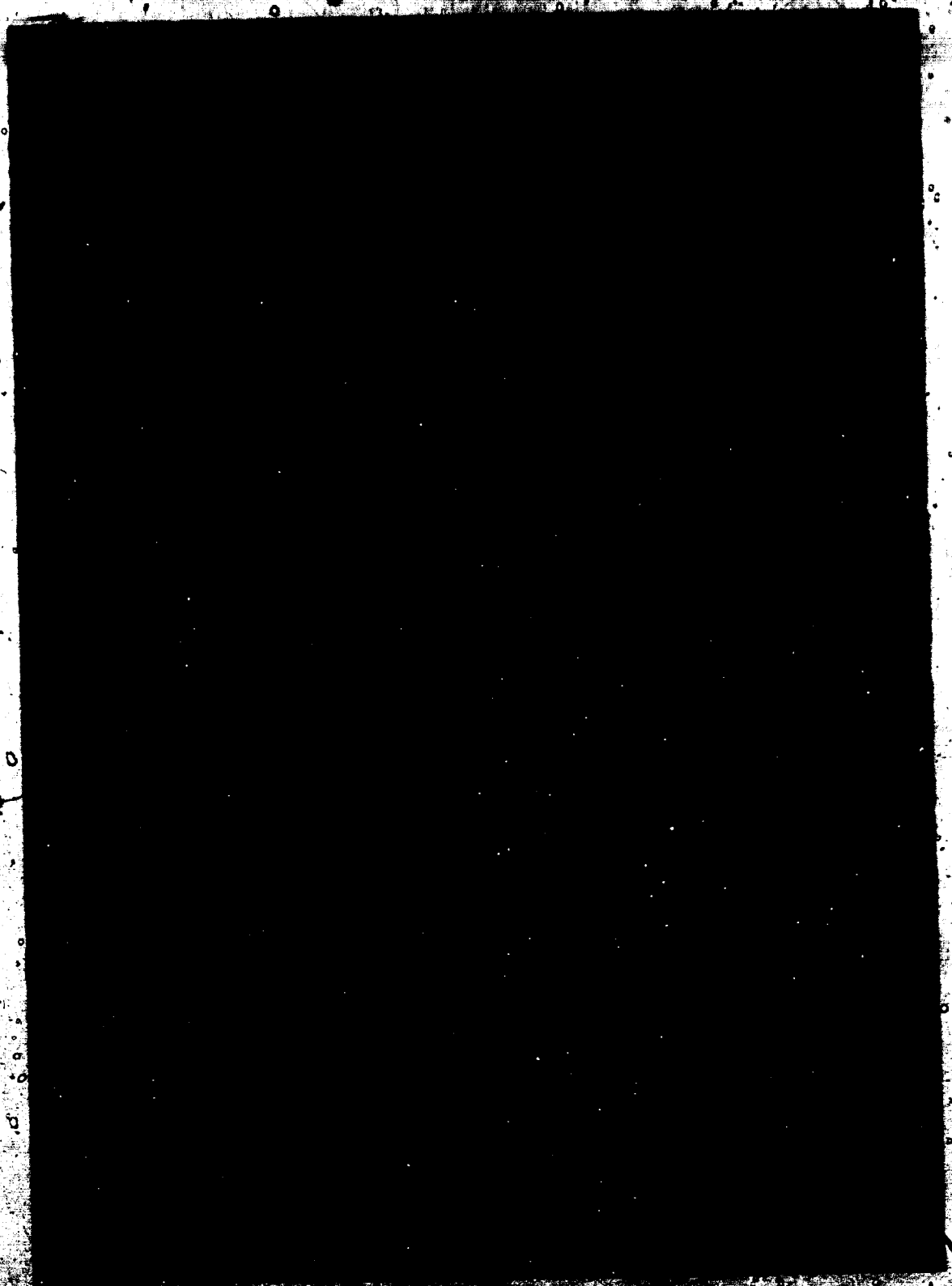


PLATE 31

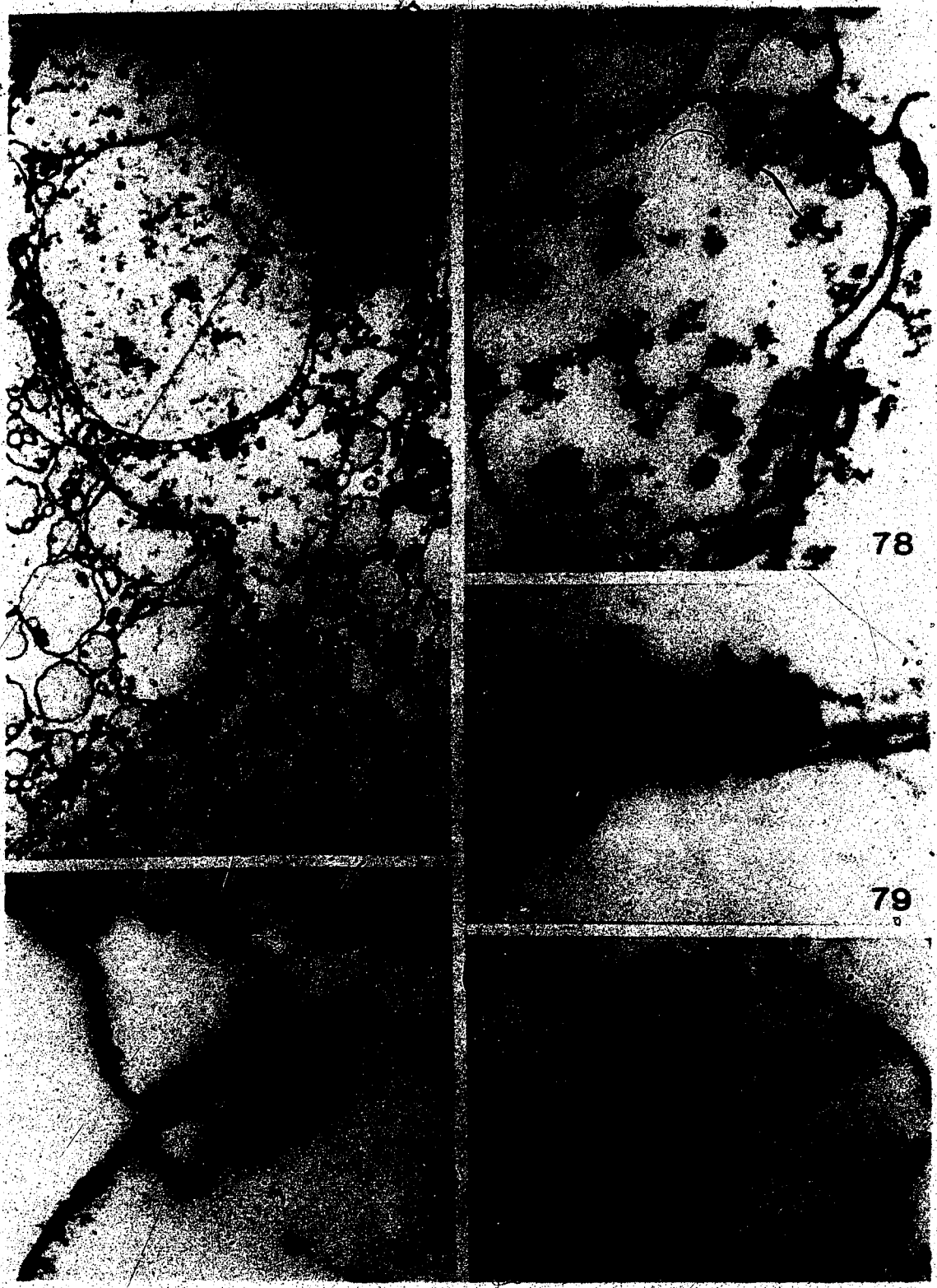
Figure 77. A cell body, lying in the lower epithelium, which bears a type 1 nerve fibre. This is seen as one line of evidence that nerve-fibre type 1 is the axon of the ciliary-cone receptor. Note the numerous vesicles in the extracellular space and within the nuclear membrane; their translocation from the cytoplasm is interpreted as an artifact caused by prolonged Mg^{++} narcotization. (Permanganate in sea water-$MgCl_2$, tentacle). X 80,000.

Figure 78. The axon and synapse of a type 1 nerve fibre. At the base of the tentacle this type of synapse has a frequency of about 50/1000 μ^2 of section area in the plexus. (Permanganate in sea water-$MgCl_2$, tentacle). X 80,000.

Figure 79. A synapse of type 1 in which dense cores have been preserved in the synaptic vesicles by permanganate fixation. (Permanganate in sea water-$MgCl_2$, tentacle). X 100,000.

Figure 80. A synapse of a type 1 fibre from tissue that was incubated in metaraminol prior to fixation. Note the decreased electron density in three synaptic vesicles (compared to those in Fig. 79) and the loss of the cores, through dissolution during sectioning, of another synaptic vesicle (arrow) and of the large dense-core vesicle (double arrow). (Permanganate in sea water-$MgCl_2$, tentacle). X 120,000.

Figure 81. A synapse of a type 1 fibre in osmium-fixed tissue. Note the intermediate band in the synaptic gap. (Westfall, oral disc). X 100,000



78

79

PLATE 32

Figure 82. A type 2 nerve fibre illustrating the three classes of vesicles which characterize the fibre. They are the synaptic vesicles (small arrows) which are often irregular in shape in aldehyde-fixed tissue, and small (large arrows) and large (*) dense-core vesicles. Note a possible synapse from a type 1 nerve fibre. (Cavey, tentacle). X 45,000.

Figure 83. A metaraminol-incubated permanganate-fixed synapse of a type 2 fibre. The cores of the synaptic vesicles have been partly removed by the metaraminol indicating that they contain a biogenic amine. (Permanganate in sea water-$MgCl_2$, tentacle). X 80,000.

Figure 84. A synapse between two type 2 fibres. Note that both the small synaptic and the small dense-core vesicles are agranular in osmium-fixed tissue. (Westfall, tentacle). X 90,000.

Figure 85. A synapse of a type 2 nerve fibre in aldehyde-fixed tissue. (4% glutaraldehyde in 0.1M phosphate at pH 7.4, tentacle). X 100,000.

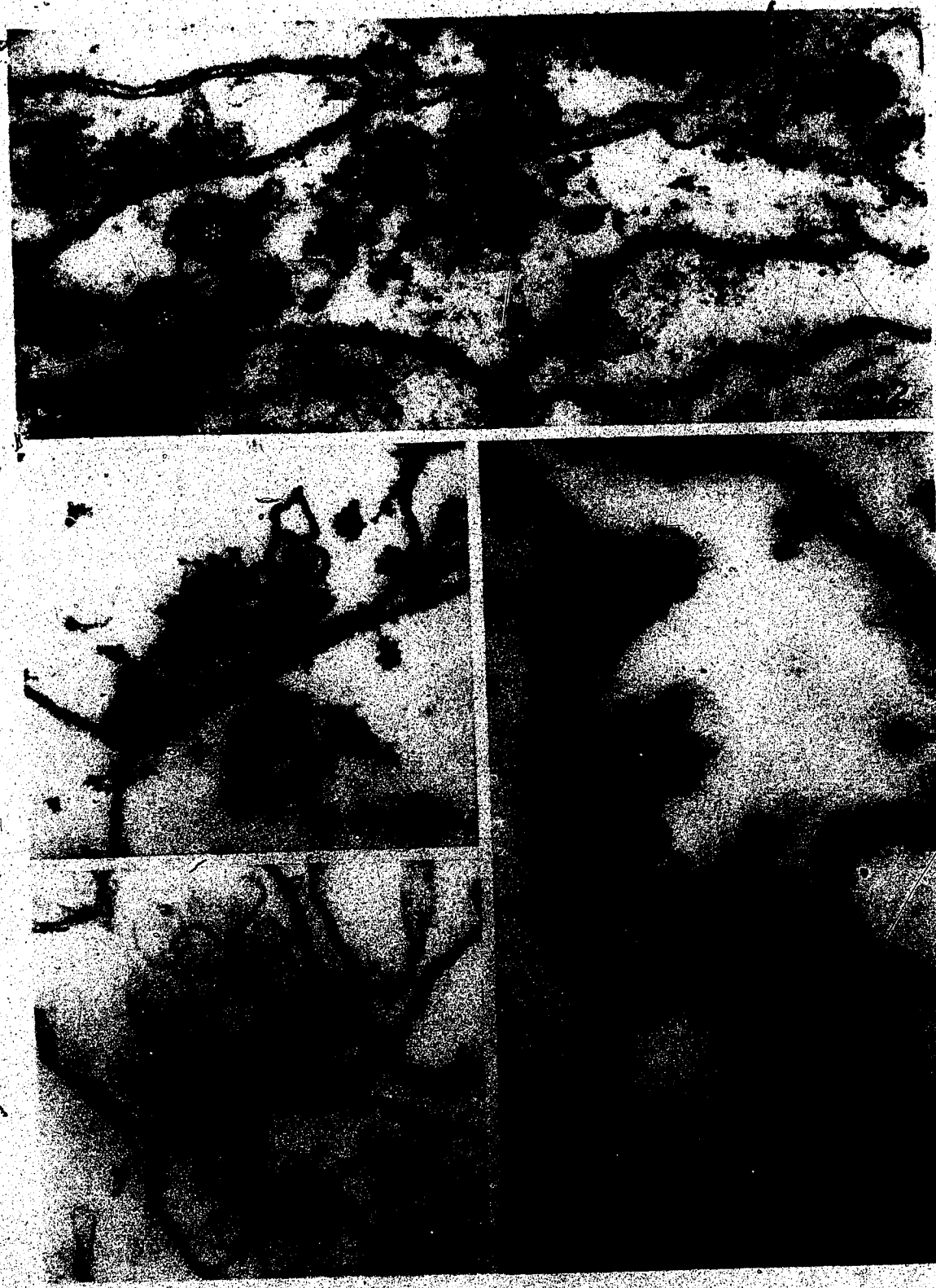


PLATE 33

Figure 86. Three sections in a series of a giant ectodermal type 3 nerve fibre which appears to be giving rise to a comparable sized branch (*). (Permanganate in sea water-$MgCl_2$, tentacle). X 5,000.

Figure 87. A giant type 3 fibre which appears to be giving rise to a small, collateral branch (*). Note also the synaptic interactions with two other small type 3 fibres, one of which has entered a deep invagination of the giant fibre. (Permanganate in sea water-$MgCl_2$, tentacle). X 25,000.

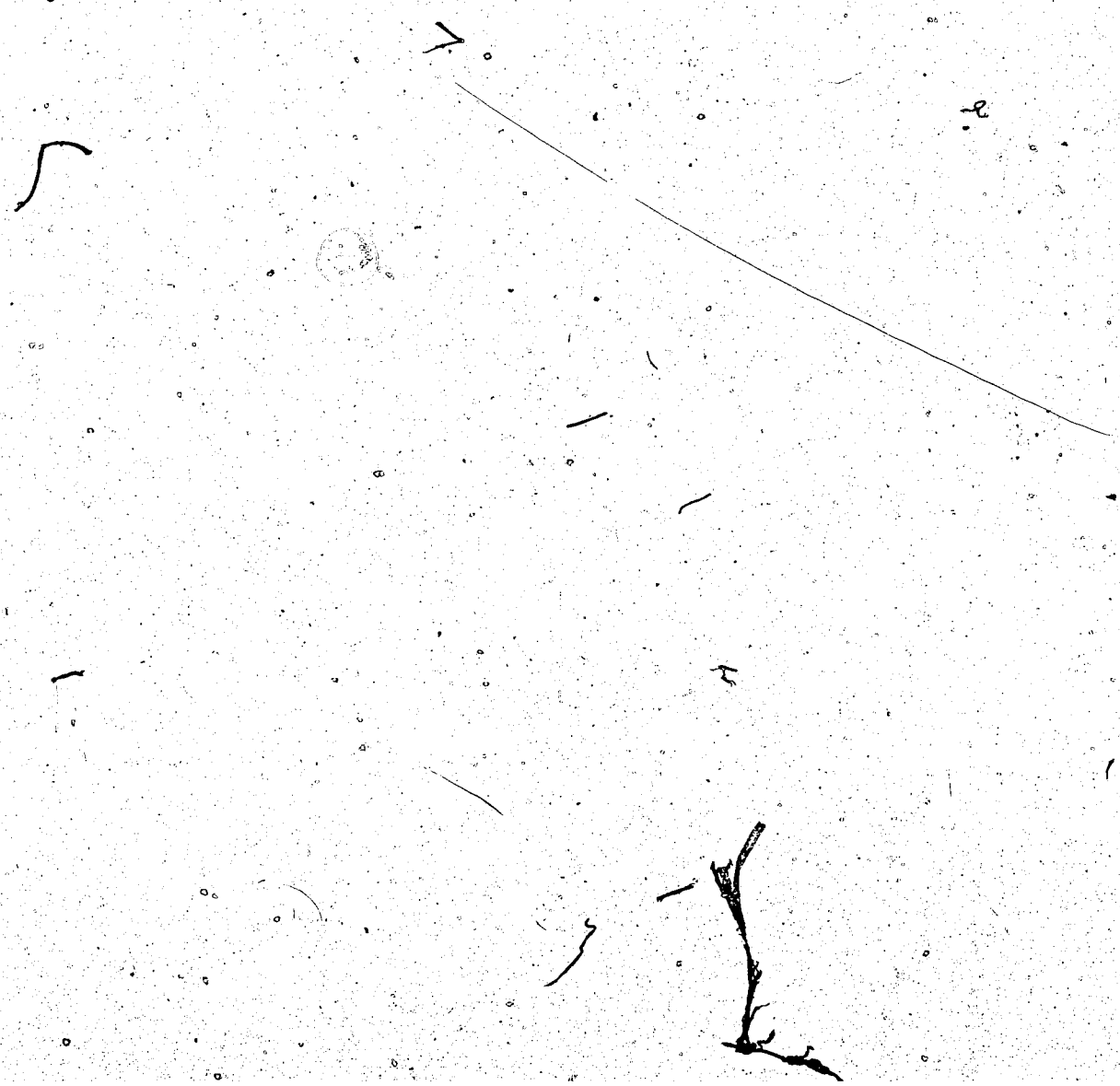


PLATE 34

Figure 88. A permanganate-fixed synapse of a type 3 nerve fibre in which the cross bars of the synaptic gap are particularly evident (arrows). (Permanganate in sea water-$MgCl_2$, tentacle). X 120,000.

Figure 89. An osmium-fixed synapse of a type 3 fibre in which the gap bars cannot be seen. Note the apposition of small flattened vesicles, or sacs of reticulum (arrows) on the postsynaptic side. (Westfall, tentacle). X 100,000.

Figure 90. A well-developed, but typical, postsynaptic structure unique to the synapses of type 3 fibres. Its organization and nature are not understood. (Permanganate in sea water-$MgCl_2$, tentacle). X 70,000.

Figure 91. An unusual synapse of type 3 nerve fibre in that the postsynaptic organelle is missing. Instead a postsynaptic web, showing periodic projections (arrows), is evident. (Permanganate in sea water-$MgCl_2$, tentacle). X 120,000.

PLATE 35

Figure 92. A type 4 ectodermal nerve fibre. Note that two of the muscle fibres have 'sarcoplasmic extensions' (*) extending into the core of the tube of muscle fibres where one receives a synapse from the nerve fibre. Note also that the nerve fibre crosses the mesoglea (M) to enter a second tube. (Cavey, tentacle). X 45,000.

Figure 93. An aldehyde-fixed neuromuscular synapse in which the synaptic vesicles are agranular. (Cavey, tentacle). X 140,000.

Figure 94. A neuromuscular synapse that has dense-core synaptic vesicles; such synapses constitute about 1% of those seen in permanganate-fixed tissue. The cores are not affected by metaraminol incubation. (Permanganate in sea water-$MgCl_2$, oral disc). X 140,000.

Figure 95. A neuromuscular synapse from tissue prepared by Martin's ZIO impregnation. In such tissue the synaptic vesicles of most of the neuromuscular synapses have a weakly stained dense core. (Martin's ZIO, tentacle). X 100,000.

Figure 96. The core of a tube of muscle fibres showing three type 4 fibres having multiple synapses with two 'sarcoplasmic extensions' of muscle fibres. (Permanganate in sea water-$MgCl_2$, tentacle). X 80,000.

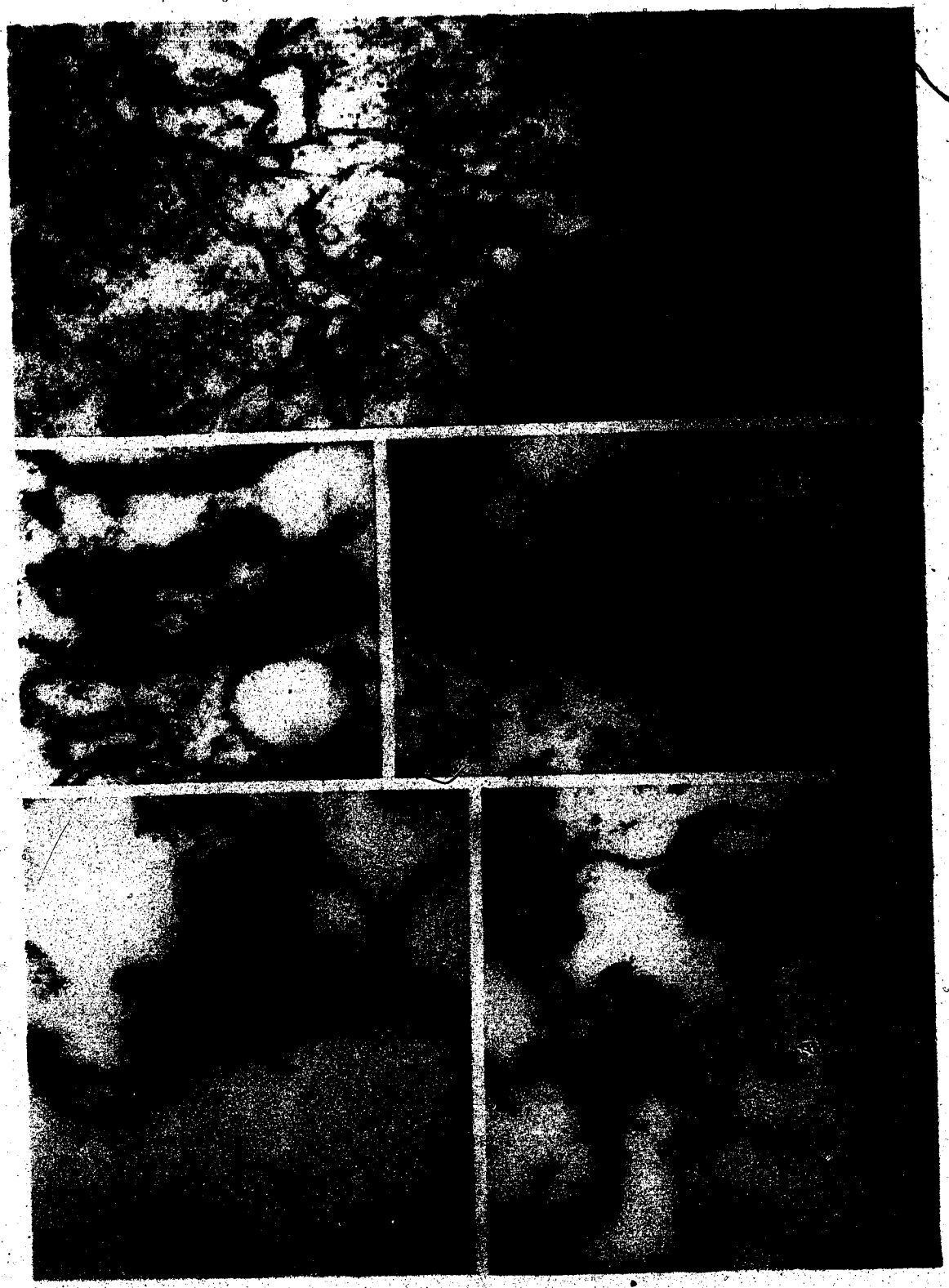


PLATE 36

Figure 97. Small multipolar cells of a type which is found throughout the tentacles and oral disc and which are found in high numbers in the inner oral disc. Although it cannot be identified as the soma of any of the types of nerve fibres identified here, it is possible that this cell type is the small multipolar neuron described by Leghissa and Robson because of similarities in their distribution and morphology. (Permanganate in sea water-$MgCl_2$, oral disc). X 18,000.

Figure 98. A cilium surrounded by 0.6 μ-diameter stereocilia found standing 5 μ above the tissue surface of the ectoderm that overlies the sphincter. The structure is distinct from the sensory apparatuses of gland cells and of cnidoblasts in *Stomphia*, and on this basis it is suggested that the structure may indicate the presence of an unidentified receptor. (Cavey, column ectoderm overlying the sphincter). X 26,000.

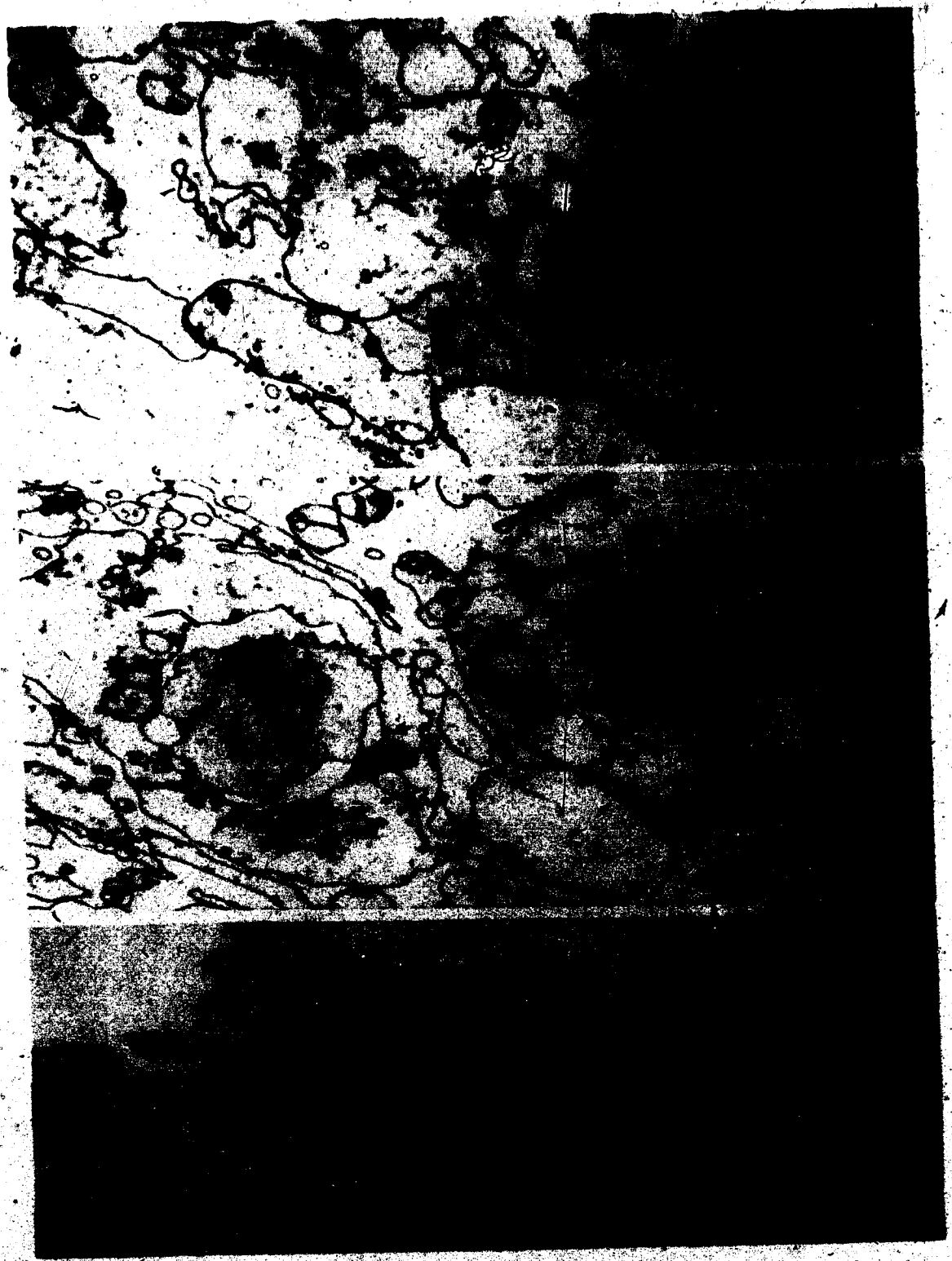


PLATE 37

Figure 99 and 100. Apparent polarized synapses which characterize nerve-fibre type 5. They have been found only in the column ectoderm that overlies the sphincter. (Cavey, column over the sphincter). 99: X 85,000; 100: X 75,000.

Figure 101. A synapse between two fibres of nerve-fibre type 2 (Cavey, column overlying the sphincter). X 100,000.

Figure 102. An apparent unpolarized synapse, of a type seen only twice, for which nerve-fibre type 6 has been created. (Cavey, column overlying the sphincter). X 75,000.

PLATE 38

Figure 103. The dendrite of a receptor that was found only in the pedal disc ectoderm. Its sensory apparatus consists of a cilium within a circle of stereocilia whose fibres run as discrete bundles for several μ into the dendrite. The adjacent supporting cells surround the sensory apparatus with microvilli creating a multicellular cone similar to that of the tentacular ciliary-cone receptors. (Cavey, pedal disc). X 25,000.

Figure 104. The dendrite and cell body of a pedal-disc receptor, identified by its sensory apparatus which is not in the plane of this section. (Cavey, pedal disc). X 7,000.

Figure 105. A tangential section through the surface ectoderm of the pedal disc showing the aggregation of supporting-cell microvilli (arrows) around the stereocilia (*) and the cilium of the receptor. (Cavey., pedal disc). X 30,000.

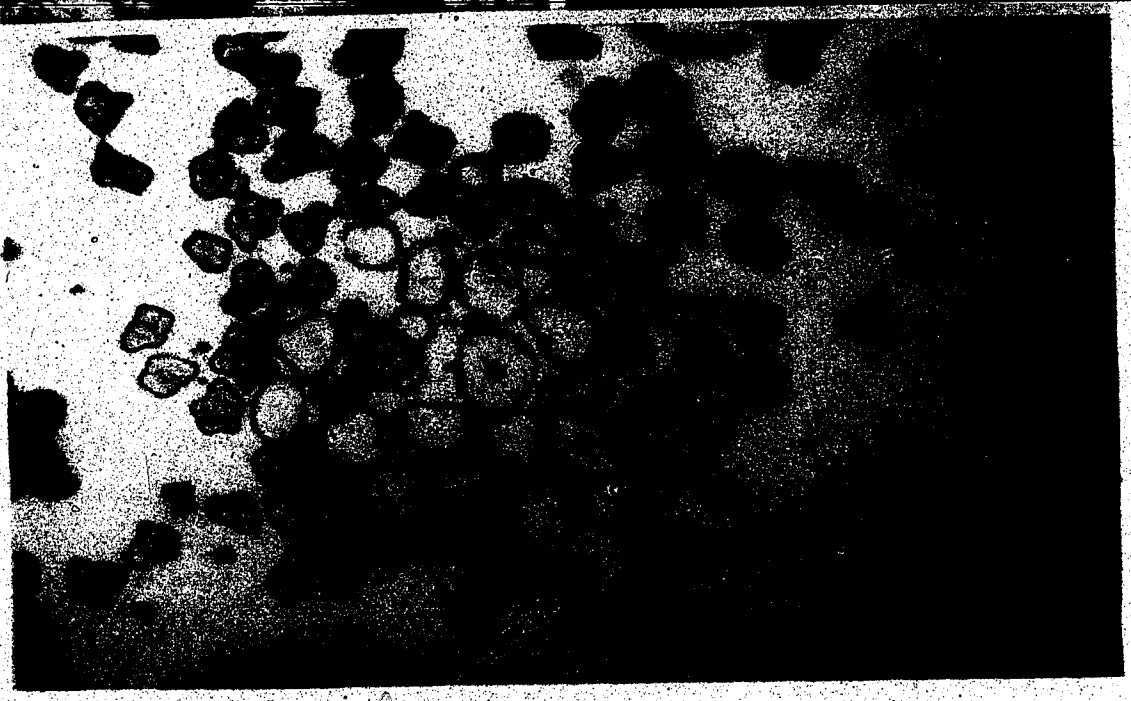
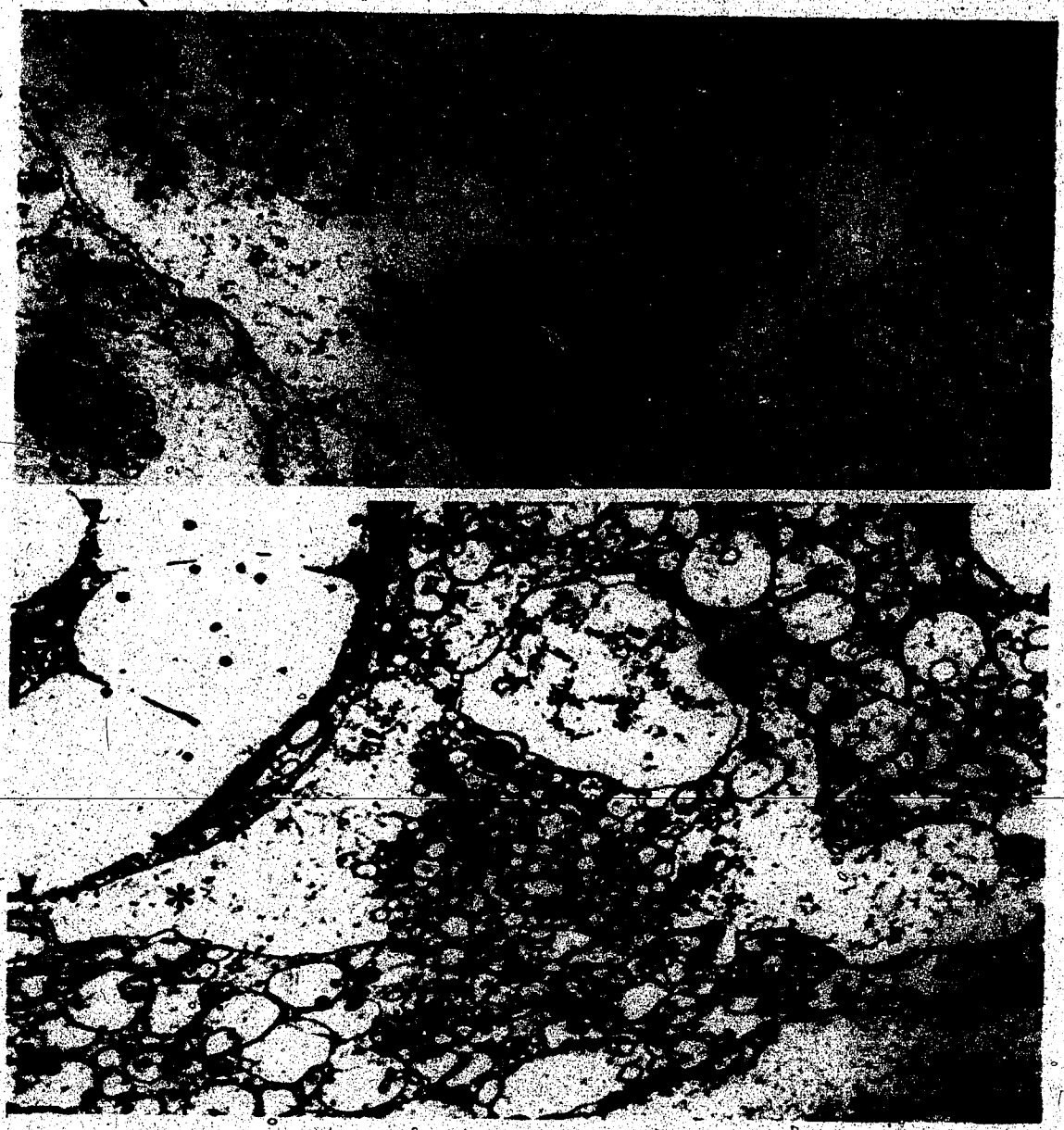


PLATE 39

Figures 106 and 107. Ectodermal amoebocytes which resemble bipolar neurons in shape because they each possess two major processes (*) in opposito-polar positions. In addition the cell in Fig. 107 closely resembles the bipolar-multipolar neuron described by Leghissa (1949) in that both its cell body and its major processes bear a smaller class of process (arrows). (106: Permanganate in sea water-$MgCl_2$, oral disc; 107: Permanganate in sea-water-$MgCl_2$, pharynx). 106: X 5,000; 107: X 10,000.



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APPENDIX 1

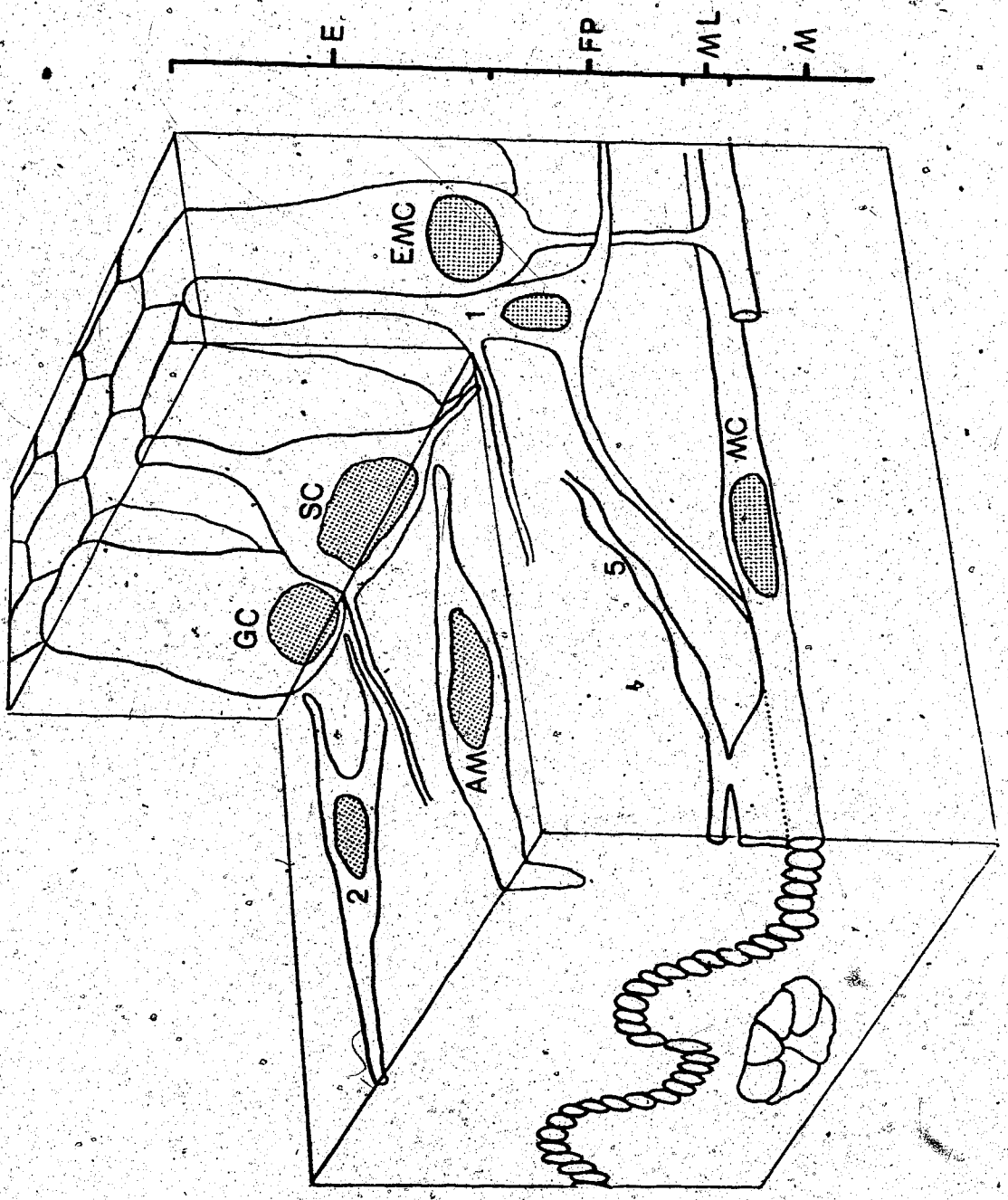
TABLE 6 Data supplemental to those presented in Table 5 (p. 133) for the distinction of ectodermal nerve-fibre types 1 and 2.

	Type 1	Type 2
Distribution	tentacles and outer oral disc	tentacles and oral disc
Diameter of synaptic apposition (average)	0.25 μ	0.50 μ
Influence of fixatives on synapse recognition:		
K_2MnO_4 following incubation in catecholamines	numerous	frequent
K_2MnO_4	rare	frequent
aldehydes	very rare	frequent
OsO_4	very rare	frequent
Fibre types receiving synapses from this class	type 2	type 2
Fibre types having synapses upon this class	none	types 1 and 2

* At the defense of this thesis it was pointed out that the data presented in Table 5 ("Distinguishing characteristics of the ectodermal nerve fibres", p. 133) provided for a distinction of types 1 and 2 only if the reader chose to believe that a particular type of vesicle was found in only one of the two types of fibre. To save such a reader from needing to resort to the text for additional characters which distinguish the type 1 and 2 ectodermal nerve fibres it was suggested that a supplemental table be provided.

A diagramatic summary of the topographical relationships (as determined by EM study) of the cell types comprising the endoderm. It should be noted that the drawing ignores the four classes of neuron, which have been observed only on a LM level, and the type 3 and 4 fibres because their somata have not been identified.
The endoderm is organized into three layers. The muscle layer (ML), apposed to the mesoglea (M), contains only the contractile muscle fibres and the sunken muscle cells (MC). Overlying this is the fibre plexus (FP) and the superficial epithelium (E). The epithelium is composed of the cell bodies of the epitheliomuscular cells (EMC) and a small number of gland cells (GC), receptors (SC), and type 1 cells (1). The fibre plexus is composed of the axons of the receptor and of the four types of neuron, and of fibre types 1, 2, 3, 4, and 5; in addition it contains the somata of the type 2 cell, amoebocytes (A), and of the four type of neurons.
An important discovery made since the writing of this thesis has been included here (it is not to be found in the thesis proper). The type 5 fibre is a "sarcoplasmic extension" of the muscle fibres. They are attached to cytoplasmic ribbons of the fibres which are filled with vesicles and tubules which may represent a reticulum.

Note: Most of the anatomical and topographical characters of the cells have been distorted, both purposely and through ignorance, in this drawing. The drawing serves no purpose but to answer the unwriten requirement that every thesis must have at least one drawing; the reader who may want some touch of reality is directed to the photographs presented in the thesis proper.


E
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