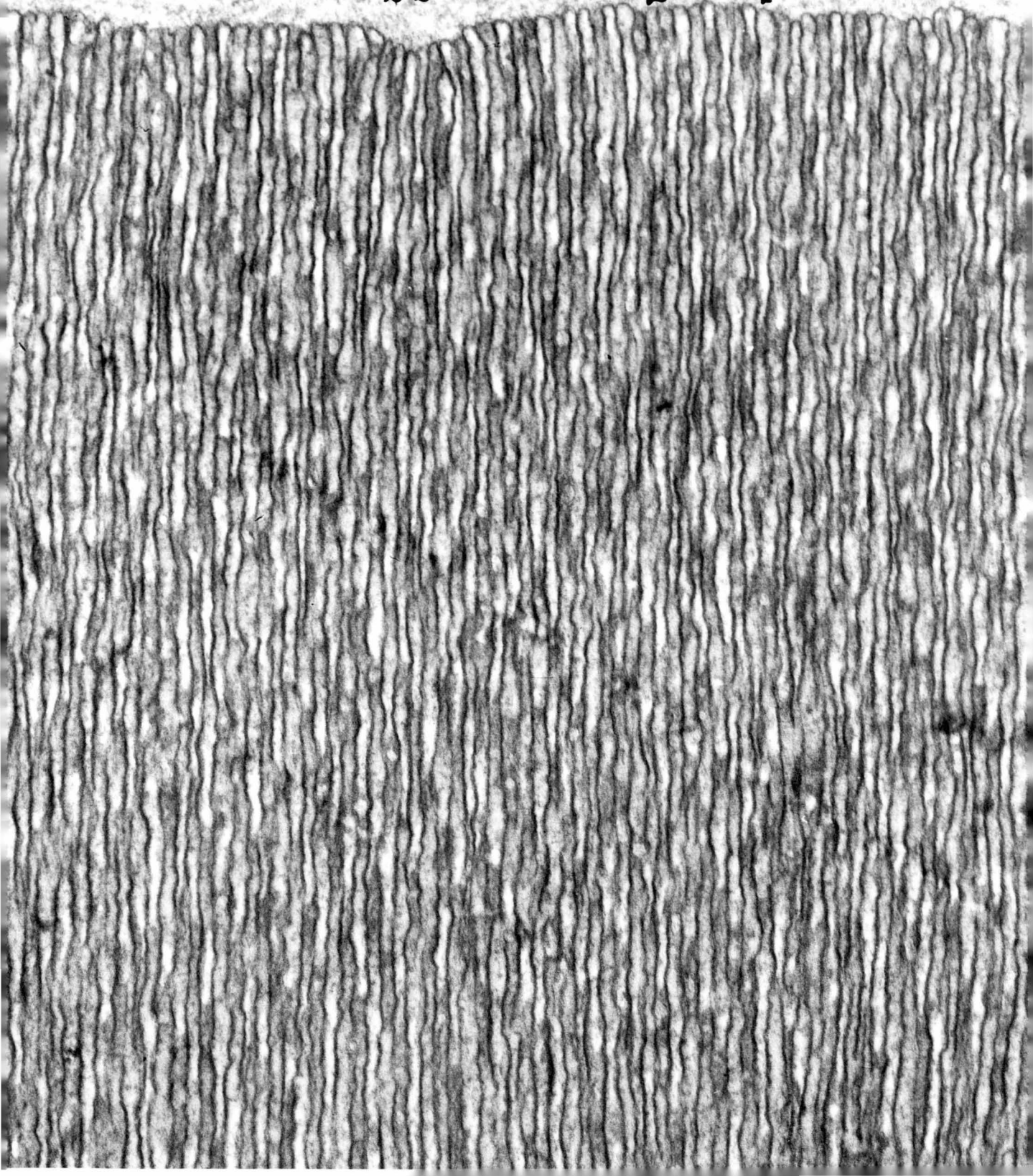"One picture is worth a thousand words"

~ ~ ~ when correctly interpreted ~

FRONTISPIECE

Electron micrograph of a section through part
of the lateral ocellus of the nauplius eye of
the marine calanoid copepod, Labidocera cervi
Krämmer.   The electron lucent horizontal
bars at the top of the electron micrograph
are possibly reflectile plates and these are
part of the tapetal cell.   There is an
amorphous layer between the tapetum and the
rhabdomeric microvilli which occupies most of
this electron micrograph.   X29,700.

ULTRASTRUCTURAL STUDIES OF THE CEPHALOSOME OF SOME

MARINE AND ESTUARINE CALANOID COPEPODS

by

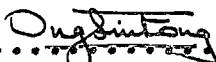ONG   JIN   EONG, B.Sc.(Melb.),B.Sc.Hons.(Tas.)

Submitted in fulfilment of the requirements

for the Degree of Doctor of Philosophy

UNIVERSITY OF TASMANIA

HOBART

July 1970.

Except as stated herein, this thesis contains no material which has been accepted for the award of any other degree or diploma in any University, and to the best of my knowledge and belief, this thesis contains no copy or paraphrase of material published or written by another person, except where due reference is made in the text of the thesis.

J. E. Ong.

CONTENTS

ACKNOWLEDGEMENTS

ULTRASTRUCTURAL STUDIES ON THE CEPHALOSOME OF SOME MARINE AND ESTUARINE CALANOID COPEPODS

ABSTRACT

The ultrastructure of the integument, sensory, neurosecretory and digestive systems are described from the marine calanoid copepods, Calanus helgolandicus (Claus) and Labidocera cervi Krämer and the estuarine calanoid copepods, Sulcanus conflictus Nicholls and Gladioferens pectinatus (Brady).

The Integument

The ultrastructure of the epidermal cells and the very well developed tegumental glands are described. The possible functions of the tegumental cells are discussed. The ultrastructural architecture of the cuticle is described. Modified cuticle such as the lens of the Pontellidae, Labidocera and sites of muscle attachment to the cuticle are also treated.

The Sensory System

Nauplius Eye: The ultrastructure of the apparently specialized moveable eye of Sulcanus is described and briefly compared to those seen in other copepods. The eye consists of two lateral ocelli and one median ocellus. The median ocellus is different from the lateral ocelli in the arrangement and organization of the retinular cells. The median

ocellus has a two-tiered arrangement of rhabdoms and it is suggested that the median ocellus functions like the Pecten (Mollusca) eye, with an "on" "off" response.

The Dorsal Frontal Organs: The dorsal frontal organs are shown to be present in the Copepoda. The micromorphology of the dorsal frontal organs of the estuarine calanoid copepod, Sulcanus are described. The organs are probably photoreceptors, as indicated by the presence of the phaosomes and the rhabdomeric-like "tubular bundles". It is suggested that the dorsal frontal organs may act as a switch involved in the perception of seasonal changes.

Mandibular Sensilla: The ultrastructure of the sensory receptors in the mandibles of Gladioferens are described. These sensilla, which also occur in the other appendages of the cephalosome, are probably chemo- and mechanoreceptors. There are also "chloride cells" in the mandibles and it is possible that associated with these are osmoreceptors. The functional significance of these sensilla with respect to the animals' feeding behaviour is discussed.

The Neurosecretory System

The ultrastructure of Claus's organ and the X-organ associated with the frontal filaments are described from the marine calanoid copepod, Calanus and from the estuarine calanoid copepod, Sulcanus. Claus's organs are apparently

closely associated with the X-organ and may be considered as part of the X-organ complex. Claus's organ may be a neurosecretory baroreceptor. The X-organ complex probably constitutes the sensory pore X-organ and is comparable to those seen in the peracaridian Malacostraca.

The Digestive System

The Labral Glands: The ultrastructure of the labral glands of Gladioferens are described. The glands show similarities to the goblet cells of mammalian stomachs and probably secrete mucus. This is supported by histochemical tests. The gland is highly organized, with well developed valves. The significance of the glands with respect to the filter feeding mechanism of the animal is discussed.

The Mid-gut Diverticulum: The ultrastructure and histochemistry of the mid-gut diverticulum of Calanus are described. This part of the gut appears to be associated with some form of active transport, as seen in the presence of so-called mitochondrial pumps. It appears unlikely that the mid-gut diverticulum is involved in the secretion of enzymes as there is an absence of rough endoplasmic reticulum.

Appendix

This is a section consisting of electron micrographs (with appropriate legends) on the eye and other organs from species of copepods other than those treated in the main part of the thesis. It is intended for comparative studies.

Electron micrograph of a frontal section
through part of the brain of the estuarine
calanoid copepod, Sulcanus conflictus
Nicholls.   Note the giant fibres towards
the bottom of the electron micrograph.
The numerous small electron opaque patches
seen scattered through the electron micro-
graph are possibly synaptic junctions.
X3,750.
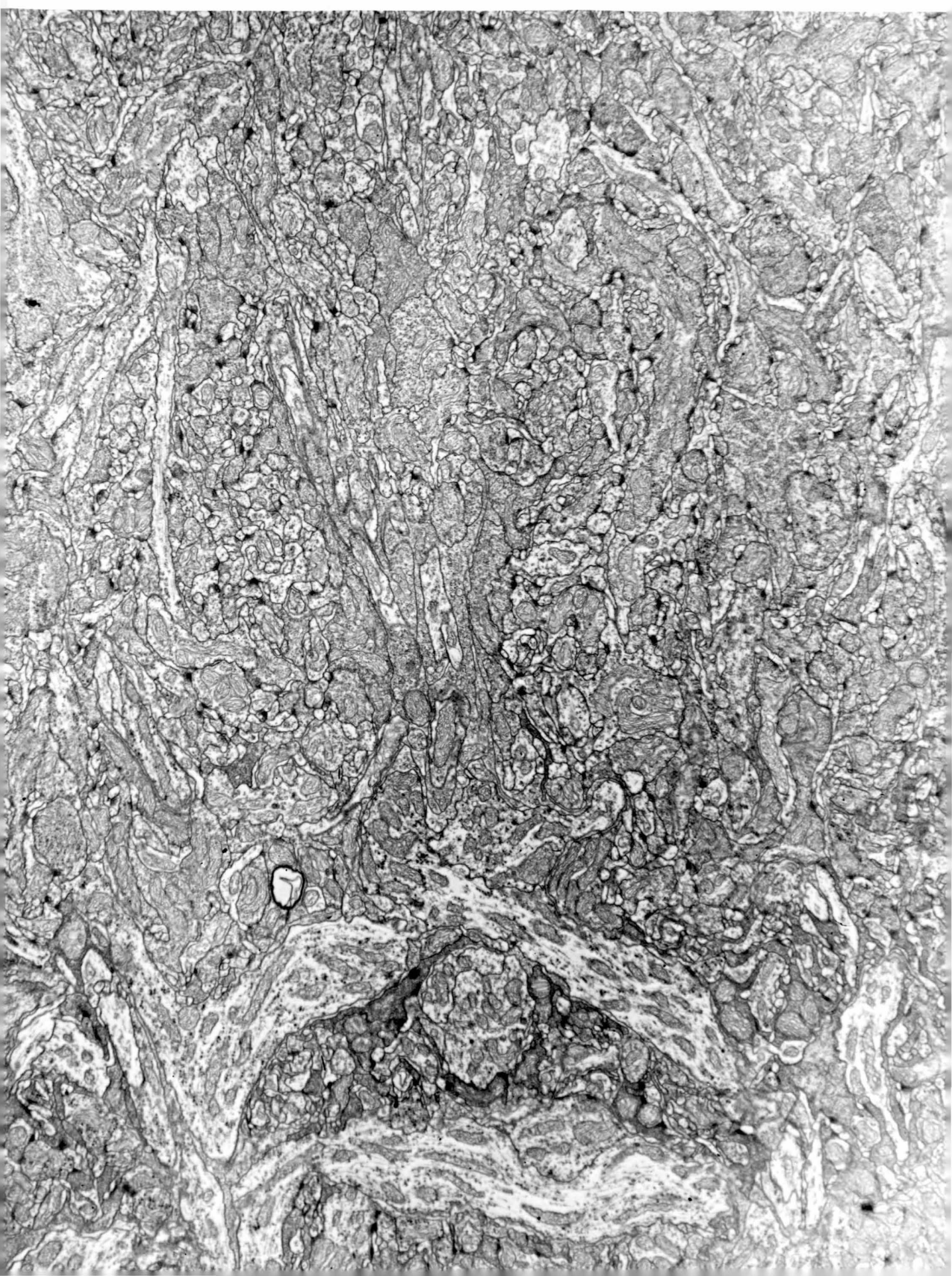
GENERAL   INTRODUCTION.

The members of the class Copepoda are one of the most inconspicuous but certainly the most numerically abundant metazoa of the aquatic environment.   These tiny arthropods are the main food organisms of the secondary consumers, although some copepods are themselves secondary consumers.   Their economic importance is closely linked with the fisheries industry and they have been the subject of many studies.   Most of these studies are ecological, and many are concerned with seasonal and spatial distribution. Because the copepods form the main bulk of the second trophic level, in the marine environment, much research has been centred on the efficiency of these crustaceans in utilizing the primary producers as well as the utilization of the copepods themselves by their predators. There is thus a reasonable amount of literature on the ecology, nutrition, metabolism and biochemical composition of copepods.   Most of the rest of the literature on copepods are taxonomic works and these form the bulk of the literature on the class.

There are only a few studies on the internal anatomy of the copepods and while these have been valuable they are nonetheless inadequate in that the detailed study of many of the structures reported is not possible with the limited resolution of the light microscope.

The development of fixing, embedding, sectioning
and staining techniques have made it possible, over the
last fifteen years or so, to obtain a resolution of a
nannometre or even less for structures of biological
material with the electron microscope. With the elec-
tron microscope, resolution is no longer the limiting
factor. It is indeed surprising that there are no more
than a handful of papers describing electron microscope
studies of copepods. While it is understandable that
copepods are not particularly ideal animals for light
microscope studies (because of their small size) they
are very suitable for electron microscope studies. Since
it is not normally possible to obtain ultrathin sections
much larger than 0.2 mm square for electron microscopy,
the small size of copepods is an advantage. The majority
of copepods are not larger than 3 mm long so that it is
possible, over a period of time, to study the fine
structure of the entire animal. This study is centred
on the cephalosome, with particular emphasis on the
sensory and neurosecretory systems.

Of the few light microscope studies on the internal
anatomy of copepods the majority are studies on the nauplius
eye and closely associated structures. (see separate
sections for references). That the copepod nauplius
eye has attracted such interest is possibly due to the fact

that in the Copepoda the nauplius eye is the only eye present in both the larval stages and in the adults (in other groups of Crustacea the compound eyes are also present although in the Cirripedia only the nauplius eye is present in the adult but compound eyes are present in the larval stages).

Two other sensory structures, apart from the nauplius eye have attracted studies. These are the frontal organs and the so-called statocysts or Claus's organ. The presence and functions of these have been repeatedly questioned, possibly because these structures are just within the limits of resolution of the light microscope (in properly fixed and reasonably thin sections). The literature on these organs, as well as on the nauplius eye has been reviewed by Elofsson (1966). Publications that have appeared since Elofsson's (1966) work are treated under the relevant sections in this thesis. While Elofsson has undoubtedly helped to clarify many concepts associated with the above-mentioned organs the picture concerning the nauplius eye, frontal organs and Claus's organ in copepods is still far from clear or complete.

It has been said that: "There is no difference between structure and function; they are the two sides of the same coin". (A. Szent-Györgyi, 1951) . Electron microscope studies over the last fifteen years or so have supported this view. The approach adopted in this study

is one based on this tenet of complementarity of structure and function. This is basically a morphological study and it is intended primarily to provide the necessary groundwork for studies of a more experimental nature.

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MATERIALS AND METHODS

The planktonic calanoid copepod, Gladioferens pectinatus (Brady) is an Australasian species common in and almost confined to estuaries. In Australia Gladioferens is nearly always found in association with another planktonic calanoid copepod, Sulcanus conflictus Nicholls. Aspects of the biology and ecology of either or both these species have been described by Bayly (1965), Ong (1967) and Thomson and Dunstan (1968).

The marine calanoid copepod, Calanus helgo-landicus (Claus) is an ubiquitous temperate water species. A slightly colder water species Calanus finmarchicus (Gunnerus) is very closely related to this species but it has only recently been shown that these two are separate species (see Manwell et al., 1967). Many of the earlier works on Calanus finmarchicus may thus actually be referring to Calanus helgolandicus (especially if the animals are known to be taken from the English Channel and lower latitudes). Calanus helgolandicus occurs in waters off Hobart and it is likely that the Calanus finmarchicus recorded by Dakin and Colefax (1940) from Melbourne Harbour, New Zealand and Tasmania are C. helgolandicus. I have never taken C. finmarchicus in Tasmanian waters.

According to Dakin and Colefax (1940) the plank-
tonic calanoid copepod, <u>Labidocera cervi</u> Krämer has
been recorded from the North Island of New Zealand,
Sydney and Melbourne with a maximum occurence from May
to July.   Little else is apparently known of the
biology or ecology of this species.

Gladioferens pectinatus and Sulcanus conflictus
were taken from the Derwent River estuary and Brown's
River estuary (an estuary near the mouth of the Derwent
River).   <u>Calanus helgolandicus</u> and <u>Labidocera cervi</u>
were taken from D'Entrecasteaux Channel ($43^{o}15'S$,
$147^{o}15'E$) near Hobart, Tasmania.   The animals were
collected with a plankton net with a mouth area of
0.25 m^{2} and a mesh pore of 200/um.   The animals were
returned alive to the laboratory where they were immed-
iately fixed.

For light microscopy the animals were fixed in
aqueous Bouin's fluid (10% buffered formalin was found
to be unreliable).   The specimens were dehydrated
through a graded series of ethanol, cleared in either
xylene or methyl benzoate and embedded in either paraffin
wax or Paraplast.   Sections were cut at about 6/um and
stained with Ehrlich's haemotoxylin-eosin, paraldehyde
fuchsin with Halmi's counterstain according to the method
of Ewen (1962), a modified Azan according to Hubschman (1962)

and alcian blue according to Steedman (1950) as modified
by Zugibe, Brown and Last (1959).

For localization of sites of halide permeability,
animals were rinsed quickly in three changes of de-
ionized water, placed in 10^{-4}M aqueous silver nitrate
solution for 10 to 30 minutes, rinsed three times in
deionized water, developed in Microdol-X (Kodak)
diluted 1:3 for five minutes and fixed in Kodak acid
fixer for five minutes before finally washing in de-
ionized water.   The animals were then fixed in 10%
neutralized formalin before examination.

For electron microscopy the animals were fixed
at 4°C for an hour in 2% OsO_4 made up in the sea or
estuarine water from which the specimens were collected
(prefixing in either buffered glutaraldehyde or
buffered formaldehyde was found to be unsatisfactory).
The animals were then cut into two and left in the fix-
ative for a further hour, at room temperature.   In
some instances the specimens were block-stained/post-
fixed in saturated uranyl acetate for an hour.   The
specimens were then dehydrated through a graded series
of ethanol, cleared in propylene oxide and embedded
in either Araldite or Epon.   Sections of about 1/um
thickness were examined mainly for the purposes of
orientation after staining in warm 1% toluidine blue
in 1% aqueous borax or with phase contrast microscopy.

Sections for electron microscopy were cut with a diamond knife on an LKB ultrotome at thicknesses between 50 - 80 nm. Some of these sections were stained in saturated aqueous uranyl acetate for 40 minutes followed by saturated lead citrate in 0.02M sodium hydroxide for 20 minutes, in a carbon dioxide free atmosphere. Sections were mounted on collodion coated copper grids and examined with an AEI EM6 electron microscope normally operating at 60 kV.

For localization of areas of halide permeability the animals were rinsed three times in deionized water and transferred to 10^{-4}M aqueous silver nitrate for half an hour (solutions of silver nitrate more concentrated that 10^{-4}M was found to be quite lethal to the animals). After rinsing three times in deionized water the animals were prepared for electron microscopy as above except that the animals were fixed in halide free phosphate buffered 2% OsO_4, osmotically adjusted with sucrose. Silver lactate and potassium antimonate were also tried but these were found to be unsuccessful in initial experiments and these methods were not pursued.

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          technique for the simultaneous demonstration of
          lipid and acid mucopolysaccharides on the same
          tissue section.
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Electron micrograph of part of the cuticle
ventral to the rostral filaments of the
marine calanoid copepod, <u>Calanus helgolandicus</u>
(Claus).   The fibrillar pattern of the
cuticle can be clearly seen.   X69,000.
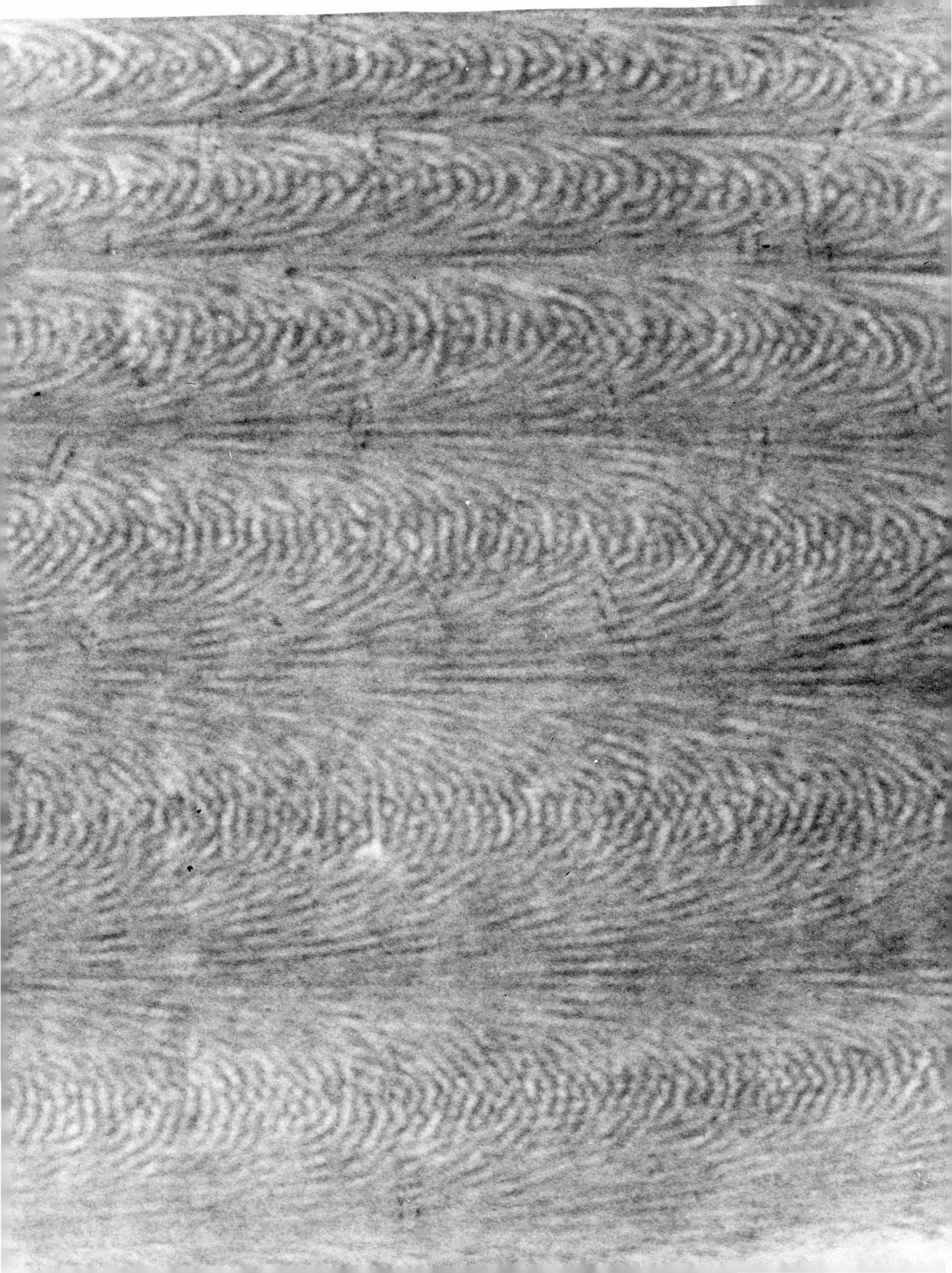
THE COPEPOD INTEGUMENT

INTRODUCTION

In common with practically all the other members of the phylum Arthropoda, the members of the class Copepoda posses cuticular exoskeletons. The cuticle, together with the epidermal cells and the modified epidermal cells that secrete and maintain it, constitutes the integument. The arthropod (especially insect) integument has attracted considerable interest and this is reflected in the numerous reviews on that topic. Amongst them are the works of Richards (1951 and 1953), Locke (1964), Hackman (1964), Bouligand (1965a and b), Wigglesworth (1965), Noble-Nesbitt (1967), Smith (1968) and Chapman (1969). It is however rather surprising that there are only a few electron microscope studies on the arthropod integument (see Smith, 1968).

Only a brief outline will be given here on the formation and structure of the arthropod integument and readers are referred to works of Richards (1951 and 1953), Locke (1964) and Noble-Nesbitt (1967) for further details. The following description is based on data from the above-mentioned reviews and the picture presented is essentially the one seen in the insects.

The main events leading to the formation of

the new cuticle are illustrated in Fig. 1.   The first
event involves changes in the epidermal cells in the
form of mitosis, increase in cell size and changes
in cytoplasmic organization.   An ecdysial membrane
is formed (by the epidermal cells) and the old cuticle
and the epidermal cells separate.   A moulting gel is
secreted into the space between the cuticle and the
epidermis.   The moulting gel is possibly membrane-
bound moulting fluid and is inactive.   The first layer
of the new cuticle, the cuticulin, is then secreted.
This is the layer that determines the eventual shape and
sculpturing of the new cuticle, but perhaps more relevant
to the moulting process is that the cuticulin protects
the epidermis from the moulting fluid which is activated
as soon as the formation of the cuticulin is complete,
or in cases where an inner epicuticle is present the
moulting fluid is activated after the completion of that
layer.   The activation of the moulting fluid is possibly
through the digestion of the membranes of the membrane-
bound moulting gel.   The endocuticle of the old
cuticle is digested by the moulting fluid and resorbed
by the epidermal cells which are at the same time form-
ing the new cuticle (the endocuticle is formed in two
phases: pre-ecydsial and post-ecdysial).   In most
instances ecdysis takes place at this stage but in cer-
tain instances ecdysis may be delayed, the so-called
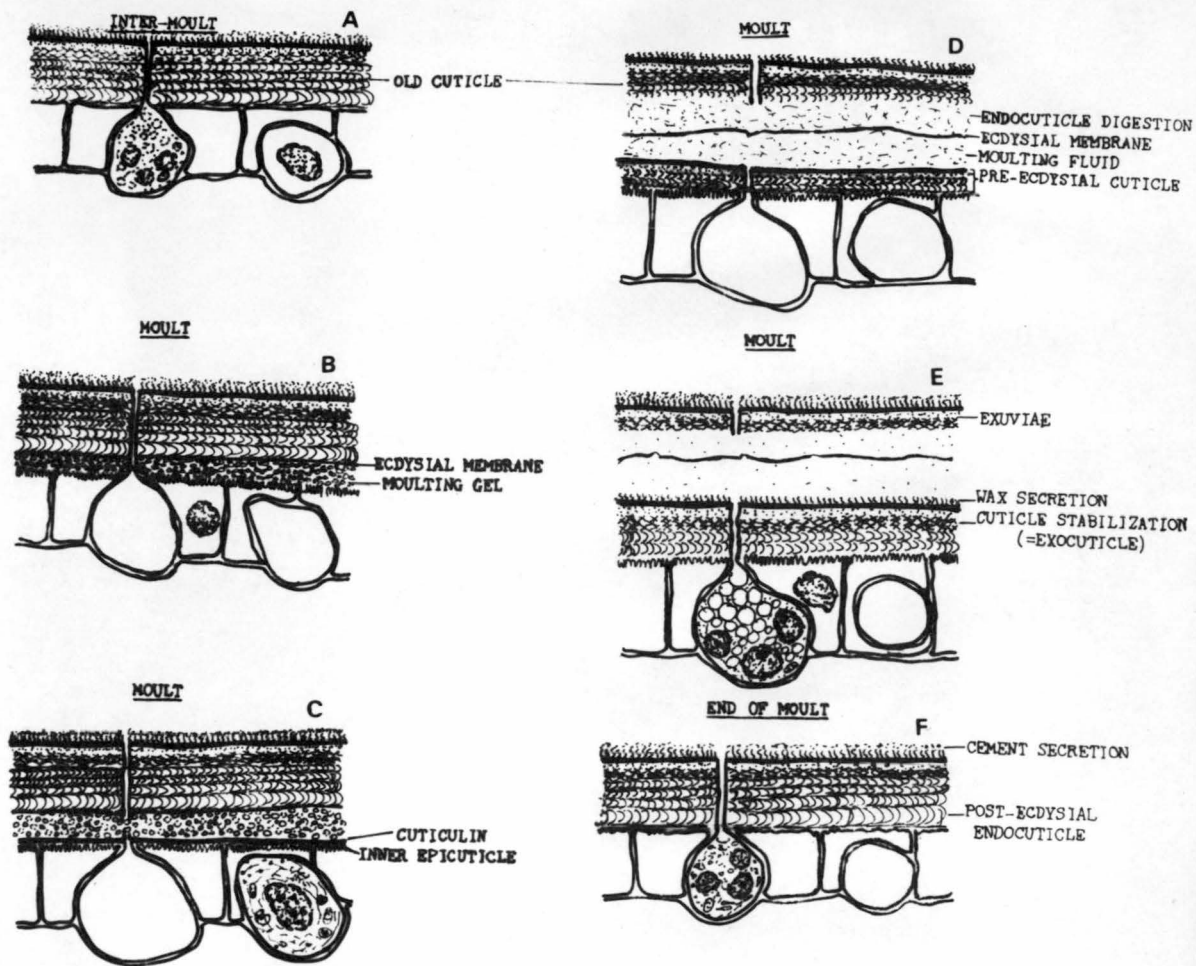
Fig. 1.

Diagrammatic illustration of the moulting cycle in a
generalized arthropod.   The data for this illustration
is taken mainly from Richards (1953) and Locke (1964).
The dermal glands reach maximum develoment just prior
to the secretion of the cement layer of the epicuticle
and the oenocytes reach maximum devlopment just prior
to the activation of the moulting gel.

pharate instar being the result (Jenkins, 1966).   Part
of the pre-ecdysial cuticle is then stabilized, usually
by the process of quinone tanning and this stabilized
cuticle is termed the exocuticle.   The wax layer is
then secreted and immediately after, the cement layer
is deposited, alledgedly by the dermal glands (Wiggles-
worth, 1965).   The rest of the endocuticle is then
secreted and the formation of the cuticle is complete.
The epidermis then reverts to the intermoult stage, a
stage which may be considered as preparatory to moult
except in cases where the terminal moult is reached.
It is noted that the endocuticle is a very labile
structure and in instances acts as stored metabolites:
parts of the endocuticle may be resorbed when external
food sources are not available.

      It must be noted that any layer or a combination
of layers of the cuticle may be absent in different
parts of the arthropod and although the cuticulin is
almost always present it is known to be absent in
areas of the cuticle associated with, for example, chemo-
receptors (see electron micrograph on pages 47 & 192 of
this thesis).   The structure of the cuticle is thus
variable from one part of the animal to the next and
certain parts may be highly modified e.g. the cuticle
forming the hinges of insect wings may have a slightly
elastic rubber-like chitin, resilin (Weis-Fogh, 1960).

As far as I am aware there are only two pub-
lished works on the ultrastructure of the copepod in-
tegument; those of Bouligand (1965a and 1966) both of
which dealt only with the cuticle. Unlike most other
arthropods, copepods do not have specialized respiratory
structures such as gills or trachae: the cuticle or
parts of it probably serve as the respiratory surface.
As well, many copepods, especially estuarine forms have
an added problem in osmotic and ionic regulation and
this is closely related to the permeability of the
cuticle.

The following description of the ultrastructure
of the integument of some marine and estuarine copepods,
with special emphasis on the structure of the cuticle
and the tegumental cells.
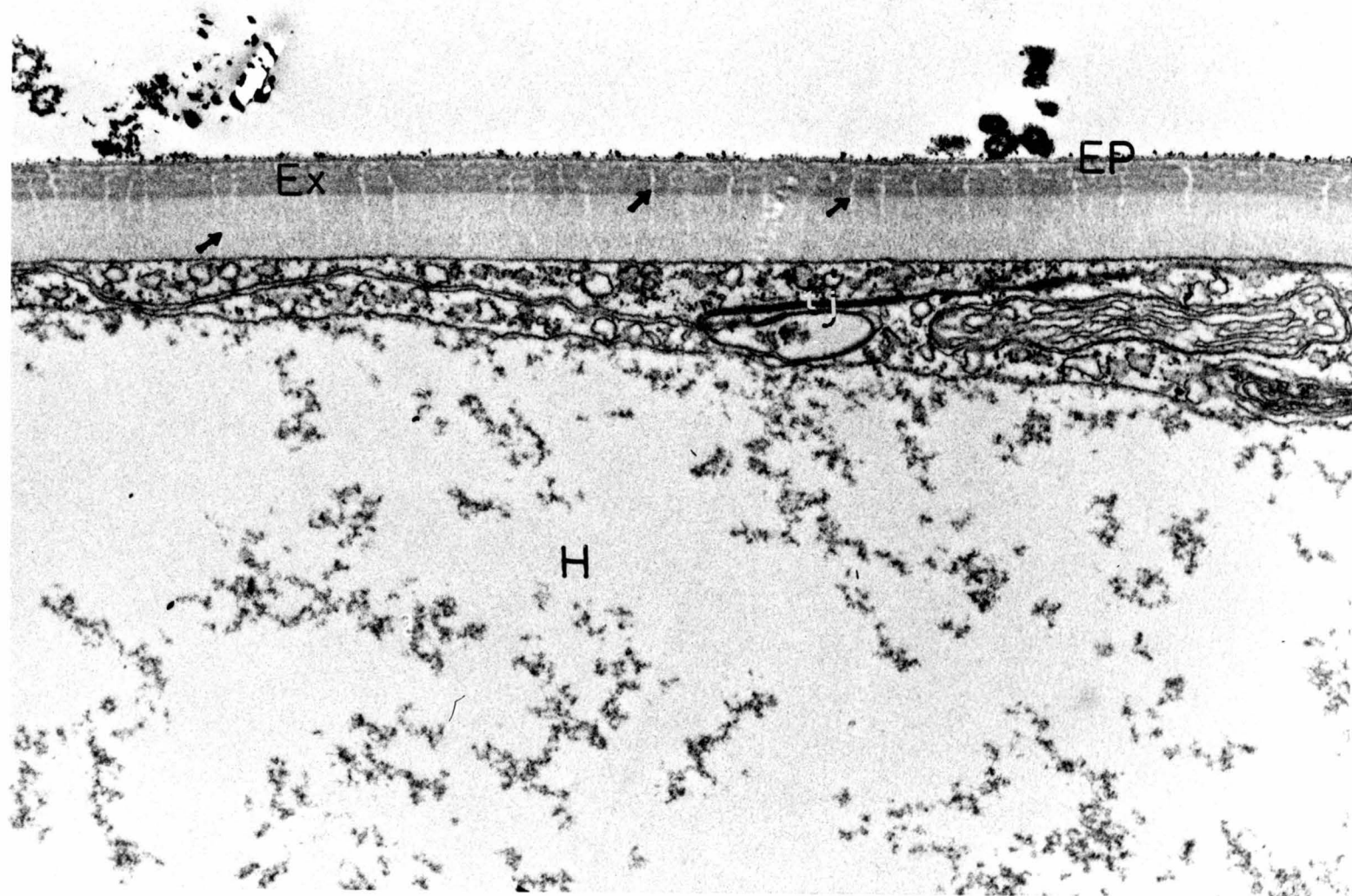
THE EPIDERMIS

INTRODUCTION

The arthropod epidermal cell, especially that at the inter-moult stage, is rather featureless in that it contains only a few cytoplasmic organelles. The epidermal cell is however probably the most versatile cell-type found in the arthropod. That the epidermal cell is a very dynamic and versatile cell is beautifully documented by Locke (1967). More so than any other cells the study of the epidermal cell is best done on a time series so as to trace its morphogenesis. The following is a very brief illustrated description of the epidermal cells, in the inter-moult stage, of some estuarine copepods.

OBSERVATIONS

The cells are situated immediately below the cuticle and are usually flattened so that in places they form a very thin sheet under the cuticle (Fig. 2). The epidermal cells have a very well developed system of endoplasmic reticulum. There are also clusters of free ribosomes apart from those associated with the endo-plasmic reticulum. The cells also contain a number of mitochondria and microvesicles (Figs. 2 and 3). One of the striking and probably characteristic features of epidermal cells is the presence of desmosomes, on the

Fig. 2.

Electron micrograph of a section through part
of the integument of <u>Sulcanus conflictus</u> showing
the flattened epidermal cells held together by
tight junctions (tj).   The procuticle consists
of an outer electron opaque exocuticle (Ex) and
a less electron opaque endocuticle.   There appear
to be pores piercing the cuticle (arrows).
EP = epicuticle,   H - haemocoel.   X 15,000.

apical side (the side just under the cuticle), between adjacent cells. The desmosomes may be simple or septate (Fig. 3). Since the shape of the newly formed cuticle depends to a great extent on the shape of the epidermis, it seems vital that the epidermal cells are tightly held together.

Epidermal cells are often modified in different parts of the cuticle that are specialized in sensory or other functions. These modified epidermal cells are treated elsewhere in this thesis.

Fig. 3.

Electron micrograph of a section near the base
of the mandible of _Gladioferens pectinatus_
showing the cuticle and the epidermal cells
below it.   The epidermal cells contain
granular endoplasmic reticulum (GER), clusters
of free ribosome (r), mitochondria (M) and
microvesicles.   A septate desmosome may be
seen at the top·· and a non-septate one to-
wards the bottom of the electron micrograph.
The exocuticle (Ex) and endocuticle (En) can
be readily discerned.   It is difficult to
pick the epicuticle which possibly consists
of only the cuticulin and a thin dense homo-
geneous layer (arrow) below it.

X 19,000.
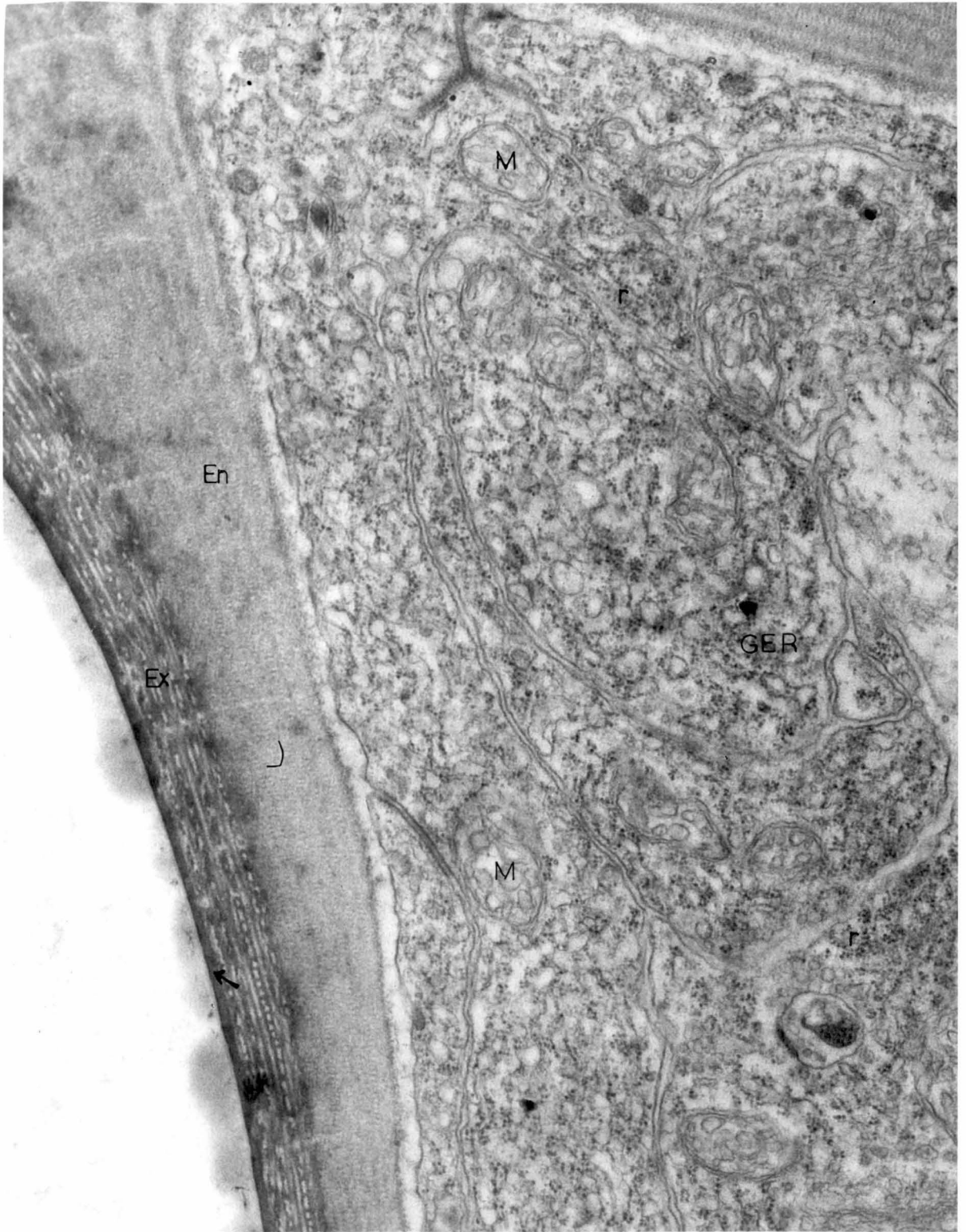
THE TEGUMENTAL GLANDS

INTRODUCTION

Scattered in a specific pattern throughout the animal and just under the epidermis are numerous distinct groups of cells. These cells constitute the tegumental glands and are also known as cutaneous glands and dermal glands, among other names (the numerous terms used to describe these glands have been reviewed by Richards, 1953). Such structures were described from copepods as early as 1881 by Claus and 1891 by Richard. With (1915) and Sewell (1932 and 1947) considered the number and distribution of these glands to be of taxonomic importance. Park (1966) recently described these glands from the calanoid copepod, Epilabidocera amphirites using improved light microscopical techniques. There has however been no ultrastructural study of them.

The following is a description of the micromorphology of the tegumental glands of the marine calanoid copepod, Calanus helgolandicus and the estuarine calanoid copepods, Sulcanus conflictus and Gladioferens pectinatus with special reference to the tegumental glands in the anterio-dorsal region of the head of the estuarine calanoid copepod, Sulcanus conflictus.

OBSERVATIONS

<u>Light microscopy</u>:   The tegumental cells are easily visible under the light microscope with paraldehyde stained material.   These are stained dark purple and appear granular, showing a resemblence to neurosecrtory material.   The positive staining reaction to paraldehyde fuchsin is an indication of some form of mucopolysaccharide.   It is however not possible to properly discern the internal morphology of the tegumental cells under the light microscope.

<u>Sulcanus</u>:   The anterio-dorsal tegumental gland of the head of <u>Sulcanus</u> consists of three multinucleate cells (Fig. 4).   It consists of a "dark" central cell and two or more "light" peripheral cells.   All these cells are multinucleate and each nucleus contains a single nucleolus.

The central "dark" cell posseses a cuticular duct which passes through the epidermal cells and opens immediately below the cuticle (Fig. 5).   The wall of the duct is extremely electron opaque and this is possibly cuticulin.   It appears that the large secretory droplets of the tegumental cells are discharge their contents into this duct.   The dark appearance of the cell is caused by the slightly more electron opaque cytoplasm (compared to that of the "light cell") but mainly by the numerous granules of free ribosomes. (Figs. 6 and 7).   Apart from the conspicuous secretory droplets

Fig. 4.

A low power electron micrograph of a
frontal section through the head of
Sulcanus conflictus showing the anterio-
dorsal tegumental gland and surrounding
tissue.   DC = "dark cell",   LC = "light
cell",   c = cuticle, N = nucleus, G =
Golgi complex, XOC = X-organ complex,
BM = basement membrane,   H = haemocoel.
X2,800.
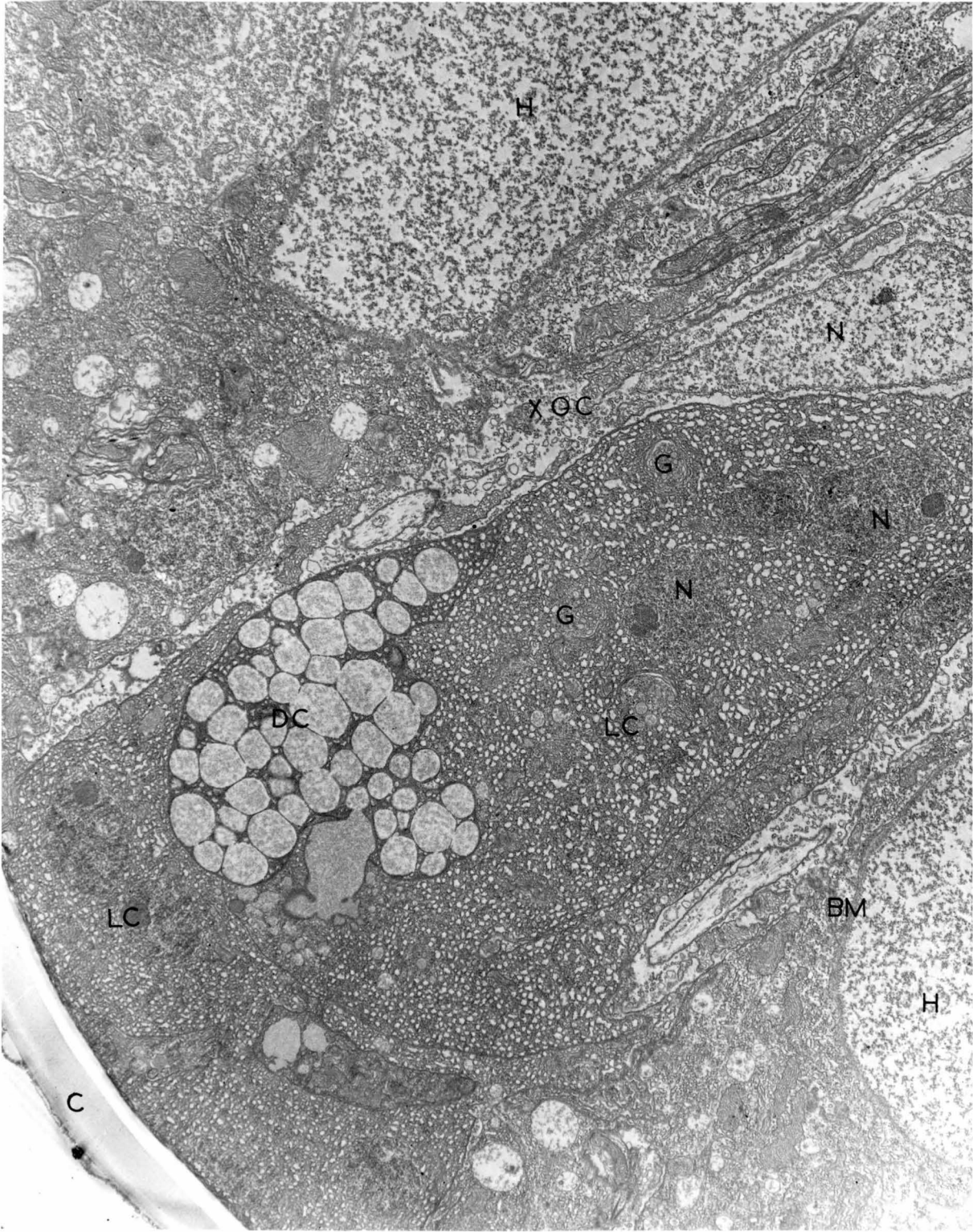
Fig. 5.

Electron micrograph of a frontal section
through the head of Sulcanus conflictus
showing the cuticular duct of the central
"dark cell" of the anterio-dorsal tegu-
mental gland. The duct passes through
an epidermal cell (E) and opens just
under the cuticle (C) (the section is cut
almost parallel to the cuticle). On
either side of the epidermal cell are the
"light cells" (LC) of the tegumental
gland. The structure on the top left-
hand corner of the electron micrograph
is part of the companion cell of the
sensory pore X-organ complex.
X15,750.
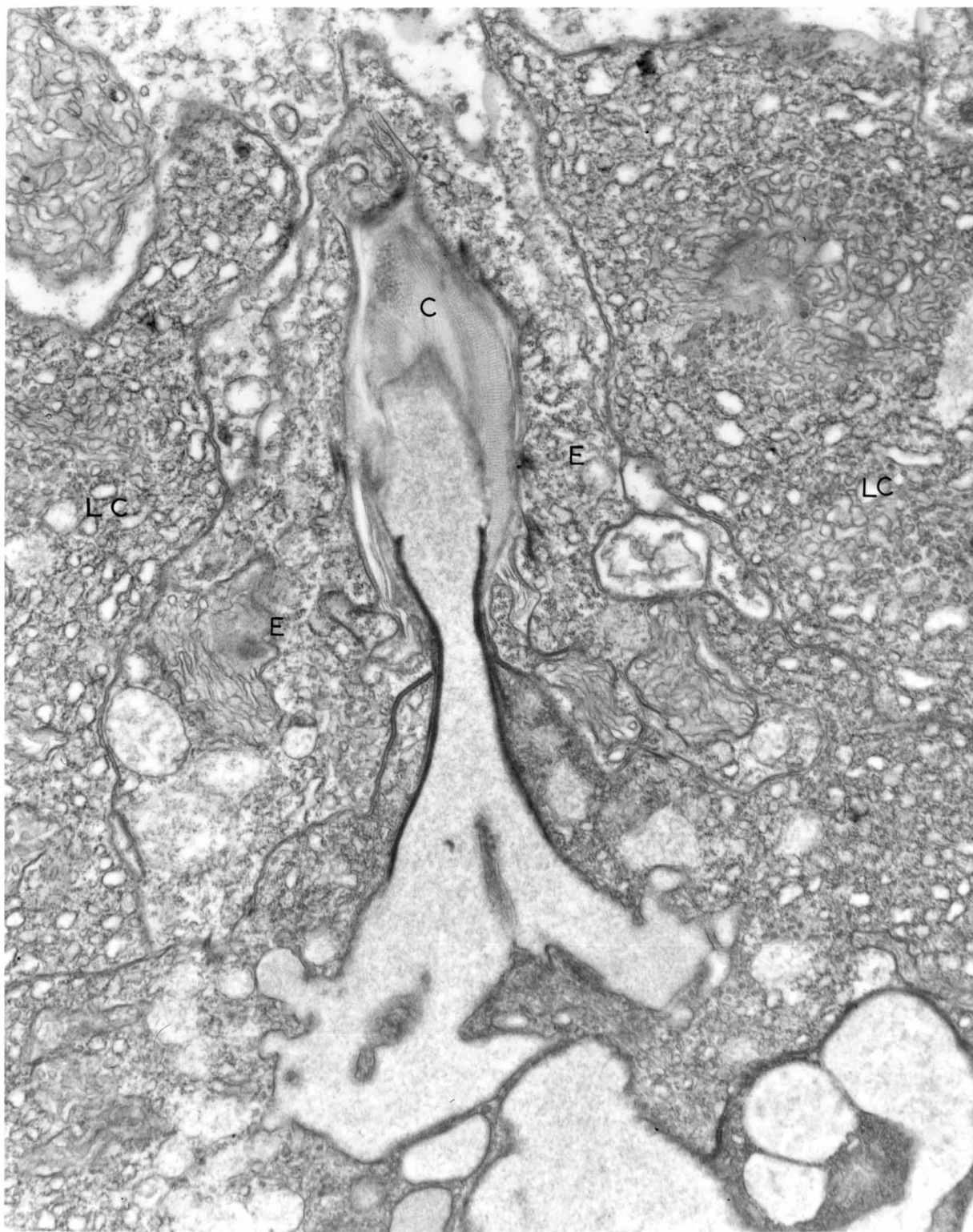
and the numerous free ribosomes the cell contains
numerous Golgi complexes, and tubular endoplasmic retic-
ulum (Fig. 8). The endoplasmic reticulum appears
rather swollen and there are only a few ribosome part-
icles on the membranes of the endoplasmic reticulum,
especially when compared to the endoplasmic reticulum
of the "light cells" (Figs. 6 and 7). The Golgi cisternae
are cup-shaped and well developed, with numerous tiny
vesicles associated with them (Figs. 6 and 8). There
are only a few mitochondria present in the "dark cell".

The peripheral "light cells" have a similar
appearance to the "dark cell" in that they are also
multinucleate and contain an extensive system of endo-
plasmic reticulum and Golgi complexes (Fig. 11).
There are however only a few Golgi complexes and these
are not as well developed as those in the "dark cell":
the Golgi complexes in the "light cell" contain only a few
cisternae. There are also very few free ribosomes in
the cell; most of the ribosomes are attached to the
membranes of the extensive tubular endoplasmic reticulum
(Figs. 6 and 7). As with the "dark cell" there are
only a few mitochondria in the "light cell". There
are no large secretory droplets in the "light cells".
The difference between "light" and "dark" cells is
possibly due to the different stage of development of
the cells.

Fig. 6.

Electron micrograph of a section through
parts of a "dark cell" and a "light cell"
(top of micrograph). The Golgi complexes
of the "dark cell" is cup-shaped and con-
tains numerous cisternae, those of the
"light cell" have only a slight concavity
and only a few cisternae. S = secretory
droplets; G = Golgi complexes; TER =
tubular endoplasmic reticulum. X18,200

Fig. 7.

Electron micrograph of a section similar
to that in Fig. 6 showing the distribution
of ribosomes in "light" and "dark" cells.
Note that the membranes of the tubular
endoplasmic reticulum (TER) of the "light
cell" (right-hand side of micrograph)
have ribosomes adhering to them whereas
those in the "dark cell" have only a few
ribosomes on the membranes of the
tubular endoplasmic reticulum. X26,800.

Fig. 8.

Electron micrograph of a section through
the anterio-dorsal tegumental gland of
Sulcanus conflictus showing both "dark"
and "light" cells.   The "dark cell" con-
tains numerous secretory droplets (S),
Golgi complexes (G) and very few mito-
chondria (M).   N = nucleus;   TER =
tubular endoplasmic reticulum;   BM =
basement membrane and H = haemocoel.
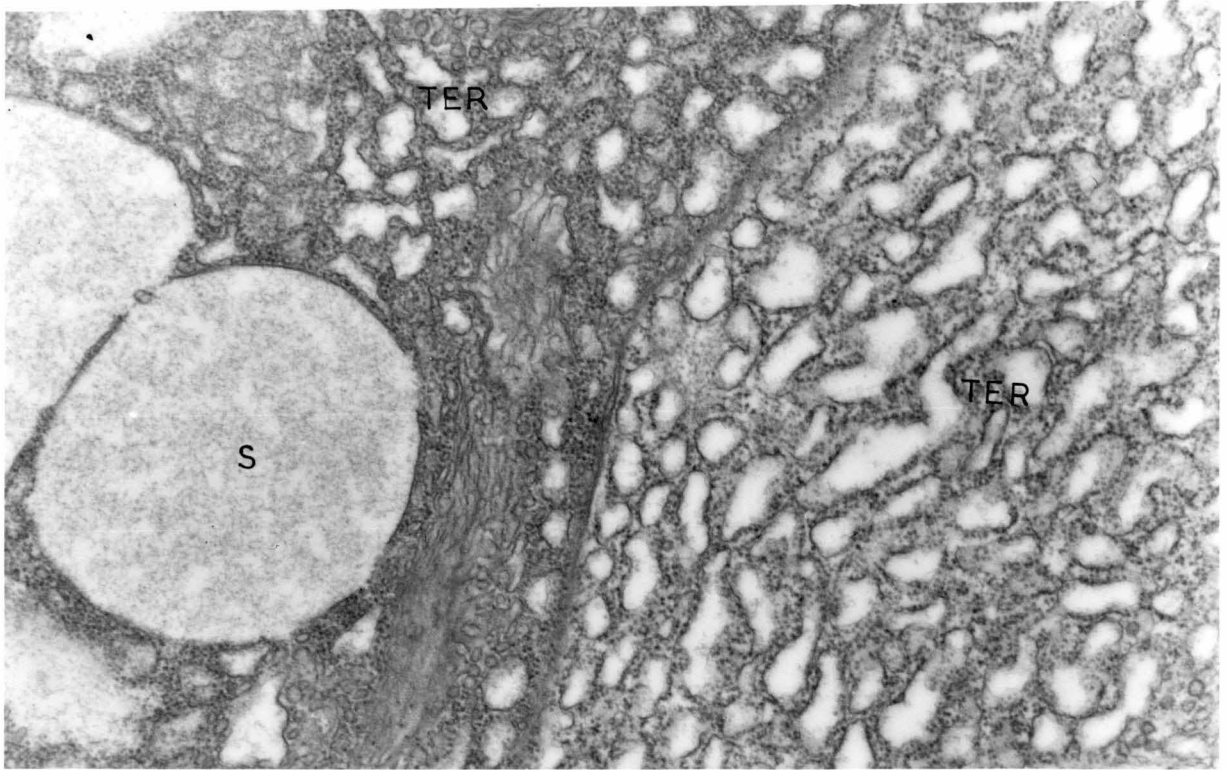X3,000.
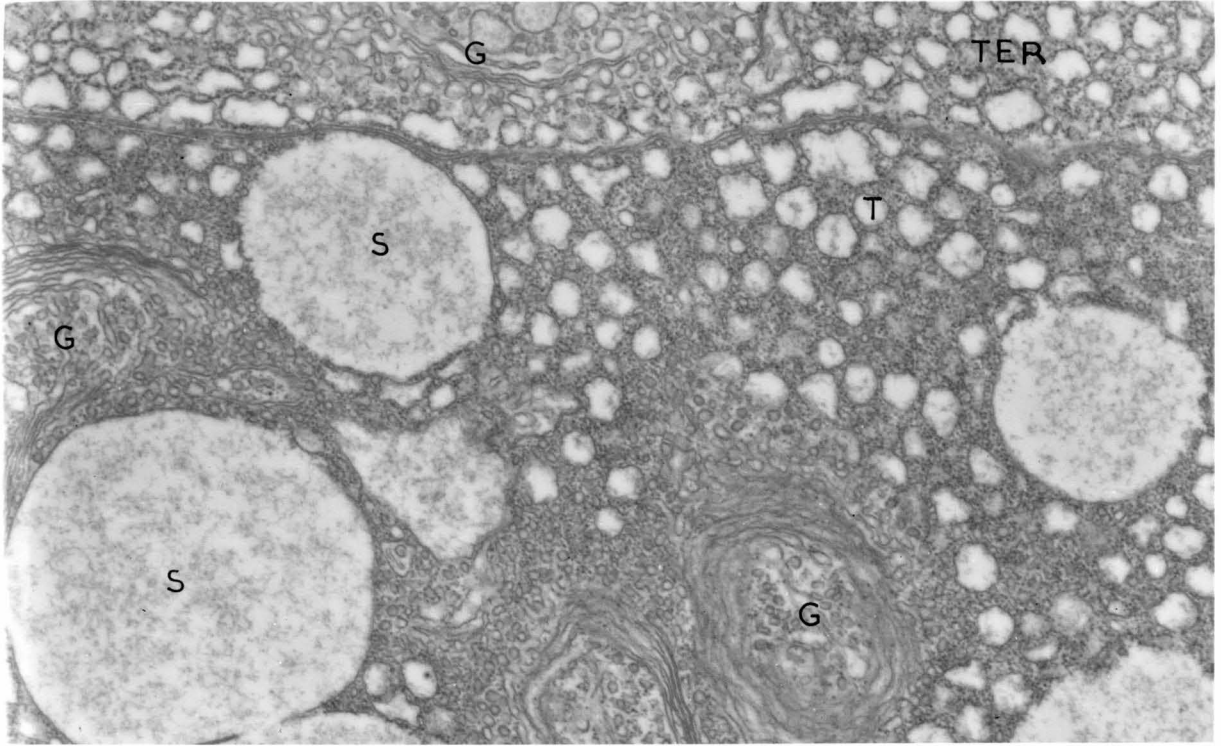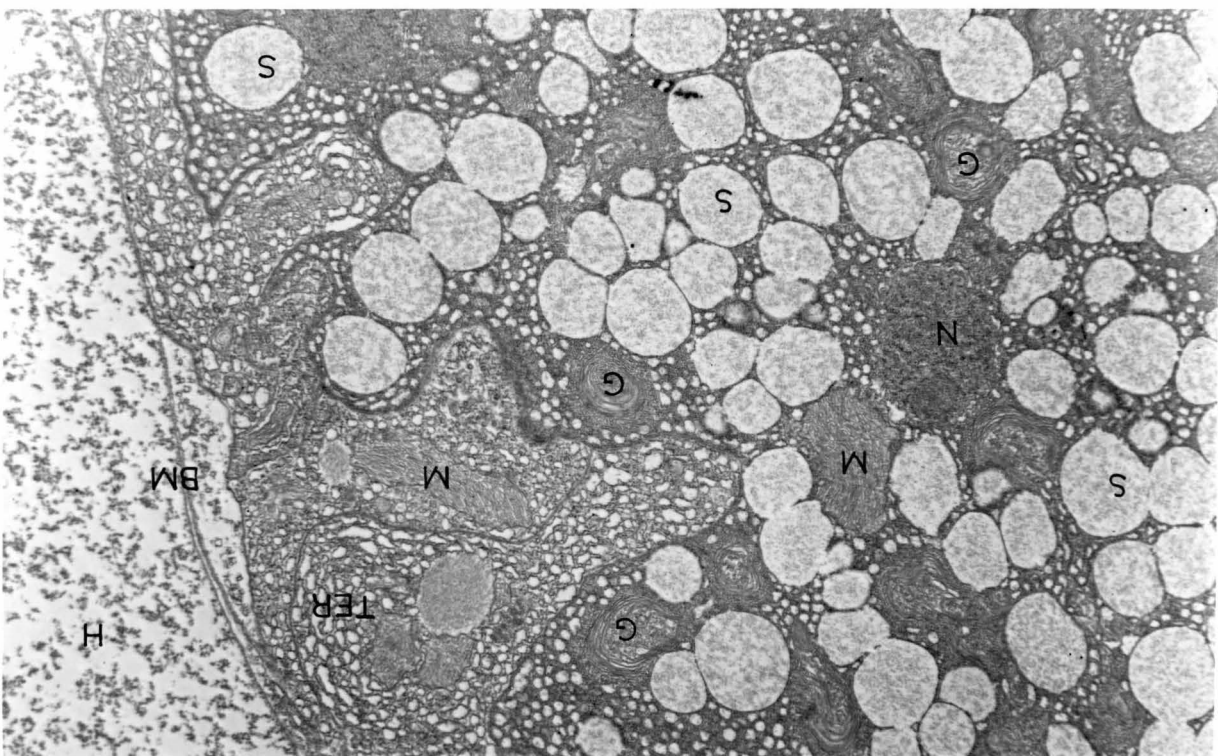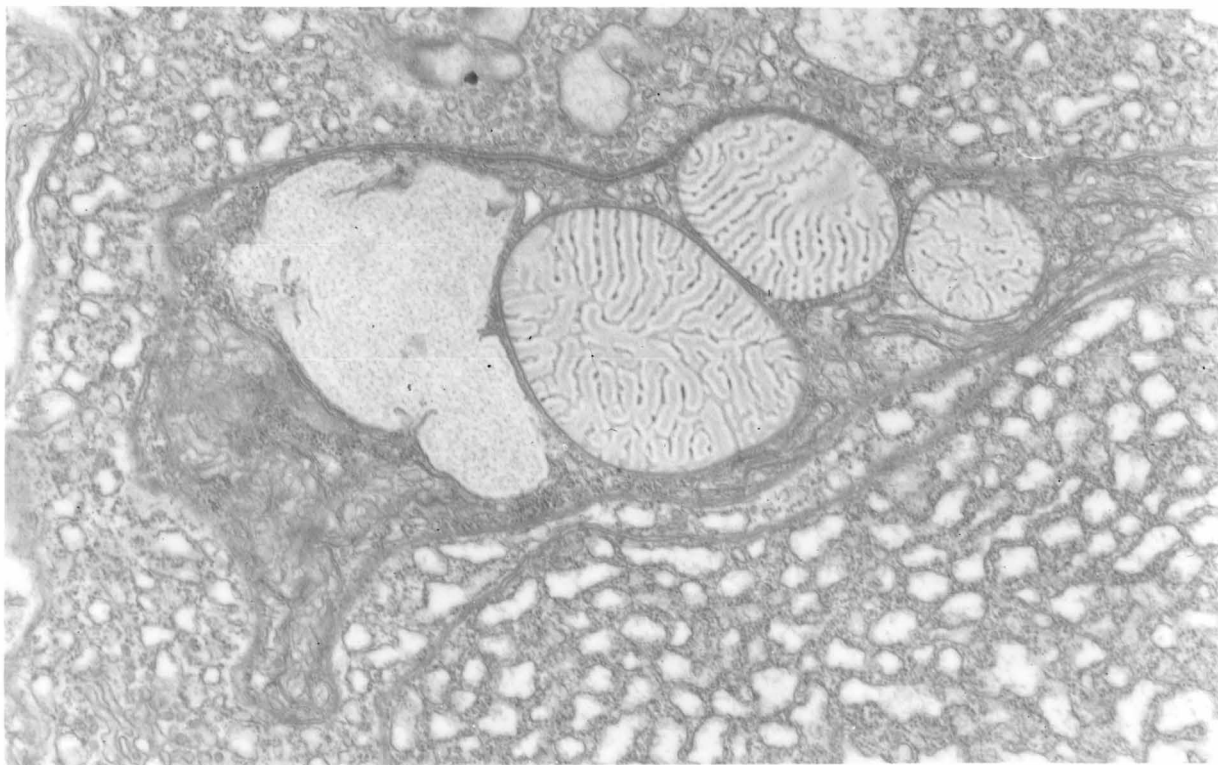
Fig. 9.

Electron micrograph of a section through
the anterio-dorsal tegumental gland of
Sulcanus conflictus showing some secretory
droplets in a "dark cell".   The secretory
droplets have peculiar "structured" con-
tents.   The cell on the top left-hand
side of the micrograph is a "light cell"
and that on the bottom of the micrograph
is an active epidermal cell.   X16,800.

The tegumental cells are in close contact with both Claus's organ and the sensory pore X-organ (Figs. 4 and 11).   The latter organs are treated elsewhere in this thesis and the possible relationships between the sensory pore X-organs and the tegumental glands are discussed there.

Gladioferens: The tegumental cells of this species are basically similar to those in Sulcanus.   The cell is multinucleate and contains numerous Golgi complexes, secretory droplets, extensive rough tubular endoplasmic reticulum, free ribosomes and very occasional mitochondria (Fig. 12).   The nuclei, like those in Sulcanus each contains a single nucleolus.   The most notable difference between the tegumental cells of Sulcanus and Gladioferens is in the structure of the Golgi cisternae.   In Sulcanus the Golgi cisternae are cup-shaped whereas the Golgi cisternae in Gladioferens are only very slightly concave.   But, like the Golgi of Sulcanus the periphery of the cisternae of the Golgi in Gladioferens forms a tubular reticulum.   I have not as yet found any "dark cells" or tegumental cells with cuticular ducts in Gladioferens.   This is possibly because the animals I have examined are not in a physiological stage where these structures occur.

Calanus: Like the tegumental cells of Gladioferens

Fig. 10.

Electron micrograph of a section through
a tegumental gland cell in the antenna
of Sulcanus.   In places like these the
tegumental cells occur singly.   G =
Golgi complexes;   S = secretory granule
and N = nucleus. X 7,500.

Fig. 11.

Electron micrograph of a slightly oblique
frontal section through the head of
Sulcanus showing a "dark cell" (DC) and
"light cell" (LC) of the anterio-dorsal
tegumental gland.   Parts of the paired
Claus's organ (CO) may also be seen.
X2,400.
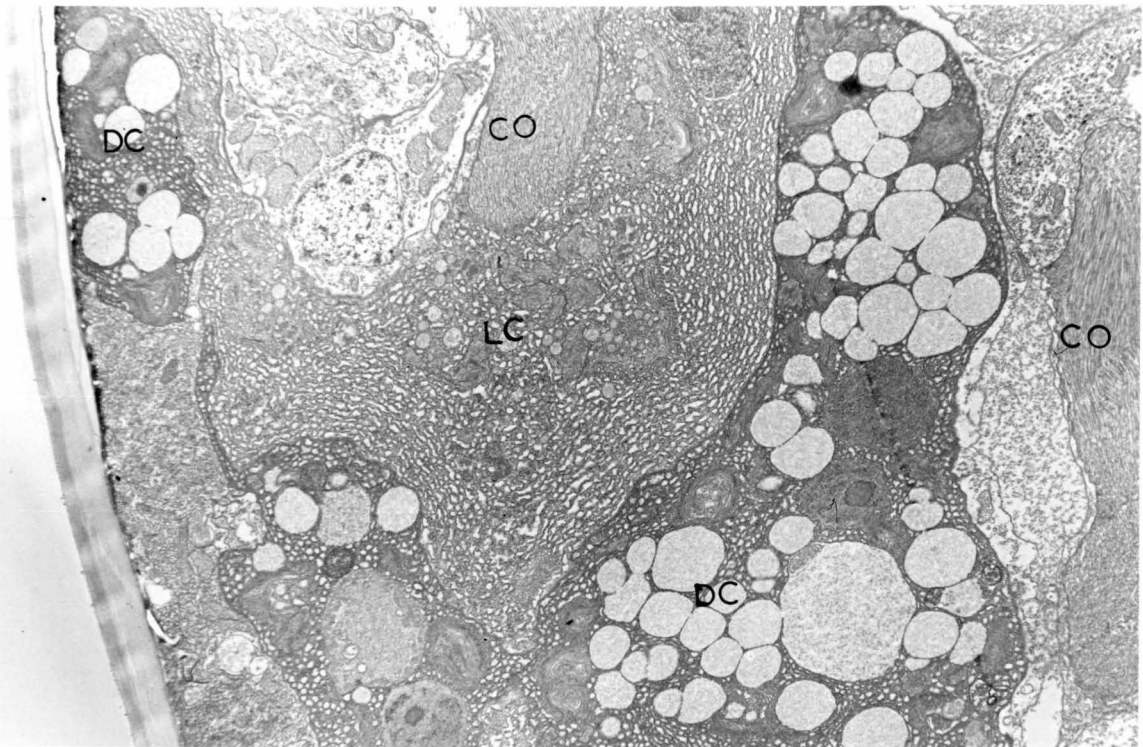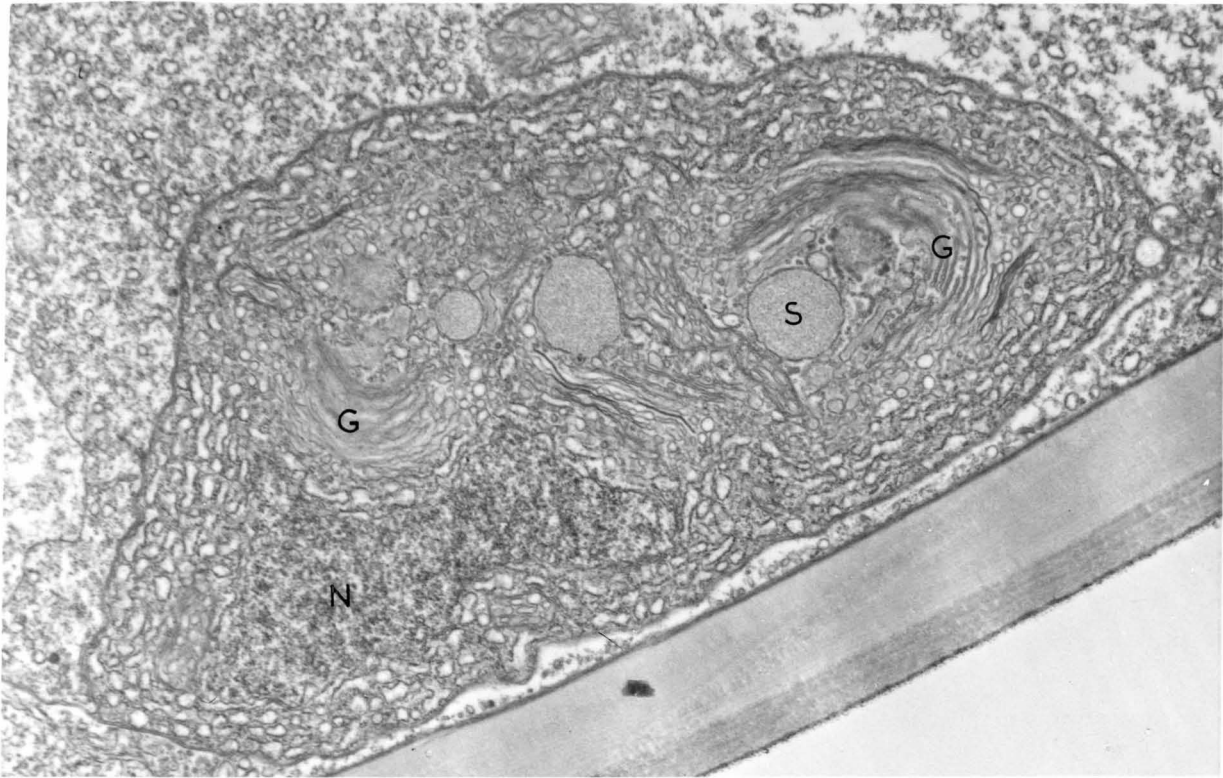
4

Fig. 12.

Electron micrograph of a section through
part of a tegumental gland of Gladioferens
showing parts of two tegumental cells.
The cells are multinucleate (N = nucleus)
and the nuclei each contain a single
nucleolus (n).   The cells contain
numerous conspicuous Golgi complexes (G),
rough endoplasmic reticulum (RER), free
ribosomes (r) and a few secretory drop-
lets (S).   X14,250.
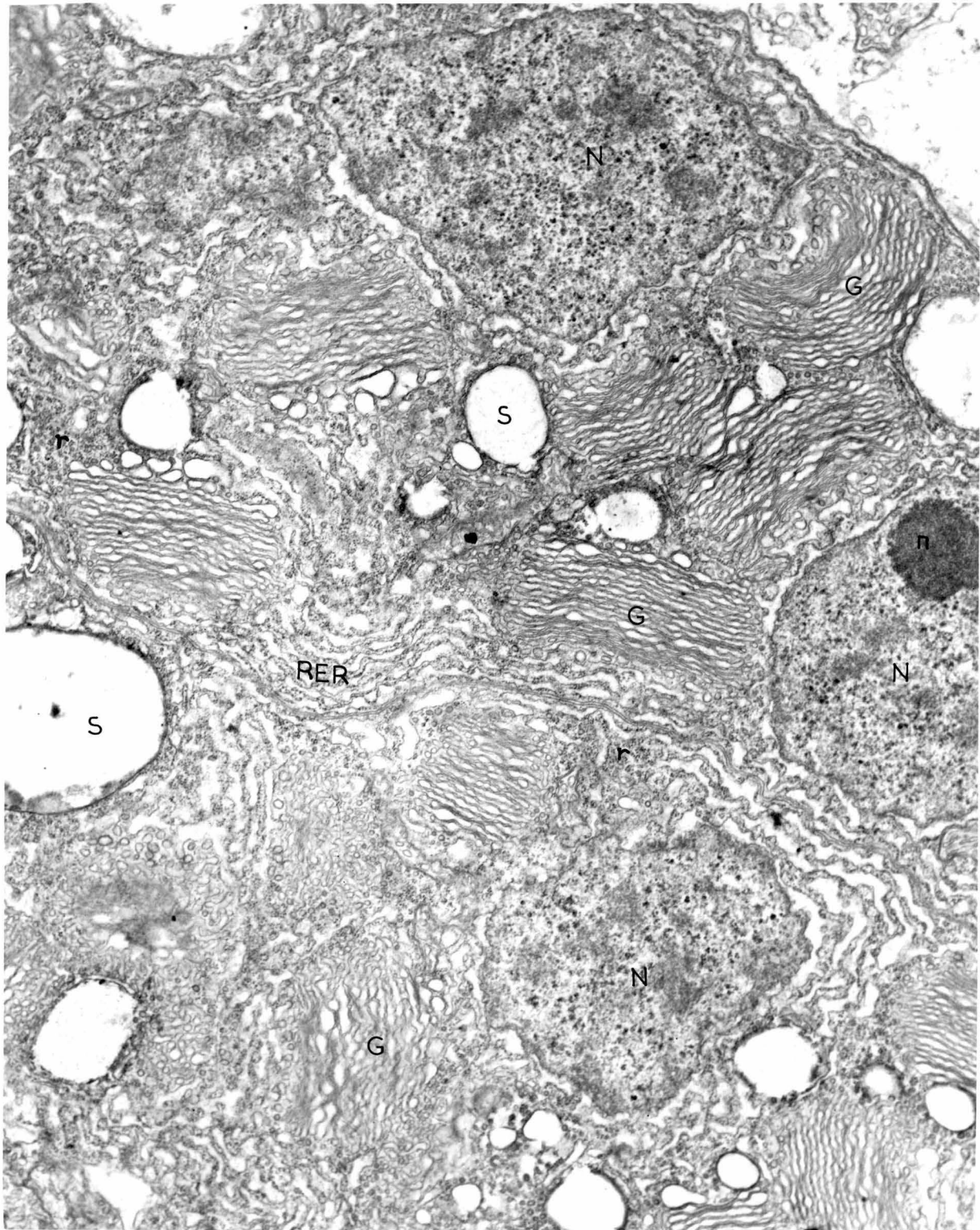
the tegumental cells of <u>Calanus</u> show a basic similarity
to those of <u>Sulcanus</u> with the biggest difference seen
in the structure of the Golgi complexes (Fig. 13).
The Golgi cisternae in <u>Calanus</u> are very expanded and the
concavity of these cisternae lies somewhere midway be-
tween those seen in <u>Sulcanus</u> and those seen in <u>Gladio-</u>
<u>ferens</u>.   As with <u>Gladioferens</u>, I have not as yet found
any "dark cells" or cuticular ducts in <u>Calanus</u>.

DISCUSSION

As stated earlier the positive staining reaction
of the tegumental cells to paraldehyde fuchsin is an
indication of mucopolysaccharides.   The presence of
extensive rough endoplasmic reticulum and numerous
Golgi complexes is further suggestion that the tegumental
cells may be involved in the synthesis of protein for
extracellular export (e.g. Beams and Kessel, 1968). The
secretory role is further confirmed by the presence of
large secretory droplets and the cuticular ducts. It
is however not possible at this stage to determine the
exact chemical nature of the secretory product.

In <u>Sulcanus</u> at least the cuticular duct opens
under the cuticle but does not pierce the cuticle. This
may be a suggestion that the secretory product of the
tegumental cells are involved in the formation of the
new cuticle.   There is no evidence to suggest that the
tegumental cells are involved in lipid synthesis as no

<u>Fig. 13.</u>

Electron micrograph of a section through
parts of a tegumental cell (TC) and an
epidermal cell (E) of <u>Calanus</u>.   The
Golgi cisternae of the <u>Golgi</u> complexes
(G) appear expanded in <u>Calanus</u>.
N = nucleus;   M = mitochondria;   C =
cuticle.   X10,000.
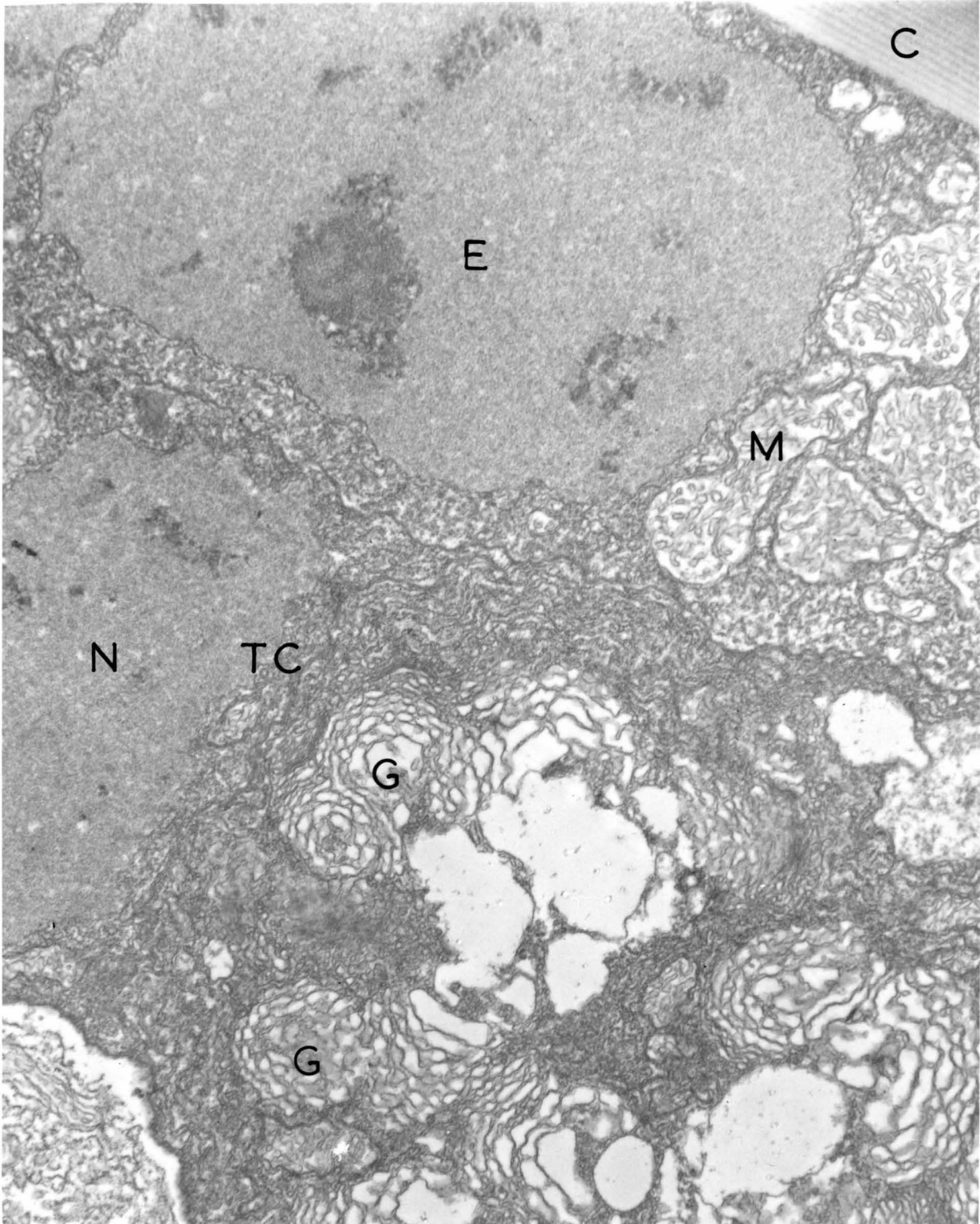
abundance of smooth tubular endoplasmic reticulum was evident. The lack of lipid synthesis would rule out the involvement of the tegumental cells being involved in the synthesis of the cuticulin or wax layers of the cuticle since these layers contain a high proportion of lipids (Wigglesworth, 1965). Two other possible functions exist: the involvement in the secretion of the endocuticle and the involvement in the ecdysial process. Since the epidermal cells are responsible for the secretion of the endocuticle it seems that the tegumental cells may be involved in the ecdysial process. These cells may secrete an enzyme. The tegumental cells could thus be either involved in the secretion of the moulting fluid, possibly in the form of moulting gel, or be involved in the secretion of the moulting hormone which activates the moulting gel. In the Crustacea, crustecdysone and deoxycrustecdysone are the hormones involved in moulting and these moulting hormones are steroids (e.g. Galbraith, Horn and Middleton, 1968). Cells which secrete such hormones have been found to have an extensive system of smooth endoplasmic reticulum (e.g. Locke, 1969). This possibly rules out the tegumental cells as being involved in moulting hormone secretion as they do not contain smooth endoplasmic reticulum. The most likely role of the tegumental cells appears to be the secretion of moulting fluid.

The likelihood of enzyme secretion by the tegumental cells has been mentioned earlier. The opening of the cuticular duct just below the cuticle would make it possible for the moulting hormone to be secreted to its site of action. On the separation of the cuticle from the epidermis the unactivated moulting fluid could pour into the space between the cuticle and the epidermis.

I have as yet not detected cells in these animals similar to the oenocytes of insects. In insects oenocytes have been shown to be involved in the synthesis and secretion of the hormone ecdysone (Locke, 1969). As previously stated, there is a close physical contact between the tegumental gland and Claus's organs and the sensory pore X-organ (also see chapter on these structures). Claus's organs contain cells with extensive smooth endoplasmic reticulum. It is thus possible that Claus's organ and the sensory pore X-organ may be involved in the secretion of the moulting hormone which activates the moulting fluid which is possibly secreted by the tegumental cells. It is also possible that the sensory pore X-organ and Claus's organ secrete substances that stimulate the tegumental cells into activity.

THE CUTICLE

INTRODUCTION

The cuticle of the copepods, like those of other arthropods, is a multilayered structure. The copepod cuticle is not only the skin of the animal but amongst other functions acts as the skeleton, respiratory surface, food store and, when modified it forms auxillary structures such as lenses and sensory structures. There have been numerous reviews on the arthropod cuticle and perhaps the most notable of these is the work of Richards (1951). This work though old is nonetheless still useful. Of the more recent reviews reference may be made to Locke (1964) and Neville (1967). The copepod cuticle is one of the few structures of this crustacean to have been studied with the electron microscope. This perhaps is not surprising since the copepod cuticle, unlike most other crustacean cuticle, is not mineralized and thus easier to section and as will be seen it is almost the ideal material for structural studies. The only published electron microscope studies on the copepod cuticle are those of Bouligand (1965a and 1966).

Light microscope studies on the arthropod cuticle have indicated that the chitin fibrils of the cuticle form a parabolic pattern. This arrangement is also evident in thick sections viewed under the

electron microscope (e.g. Locke, 1964 and Bouligand, 1966). The reality of these parabolic fibrils were in doubt when Bouligand (1965), in an elegant elaboration of Schmidt's (1924) explanation of the structure of the lobster cuticle, put forward the hypothesis that the parabolic patterns seen in thick sections of cuticle were artefacts, caused by Moiré patterns. According to this hypothesis the fibrils were laid down in layers parallel to each other and to the epicuticle. The fibrils within each layer are also parallel to each other. There is however a slight change in the angle between the fibrils of adjacent layers. There is thus a rotation of the fibrils through succeeding layers such that a rotation through 180° results in a complete lamella and the apparent parabola (Fig. 16). Such a hypothesis would provide a uniform basis to explain the various structural features of the cuticle, e.g. the thickness of cuticle and pore canals (Neville, 1967 and Neville et al., 1969). While the Bouligand hypothesis has been generally accepted (e.g. see Neville, 1967), there appears to date, to be a lack of direct support. In the cuticles so far studied, individual chitin fibrils cannot be properly discerned in thin sections.

During this study of the cephalosome of marine and estuarine copepods it was noticed that the cuticle in certain parts of these copepods were thickened and that individual chitin fibrils could be discerned,

especially in very thin sections, of less than 50 nm.
It was thus possible for the architecture of the fibrils
to be resolved.

The following is a description of the structure
of the cuticle, including an illustrated description of
some specialized cuticular structures such as hinges,
muscle attachment and lenses.

OBSERVATIONS

        The structure of the cuticle ventral to the
rostral filaments of <u>Calanus</u> is illustrated in Fig. 14.
The cuticle may be divided into three basic layers:
The Epicuticle.   This layer may be sub-divided into
three further layers (Fig. 14) although a fourth layer
may sometimes be present (Fig. 3).   This fourth layer
is the innermost layer of the cuticle and is similar to
the homogeneous dense layer seen in insects.   The layer
next to this one is the cuticulin, an extremely electron
opaque layer about 20 nm thick.   The cuticulin,
unlike the other layers of the cuticle is almost always
present, except in small areas over certain chemoreceptors
(Fig.. 22 , pp. 47.).   There are two layers outside
the cuticulin.   The inner of these is more or less
homogeneous and slightly less electron opaque than the
cuticulin.   This layer is about 60 nm thick.   The outer
layer appears to be of a similar electron opacity to
the inner layer but is very diffuse.   This layer is about
180 nm thick.   It is not known if these two layers re-
present the orientated and diffuse wax layers seen in
some insect cuticles, (e.g. Locke, 1964).
The Exocuticle.   The exocuticle is possibly best defined
as that part of the procuticle (the cuticle minus the
epicuticle) that is shed with the exuviae of the animal

Fig. 14.

Electron micrograph of a very slightly
oblique section through the cuticle
ventral to the rostral filaments of
Calanus showing the various layers of
the cuticle.   Ep = epicuticle;
Ex = exocuticle; En = endocuticle
and E = epidermis.   X15,000.

Ep

Ex

En

E
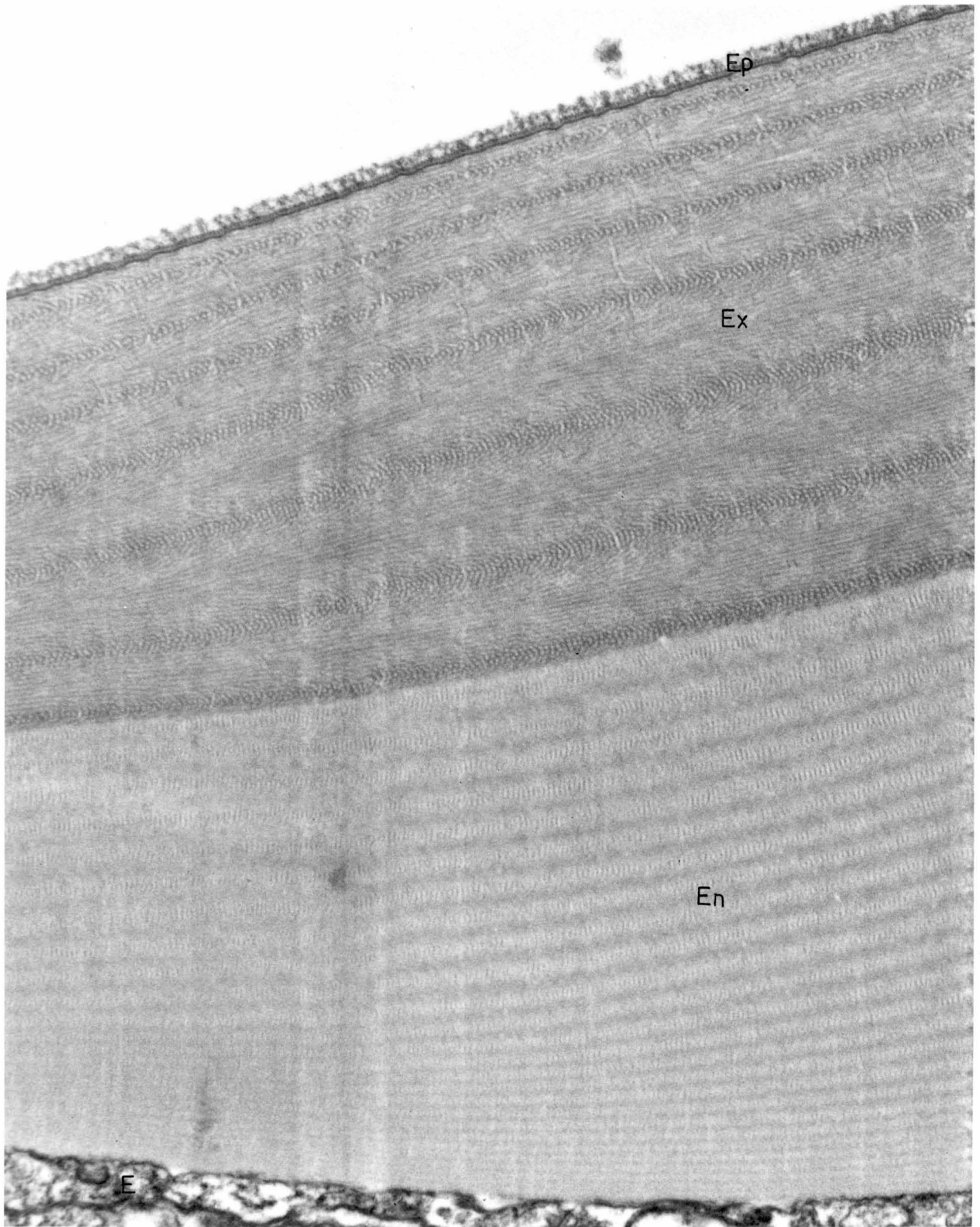
during ecdysis (Richards, 1951). It is difficult to
morphologically define this region of the cuticle in
those arthropods, like the copepods, where tanning is not
evident. A more arbitrary definition is thus used here.
It is generally possible to discern at least two layers
in the procuticle so that the outer layer (the layer
next to the epicuticle) may be termed the exocuticle and
the inner layer the endocuticle. Where a third layer is
present, the middle layer may be termed the mesocuticle.
This is purely a morphological definition (for further
references on terminologies, see Richards, 1951 and 1953).
In many parts of Calanus, like in the other copepods ex-
amined, the exocuticle appears more opaque than the endo-
cuticle and it is not possible to discern any structural
components (Fig. 2) but in other parts of the copepod
a fibrillar component can be discerned in a homogeneous
matrix (Figs. 14 and 15). In the latter the exocuticle
shows an apparent parabolic pattern. It is seen from
Fig. 15 that the apparent parabolic pattern is caused
by electron lucent (possibly chitin) fibrils about 25 nm
in diameter embedded in an electron opaque (possibly
proteinaceous) matrix. Closer examination reveals that
the fibrils are laid down in parallel layers. Six com-
plete lamellae may be discerned in Fig. 15 and the lamellae

Fig. 15.

A higher power electron micrograph of
the section shown in Fig. 14 showing
the layers in the epicuticle and the
fibrillar components of the exocuticle
and the endocuticle.   The cuticulin
is the extremely electron opaque line
(arrows) and above this are a compact
layer and a diffuse layer.   The fibrils
in both the exocuticle (Ex) and the
endocuticle (En) are electron lucent.
Pores (P.) may be seen in the exocuticle.
X25,000.
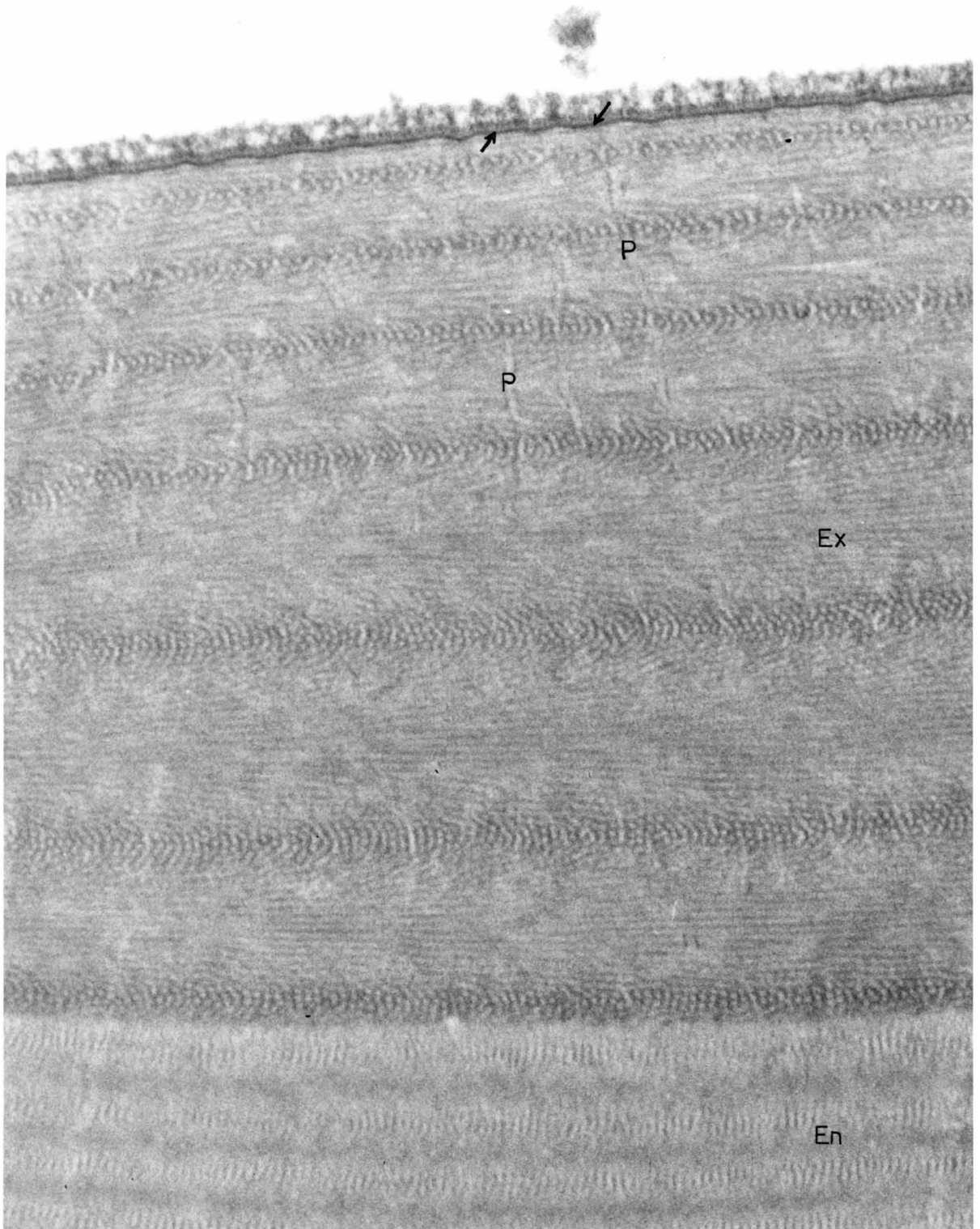
increase in thickness from 0.4 μm next to the epicuticle
to 1.2 μm towards the endocuticle.   The 0.4 μm layer
may be seen to contain seven layers of fibrils and the
1.2 μm layer, twenty-one.   The fibrils are of the same
diameter throughout the endocuticle and there is no
increase in the thickness of the matrix between layers
of fibrils from one lamella to the next.   The thickness
of the lamellae is directly proportional to the number
of fibril layers.

The Endocuticle.   The endocuticle, where the fibrillar
structures cannot be discerned (Fig. 2) appears less
electron opaque than the exocuticle.   In regions of the
cuticle where the fibrillar structures can be discerned
(Figs. 14 and 15) the endocuticle is layered but does
not exhibit the apparent parabolic pattern.   The elec-
tron lucent fibrils are about 0.2 μm long and are
arranged perpendicular to the epicuticle.   There is a
rather thick electron opaque, homogeneous layer of
about 0.1 μm thick between the perpendicular fibrillar
stacks.   This stack arrangement is reminiscent of bipolar
micelle arrangement seen in bipolar structures e.g. the
wax layer in insect cuticle (Locke, 1964).   The fibrils
are of the same diameter as those in the exocuticle but it
is not known if they have a similar chemical composition.

DISCUSSION

   A diagramatic three dimensional reconstruction
of the exocuticle as seen in the copepods is shown in
Fig. 16. This reconstruction is based on the structures
discernable in Fig. 15 and on the Bouligand model. The
fibrils appear to be laid down in parallel layers which
are parallel to the epicuticle. The fibrils are parallel
to each other within the same horizontal layer but there
is a small change in the angle of orientation of the fib-
rils from one horizontal layer to the next. There is
thus a progressive horizontal rotation of the fibrils
from layer to layer such that a rotation through 180°
results in a complete lamella or the apparent parabola
seen in thick sections. The relationship between the
number of fibril layers (or laminae) and the change in
the angle of orientation of fibrils between adjacent
fibril layers is given by the expression: $n - 1 = \frac{180^O}{\alpha}$,
where n = number of laminae (or fibril layers) in the lam-
ella and α = the change in the angle of orientation
of the fibrils between adjacent laminae. Thus the 0.4 um
lamella with its seven layers of fibrils undergoes a
rotation of 30° per lamina and the 1.2 um lamella with
its twenty-one laminae goes through a rotation of 9°
per layer of fibrils. The fibril structure described
here in the exocuticle provides direct support for the
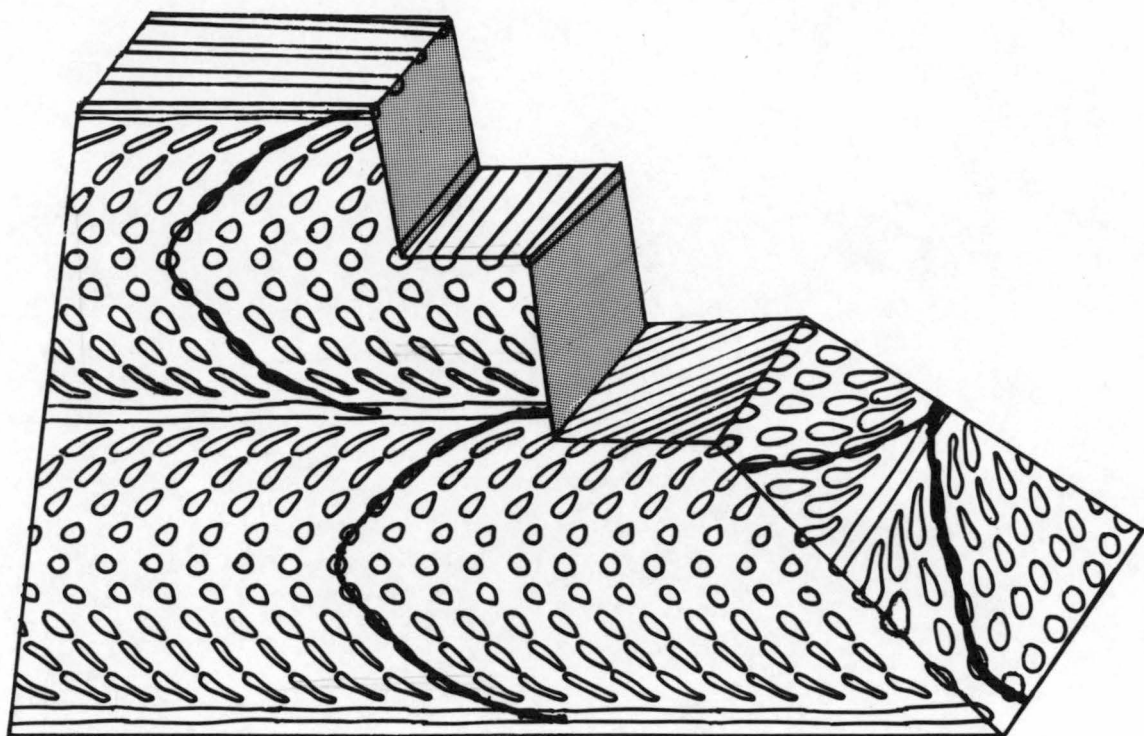
Fig. 16.

A diagramatic three dimensional reconstruction
of the cuticle of Calanus showing the arrangement
of fibrils, based on Fig. 15 and on the Bouligand
model.   The oblique cross-sections of the fibrils
are exaggerated.   The fibrils are laid down in
layers (with fibrils within each layer parallel
to each other) parallel to each other and to the
surface of the cuticle but there is a small
horizontal rotation of the fibrils from layer
to layer.   In this diagram there are eleven fib-
ril layers (or laminae) per lamella such that the
angle or rotation from one lamina to the next is 18°.
The front and side faces are obliquely cut.

Bouligand hypothesis.   The observations in this

study do not support the alternative hypothesis based

on parabolic fibrils.   It must however be noted that

the fibrillar exocuticle discussed here may be different

in other parts of the animal or in other species (e.g.

see Neville, 1967 on the structure of the tunicin

of the Tunicata).

Fig. 18.

Electron micrograph of a section through
the hinge of a seta of the maxilliped
of Gladioferens.   Note that the arthrodial
membrane is thinner than the surrounding
cuticle.   The epicuticle (arrow) is
continuous but the "membrane" is of a
different composition to the other parts
of the cuticle, being less electron opaque.
Ci = cilium of sensory neuron of seta.
X7,000.

Fig. 19.

Electron micrograph of a section of part
of the hinge of the rostral filament of
Calanus.   X7,500.
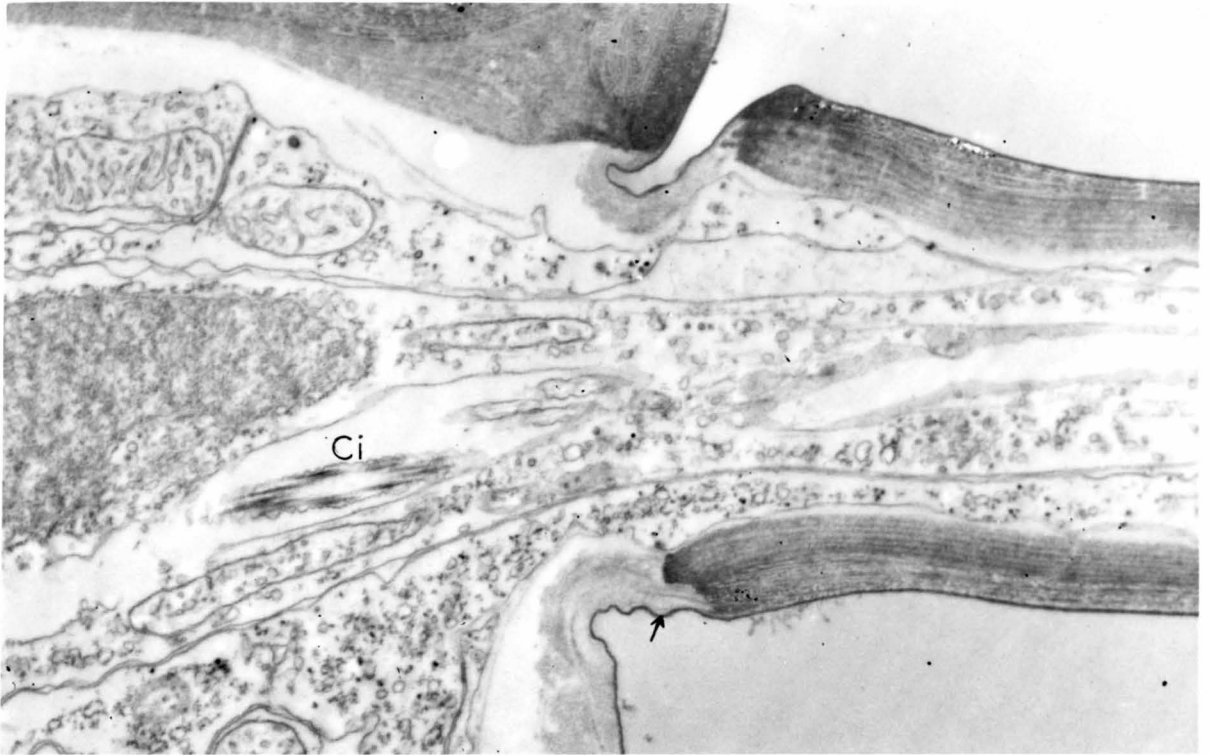
Fig. 20.

Electron micrograph of a section through
the cuticle of Calanus showing one type
of muscle attachment.   Here the fibrils
are embedded in the cuticle, the cuticle
showing no apparent specialization.
X7,500.

Fig. 21.

Electron micrograph of a section through
part of the cuticle of Calanus showing a
type of muscle attachment different to
that seen in Fig. 20.   The cuticle is
modified and the muscle is attached to
projections of the endocuticle via
tonofibrils.   X34,500.
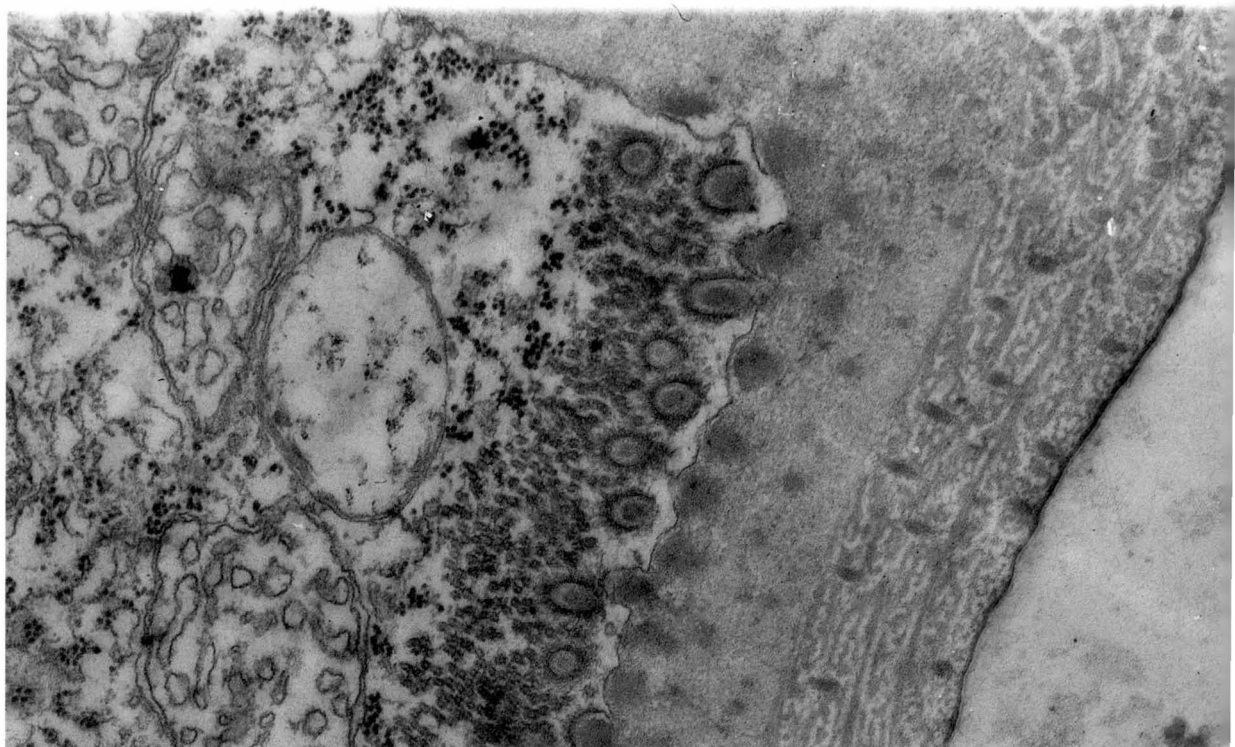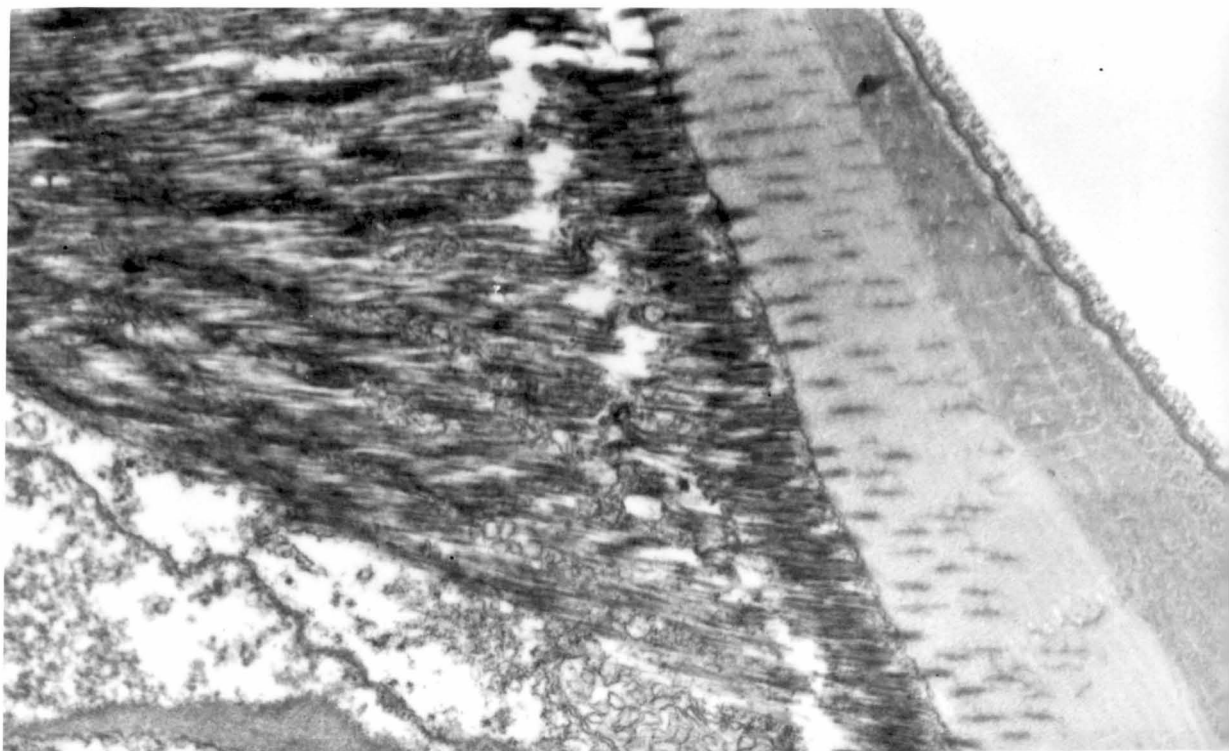
Fig. 22.

Electron micrograph of a section of the
cuticle of Sulcanus, over the chemo-
receptors of the sensory pore X-organ
showing the pores (arrows) in the cuticle
and the absence of the epicuticle. This
appears to be the only part of the animal
where the epicuticle is absent.
X42,000.

Fig. 23.

Electron micrograph of a section through
part of the dorsal lens of the pontellid
copepod, Labidocera.   The lens appears
to be laid down in layers similar to the
cuticle.   X13,200.
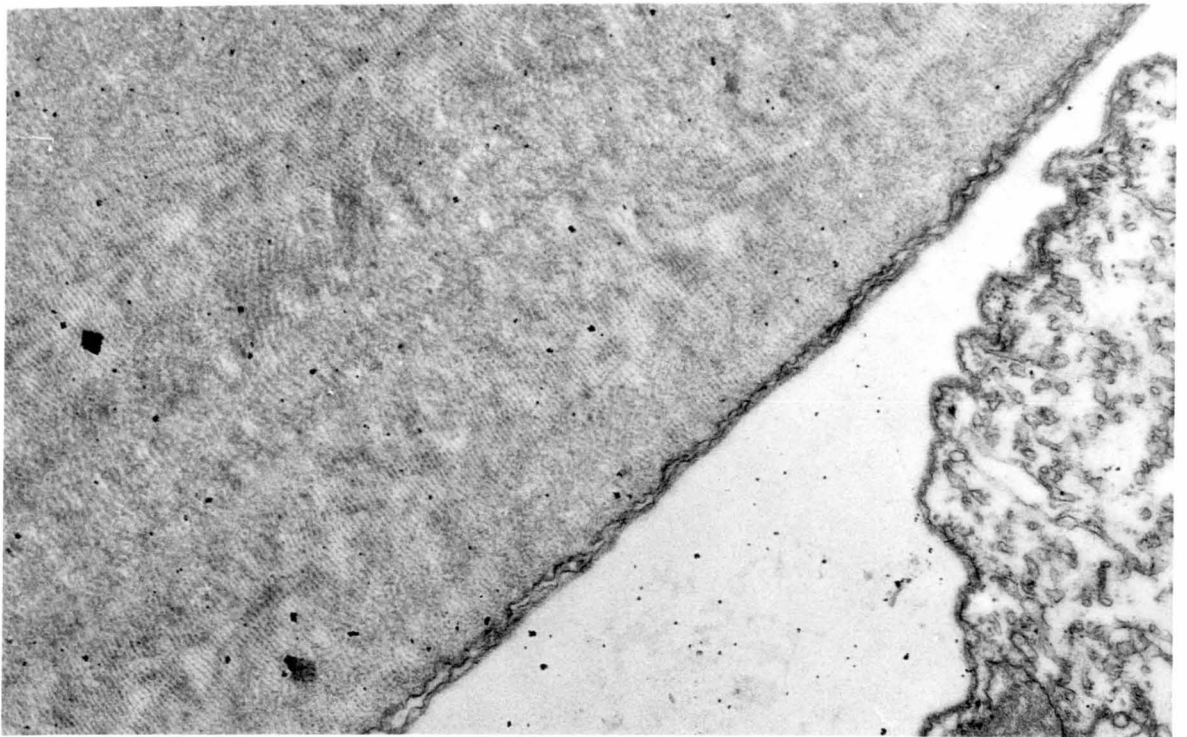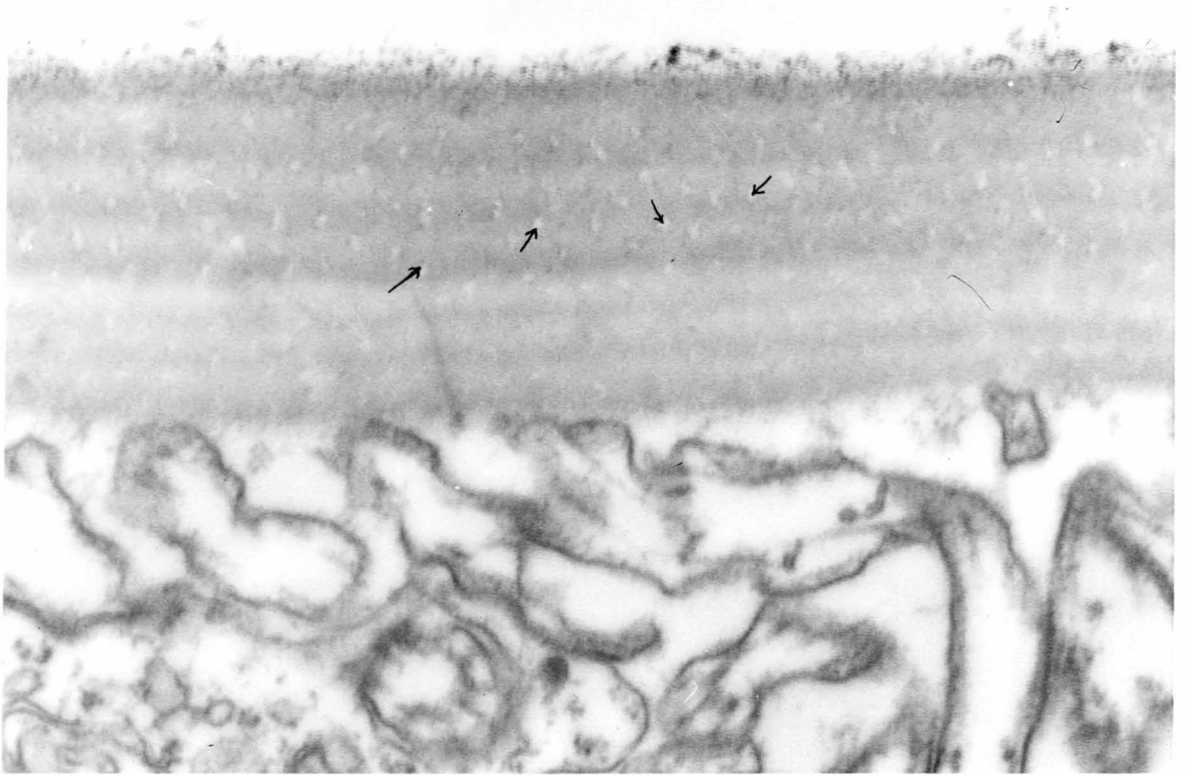
Fig. 24.

Electron micrograph of a section through
the tip of the mandible of Gladioferens.
The electron opaque substance at the tip
of the mandible is extremely brittle as
seen by the tears in the section.   The
hardness of this material is possibly
due to impregnation with Ca^{++}.
X9,000.

Fig. 25.

Electron micrograph of the cuticle in the
mandible of Gladioferens, cut almost
parallel to the surface of the cuticle.
The fine fibrils seen in the middle of
the micrograph are possibly those in the
endocuticle.   The fibrils of the exo-
cuticle can be seen on either side of
the micrograph (parabolic pattern).
X12,500.
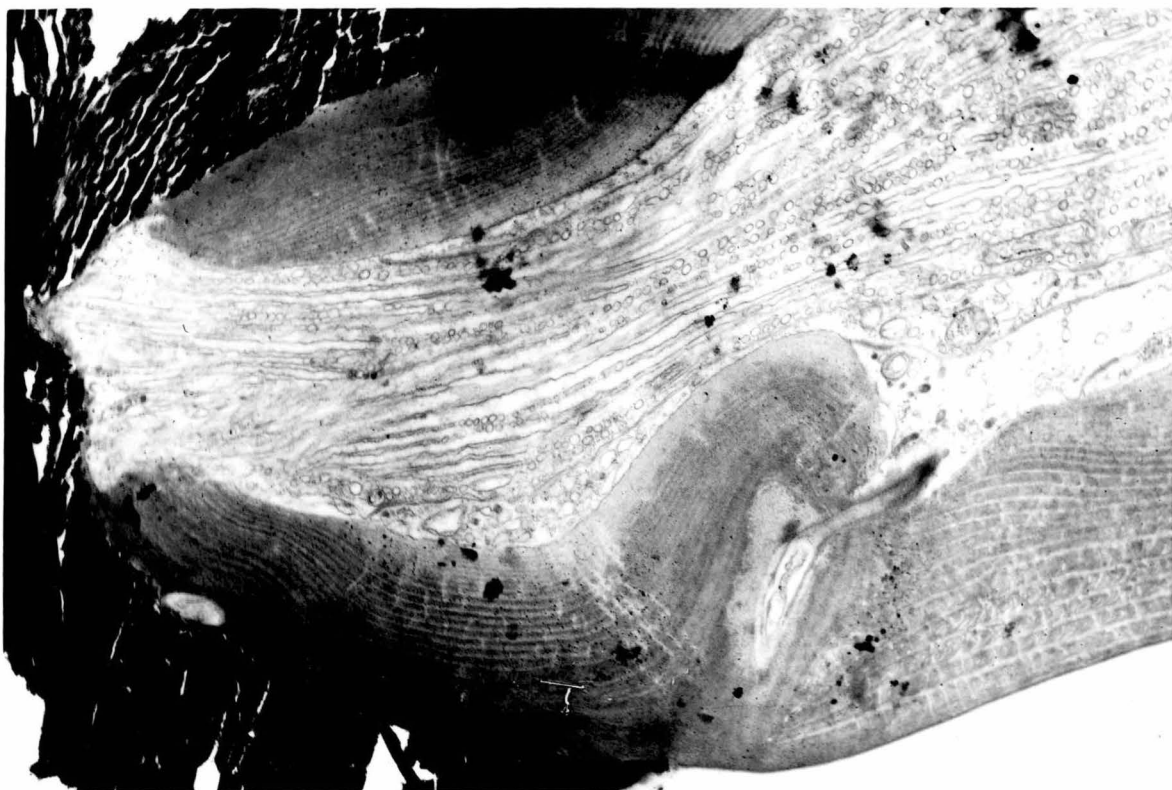
PERMEABILITY OF THE CUTICLE TO HALIDES

When live Gladioferens pectinatus were treated with silver nitrate to determine the possible sites of permeability to halides it was found that silver was deposited in patches in different parts of the animal. One of the areas at which silver was deposited was near the tip of the head of the animals. This appears to coincide with the position of the anterio-dorsal tegumental gland (the structure of which has been treated in this chapter).

These silver granules are also found in the epidermal cells overlying the tegumental cell as well as in the tegumental cells themselves (Fig. 26). In the tegumental cell the silver granules occur in the cytoplasm around the nuclei and within the cisternae of the endoplasmic reticulum but there are no silver granules in the Golgi complex. No silver granules can be seen in the haemocoel and this suggests that the concentration of halides in the haemocoel is low, the basement membrane of the integument is impermeable to silver ions or that it takes more than thirty minutes (the period of time the animals were kept in silver nitrate solution) for the silver ions to get into the haemocoel from the exterior of the animal. This experiment is too preliminary to

Fig. 26.

Electron micrograph of a section through
part of the anterio-dorsal tegumental gland
of the head of Gladioferens. The living
animal was treated with 10^{-4} M silver nit-
rate solution. The highly electron opaque
granules are silver particles. These
particles can be seen in the cuticle (C),
the epidermal cell (Ep) and the tegumental
cell (TC) but not in the haemocoel (H).
Note that the granules occur in the endo-
plasmic reticulum (ER) but not in the
Golgi cisternae (G) or in the secretory
droplets (S) of the tegumental cell.
N = nuclei.   X19,500.
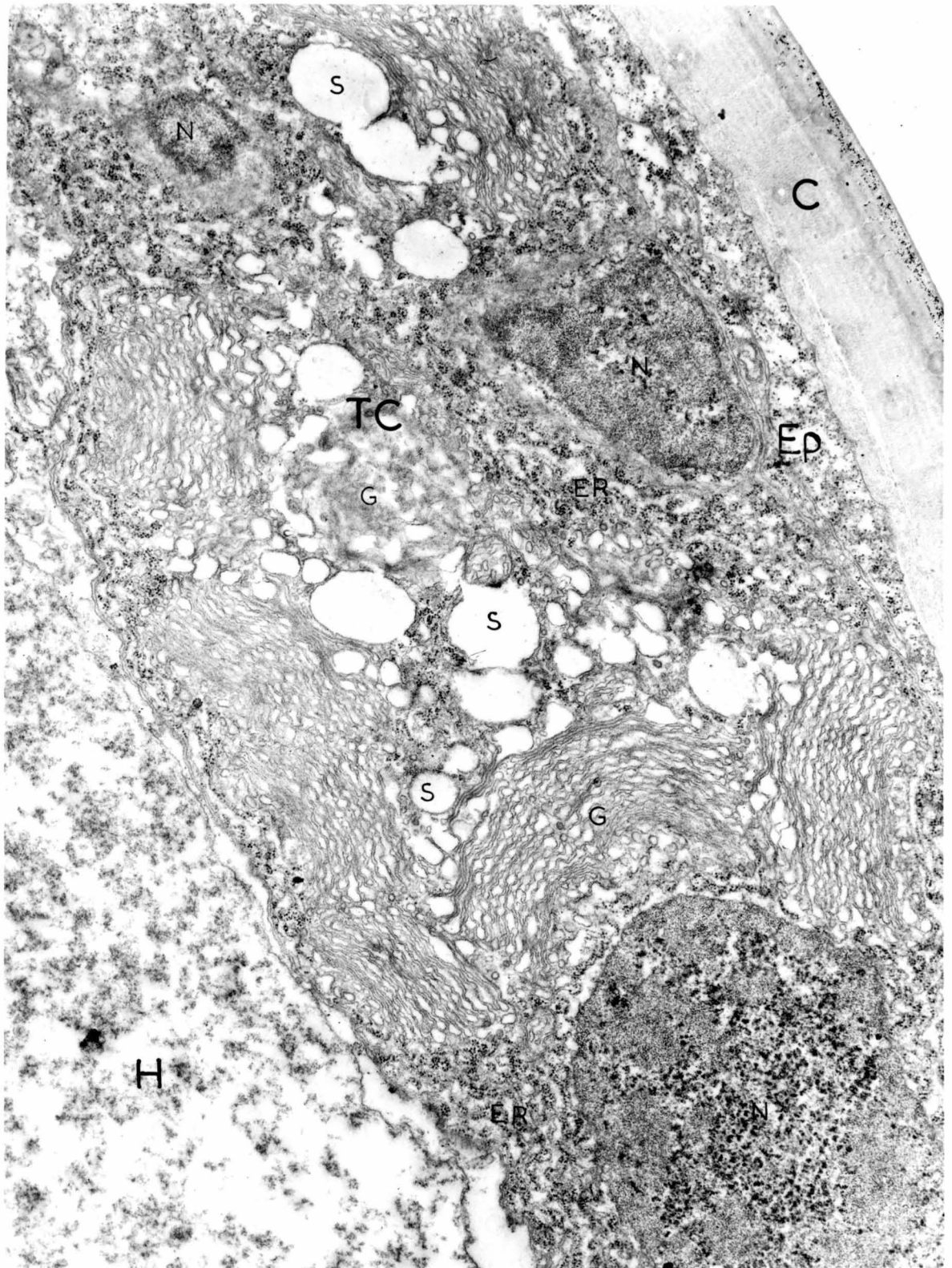
specify which of the above is true and only demonstrates that there are areas of the copepod cuticle that are permeable to such common ions as sodium and chloride which are the common ions found in their natural environment.

CONCLUDING REMARKS

The copepod integument appears to be basically similar to the integument of other arthropods, especially those of insects. The exocuticle in the copepods do not appear to be as deeply tanned as in most insect cuticle and it is not mineralized, apart from the tips of the mandibles (Fig.24 pp. 48.). The difference between the copepod integument and the generalized insect integument is illustrated in Fig. 17. In the insects the dermal glands have been associated with the secretion of the cement layer of the epicuticle (Wigglesworth, 1965). The tegumental glands of copepods appear to be morphologically similar to the insect dermal glands but it appears that the tegumental glands in copepods are more likely to be involved in the secretion of moulting enzymes.

Locke (1969) has shown that in the insect, Calpodes ethlius the oenocytes are possibly the site of ecdysone synthesis which provides the stimulus for moulting. I have not yet found any oenocyte-like cells in the copepods but some of the cells of Claus' organs and the sensory pore X-organ contain extensive smooth tubular endoplasmic reticulum reminiscent of insect oenocytes. These cells are either in contact or in close proximity to the anterio-dorsal tegumental

A.

CEMENT — ?

WAX

CUTICULIN
DENSE HOMOGENEOUS
INNER EPICUTICLE

STABILIZED ENDOCUTICLE —
= EXOCUTICLE

MESOCUTICLE ?

ENDOCUTICLE

EPIDERMIS

OENOCYTE ?

DERMAL GLAND

B.

TEGUMENTAL GLAND
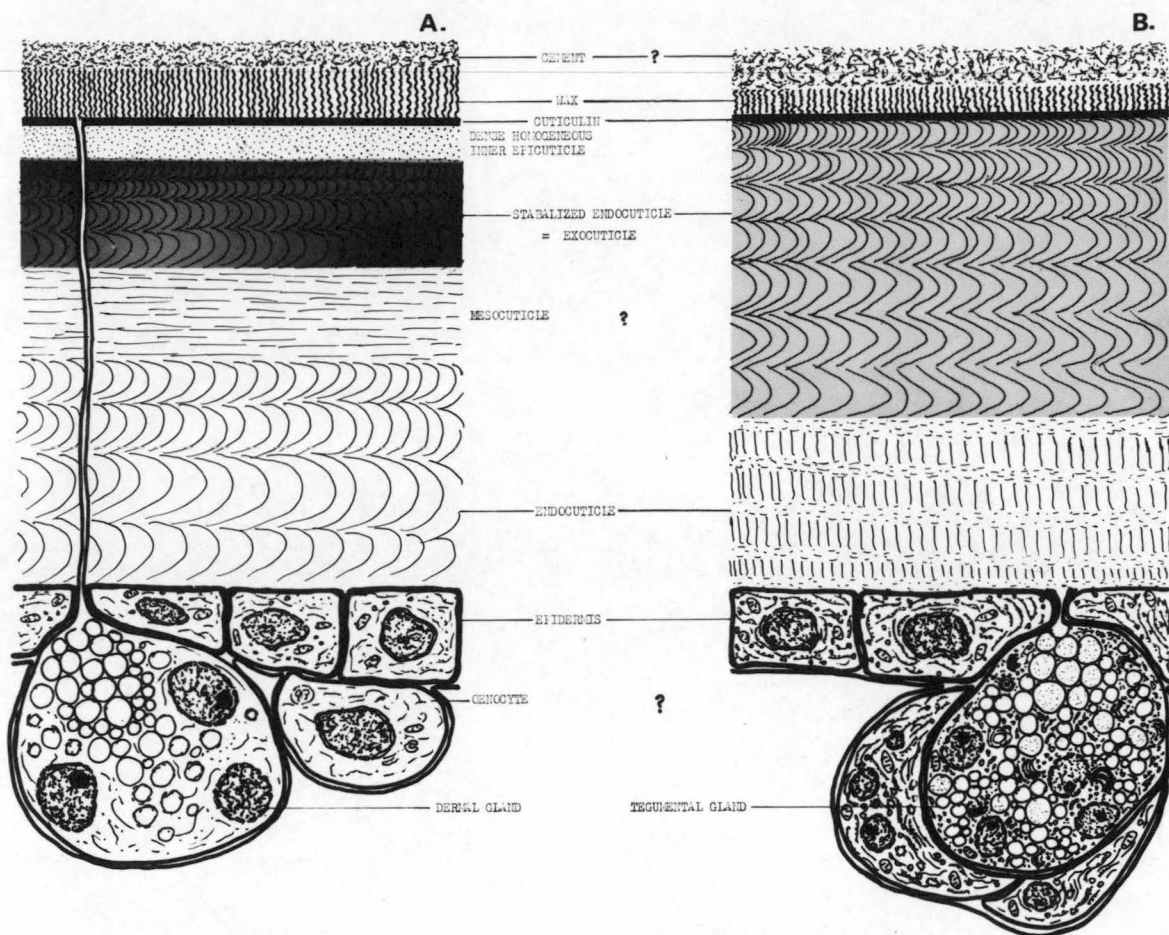
Fig. 17.

Diagram comparing the idealized insect integument (A)
with the copepod integument (B). The copepod cuticle
illustrated is one where the fibrillar structure
of the procuticle (cuticle minus the epicuticle)
can be discerned.   The structure of the cuticle
varies from one part of the animal to the next.
Further description of this diagram is given in
the text.

cells of the head of the copepod.   It is possible that these cells secrete the moulting hormone or other substances that stimulate both moulting and the tegumental cells into activity.

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Electron micrograph of a section through
the nauplius eye of the marine calanoid
copepod, Calanus helgolandicus (Claus).
The boundary between parts of two rhab-
domeres can be seen.   The rhabdomeric
microvilli are cut in transverse section
and the tightly packed arrays of micro-
villi is most evident.   The rhabdomere
is thought to be the part of the eye
sensitive to light.   X30,000
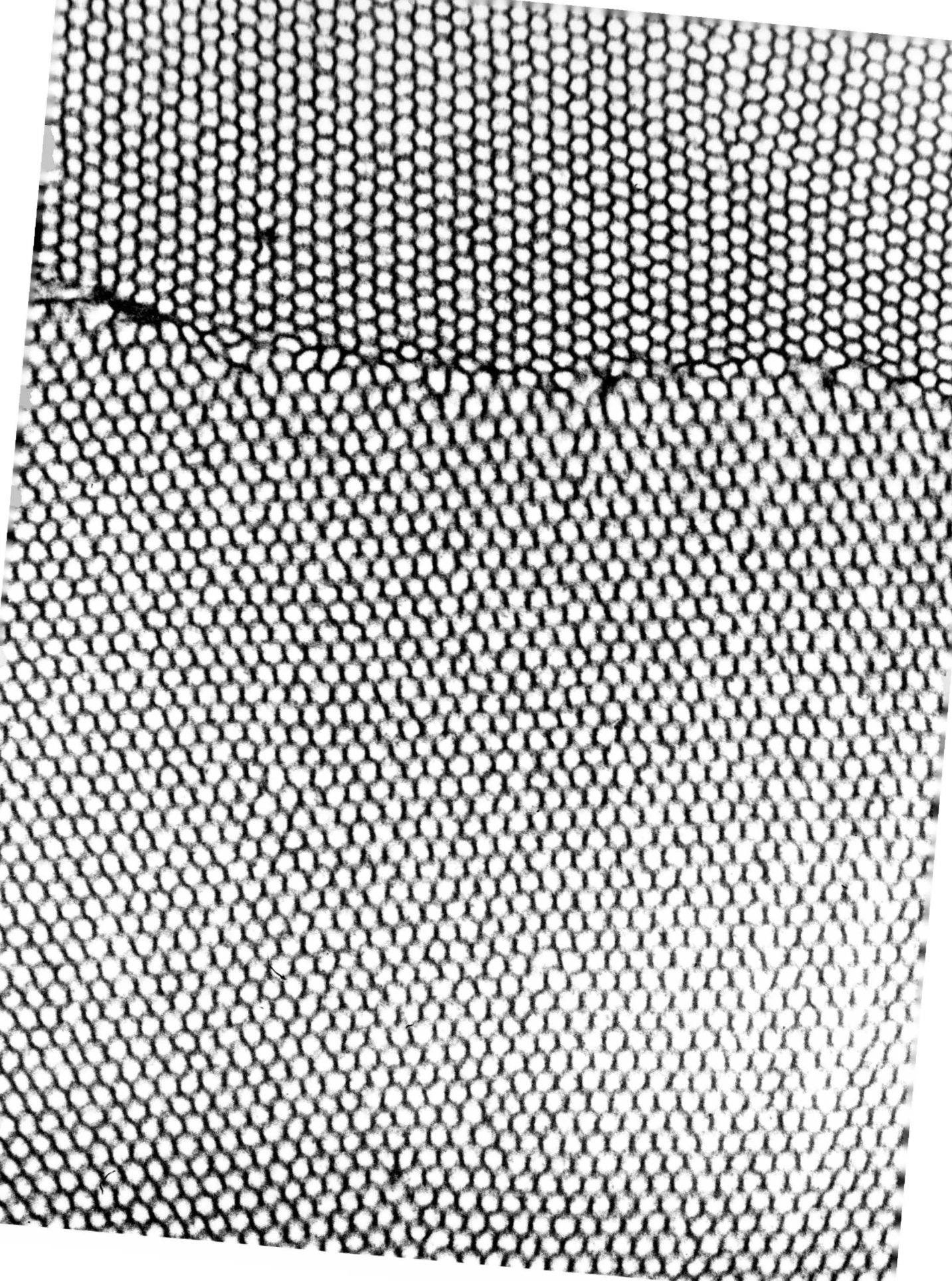
THE SENSORY SYSTEM

INTRODUCTION

Apart from the nauplius eye, little is known of the other sensory structures in the copepods. Structures such as frontal organs and statocysts have been reported but doubts have been expressed as to their existence (see Elofsson, 1966). Still less is known of the minute sensilla of the integuments of the copepods.

Although the gross anatomy of the copepod eye is well known (e.g. Claus, 1863; Elofsson, 1966 and Park, 1966), there was until recently only a single published work on the ultrastructure of the nauplius eye (Fahrenbach, 1965). Two additional studies have been published in the last year; Wolken and Florida (1969) and Elofsson (1969). These two studies are on Copilia and Sapphirina, both of which have apparently aberrent and specialized eyes (see Elofsson 1966a).

In his comprehensive study of the nauplius eye and the frontal organs of the Entomostraca, Elofsson (1966) was unable to locate the presumed statocysts (or Claus's organs) in the copepods he studied. These included Calanus finmarchicus, a species from which the statocysts had previously been reported to occur. Elofsson, in the same study, also expressed the opinion that dorsal

frontal organs (=reduced photoreceptors) do not occur in the class Copepoda.

Bouligand (1966) figured and very briefly described what may possibly be the terminal portion of a sensillum in the parasitic copepod, <u>Lamippe rubicunda</u>. Krishnaswamy et al. (1967) were unable to locate any sensory structure on the maxillae and maxillipeds of the bathypelagic calanoid copepod, <u>Centraugaptilus horridus</u>. There were thus doubts as to the existence of integumental sensilla in the copepods.

The following are the results of electron microscope studies undertaken in an attempt to resolve the problems stated above. The ultrastructure of the nauplius eye, the dorsal frontal organs and the integumental sensilla of some marine and estuarine calanoid copepods are described, with discussions on their possible functions. The alleged statocysts or Claus's organs are described elsewhere in this thesis.

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The other references cited are given at the end of each of the sections.

THE NAUPLIUS EYE.

INTRODUCTION

The photoreceptors in Crustacea are represented by two highly developed systems: the compound eyes as seen in the Decapoda and the nauplius eyes as seen in the Copepoda.   In most groups both eye types are present but in the Copepoda only the nauplius eye is seen and this is the functional photoreceptor in both the larval and adult forms.   Within the Copepoda two lines of evolution of the eye are apparent;   as illustrated by one line leading to the apparently well developed eye of the Pontellids and the other line leading to the apparently aberrant eye of the Corycaeids and the Sapphirinids.   Since the early works of Claus (1859 and 1863) the nauplius eye of the copepods has interested numerous researchers.   An historical review of the various interpretations of the copepod nauplius eye is given by Elofsson (1966).   It is evident from the review of Elofsson (1966) and the earlier review of Vassiere (1961) that a considerable number of discrepancies, most of them interpretative, exist.   This is due mainly to the limited resolution of the light microscope and it is indeed surprising that to date there appear to be only three published studies of the copepod nauplius eye using the electron microscope.   These are Farenbach's (1964) work on

the Cyclopoida <u>Macrocyclops albidus</u>, Wolken and Florida's (1969) work on <u>Copilia</u> and the work of Elofsson (1969) on <u>Sapphirina</u>.

It is noted that the cited works are all on the Cyclopoida and while these have helped clear many ambiguities they have also raised new problems. It was thus felt that an ultrastructural study of a Calanoida might serve as a useful comparison. The following is an ultrastructural study of the morphology, including the innervation of the nauplius eye of the Australian estuarine calanoid copepod, <u>Sulcanus conflictus</u> Nicholls.

<u>OBSERVATIONS</u>.

<u>Gross Morphology</u>

The position of the eye within the cephalosome of the copepod is shown in Fig. 1. The eye is shown in its relation to the brain and the X-organ complex. Fig. 1A depicts the position of the eye as seen in the dead or preserved animal and Fig. 1B depicts the position of the eye in the live animal. It is seen that the so-called ventral ocellus is actually in the anterior and anteriorly directed position in the live animal. Thus the so-called ventral ocellus is in effect more correctly the anterior ocellus. To avoid confusion the terms median and lateral will be used in place of the terms ventral and dorsal respectively. The proposed terms are neutral with respect to
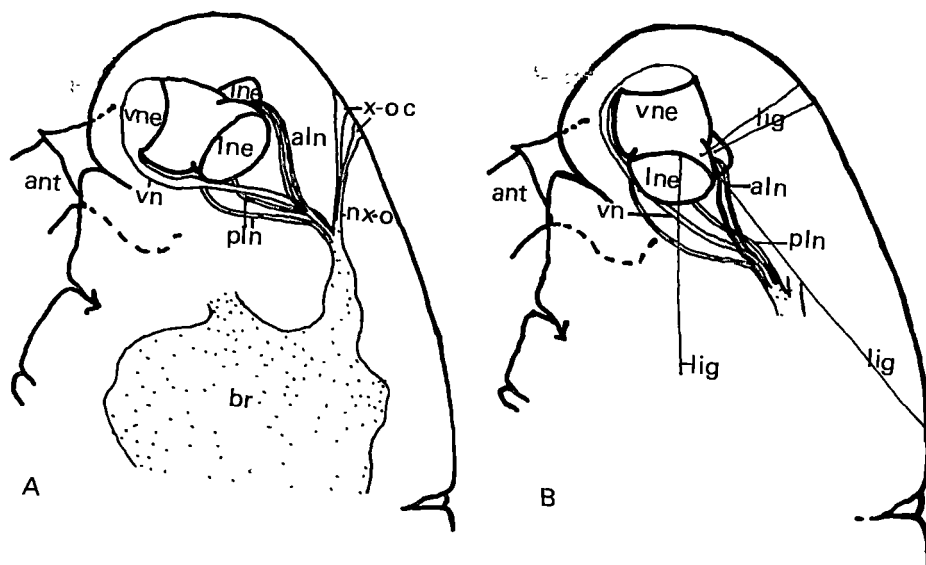
Fig. 1.

Diagram of the cephalosome of Sulcanus showing the positions of the nauplius eye and the nerve bundles leading away from the eye. Fig. 1 A shows the usual position of the eye as seen in formalin fixed material and in the dead animal. Fig. 1 B shows the usual position of the eye as seen in the living animal. The living animal is able to direct the median ocellus through an arc of about 90°, i.e. from the position shown in Fig. 1 A to that shown in Fig. 1 B and vice versa.

aln	anterior lateral nerve to lateral ocellus
ant	first antenna
br	brain
lig	ligament
lno	lateral ocellus
mn	nerve bundle to median ocellus
mno	median ocellus
nx-o	nerve to X-organs
pln	posterior lateral nerve to lateral ocellus
x-oc	X-organ complex.

the orientation of the eye and thus less confusing. Positions of the eye subsequently referred to in this paper refer to the orientation shown in Fig. 1A.

The eye in Sulcanus is very conspicuous and can be readily seen without the use of a microscope. The tri-partite nature of the eye is evident under a binocular dissecting microscope but it normally appears red in colour. Animals with blue eyes[*] have been observed and this could be due to genetical variation. The red colour could be a pigment similar to the visual pigment rhodopsin. The pig-ment cups of the eye are black and the red colouration comes from the retinular cells.

The nerves of the eye are not readily visible in whole mounts of live animals examined under the light micro-scope but ligaments suspending the eye in the haemocoel are quite evident. In the live animal the movements of the eye can be easily seen under the microscope. There is an anterior-ventral swing of the eye with the pivoting axis through the two lateral ocelli. The animal is thus able to move its eye from the position figures in Fig. 1A to that shown in Fig. 1B and vice versa. Observations on

[*]ADDENDUM

The blue colour observed in the eyes of some of the animals may be due to reflection from the tapetum. A similar phenomenon was recently described by E. J. Denton (Phil. Trans. Roy. Soc., Lond. 258 : 285-313. 1970) from the eye of Pecten (Mollusca).
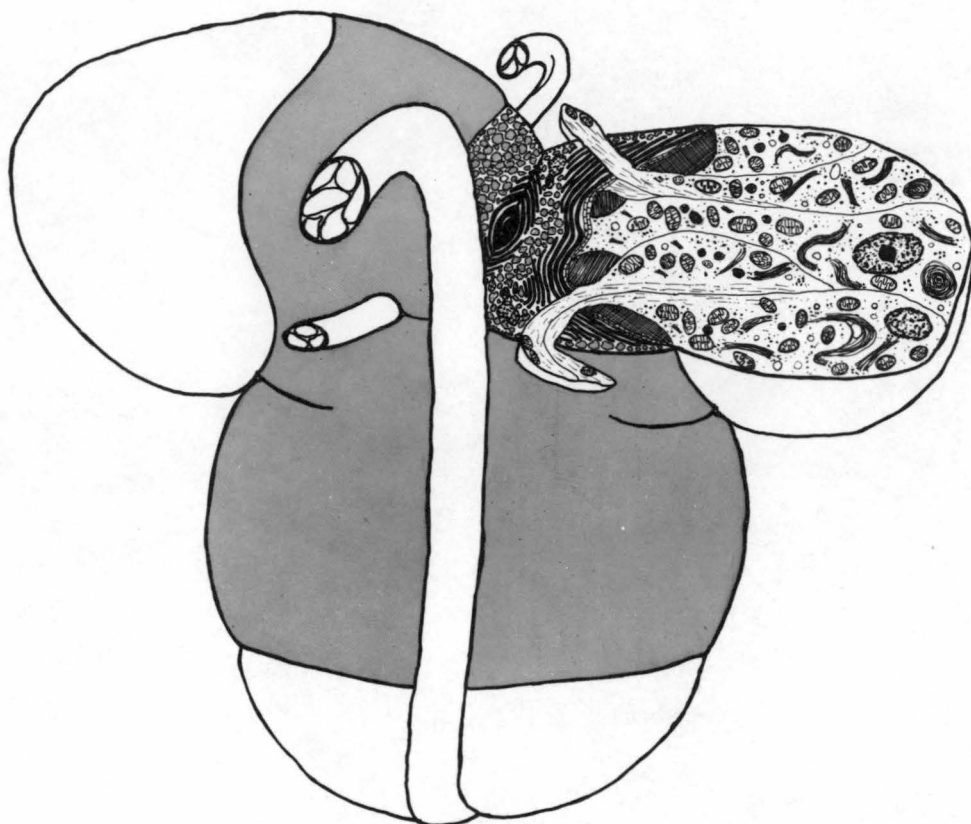
Fig. 2.

Schematic reconstruction of the nauplius eye of
Sulcanus showing one of the lateral ocelli in
section.   The grey area represents the pigment cups.
The whole eye as well as the nerve bundles are enve-
loped in a very thin sheath.   The nerve from the
median ocellus leaves the ocellus posteriorly, in
a single nerve bundle.

<u>Innervation.</u>

A total of five nerve bundles run from the eye to the brain (Fig. 1). Two nerve bundles leave from each of the two laterall ocelli and a single nerve bundle leaves from the median ocellus (Fig. 2). Each of the nerve bundles from the lateral ocelli consists of three axons and the bundle from the median ocellus contains six axons. The nerve bundles from the lateral ocelli leave the ocelli through the anterior and posterior parts of the pigment cups, through "breaks" in the pigment cups but the nerve bundle of the median ocellus leaves the ocellus not through a "break" in the pigment cup but via the posterior rim of the pigment cup. Posteriorly the nerve bundle from the anterior part of the lateral ocellus joins up with the nerve bundle from the posterior part such that at this region only three nerve bundles can be discerned. Slightly posterior to this region the nerve bundles from the median ocellus joins up with the bundles from the lateral ocelli to form a single optic nerve. The optic nerve then joins up with the nerves from the X-organ complex (which comes in dorsally) and together these return to the protocerebrum. There is no efferent nerve from the brain to the eye.

<u>Ultrastructure.</u>

<u>General.</u> While all three ocelli are closely associated with one another the median ocellus is structurally different from the two identical lateral ocelli. The close

Fig. 3.

Diagramatic reconstruction of a transverse section
through the nauplius eye of Sulcanus showing the
two pigment cells (PC) which forms the cups of the
lateral ocelli (LO) and the median ocellus, and the
arrangements of the cells of the median ocellus.
S_1, S_2 and S_3 form the superior group of retinular
cells of the median ocellus.   The inferior group of
retinular cells consists of a central retinular cell
(S_4), with its terminal cells (S_5 and S_6), with their
hemi-annulate rhabdomeres (R_a).   The pigment cups are
lined by the "reflectile plates" of the tapetal cells
(T).   CC = conjunctive cell.   X1,500.
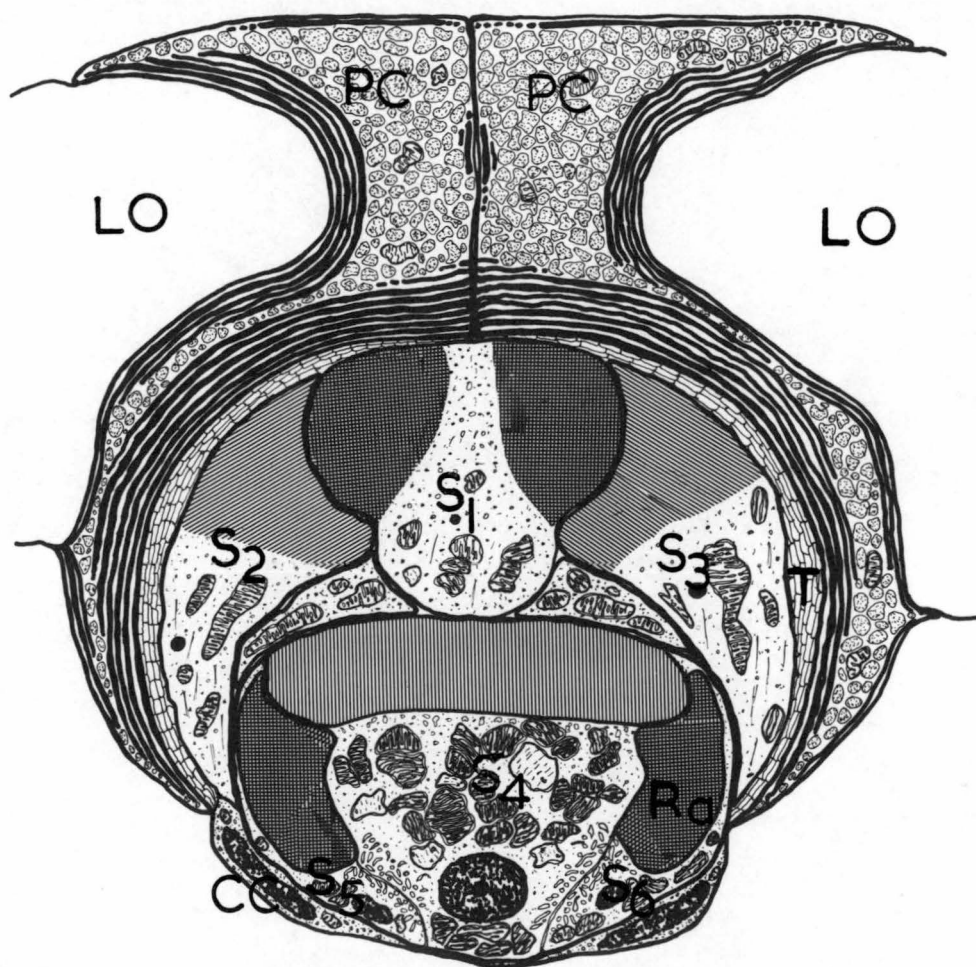
association of the three ocelli is seen especially by the fact that the three pigment cups are formed from only two cells and each of the cells that forms each of the lateral ocelli contributes to the formation of half of the pigment cup of the median ocellus. For ease of description the pigment cups, the lateral ocelli and the median ocellus will be described separately.

The Pigment Cups. Two large syncitial cells make up the three pigment cups; each cell forming the whole of one lateral cup and half of the median cup (Figs. 3 and 4). These cells contain at least six and possibly ten nuclei per cell (the exact number of nuclei present per cell is not clear because the nuclei are extremely irregular in shape and I have not been able to obtain complete serial sections for electron microscopy).

The pigment cups show an apparently high degree of orientation. The parts of the cells that form the inner surfaces of the pigment cups consist of layers of tightly packed and extremely electron dense spheres. These are packed in membrane bounded layers (Fig. 5). A section taken parallel to and in the centre of the pigment layer shows the pigment "spheres" to be hexagonal in section, illustrating the extremely tight packing. There are up to fifteen of these pigment layers towards the bottom of the pigment

Fig. 4:

Electromicromontage of a frontal section
through the nauplius eye showing the two
symmetrical lateral ocelli.   The section
is slightly oblique (anterio-posteriorly)
such that only the axons (a) leaving from
the anterior of the ocelli, through the
pigment cups (PC) can be seen.   The spaces
between the tapetal layer (T) and the pig-
ment layers (pl) of the pigment cup are
dehydration artefacts.   The rhabdomeres
(R) are next to the tapetal layer and the
nuclei (N) at the opposite ends to the
rhabdomeres.   The whole eye is enclosed
in a thin sheath (sh).
CC = conjunctive cell, lp = lipid pigment,
P = phaososome, M = mitochondrion and
H = haemocoel X 2,500.
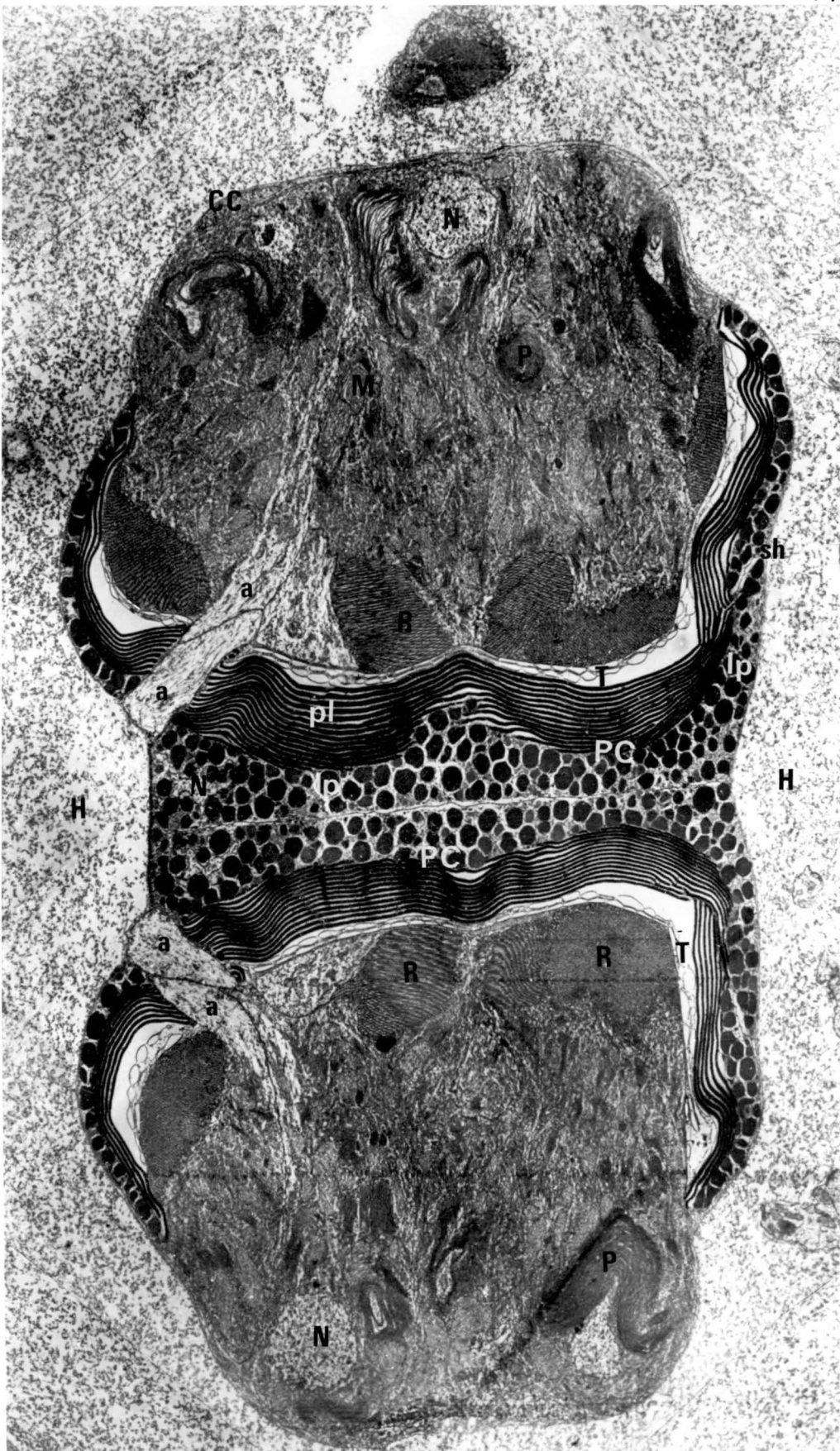
cups and a single layer towards the rims of the cups. The outer surface of the cell forming the ventral half-cup is also lined by up to three layers of pigment. The rest of the pigment cell is filled with what appears to be lipid bodies (Fig. 5), a few scattered mitochondria and the nuclei. The lipid bodies are of moderate electron density and possibly contain lipid soluble pigments or caroteinoids. The nuclei are present in the anterior and posterior regions of the pigment cells.

The Tapetal Cells. Closely associated with the pigment cups and lining the insides of the pigment cups are layers of what appear to be crystalline reflectile bodies (Figs. 3 and 4) of the tapetal cells (Fig. 6). These apparent reflectile bodies appear to be crystalline structures measuring about 1.1/um by 0.6/um by 0.15/um thick (Fig. 7). These "crystals" appear to be leached out during the preparation of the tissue for electron microscopy as I have been unable to obtain defraction patterns from this layer. The number of tapetal cells to each ocellus has not been determined but there are at least two per ocellus. In some of the micrographs (e.g. Figs. 4 and 11) a space can be seen between the pigment cell and the tapetal cell: this is a dehydration artefact. This artefact ·is difficult to eliminate even though care was taken to ensure

Fig. 5:

Electronmicrograph of part of the pigment cup
showing the two types of pigments.   The
extremely electron dense pigment granules
are arranged in membrane bonded layers (top
of electronmicrograph) while the moderately
electron dense type are in the lipid drop-
lets (lp), seen at the bottom of the
electronmicrograph.   X 24,750.

Fig. 6:

Electronmicrograph of a frontal section
through part of a lateral ocellus showing
parts of two retinular cells (a),
part of the pigment cup (PC), and the cell
bodies of a tapetal cell (TC) and a conjun-
ctive cell (CC).   The mitochondria (M)
and nuclei (N) and phaosomes (P) of the
retinular cells are quite conspicuous.
X 4,300.

Fig. 7:

Electronmicrograph of a section through the
"reflectile layers" of the tapetal cell. The
"crystals" appear as flat membrane bonded
rectangular plates and are apparently
leached out of the tissue during preparation
for electron microscopy. Some of the
"crystals" have a striated appearance.
X 14,900.

Fig. 8:

Electronmicromontage of a frontal section
through the right lateral ocellus of the
nauplius eye of Sulcanus. Axons (a) can
be seen leaving the ocellus through "breaks"
in the anterior and posterior regions of the
pigment cup (PC). One of the nuclei (N)
of the multinucleate pigment cell can be
seen in the posterior part of the pigment
cell (bottom, right of electronmicromontage).
Part of the nerve bundle from the median
ocellus (arrowed) can also be seen in the
bottom right-hand corner of the electron-
micromontage. X 2,100.

Fig. 9:

Electronmicrograph of a section through the
base of part of a rhabdomere shoing the
rhabdomeric microvilli in longitudinal
section and the presence of "bristle
coated" microvesicles (arrowed). X 23,300.
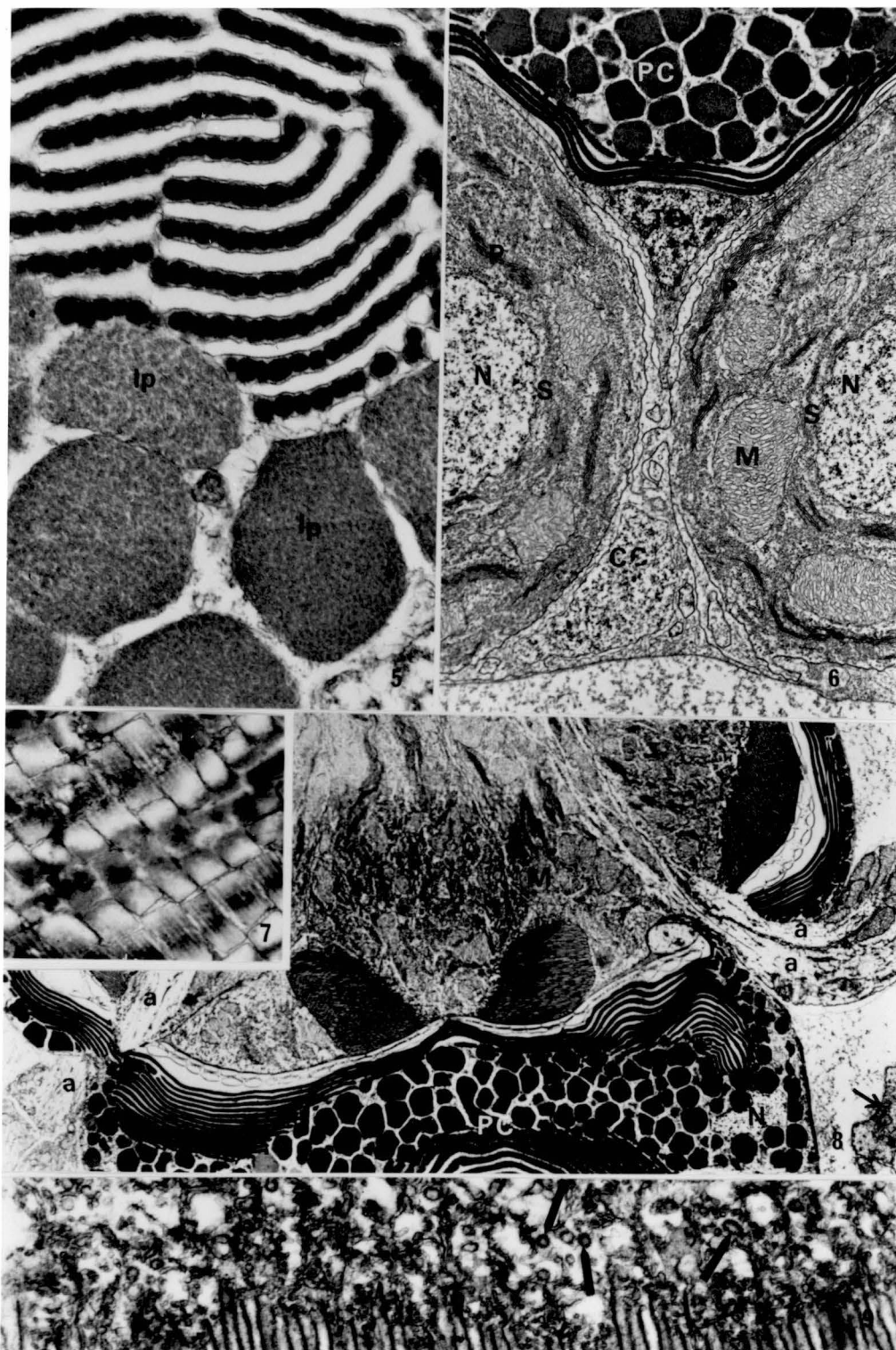
a gradual dehydration of the tissue during preparation.

The Lateral Ocelli.   The two lateral ocelli are struct-
urally identical (Fig. 4).   Each is made up of six sensory
or retinular cells.   The axons of three of the retinular
cells leave the ocellus via the posterior region of the
pigment cup while the other three leave by the anterior
region of the pigment cup (Fig. 8).   There are no other
nerves to or from these ocelli.   The arrangement of the
lateral ocellus is shown in Fig. 2.   The rhabdomeres
are situated next to the tapetal cell layer with the more
or less spherical nucleus of the retinular cell on the
opposite end (Fig. 4).   The end on which the rhabdomeres
are situated are here termed the apical or terminal end of
the retinular cell so that by this definition the nucleus
is situated at the base of the retinular cell.   All six
retinular cells appear to be structurally similar.   The
axons appear to leave the ocelli by the rhabdomeric end
through the pigment cells but a closer examination shows
the origins of the axons are the bases of the retinular cells
(Fig. 4).   The cytoplasm of the retinular cell contains
a multitude of organelles and inclusions and among these
are (i) the rhabdomeric microvilli, (ii) phaosomes, (iii)
multivesiculate bodies, (iv) microbodies, (v) microvesicles,
(vi) mitochondria, (vii) paracrystalline bodies, (viii)
microtubules, and (ix) endoplasmic reticulum.

(i) Rhabdomeric microvilli.   These regularly and tightly packed tubular structures are situated on the apical end of the retinular cell.   There is a pair to each retinular cell, one rhabdomere opposite the other (Figs. 4 and 8). The rhabdomeres are thus lateroterminally situated and of the straight lateral type of Eakin (1965).   The microvilli are about 90 nm in diameter and up to 7 μm long and within them are granules of moderate electron density of about 15 nm in diameter (Fig. 10).   The rhabdomeres are situated next to the reflectile layers of the tapetal cells. Microvesicles ranging in size from 35 nm to 80 nm in diameter are found at the bases of the rhabdomeric microvilli (Fig. 9). The 80 nm diameter microvesicles are similar to the "bristle coated vesicles" at the base of the rhabdomeres of the compound eye of <u>Daphnia magna</u> (vide Röhlich, 1967).   The retinular cells of the lateral ocelli are arranged such that the rhabdomeres of adjacent cells lie next to each other with the rhabdomeric microvilli of adjacent cells at right angles to each other (the functional significance of this arrangement with respect to perception of polarized light has been reviewed by Elofsson, 1969).   Rhabdomeres from two adjacent cells form the rhabdom.   The rhabdomeres are believed to be the photoreceptive organelle of the retinular cells.

(ii)   Phaosomes. These rather conspicuous bodies were seen as shiny or reflectile bodies by early light microscopists and thus the name phaosomes ("phao" :"to shine"). They normally appear as lens shaped bodies but this configuration apparently changes under different light regimes and physiological conditions.   These structures look like giant sinuous Golgi apparatuses and are located towards the bases of the retinular cells and are usually situated near the nuclei (Figs. 4 and 6).   The thickness of the cisternae or saccules is only about 8 nm but they are up to 10 µm in length (Fig. 11).   The thickness of the saccules is constant and unlike the Golgi apparatus the saccules of the phaosomes are non-polar.   At their periphery the phaosomes appear to be vesiculate (Fig. 11) and it is possible that the numerous microvesicles of the retinular cells originate from the phaosomes.   The exact function of the phaosomes is not known but the phaosomes are not unique to the nauplius eye and have been reported in the pigment epithelium of the vertebrate eyes as myeloid bodies (Porter and Yamada, 1960).

(iii) Multivesiculate bodies.   These structures are scattered all over the retinular cells including the preaxonal regions (Fig. 16).   They consist of tightly packed groups of microvesicles of between 30 nm and 50 nm in diameter and these are enclosed within a second membrane. The whole spherical structure is about 0.3 µm in diameter. These structures are not unique to the retinular cells and

Fig. 10;

Electronmicrograph of a section of parts of
two adjacent rhabdomeres (= rhabdom) showing
the arrangement of the rhabdomeric micro-
villi, perpendicular to each other. Moderately
electron dense inclusions can be seen in some
of the rhabdomeric microvilli (arrowed).
X 52,500.

Fig. 11:

Electronmicrograph of a section through
a retinular cell of the lateral ocellus
showing the nucleus (N), phaesomes(P),
and groups of ribosomes (r). Micro-
tubules (arrowed), endoplasmic reticulum (er)
and numerous microvesicles can also be seen.
Some of the endoplasmic reticulum (the
differentiation between these and micro-
vesicles is not very clear) have ribo-
somes on their membrane surfaces.
X 10,200.

Fig. 12:

Low power electronmicrograph of a transverse
section through the nauplius eye showing
the median ocellus and parts of the lateral
ocelli (LO). It can be seen that the pig-
ment cup of the median ocellus is made up
from parts of two pigment cells. The
superior group of retinular cells consists
of the central retinular cell (S_1) and two
peripheral retinular cells (S_2 and S_3) and
these form two rhabdoms (rh) situated next to
the pigment cup (note the near absence of the
reflectile places of the tapetum next to
these rhabdoms). The inferior group of re-
tinular cells consists of a large central
cell (S_4) with its terminal rhabdomere (R_t)
and two cup-shaped peripheral cells (S_5 and S_6)
with their hemi-annulate rhabdomere (R_a). The
inferior group of cells appear to be held to-
gether by the double walled tubular elements
(dte). X 2,100.

10

11

12
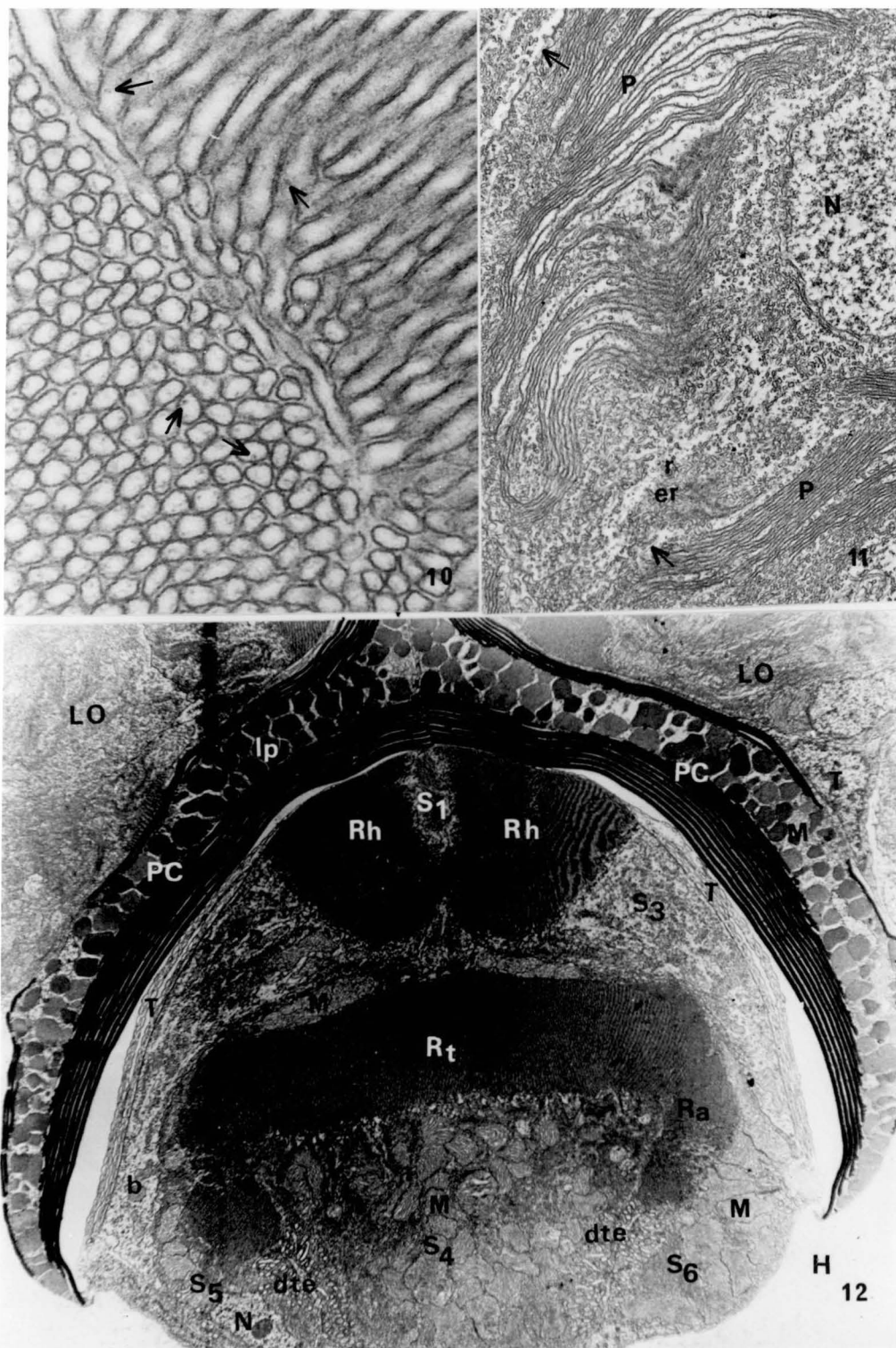
Friend (1969) has shown that in the epithelial cells of
the epididymis of the rat the vesicles of the multi-
vesiculate bodies show a similar cytochemical reaction to
the microvesicles budded off on the concavity of the Golgi
complex and are probably derived from these. The multi-
vesiculate bodies were also shown to be neither lysosomes
nor were they involved in protein uptake. It is thus
reasonable to suggest that the vesicles of the multivesic-
ulate bodies here are possibly derived from the microvesicles
of the Golgi in the retinular cells or even from the micro-
vesicles of the phaosomes.

(iv) Microbodies. Scattered within the cytoplasm of the
retinular cells are numerous more or less ovate shaped
bodies of moderate electron density (Fig. 15). These are
microbodies or peroxisomes (e.g. vide Beard and Novikoof,
1969). The microbodies are up to 2μum in diameter and
contain electron dense and apparently granular material
similar to the material that compose the nucleolus (Fig. 15).
The nature and function of these structures have been
described by Beard and Novikoof (1969) and de Duve (1969).
It is interesting to note that these structures have been
linked to photorespiration in leaves but its significance
here in a photoreceptor is not known.

(v)   Microvesicles.   These range in size from 30 nm to
60 nm in diameter and are present in large numbers in the
retinular cells (Figs. 6, 9 and 11).   As previously sug-
gested, they could possibly have been derived from either
the phaosomes or the Golgi apparatus.

(vi)   Mitochondria.   Numerous mitochondria are scattered
throughout the retinular cells and these comprise one of
the conspicuous cytoplasmic inclusions of the retinular
cells (Figs. 4, 6 and 8).   They range in size from 0.5μm
in diameter to elongated ones measuring up to 3μm by 6μm,
the majority being the upper size range.   The cisternae
of these mitochondria are of the lamellate type.   The large
numbers suggest the active metabolic state of the retinular
cells.

(vii)   Paracrystalline bodies.   These are lamellate
structures about 1μm in length, of high electron density
and are more common in the retinular cells of the median
ocellus (Figs. 15 and 16).   They occur singly, in pairs
or in threes and are apparently similar to the paracrystalline
bodies described by Nørrevang (1968) in oocytes.   The
function of the paracrystalline bodies found in the retinular
cells is unknown.

(viii) Microtubules.   These are present mainly in the axons
and at the bases of the retinular cells near the origins
of the axons.   They are thus more specifically neuro-
tubules.   There are however a few microtubules thinly

scattered throughout the retinular cells but these are very sparse and inconspicuous (e.g. Fig. 11).

(ix) Endoplasmic reticulum. The tubular type of endoplasmic reticulum forms a rather extensive network in the retinular cells. Both the smooth and rough types of endoplasmic reticulum are present in the cells but the rough endoplasmic reticulum is not at all widespread and is confined mainly to the region around the nuclei of the retinular cells (Fig. 11). Ribosomes are found not only associated with the rough endoplasmic reticulum but also freely and in clusters (Fig. 11).

The Nucleus. The nucleus of the retinular cell is nearly spherical in shape and situated towards the base of the cell, away from the rhabdomeric end (Figs. 4 and 6). It measures about 20 um across and contains a single nucleolus. Phaosomes, rough endoplasmic reticulum and an occasional Golgi apparatus are found around the nucleus (Fig. 11).

The Median Ocellus. Morphologically the median ocellus is a complex structure. There appear to be two distinct groups of cells which for descriptive purposes are termed the superior group and the inferior group (Figs. 3 and 12). The superior group, like the inferior group is made up of three cells and these are situated within the pigment cup and next to the tapetal and pigment layers (Fig. 13). The three retinular cells are arranged in one layer but with

Fig. 13:

Electronmicromontage of a frontal section through the nauplius eye showing parts of the lateral ocelli (LO) the superior group of retinular cells of the median ocellus and the nerve bundle (mn) from the median ocellus. The three pigment cups are formed from two pigment cells (PC). At least two nuclei (N) can be seen in each of the pigment cell. The central retinular cell (S_1) of the superior group of the median ocellus is linked to the two peripheral cells (S_2 and S_3) by desmosome-like structures (D). X1,320.

Fig. 14:

Electronmicromontage of a frontal section through the median ocellus of the nauplius eye, near the rim of the pigment cup of the median ocellus. The hemi-annulate rhabdomeres (Ra) of the peripheral retinular cells (S_5 and S_6) of the inferior group has a scalloped appearance. The central retinular cell (S_4) occupies the centre of the electronmicromontage and is filled with numerous mitochondria (M). The double-walled tubular elements (dte) are seen at the bottom of the electron-micromontage (at a level below the rhabdomeres). S_1,S_2 and S_3 are processes of the superior group of retinular cells. The space between the pigment cells and the tapetal cells (T) is a dehydration artefact. X 2,000.
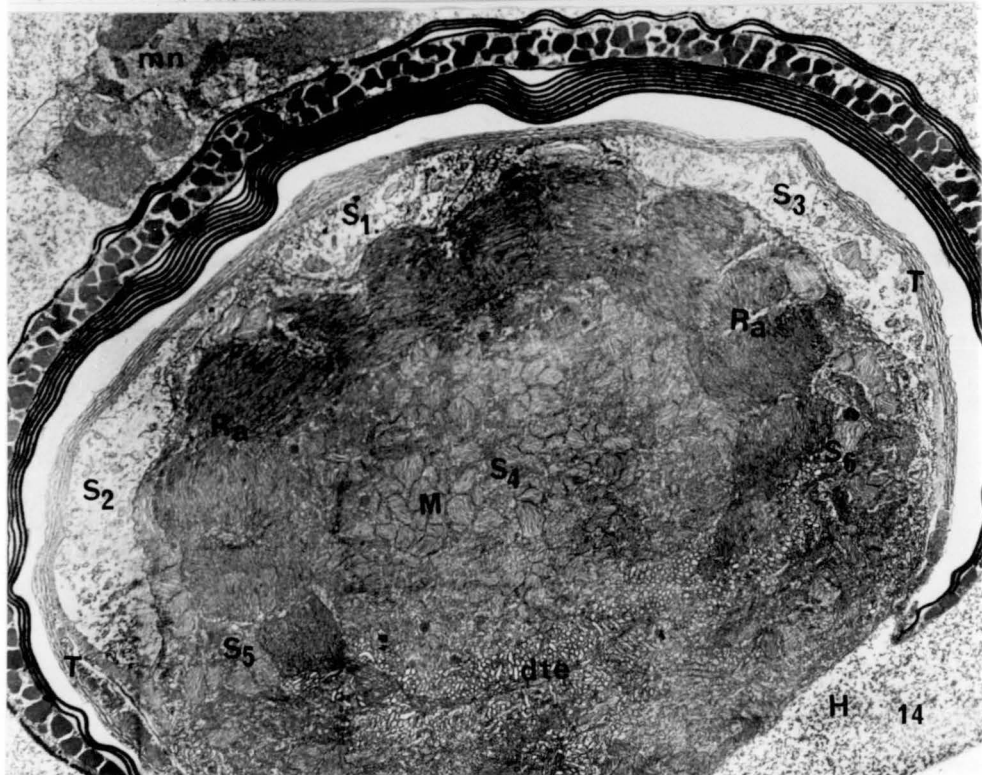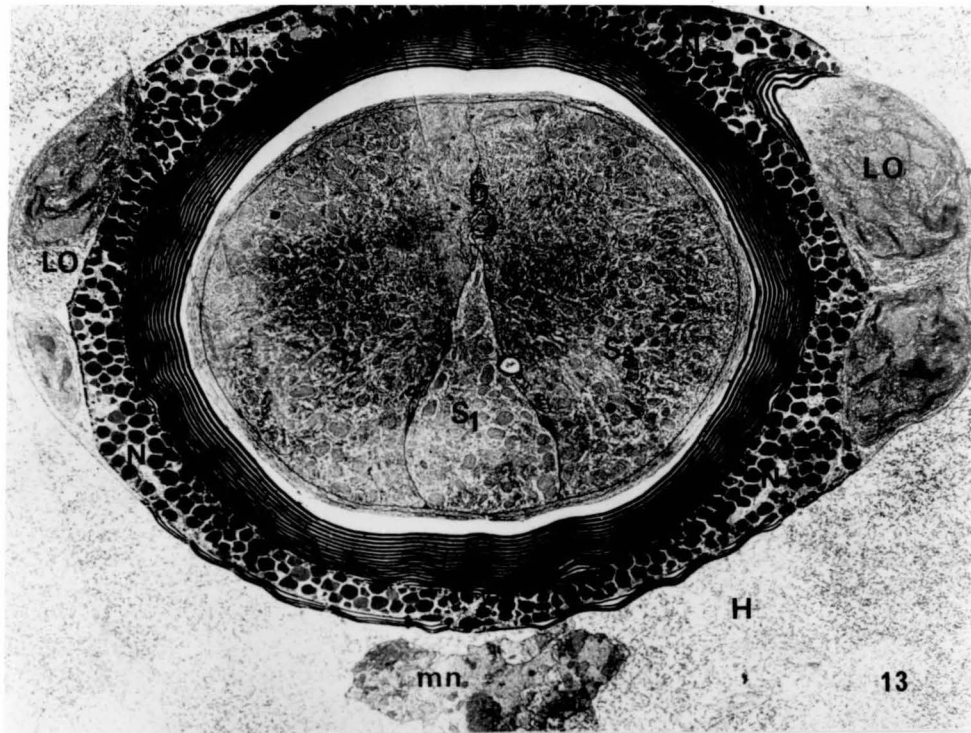
one of the cells more dorsally and centrally situated than the other two more peripheral cells. The more centrally situated cell is slightly larger than the other two cells. The centrally situated cell contains a set of two latero-terminally situated rhabdomeres and these form two complete rhabdoms with the single straight terminal rhabdomere of each of the more peripherally situated retinular cells (Fig. 13). Compared to the retinular cells of the lateral ocelli the cytoplasm of the retinular cells of the ventral ocellus contains very few organelles and cytoplasmic inclusions; they do not contain any phaosomes. The more proximal part of the retinular cells of the superior group of the ventral ocellus extends peripherally and skirts the periphery of the inferior group of cells (Figs. 3 and 12). It is in this proximal part that the nuclei of the retinular cells of the ventral ocellus are situated, such that the nuclei of all the six retinular cells are situated in the same frontal plane. The three cells of the superior group appear to be held to each other as well as to the inferior group rather tightly as seen from the apparently peculiar structure of the interdigitating cell membranes between adjacent cells. The structure is vaguely reminiscent of septate desmosomes. This arrangement of interdigitating membranes is seen in Fig. 16.

The inferior group is made up of one conspicuous central cell and two peripheral cup-shaped cells (Figs. 12

and 14) which completely surround the central cell. A reconstruction of the peripheral cup-shaped cell is shown in Fig. 19. The central cell of the inferior group contains a single large terminal rhabdomere. The rhabdomeres of the two peripheral cells (Fig. 14) together form an annulate structure (from two hemi-annulate rhabdomeres) which rings the central rhabdomere of the central cell. The hemi-annulus of each of the peripheral retinular cells apparently consists of eight units, imparting to the annulus a scalloped effect as seen in Fig. 14. The microvilli of the annulate rhabdomere is orientated such that its long axis runs circularly and at right angles to the microvilli of the terminal rhabdomere of the central cell. The two hemi-annulate rhabdomeres are so closely associated that it is not possible to visually separate them from one another. The three cells of the inferior group are thus very intimately associated.

The "core" of the central cell is filled with numerous large mitochondria (Figs. 12 and 14). These mitochondria measure about 6/um by 7/um and have lamellate cristae. The peripheral regions of the central cell below the rhabdomere contain peculiar double-walled tubular elements (Figs. 12 and 17). These elements are also present in the cup-shaped peripheral cells; in the

Fig. 15:

Electronmicrograph of a sectionof part of
a cell of the superior group of retinular
cells of the median ocellus showing the
mitochondria (M), microbodies (b), para-
crystalline bodies (arrowed) and micro-
vesicles). X7,400.

Fig. 16:

Electronmicrograph of frontal section through
the superior group of retinular cells of
the median ocellus showing the desmosome-
like structures that link the retinular
cells.   Multivesiculate bodies (mvb),
mitochondria (M) paracrystalline bodies
(arrowed) and microvesicles are scattered
in the cells. X 9,400.

Fig. 17:

Electronmicrograph of a section through
the inferior group of retinular cells
of the median ocellus showing the double-
walled tubular elements. Note the close-
ness of the membranes of the double walls
and the inclusions within the tubular
elements.   X 32,100.

Fig. 18:

Electronmicrograph of a frontal section
through the nerve bundle from the median
ocellus. Part of a glial cell (Gl)
associated with the nerve bundle can be
seen.   This is the only part of the nerve
bundle with a neuroglail association.
Note that there are six axons (the same
as the number of retinular cells in
the median ocellus). X 4,200.
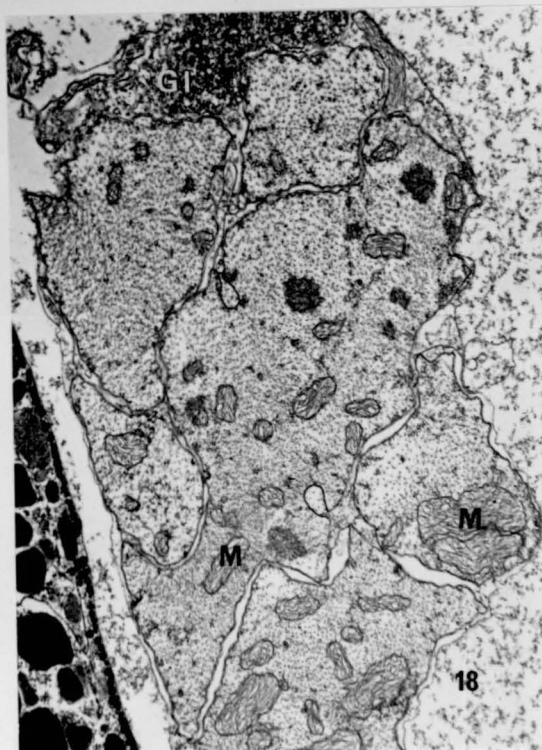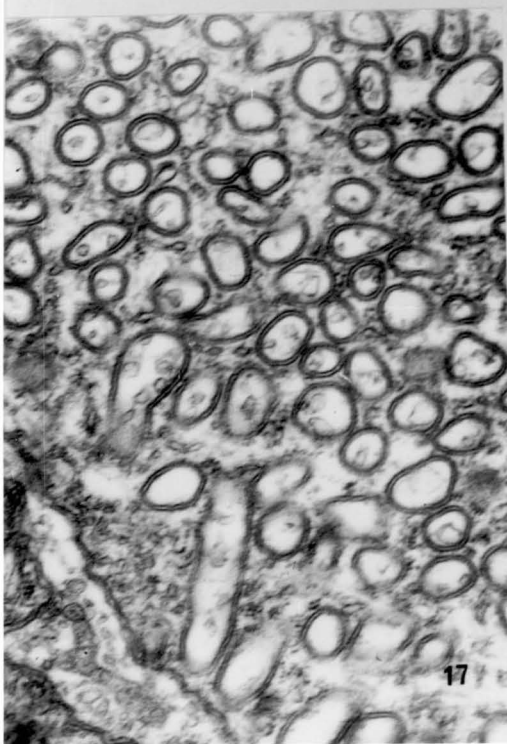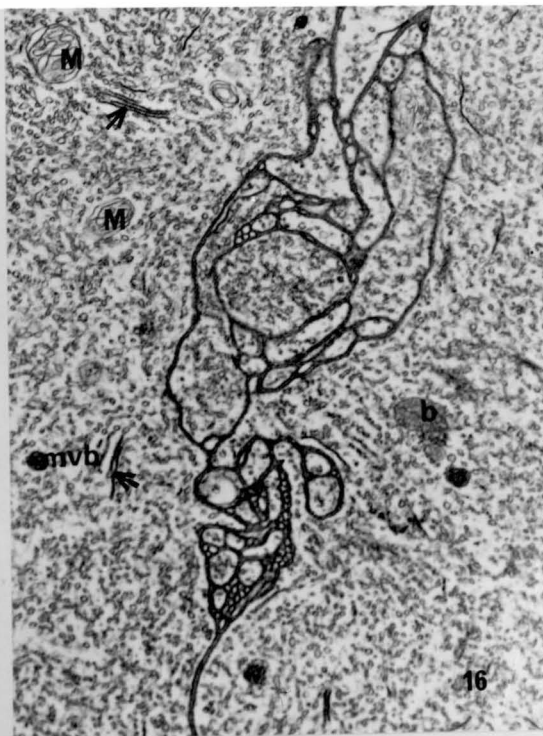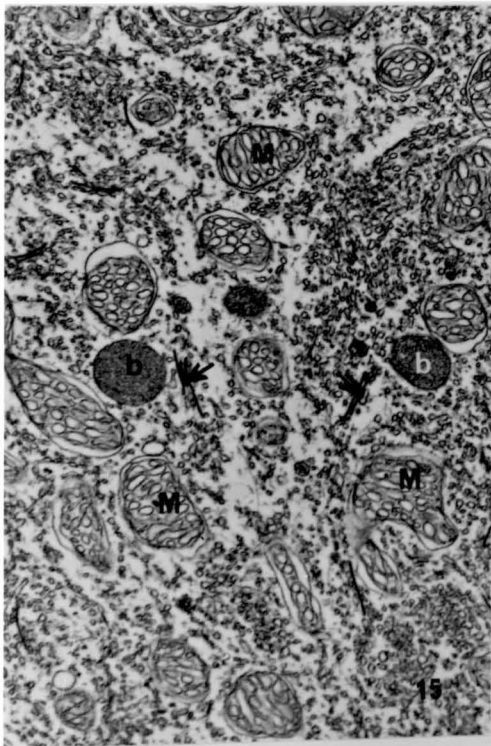
region next to where the tubular elements are situated in the central cell, i.e. in the inner border of the peripheral cells, as well as in the outer peripheral region next to the rim of the pigment cup (Fig. 12). These tubular elements have an internal tube diameter of about 0.21 μm and an external tube diameter of about 0.25 μm. The inner tubules contain vesicles of about 90 nm diameter but these are not numerous (Fig. 17). It appears that these double-walled structures are formed by impushings (not interdigitations) of microvilli into adjacent cells, reminiscent of those seen in the chaetograth eye (Eakin and Westfall, 1964). The membranes of the microvilli and that of the inpushed adjacent cell are very close together (Fig. 17) compared to those seen in the chaetognath eye.

The peripheral cells also contain a large number of mitochondria similar to those of the central cell. Like all the other cells of the median ocellus there are no phaosomes in the peripheral cells. The nuclei of the peripheral cells are situated at the proximal end of the cells and are at the same horizontal plane as the nuclei of the other retinular cells of the median ocellus. The axons of the peripheral as well as the other cells of the median ocellus leave the cells proximally and form a bundle of six axons on the posterior aspect of the median ocellus (Fig. 18).
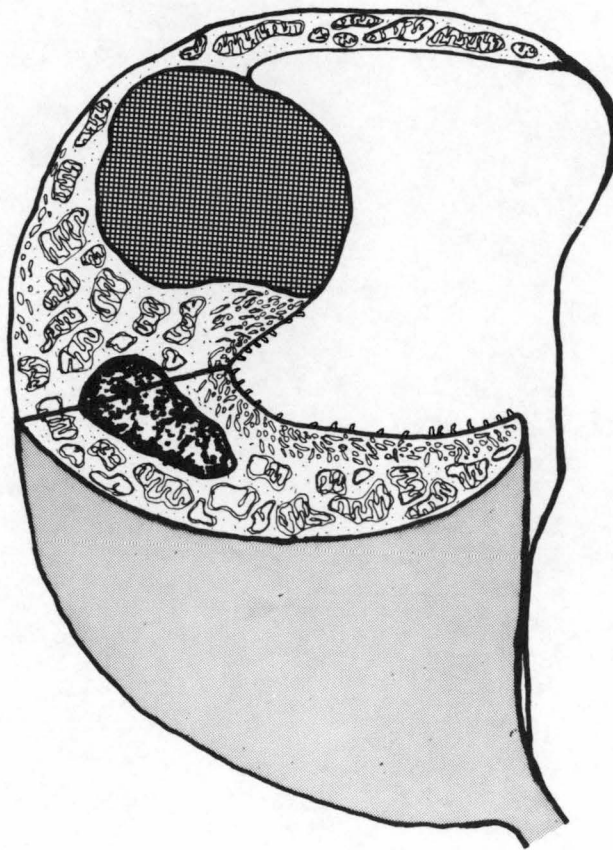
Fig. 19.

Schematic three-dimensional reconstruction
of the cup-shaped peripheral cell of the
inferior group of retinular cells of the
median ocellus.

There is a group of about six glial cells associated with the axon bundle a short distance from where the bundle leaves the median ocellus (Fig. 18). This is the only region of any of the axon bundles from the eye with surrounding glial cells. In the other nerve bundles and other regions of the median nerve bundle the glial associations are very inconspicuous or absent. As in the lateral ocelli there are no efferent axons from the brain.

DISCUSSION.

It is evident from the reviews of Vaisiere (1961) and Elofsson (1966) that most of the discrepancies in the interpretation of the structures of the nauplius eye in copepods were due to the limited resolution of the light microscope. Fahrenbach (1964) recognized this and devoted much of his discussion on the fine structure of the nauplius eye of Macrocyclops albidus to a comparison of his electron microscope study to previous light microscope studies.

The striking feature of the nauplius eye of Sulcanus is the structural difference between the lateral ocelli and the median ocellus. The difference as seen in Sulcanus was not noted by Fahrenbach for Macrocyclops albidus in which it appears that the three ocelli are basically similar. In Sulcanus the morphological difference between the lateral and the median ocelli appears to be marked enough to suggest a possible functional difference.

While the lateral ocelli are typified by the pre-
sence of a multitude of organelles and cytoplasmic inclusions
in their retinular cells there is only a limited variety
of organelles and inclusions in the retinular cells of
the median ocellus.   The absence of phaosomes from the
retinular cells of the median ocellus is notable.
Phaosomes are of course not unique to the retinular cells
of the nauplius eye: the structures termed myeloid bodies,
present in the retinal epithelial cells of some certebrates
appear to be identical to phaosomes.   These myeloid bodies
of the retinal pigment epithelial cells have been des-
cribed in some detail in the frog Rana pipiens by Porter
and Yamada (1960).   These authors suggested that the
myeloid bodies could possibly be light receptor units,
and are possibly involved in the control of the migration
of the pigment granules of the epithelial cells.   The
structure of the phaosomes in Sulcanus completely fits the
description of the myeloid bodies of Porter and Yamada and
I agree with these authors as to their possible function.

The phaosomes were first described in any detail
in the nauplius eye by Fahrenbach (1964).   He described
them in Macrocyclops as "....highly flattened cisternae,
each delimited by ribosome covered membranes,.....".   The
cisternae of the phaosomes in Sulcanus are highly flattened

(about 8 nm separation of membranes) but they are not
covered by ribosomes: they are of the smooth or agranular
type similar to those described in the myeloid bodies
by Porter and Yamada (1960). My unpublished observations
on two other calanoid copepods, Gladioferens pectinatus
and Calanus helgolandicus showed the phaosome membranes of
these also to be of the agranular type. If the inter-
pretation of Fahrenbach turns out to be correct it implies
that the phaosomes in Macrocyclops may be associated
with some form of protein synthesis and are analogous
with rough endoplasmic reticulum whereas the smooth mem-
brane phaosomes of Sulcanus, Gladioferens and Calanus are
more analogous to smooth endoplasmic reticulum or Golgi
cisternae.

Vaissiere (1961) suggested that the normally len-
ticular shape of the phaosomes, which show considerable
reflectility in the living cell, may act as light con-
centrating structures and Fahrenbach (1964) went on to
suggest that the unravelling of the phaosomes "....(in
the light adapted eye?) would favour greater metabolic
activity, that is, protein synthesis. This indication
of a high protein turnover rate may be associated with the
synthesis or resynthesis of the visual pigment".

It is interesting to note that phaosomes were not
reported from the nauplius eye of Sapphirina by Elofsson

(1969). It is not possible to tell if the "large lamellar body" figured by Elofsson are homologous to the phaosomes.

A striking feature of the median ocellus of Sulcanus is the arrangement of the rhabdomeres of its retinular cells. There are two sets of rhabdomes: a superior set of two formed by the three retinular cells of the superior group of retinular cells and the inferior set formed by the three retinular cells of the inferior group. Thus, the superior set is situated behind the inferior set in relation to the light source: the same beam of light would have passed through the inferior set of rhabdomes before getting to the superior set. As far as I am aware this situation is unique to Sulcanus within the Copepoda. This two tiered arrangement of rhabdomes is not seen in the pontellid Epilabidocera amphirites (Park, 1966). The closest comparison I am aware of is that seen in the mollusc Pecten where two retinal layers, the proximal and distal layers, are present in the eye (Barber et al.,1967). If indeed the superior and inferior rhabdomes of the median ocellus of Sulcanus are comparable to the proximal and distal retinae of Pecten then the implications are significant. In Pecten, Hartline (1938) found that the fibres from the proximal retinal layer discharged only upon illumination, while those from the distal retinal layer discharged upon reduction of illumination (giving the "on" and "off"

response) and more recently Gorman and McReynolds (1969)
were able to show that depolarizing responses arose from
cells in the proximal retinal layer while hyperpolarizing
responses arose from cells in the distal retinal layer
and that both types of potentials appear to be primary
effects of light on the photoreceptor cells. Barber et al.,
(1967) has shown the proximal and distal retinal layers
to be morphologically different: the receptor surface of
the distal cells consist of lamellae formed from modified
cilia while the receptor surface of the proximal cells con-
sist of arrays of microvilli, although the proximal cells
also possesses one or two cilia. In Sulcanus the rhab-
domeric microvilli of the superior and inferior sets of
rhabdomes are similar although the annulate rhabdomeres
of the inferior group is certainly unique. As well,
the inferior group of retinular cells differ from those
of the superior group in the presence of the peculiar double-
walled tubular elements in the retinular cells of the
inferior group.

It appears evident that the superior group of
retinular cells of the median ocellus are held together
by an elaborate system of inter-digitating membranous
process (giving an appearance reminiscent of septate des-
mosomes). This structure is lacking in the inferior group

of retinular cells. Here, the double-walled tubular elements are seen and identical structures are also seen in the ventral (median) ocellus of the pontellid, Labidocera cervi where they occur extensively and are arranged in closely packed arrays (unpublished personal observations). It appears that these structures are a specialised means of linking and holding together adjacent cells. The microvilli inpushings are possibly a means of increasing area of membrane contact between adjacent cells which suggests that the cells are in close chemical or electrical communication. This is suggestive of a synchrony reminiscent of the electrical coupling between retinular cells as seen in the compound eyes (e.g. Smith et al., 1965) but whether or not this is so can only be ascertained through electrophysiological study.

The absence of any form of lens system may be an indication that the ocelli in Sulcanus are not able to detect images but this of course does not completely rule out the possibility of the ocelli to form images as Land (1965) has demonstrated the ability of image formation by the argentea or reflectile layer of the eye of Pecten. In Sulcanus the corresponding layer is the reflectile layer of the tapetum and it seems that if this does act as a concave reflector, images can be focussed on the rhabdomes

of the inferior group of retinular cells of the median

ocellus.   The rhabdomes of the superior group as well

as those in the lateral ocelli are directly apposed to

the reflectile layer of the tapetum and thus unable to

utilize the reflected image (if any) formed by the tapetum.

That copepod eyes may possibly be able to function like

the Pecten eye was also suggested by Elofsson (1969).

The position of the axons of the retinular cells

in relation to the rhabdomeres is different in the median

and the lateral ocelli.   In the lateral ocelli the axons

appear to leave by the rhabdomeric end whereas in the

median ocellus the axons leave by the end proximal to the

rhabdomeres.   It is evident from Elofsson's (1966) review

that the everse/inverse nature of the retinular cells of

the nauplius eye of copepods is a controversial one.

This may be attributed to the limited resolution of the

light microscope but perhaps the main contributing factor

lies in the lack of consistency in the terms everse (or

everted) and inverse (or inverted) used by various workers.

I consider the definition of these terms to be entirely

dependent on one's definition of the orientation of the

retinular cell.   Here I define the terminal end of the

retinular cell as that end bearing the rhabdomeres.   From

this premise the inverse(or inverted) eye may be defined

as one with its terminal (i.e. rhabdomeric) end facing

away from the light and the everse (or everted) eye as

one with its terminal end facing towards or into the
light.   I have adopted the above definitions in pre-
ference to an alternative definition where the end from
which the axons leave may be termed the distal end of
the retinular cell because I believe that the latter
definition could be confusing as I will illustrate below.
It was noted that in the lateral ocelli of Sulcanus the
axons of the retinular cell (and this is possible only
at the electron microscope level) will reveal that the
axons in fact leave by the end proximal to the rhabdomeres
and the axons have folded through 180° onto the retinular
cell bodies giving the impression that the axons leave from
the end where the rhabdomeres are (Figs. 4 and 20).   This
I believe is perhaps the reason some earlier workers (with
the limited resolution of the light microscope) have found
the retinular cells of the eye of copepods to be of the
everse or everted type.   In Sulcanus at least there is
little doubt that the retinular cells are of the inverse
or inverted type and this is probably so with all the
other members of the Copepoda.

There is a lack of agreement as to the number of
nerve bundles from the eye to the brain although Elofsson
(1966) concluded that there were three.   It appears more
likely that the number of nerve bundles varies from species
to species.   The nerve bundles are enveloped in the
sheath that covers the whole eye but this sheath does
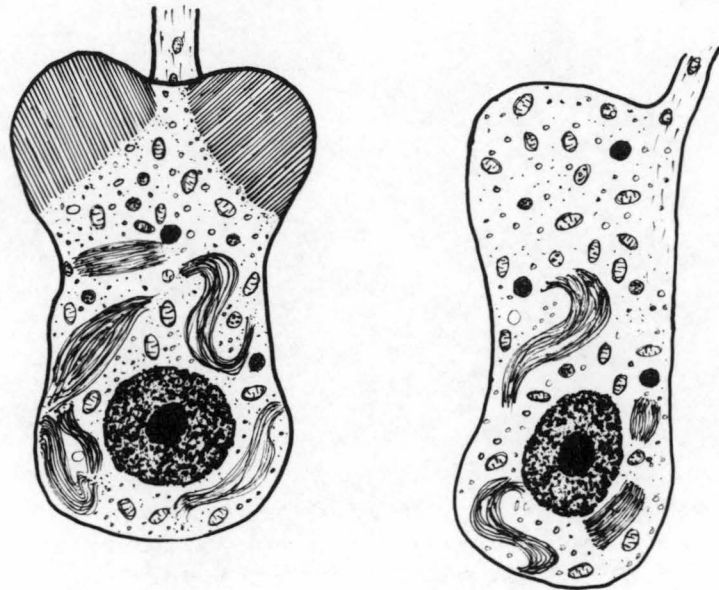not extend all the way back to the brain.   Processes of

Fig. 20.

Diagrams of the retinular cell of the lateral
ocelli seen in two aspects. There is a
suggestion (as seen from the neurotubules in
Fig. 4) that the axon has folded through 180°
back onto the cell body and thus giving
the impression that the axon leaves directly
from the rhabdomeric end of the retinular
cell.

glial cells are associated with the nerve bundles but the glial processes are extremely fine and not easily discerned. This however is not so with a part of the nerve bundle from the ventral ocellus. A short distance from the rim of the ventral cup there is a group of about six glial cells associated with the ventral nerve bundle. This is the only conspicuous group of glial cells associated with any of the nerve bundles. Its exact significance is not known but could be functionally significant.

Fahrenbach (1964) described a single "afferent" fibre that enters each ocellus in Macrocyclops, and as stated by Fahrenbach represents significant functional implications. This type of fibre was not found in Sulcanus: six axons leave from each of the three ocelli and each ocellus contains six retinular cells. Neither are there signs of any nerve fibre connecting the median and lateral ocelli as reported by Park (1966) in Epilabidocera amphirites.

One interesting feature found in this study is the structure of the pigment cups. Various workers have reported different numbers of cells making up the pigment cups of the ocelli. In Sulcanus it can be readily seen that the three pigment cups are made up from two multinucleate pigment cells. It is interesting to note that

while the lateral and the median ocelli are morpho-
logically and possibly functionally different the pig-
ment cups are most intimately associated (but see
Elofsson, 1969). This leads one to the question if there
is a direct line in the evolution of the nauplius eye
from the type seen here in Sulcanus to that seen in the
Pontellids where the median (ventral) eye is completely
separated from the lateral (dorsal) ocelli although
as previously stated the ventral eye of the Pontellid
Labidocera cervi has at least one feature common with
the ventral ocellus of Sulcanus, the double-walled tub-
ular elements. It is also noted that the pigment cells
of the ocelli of the eye of Epilabidocera amphirites are
also multi-nucleate (Park, 1966) although the ventral
(median) ocellus is completely removed from the dorsal
(lateral) ones. The pigment cells are apparently
more complex than those seen in Macrocyclops. The dark
pigment is apparently highly organized into tightly
packed regular membrane limited sheets in Sulcanus. A
similar arrangement is seen in the eye of Gladioferens
pectinatus but in Calanus helgolandicus the pigment is
apparently not as highly organized (unpublished personal
observations) and is more similar to that described by
Fahrenbach for Macrocyclops.

The tapetal cell layer containing the apparently reflectile crystals is not as well developed as seen in Macrocyclops. The possible nature of the crystalline plates have been discussed by Fahrenbach who suggested the reflectile role of the crystals and that they could function as an image forming reflector in the median ocellus. The exact function of the layer is unknown but a study of their physical properties such as polaroid properties may illucidate its functional role.

In Macrocyclops Fahrenbach described large and small retinular cells but in Sulcanus all the retinular cells of the lateral ocelli appear to be of a similar size. The only difference in the retinular cells is in the ventral ocellus where the size as well as the morphology of the cells are different.

The multivesiculate bodies described earlier are apparently different from the fenestrated bodies of the retinular cells of Macrocyclops described by Fahrenbach. The fenestrated bodies according to Fahrenbach contain vesicles with double walls while the vesicles of the multivesiculate bodies in Sulcanus are single walled. Since the material here as well as that of Fahrenbach's were osmium fixed it is unlikely that the difference seen between the mvb Sulcanus and the fenestrated bodies in Macrocyclops is due to fixation differences. The function of these structures is unknown.

From observations on the live animal it appears
that the lateral ocelli may be functionally different
from the median ocellus.   The lateral ocelli are limited
in their movement and are essentially fixed in a later-
ally directed position.   The median ocellus is however
endowed with a degree of movement in that it can be
directed in an anterio-ventral direction, describing an
arc of about 90°.   The fixed position of the lateral
ocelli and the ability of the median ocellus to be moved
may be taken to indicate that the lateral ocelli are in-
volved in general photoreception (possibly of changes in
light polarity and intensity) whereas the moveable median
ocellus may be involved in a more specialized form of
photoreception (possibly in the detection of movement, colour
and even images).   From various works dealing with
the eye of Pontellidae, it has come to be accepted that
the dorsal (lateral) eyes are more complicated and there
has been speculation that this may indicate an evolution
leading to the compound eye (see the review of Elofsson,
1966).   However, it is evident that in Sulcanus the over-
all organization of the median ocellus is far more com-
plex than the lateral ocelli and if there is greater
specialization it is the median ocellus rather than the
lateral ocelli that show it.   There is little doubt that
there is no direct lineage to the compound eye as seen in
any of the other crustacean groups let alone the insects.

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DORSAL FRONTAL ORGANS

INTRODUCTION

Frontal organs are present in most of the main groups of Crustacea which have been studied. There is however some confusion over the identification of the frontal organs in the Copepoda and this is due mainly to the limited resolution of the light microscope and, until recently, the confusion of terminologies.

During an attempt to locate the sites of ionic transport in the estuarine calanoid copepod _Sulcanus conflictus_, a pair of cells containing various morphological elements of the eye and located one on each side of the dorsal surface of the brain, posterior and dorsal to the nauplius eye was found. There is little doubt that these are modified or reduced eyes and since recent electron microscopical studies have shown that frontal organs are either modified or reduced photoreceptors (Elofsson, 1966b and Scharrer, 1964a, 1964b), I consider the structures in _Sulcanus_ to be the dorsal frontal organs thus necessitating my rejection of Elofsson's (1966a) contention that frontal organs (=reduced eyes) are absent in the Copepoda.

The following is a description of the dorsal frontal organs of the estuarine calanoid copepod _Sulcanus conflictus_ with a discussion on their possible function.
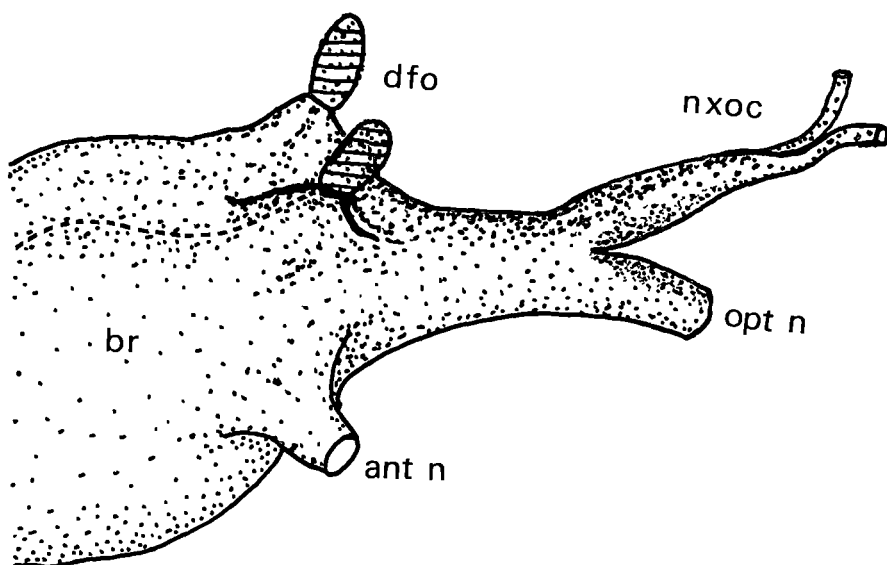
Diagram of the brain (br) of Sulcanus
showing the position of the dorsal frontal
organs (dfo) in relation to the optic nerve
(opt n), the nerves to the X-organ complex
(nxoc) and the nerves to the first antennae
(ant. n).

OBSERVATIONS

The dorsal frontal organs are a pair of large cells situated one on each side of the dorsal surface of the brain, posterior and dorsal to the nauplius eye (Fig. 1). The distal part of the cells are situated next to the cell body of the sheath cell which in turn is next to the epithelial cell adjacent to the cuticle of the dorsal part of the animal. The proximal part of the cell is very slightly tapered, rather indented and from this end the axons leave the cell (Fig. 2). The cell is roughly cylindrical in shape, about 45/um by 50/um in cross-section and about 60/um long, up to where the axons leave it. The cell itself is surrounded by a thin envelope formed by the sheath cell. The nucleus of the sheath cell is located at the distal end of the frontal organ, and the sheath encloses the whole of the organ as well as the laterodorsal projection of the brain onto which the frontal organ abutts. The sheath cell is possibly analogous to the conjunctiva cell of the nauplius eye and contains only a few cytoplasmic inclusions the more conspicuous being the few scattered mitochondria and microvesicles. A few axons containing electron dense vesicles (elementary neurosecretory granules?) may be seen closely apressed to the sheath cell towards the proximal part of the frontal
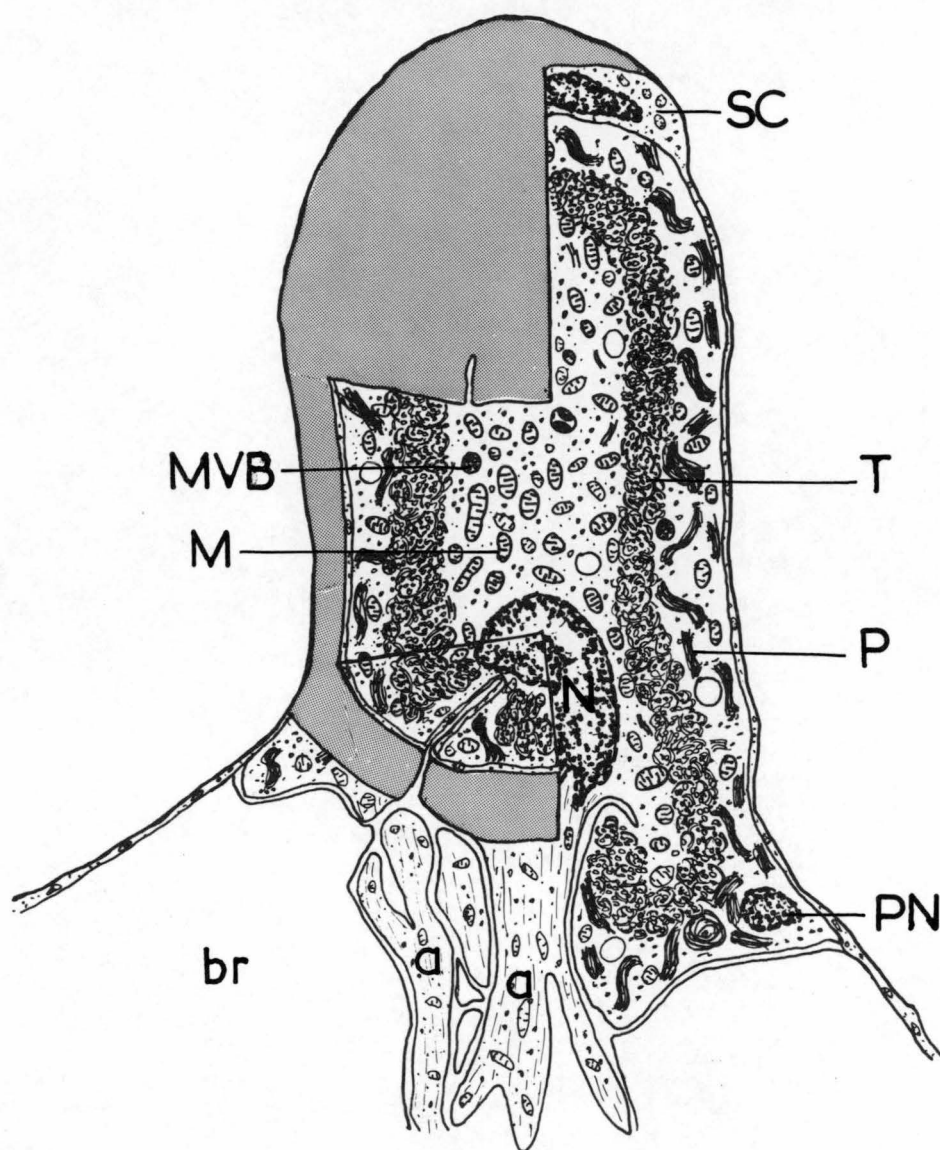
Fig. 2.

Diagram of a three-dimensional reconstruction of the
dorsal frontal organ of <u>Sulcanus conflictus</u> showing
the sheath cell (SC) that envelopes the sensory cell.
The organ is situated on the dorsal surface of the
brain (br) and the axons (a) of the dorsal frontal organ
can be seen going into the brain. The outer region of
the sensory cell of the frontal organ contains the peri-
pheral nucleus (PN) and the conspicuous phaosomes (P).
The peripheral region is separated from the core by the
"tubular bundles" (T). The core contains numerous
mitochondria (M), the central nucleus (N) as well as
multivesiculate bodies (MVB).

organ (Fig. 3).   It is not known if there are any fun-

ctional association between these axons and the frontal

organ.   The sides of the frontal organ are surrounded

by the haemolymph.

For ease of description the frontal organ will

be divided into   i) the peripheral region, ii) the

"tubular bundle" region, iii) the core or central

region and iv) the axons.

i) The periphal region.   This region contains

phaosomes, mitochondria, microvesicles, microbodies,

multivesiculate bodies and the peripheral nucleus.   The

phaosomes are the most conspicuous of the inclusions of

the peripheral region.   Structures similar to the

phaosomes, termed myeloid bodies, were first described

at the electron microscopical level in the pigment epithelial

of the retina of the fron Rana pipiens by Porter and

Yamada (1960).   These authors suggested that the

myeloid bodies may be invloved in photoreception and.

associated with the control of pigment movement in the pig-

ment epithelial cells.

The phaosomes have also been described at the

electron microscopical level in the nauplius eye of

Macrocylops albidus by Fahrenbach (1964).   The membranes

of the myeloid bodies and those of the phaosomes described

here are agranular but Fahrenbach (1964) described the

phaosome membranes in the eye of Macrocyclops as covered

with ribosomes.

Fig. 3.

Electromicromontage showing a transverse
section through the dorsal frontal organ.
The sensory cell is sheathed by the highly
flattened sheath cell (SC) on which some
neurosecretory (?) axons (NS) are apressed
on the haemocoel (H) side.   The peripheral
region of the sensory cell contains the
conspicuous phaosomes (P), mitochondria
(M), microbodies (B) and other inclusions
and is separated from the core by the
"tubular bundles" (T).   Numerous mito-
chondria (M) and the central nucleus (N)
may be seen in the cores.   X4,500.

The structure of the phaosomes seen here (Fig. 6) is in agreement with the description of the myeloid bodies described by Porter and Yamada (1960) and are identical to the phaosomes in the eye of <u>Sulcanus</u>.   Since these structures have to date been reported in photoreceptors only they are probably associated either primarily or secondarily with the photoreceptor mechanism.

Numerous mitochondria are present in the peripheral region of the cell and these range in size from 0.5/umby 1.0/umto 3/umby 6/um, with the majority in the upper size range (Fig. 6).   The cisternae of these mitochondria are of the lamellate type.   The high frequency of mitochondria as well as the numerous electron dense granules suggestive of glycogen indicates that the cell is metabolically active.

Also present in this region are microbodies (pero-xisomes).   These slightly ovoid structures are about 3/um across and contain material with an appearance similar to but not quite as dense as that of the nucleus (Fig. 3). The function here is unknown, but structure and functions of these organelles have been recently reviewed by de Duve (1969).

Sparsely scattered in this region are multivesiculate bodies.   These are clusters of microvesicles tightly bounded by a membrane into spheres of about 0.3/um diameter. The microvesicles of the multivesiculate bodies are between 30 and 65 nm in diameter.   The occurence of these structures

Fig. 4.

Electron micromontage showing a longitudinal
section of the dorsal frontal organ of
Sulcanus conflictus showing its positional
relationship to the epidermal cell (Ep)
and cuticle (C), the mitochondria of the
anterior muscle strand (MS), the haemocoel
(H) and the cells of the brain (br). The
nucleus of the sheath cell (SCN) is seen
next to the epidermal cell. The peri-
pheral region as well as the core contains
numerous mitochondria (M), microvesicles
(mv) and microbodies (B) but the phaosomes
(P) are found only in the peripheral
region. The central nucleus (N) may be
seen at the base of the sensory cell in
the core. The "tubular bundle" region
intervened between the peripheral region
and the core. X4,500.
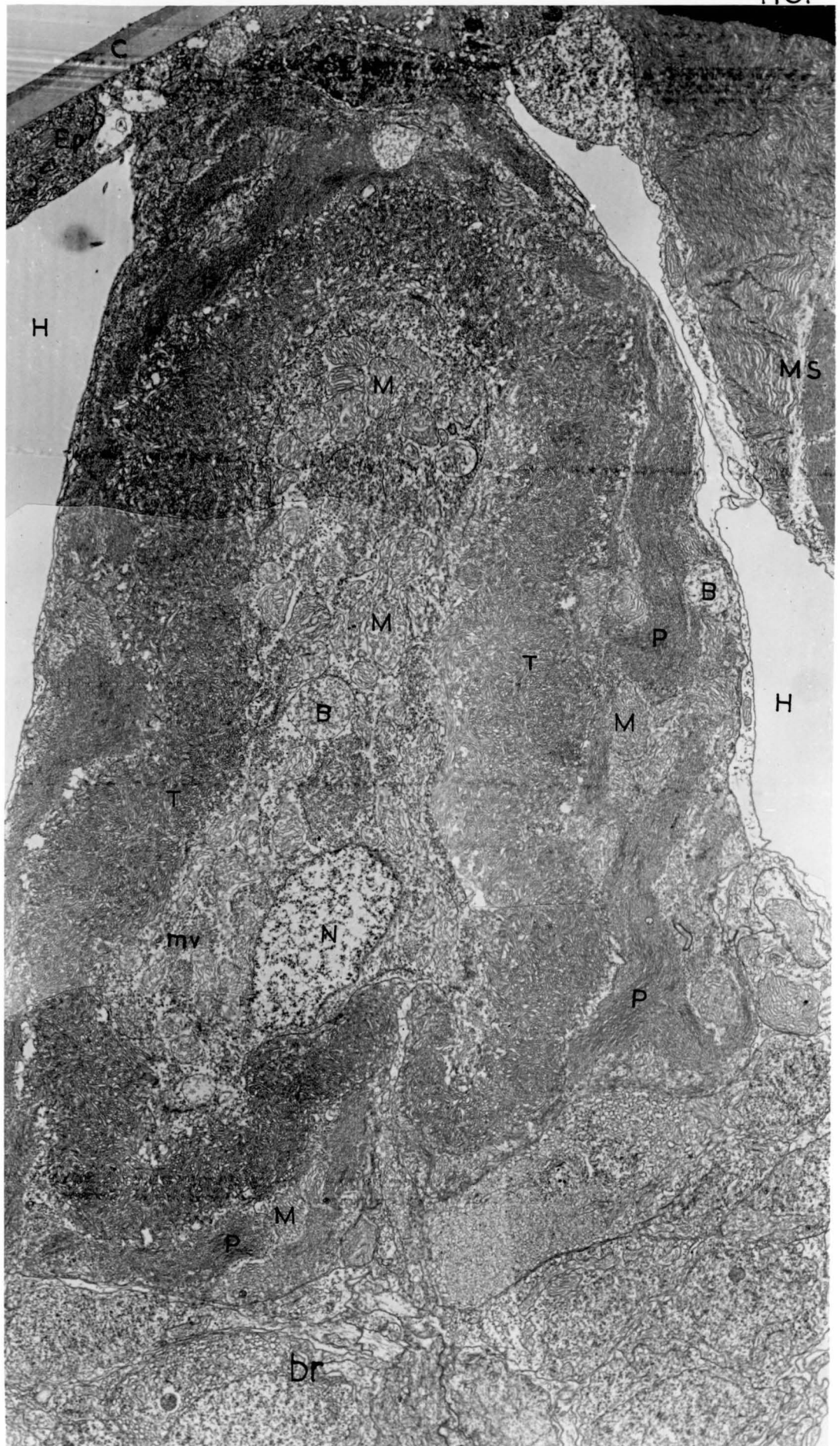
in the frontal organs is scant compared to their occurence in both the nauplius and the compound eyes of arthropods where they are believed to be intimately associated with the photoreceptor mechanism. Multivesiculate bodies are however by no means confined to photoreceptor cells, being found for example, in the statocysts of molluscs (Barber and Dilly, 1969) and in the epithelial cells of the epididymis of rats (Friend, 1969).

The numerous microvesicles found in the peripheral region are between 30 nm and 60 nm in diameter and it is possible that at least some of these are derived from the budded off ends of the phaosomes (Fig. 6.). Their function is unknown.

One of the nuclei of this binucleate cell is situated in the peripheral region towards the proximal end of the cell. This peripheral nucleus is ovoid in shape and apart from being more irregular than the central nucleus there is apparently no difference between the nuclei.

Present occasionally in the peripheral region are more or less spherical multilamellate bodies (Fig. 5). These are probably a type of lysosome.

ii) The "tubular bundle" region (Figs. 3 - 8).

Fig. 5.

Electron micrograph of a longitudinal
section through the basal part of the
dorsal frontal organ of <u>Sulcanus</u>. The
axons (a) can be seen leaving the sensory
cell from its origin just below the central
nucleus (N).   The cells of the brain (br)
are seen on the bottom of the micrograph.
As well as phaosomes (P) and mitochondria
(M) a multilamellate, more or less
spherical structure (L) can be seen in
the peripheral region of the sensory
cell (bottom right-hand corner).
Microtubules (t) may also be seen in
the core of the sensory cell.   X7,500.
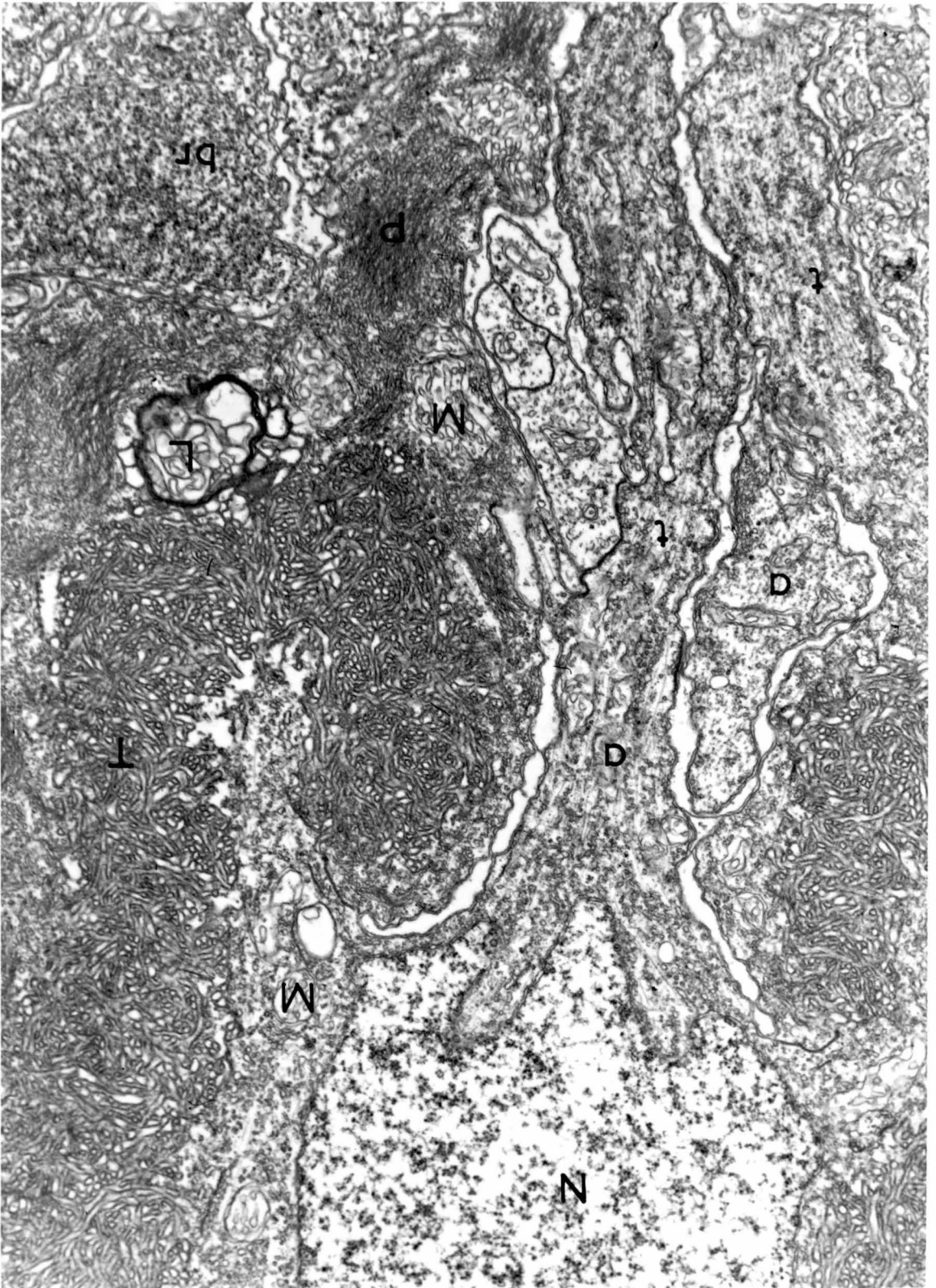
This is the most striking feature of the dorsal frontal organ. This region consists of bundles of tubular elements and these appear to be wound up in discrete balls of about 2 um in diameter. The tubules are between 60 nm and 90 nm in diameter. Spools of these tubules are arranged three to four deep to form a cylinder which is open on the proximal end and with two slits running along nearly its whole length to the dome-shaped distal end. These tubular elements are not found in the nauplius eye of this animal nor have they been reported in the eye of other copepods. Their function is unknown.

iii) The core or central region. The conspicuous inclusions of this region are the numerous large mitochondria and the central nucleus (Fig. 3). The mitochondria range in size from about 0.5 um by 1.0 um to those up to 2 um by 5 um with the majority, as in the peripheral region, in the upper size range. As well as the mitochondria the core contains microbodies and microvesicles (Figs. 4 and 6). These are similar to those described in the peripheral region of the cell. Sparsely scattered in the core are electron dense lamellate structures (Fig. 6) similar

Fig. 6.

Electron micrograph of a transverse section
through the dorsal frontal organ of
Sulcanus, showing the "tubular bundle" (T),
phaosomes (P), mitochondria (M), microbodies
(b), multivesiculate bodies (MVB) and
paracrystalline body (arrow).
H = haemocoel; Mu = muscle band and SH =
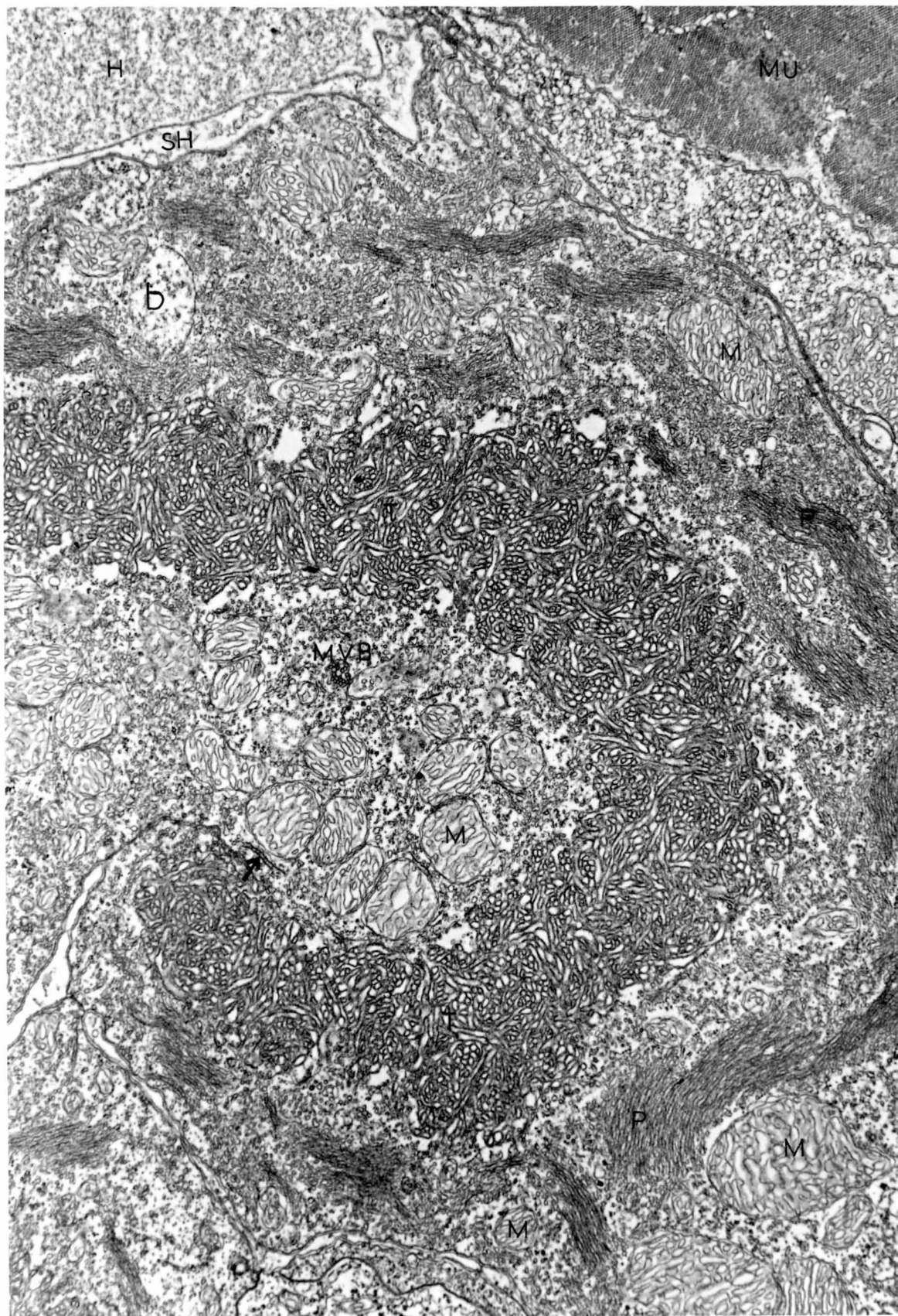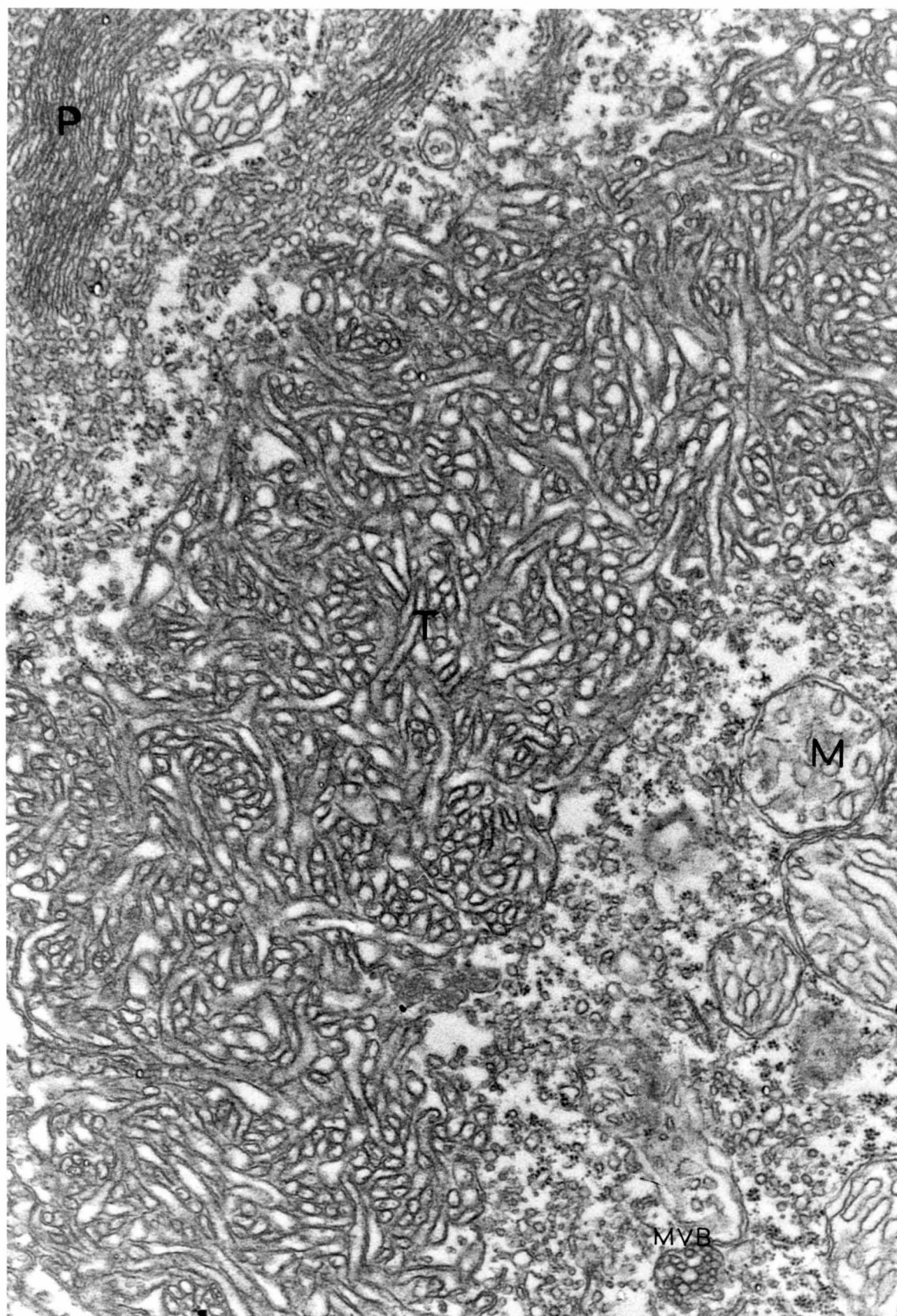sheath cell.   X85,00.

Fig. 7.

Electronmicrograph showing the structure
of the "tubular bundle" (T). A slightly
disorganized multivesiculate body (MVB) can
be seen on the bottom right-hand corner of
the electronmicrograph.   X22,000.

to the paracrystalline bodies found in mammalian oocyte (Nørrevang, 1968). Similar structures are found in the retinular cells of the nauplius eyes of Sulcanus (see section on nauplius eye). Their function is unknown. Also present in the core are scattered microtubules. The central nucleus is nearly spherical and measures about 10 µm across (Figs. 3 and 5). It is situated centrally at the base of the cell, just above the region where the axons leave the cell. The nucleus contains a small nucleolus.

iv) The axons. The base of the frontal organ tapers very slightly and is rather indented and interdigitated with the cells of the brain onto which the organ abutts (Fig. 5). The axons originate from this part of the cell. There appears to be a central axon which leaves the base a short distance from the central nucleus and about three other axons which leave from the more peripheral part of the cell. The peripheral axons are slightly smaller in diameter than the central axon. All axons branch as they enter the brain.

The axons contain numerous neurotubules, mito-

chondria and what appears to be glycogen granules.
It is not known if these axons meet with the axons from
the nauplius on entering the brain although they appear
to run in that general direction.

DISCUSSION

There is confusion over the identification of the
frontal organs in the Copepoda in that the organs associated
with the frontal filaments have been termed the frontal
organs.   Dahl (1963 and 1965) has shown that these organs
associated with the frontal filaments in Copepoda were not
frontal organs but more probably the homologue of the
X-organs of other groups of Crustacea.   This same opinion
was shared by Elofsson (1966a) who was also of the opinion
that frontal organs (=reduced eyes) were absent in the
Copepoda.

Elofsson (1963), in his discussion on the dorsal
frontal organs of the Decapoda considered that "....the
most important criterion is the innervation.   The organ
always ends in the nauplius eye centre."   He went on to
say that ".....the nomenclature of this part of the brain
is not adequate....".   This is certainly so in the
Copepoda.   It would thus be more practical and certainly
less confusing to define the frontal organs as modified or
reduced photoreceptors associated with the anterior part of
the brain (and in particular the nauplius eye centre, if
this region can be properly identified).   Elofsson (1966b)

himself has shown that the frontal organs of the Decapoda
Pandalus borealis are in fact reduced eyes. This is
probably the case with the frontal organs of the Cladecera,
Leptodora kindtii although they were not interpreted as such
by Scharrer (1964a and b).

In a recent study, Park (1966) described two groups
of cells situated dorsal to the brain and posterio-dorsal
to the nauplius eye of the calanoid copepod, _Epilabidocera
amphirites_ which he termed the "ascessory retinular groups".
These cells contained at least two distinctive components
of retinular cells; rhabdomeric microbilli and phaosomes
and their axons terminated in the nauplius eye centre.
There is little doubt that these are the dorsal frontal
organs although its true nature or homology was not clear
to Park (1966) who termed the structures associated with
the frontal filaments the suprafrontal nerves and the pair
of structures closely associated with and ventral to the
supraventral nerves, the frontal organs, being aware that
this so-called frontal organs may be X-organs.

The position of the frontal organs in relation to
the nauplius eye or to the animal as a whole should then
be the criterion for the terms "dorsal" or "ventral" as
suggested by Elofsson (1963). I agree with Elofsson
that the synonyms "median" or "unpaired" for the ventral
frontal organ and "lateral" or "paired" for the dorsal
frontal organ be dropped. Based on the above criteria

the structures described in this thesis are the dorsal frontal organs.

The dorsal frontal organs in _Sulcanus_ show a superficial resemblence to the organization of the central retinular cell of the inferior group of the median ocellus of the nauplius eye except that in the retinular cell of the median ocellus the phaosomes are absent. The similarity in the structural organization of the frontal organs of _Sulcanus_ to that of the photoreceptor cells of the segmental ocelli of the polychaete worm, _Armandia brevis_ (Hermans, 1969) and to those of the retinal club of the photoreceptors of the planarian worm, _Dendrocoelum lacteum_ (Röhlich and Török, 1962), is striking.

The most conspicuous feature of the dorsal frontal organs of _Sulcanus_ is the "tubular bundle" region. This structure appears to resemble the rhabdomeric micro-villi of the photoreceptors of the polychaete worm _Armandia_ (Hermans, 1969) as well as the microvilli of the photoreceptors of the mollusc _Helix pomatia_ (Röhlich and Tar, 1968). The "tubular bundles" appear to be identical to the "X-body" described by Scharrer (1969a) in the specialized trophospongium of the Cladocera _Leptodora kindtii_ and also to the microvilli of the dorsal frontal organs of the Decapoda _Pandalus borealis_ (Elofsson, 1966b). Scharrer (1964b) in a subsequent paper redescribed the "X-body" as microvillous borders but he was not clear as to its true

nature although he did suggest its possible function
as a transducer of photic stimuli.   Elofsson (1966a)
interpreted the structure described by Scharrer (1964a
and b) as remnants of the nauplius eye "....(and may also
be the frontal organ)".   I subscribe to Elofsson's latter
interpretation.   It thus appears that the microvillous
structures of the frontal organ of <u>Pandalus</u>, the frontal
organ of <u>Lepotodora</u> and the frontal organ of <u>Sulcanus</u> may
be a form of rhabdomeric microvilli.

It must be pointed out here that Tormey (1964)
has shown that artificial vesiculations of the plasma
membranes, especially at the infoldings of some epithelial
cells, were produced by osmium fixation. Röhlich (1966)
was however able to show that in the microvilli of the
photoreceptors of planarians the vesiculation and dis-
organization of the microvilli membranes caused by osmium
fixation was a "meaningful artefact" in that the vesicula-
tion and disorganization of the microvilli membranes
occurs only in animals subjected to light deprivation and
not in the control animals.   The material here, as were
Scharrer's (1964a and b) were osmium fixed and this possibly
at least partly accounts for the likeness of the micro-
villous borders of Scharrer's and the "tubular bundles"
seen here.   It must also be noted that the rhabdomeric
microvilli of the retinular cells of the nauplius eye (of

identically fixed material) of <u>Sulcanus</u> have never been
found to show the slighest disorganization or vesiculation.
It is for this reason that I am of the opinion that the con-
figuration of the membranes as seen in the "tubular bundle"
are possibly "meaningful artefacts" in the sense as used by
Röhlich (1966) (if indeed they are artefacts) in that this
"artefact" is probably a reflection of the physico-chemical
condition of the membranes which may possibly vary with
season. The animals here were taken in winter and it is
possible that the "tubular bundles" may assume a different
configuration in animals taken for example in summer.

It is probable that an ultrastructural study of
the paired binucleate Organ of Glicklhorn described by
Elofsson (1966a) in <u>Pareuchaeta norvegica</u> would reveal
them to be the dorsal frontal organs in <u>Calanus</u>
<u>helgolandicus</u> with the electron microscope and from
Elofsson's (1966a) brief description of the Organ of
Glicklhorn in that genus it seems probable that the Organ
of Glicklhorn are indeed the dorsal frontal organs (as
Glicklhorn (1930) himself had originally designated in
<u>Cyclops strenuus</u>). The dorsal frontal organs of <u>Calanus</u>
are similar to those of <u>Sulcanus</u> in that they contain
phaosomes and the distinctive "tubular bundles" (the
<u>Calanus</u> examined were taken in winter). As stated earlier,

the structures termed "ascessory retinular groups" in the calanoid _Epilabidocera amphirites_ by Park (1966) are without doubt the dorsal frontal organs: the structures contain phaosomes and rhabdomeres (it is not known if the membrane configuration of the rhabdomeres are similar to those of the "tubular bundle") and their axons return to the nauplius eye centre (the latter characteristic being considered by Elofsson (1966a) as the primary criterion for the frontal organ).

The actual function of the dorsal frontal organs is unknown but there is little doubt that they are reduced or modified photoreceptors. It is suggested here that they act as a special type of photoreceptor and in such a fashion as to allow the animal to accurately determine the climatic season and they would thus be closely involved in the regulation of the seasonal physiological cycle of the animal.

It has been shown that various photoreceptors assume different morphological configurations when subjected to different light regimes. Kuwabara (1966) has shown that in the rat, exposure to light of moderate intensity for as short as 24 hours resulted in the transformation of the compact lamellar structure very similar to that of the "tubular bundle" of the dorsal frontal organs of _Sulcanus._ Conversely, Röhlich and Tar (1968) have shown that the microvilli membrane of the retinal clubs of osmium-

fixed planariun, <u>Dugesia tigrina</u> progressively disintegrate
into vesicles and tubules when subjected to increasing
light deprivation.   If one considers the increase in
daylight hours as one progress from winter into summer
it is possible to envisage that as the daylight hours
exceed a certain critical period (say 11 hours) this will
cause a change in the morphological or chemical config-
uration of the light perceiving apparatus of the frontal
organs (possibly from the morphological configuration as
seen in the "tubular bundle" to one of the orderly rhab-
domeric microvilli as seen in the nauplius eye).   This is
analogous to tripping a switch which controls the physio-
logical cycle of the animal.   Then as the period of
daylight hours decreases from summer into winter and reach
the critical period (i.e. 11 hours) the "switch" is again
tripped but this time in the opposite direction, causing
a reversion to the original physiological condition.
The frontal organs could thus act as a photochemical switch,
the switch being tripped by a certain critical scoto- or
photo-phase (this light/dark regime being in fact the
"zeitgeber" (Aschoff, 1960) or synchronizer).   This would
provide the animal with an accurate mechanism for relating
its physiological condtion to the climatic season, since
the length of daylight or darkness (the "zeitgeber") is by
far the most consistent of all the environmental factors
that undergo seasonal change.   It is possible that the

animal has an intrinsic or endogenous biological clock in
the regulation of its seasonal or physiological cycle but
this does not rule out the need for a mechanism such
as that proposed, as the frontal organs could act as a
mediator between the "zeitgeber" (i.e. the scoto- and
photo-phase) and the intrinsic clock.

This hypothesis can be tested by studying the
morphology of the frontal organs at different times of the
year as well as those subjected to different light regimes.
A chemo-anatomical study would also be useful in deter-
mining the function of the frontal organs.

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MANDIBULAR SENSILLA

INTRODUCTION.

The question of whether calanoid copepods can filter feed selectively is unresolved (vide Marshall and Orr, 1955). Esterly (1916) showed that _Calanus finmarchicus_ was able to reject food pellets by a backward flick of the maxillae. Cushing (1959) suggested from indirect evidence that _Calanus_ may detect food particles with their first antennae. However, Mullin and Brooks (1967), using amputation experiments with _Rhincalanus nasatus_ found that the first antennae were not necessary for selective filter feeding in that calanoid. It must be pointed out though, that Lowndes (1935) observed that even within the calanoid copepods the feeding mechanism is variable.

To date there appears to be a complete lack of morphological evidence to support the hypothesis of selective filter feeding in the calanoid copepods. Indeed, apart from the excellent work of Lowe (1935) on the internal anatomy of _Calanus_ morphological work on these animals is lacking. Even so, these animals are so small that sensory structures could easily be missed. Thus, the electron microscope study of Krishnaswamy et al.

(1967) on the "buttons" on the setae of the maxillae
and maxillipeds of the bathypelagic calanoid copepod
Centraugaptilus horridus is relevant.   These workers
were not able to find any sensory structures in either
the maxillae or the maxillipeds.

Numerous sensory structures have been located in
the various appendages of the calanoid copepod,
Gladioferens pectinatus and this is significant in
relation to the feeding behaviour of the calanoid
copepods especially in view of the work of Krishnaswamy
et al. (1967).   The following is a description of the
mandibular sensilla of the brackish water calanoid
copepod, Gladioferens pectinatus with a discussion on
the significance of these sensilla with respect to sel-
ective filter feeding.

OBSERVATIONS.

Gross Morphology of the Mandible (Fig. 1A - D)

The mandibles are situated on either side of the
mouth and sandwiched between the labrum and the labium.
Apart from the mandibular palp these appendages are
flattened anterio-posteriorly.   The cuticle of the
biting edge of the mandible is thickened and formed into
a row of teeth.   The tip of these teeth when viewed
under phase-contrast appear to bear minute openings.
These "openings" probably correspond to the tips of the

<u>Fig. 1A-D.</u>

The mandible in its various orientations.
A Ventral view of the anterior region of
<u>Gladioferens</u>, showing the position of the
various oral appendages. B Schematic
transverse section of <u>Gladioferens</u> at the
region of the mandible, showing its in-
nervation. C Longitudinal section
(lateral to the midline) of <u>Gladioferens</u>,
showing the labrum, mandible and labium.
D The mandible. ant_1 first antenna;
ant_2 second antenna; br brain; clc
"chloride" cell; ep epithelial cell;
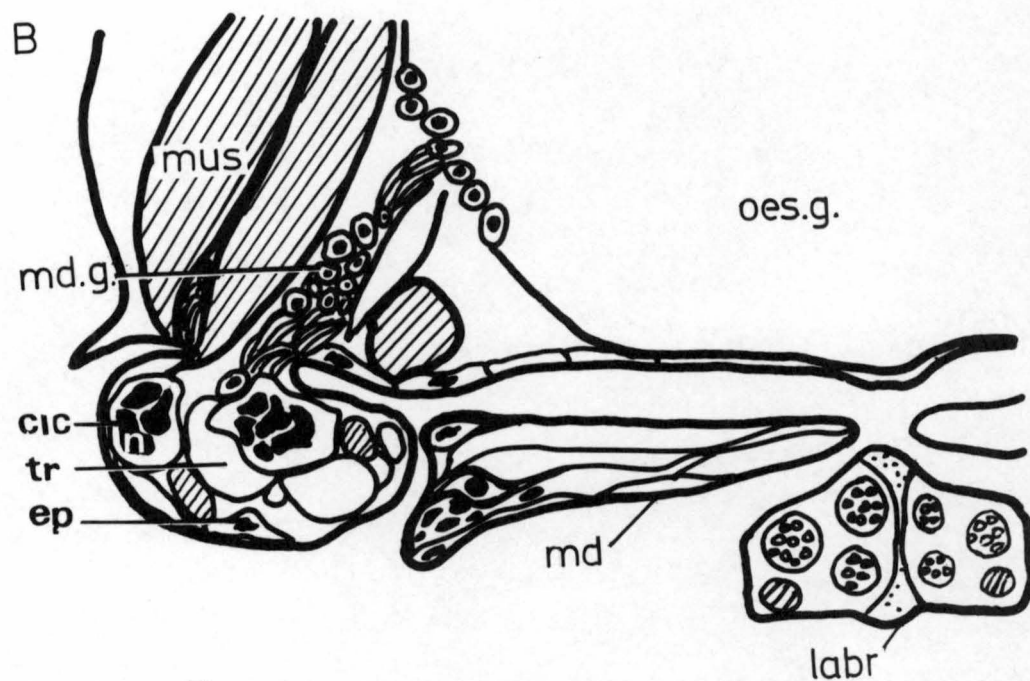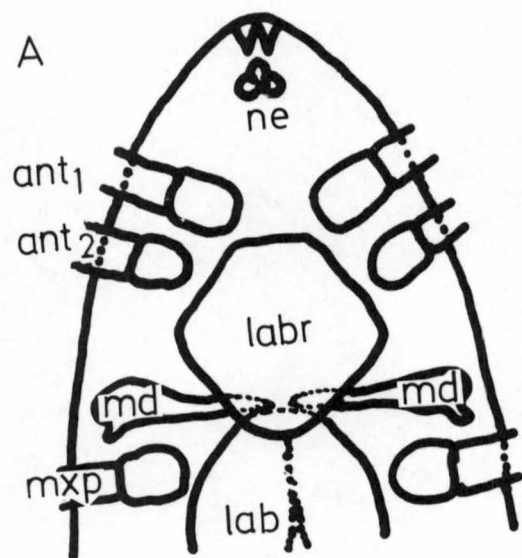lab labium; labr labrum; lg labral
gland; md mandible; mdg mandibular ganglion;
mg mid gut; mgd mid gut diverticulum;
mus muscle; mxp maxilliped; n nucleus;
ne nauplius eyes; oes oesophagus; oes.g.
sub-oesophageal ganglion; tr "trichogen
type" cell.
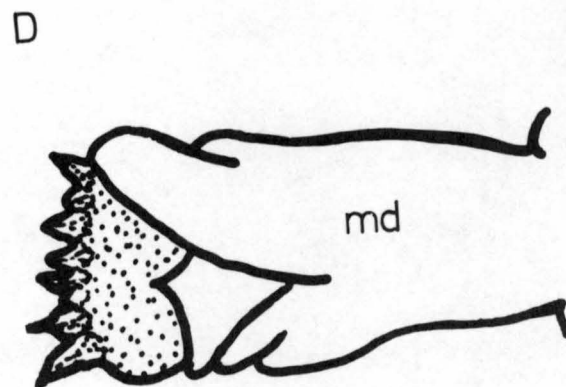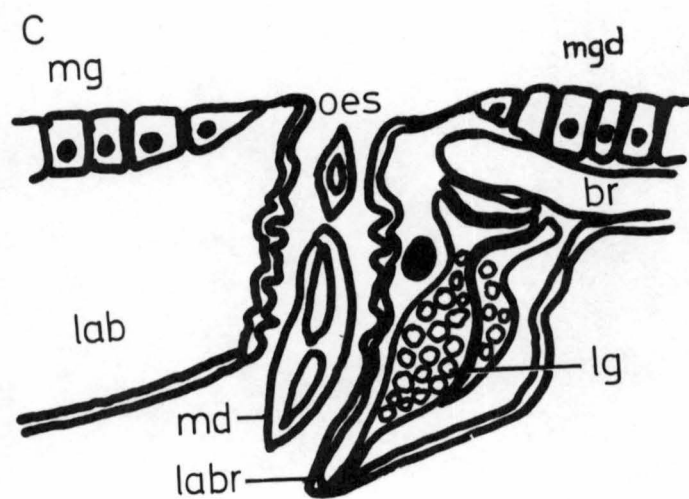
"trichogen type" cell as seen under the electron micro-scope.

The appendage as articulated allows both a lateral and a dorsoventral movement; the animal is thus able to grasp particles and either ingest or eject these as they reach the mandibles through the filter feeding currents. The function of these appendages does not appear to be one of mechanical chewing of the food particles as examination of the gut content showed that the frustules of diatoms and other phytoplankters are mostly undamaged. The actual feeding mechanism of this animal has never been studied but it is probable that the mechanism is similar to that described by Lowndes (1935) for the brackish water calanoid <u>Eurytemora velox</u> (ecologically <u>Eurytemora velox</u> appears to be very similar to <u>Gladioferens pectinatus</u>).

<u>Innervation of the Mandible</u>

The innervation of the mandible of <u>Gladioferens</u> is similar to that described in <u>Calanus</u> by Lowe (1935). It consists of the dorsal and ventral roots of the mandibular nerve, which arise from the sub-oesophageal ganglion. The ventral (sensory) root is formed by the sensory neurones of the mandibles. The perikarya of one of the two types of sensory neurones (the ciliary type) are bcated in the body of the animal, ventral to the base of the mandibles. These form the mandibular ganglion, as

<u>Fig. 2A.-E.</u>

Reconstruction of the non-ciliary sensillum.
A Longitudinal section. B - E Transverse
sections at positions indicated in A. Num-
bers refer to electron micrographs.

Fig. 2A - E are not on the same scale.
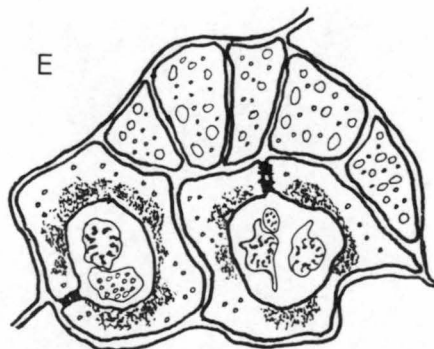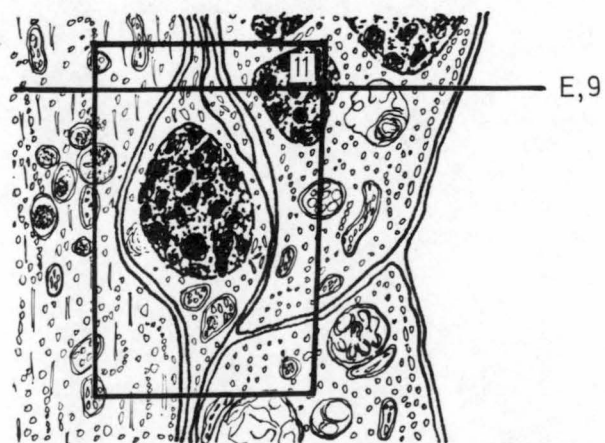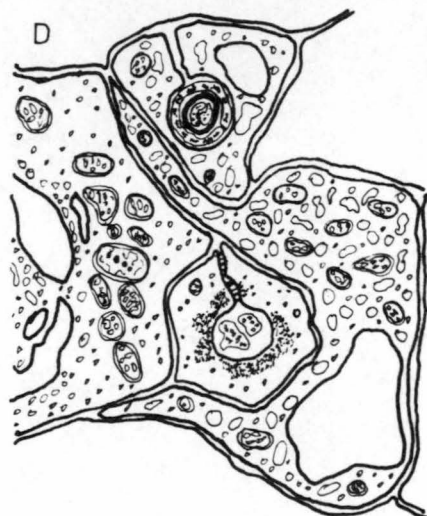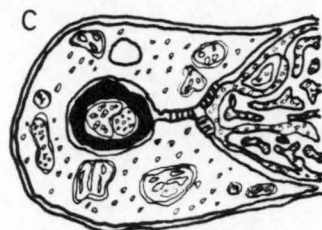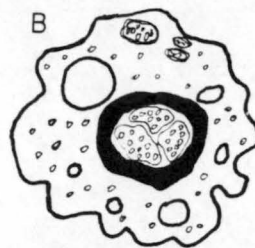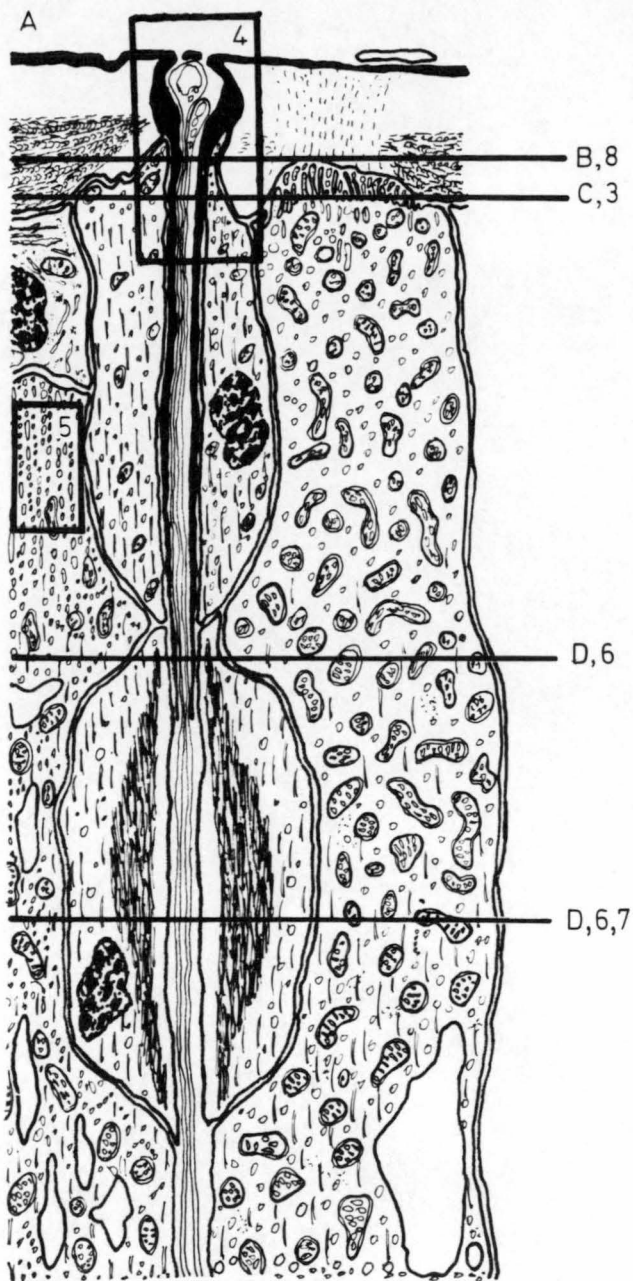
described in <u>Calanus</u> by Lowe (1935). The non-ciliary type neurones have their perikarya located at the base of and within the mandibles. The dorsal (motor) root of the mandibular nerve consists of axons running to the mandibles, the perikarya of these neurones being located within the sub-oesophageal ganglion.

Cell Types in the Mandibles

The cell types in the mandible may be divided into two groups: those associated directly with the sensilla and those that are not. The cell types that appear to be directly associated with the sensilla will be described in the next section. A total of seven cell types were discerned in the mandibles viz, the ciliary neurones, the non-ciliary neurones, the tormogen cells, the scolopale cells, the "trichogen type" cells, the epithelial cells and the "chloride" cells. The latter two of these cell types appear to be least associated with sensilla proper. These will be described briefly here.

The Epithelial Cells (Fig. 12). These cells occur under the cuticle and are probably associated with the maintenance or even the secretion of the cuticle. Processes of these cells extend into the mandible between the other cell types, and they are reminiscent of the processes of glial cells. The nuclei of those cells are also reminiscent of glial cell nuclei in morphology. The cytoplasmic inclusions include mitochondria, abundant vesicles, endoplasmic reticulum and ribosomes.

The "Chloride" Cells (Figs. 3,6).   These are
extremely large cells, extending the whole length of the
mandibles and some of them extend a short distance into
the body of the animal. The tips of these cells are
folded into microvilli and are situated below what appears
to be modified cuticle: the cuticle in this region is
much thinner than that in the surrounding regions and there
appear to be pores in it.   The cells are heavily endowed
with smooth endoplasmic reticulum, packed with mitochon-
dria and in some cells, there are conspicuous lysosomes.
Many of the mitochondria at the base of the cells contain
numerous electron dense granules, which may be calcium
or other divalent ion (vide Peachy, 1964).   Rouiller (1960)
has shown that cells containing mitochondria with these
granules are usually involved in either water or ionic
transport.   These cells are very similar to the chloride
cells found in the gills of Fundulus (Philpott and Cope-
land, 1963).   The function of these cells in Gladioferens
is now being investigated.

The Sensillum.

The sensillum consists of a single neurone or a
group of neurones with associated supporting or satellite
cells.   Three types of sensilla are present and these
appear to differ only in the type or types of sensory
neurones innervating them.   The first type contains

Fig. 3.

Section of the tip of the mandible showing
a transverse section of the terminal part
of a sensillum at the top of the electron
micrograph.   The electron dense cuticular
sheath (CS) encloses two dendrites (D)
with their neurotubules (NT).   The cuticular
sheath is surrounded by the tormogen cell
(To) which is held by the conspicuous
septate desmosome (Des) and contains elong-
ated figure of eight mitochondria (M_8).   The
structure in the centre of the electron
micrograph are the villi of the "chloride"
cell (ClC), containing microtubules (Mt).
The surrounding material is cuticle (C).
Magn. X31,800
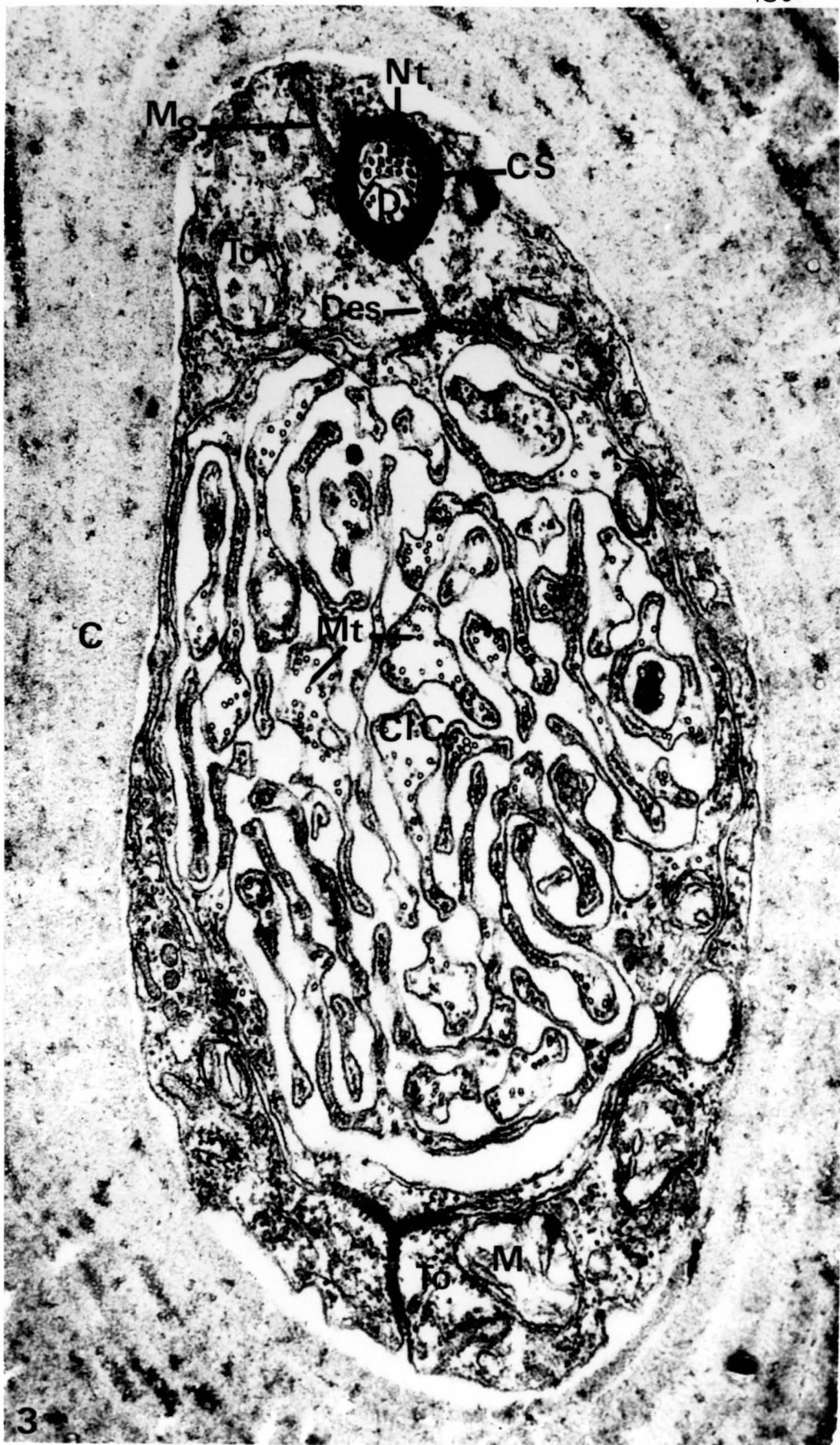
only the non-ciliary type neurones, the second type contains only the ciliary type neurones and the third type contains both the ciliary and the non-ciliary type. Each sensillum contains one to five sensory neurones, the usual number being two. The satellite cells and their arrangement about the sensilla are essentially identical in all the three types of sensilla. The ciliary type sensory neurones have their perikarya located ventral to the base of the mandible and these form the mandibular ganglion. The perikarya of the non-ciliary sensory neurones are located at the base of and within the mandibles.

The Satellite Cells of the Sensillum

The satellite cells are cells that partially or completely surround parts of the dendrites and perikarya of the sensory neurones or the so called cuticular sheath. The arrangement of the satellite cells about the sensory neurones is shown in the schematic reconstruction in Fig. 2A. Because of technical difficulties involved in obtaining orientated specimens the reconstruction inFig. 2A must remain tentative.

The Cuticular Sheath.(Figs. 3, 4, 8). This is a tubular structure extending from the cuticle into the mandible. It is composed of an electron dense material and ensheaths the terminal part of the sensory neurones This structure is apparently similar to the cuticular sheath found in the thick walled peg from the antennal

Fig. 4.

Longitudinal section of the terminal
segment of the non-ciliary type sensory
neurones and cuticular sheath (CS).
The dendrite (D) is bulbous at the tip
and is naked and exposed to the exter-
ior.   The cuticular sheath is seen
as a continuation of cuticle and is
apparently similar to the epicuticle
(Cep) material. Unstained Magn.
X159,000.

Fig. 5.

Longitudinal section of the "trichogen
type" cell near its tip, showing the
almost parallel strings of vesicles
and tubular elements.   Some of the
vesicles appear to be coated (Vec.)
Unstained. Magn. X31,500.
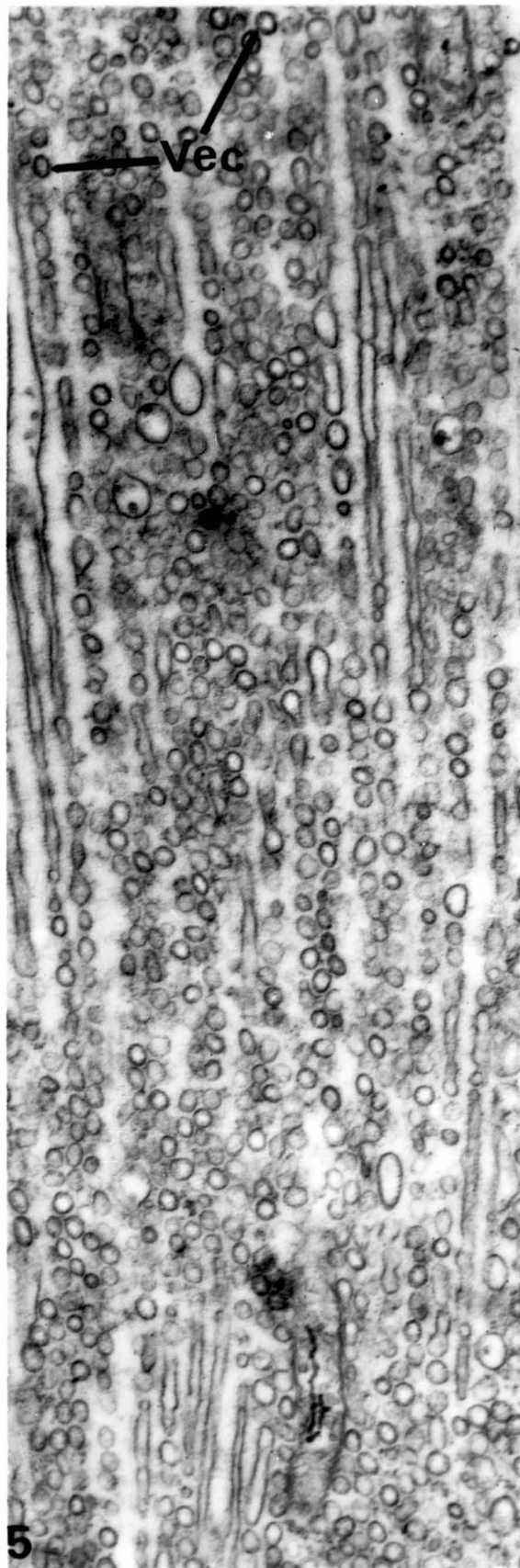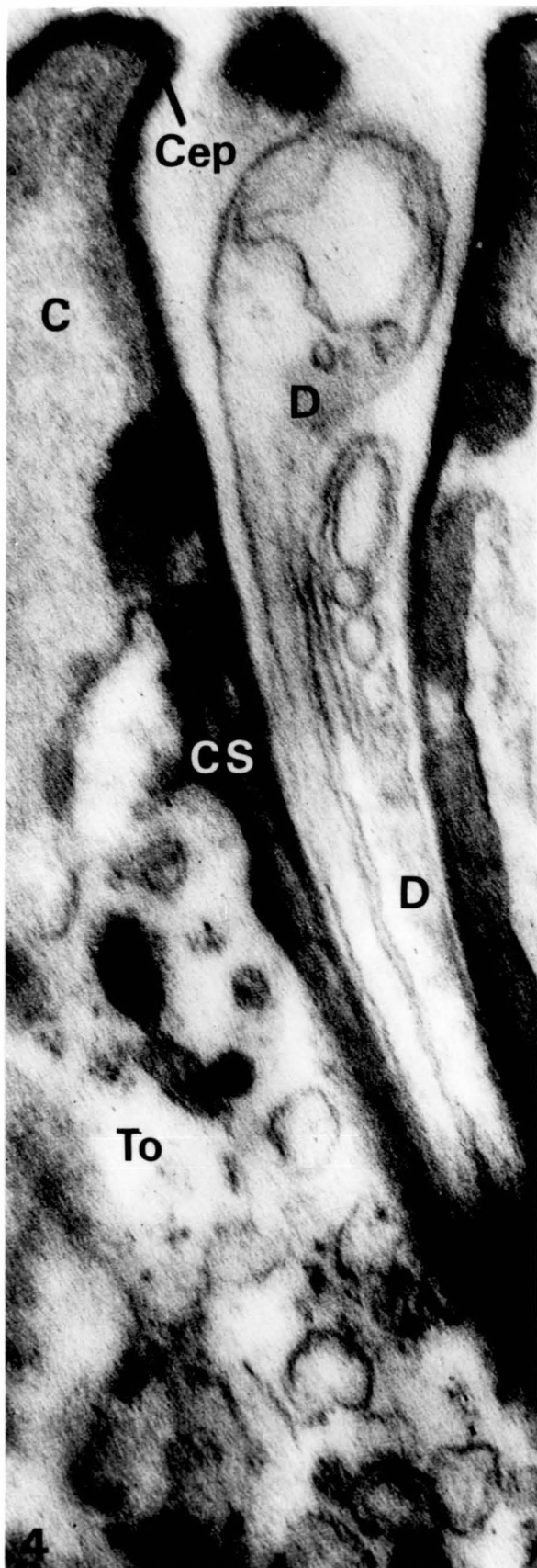
flagellum of the grasshopper, <u>Romalea microptera</u> (Slifer,
1961) and also to the electron dense "tube" of the hetero-
dynal scolopidia found in the chordotonal organs of
<u>Carcinus maenas</u> (Whitear, 1962). The material of the
sheath appears to be of a cuticular nature as the tube
itself is a continuation of the cuticle. The material
is probably identical to the material of the epicuticle
(Fig. 4). Thus the term epicuticular sheath might have
been more appropriate. Seen in transverse section the
cuticular sheath appears to resemble myelin sheath but
the cuticular sheath here is definitely not lamellate as
the myelin sheath is.

<u>The Tormogen Cell</u> (Figs. 1,5,8,11). There is
only a single tormogen cell to each sensillum. The cell
encloses practically the whole length of the cuticular
sheath which is about 15/^{um} long. It completely surrounds
the cuticular sheath and has very conspicuous septate
desmosomes. There are what appear to be two types of
mitochondria; a smaller and apparently more condensed
figure of eight type and a more "normal" larger type. The
cell appears to be lacking in the type of endoplasmic
reticulum and ribosomes which are characteristic of the
epithelial cells. This cell also contains numerous micro-
tubules and small vesicles. Since the cuticular sheath
would offer the structures adjacent to it a high degree of

support it would seem that the microtubules here play a role other than that of support. The cytoplasm of this cell appears to be similar to that of epithelial cells and it is probable that these tormogen cells are indeed modified epithelial cells.

The Scolopale Cell (Figs. 6,7,9). This cell is situated below the tormogen cell. For simplicity and ease of description the terminal parts of the dendrites of the sensory cells will be considered the top of the sensillum. Like the tormogen cell there is only one scolopale cell per sensillum. The cell completely surrounds the part of the cuticular sheath not already enclosed by the tormogen cell as well as parts of the dendrites of the sensory neurones. As with the tormogen cell the scolopale cell has many demosomes.

The main feature of the scolopale cell are the so called scolopale rods (Gray, 1960). These scolopale rods are similar but apparently not identical to the scolopale rods described by Gray (1960) from the auditory organ of the lucust, Locusta migratoria migratorioides. Gray described the scolopale membrane in the locust auditory organ; this membrane cannot be discerned in the mandibular sensilla of the present animal. The scolopale rods here appear to consist of dense aggregations of microtubules and what appear to be microtubule elements (Fig. 7). Gray described the scolopale rods of the auditory organ of locust as being

<u>Fig. 6.</u>

"Chloride" cells (ClC) and transverse
sections of two sensilla at the level of
the scolopale cell (Sc).   The villi
of the "chloride" cell at the top of the
electron micrograph are situated below a
thinner cuticle (C) which appears to bear
pores.   The cell on the right is a
"trichogen type" cell (Tr) and contains
vacuoles (V), microtubules (Mt),
numerous coated vesicles (Vec) and mito-
chondria (M) with electron dense granules
(g). The cell at the bottom of the
electron micrograph is an epithelial
cell (Ep). Magn. X23,800.

C

vi

ClC

Vec

V

g

M

D

Sc

Mt

Tr

Sc

Ep

6
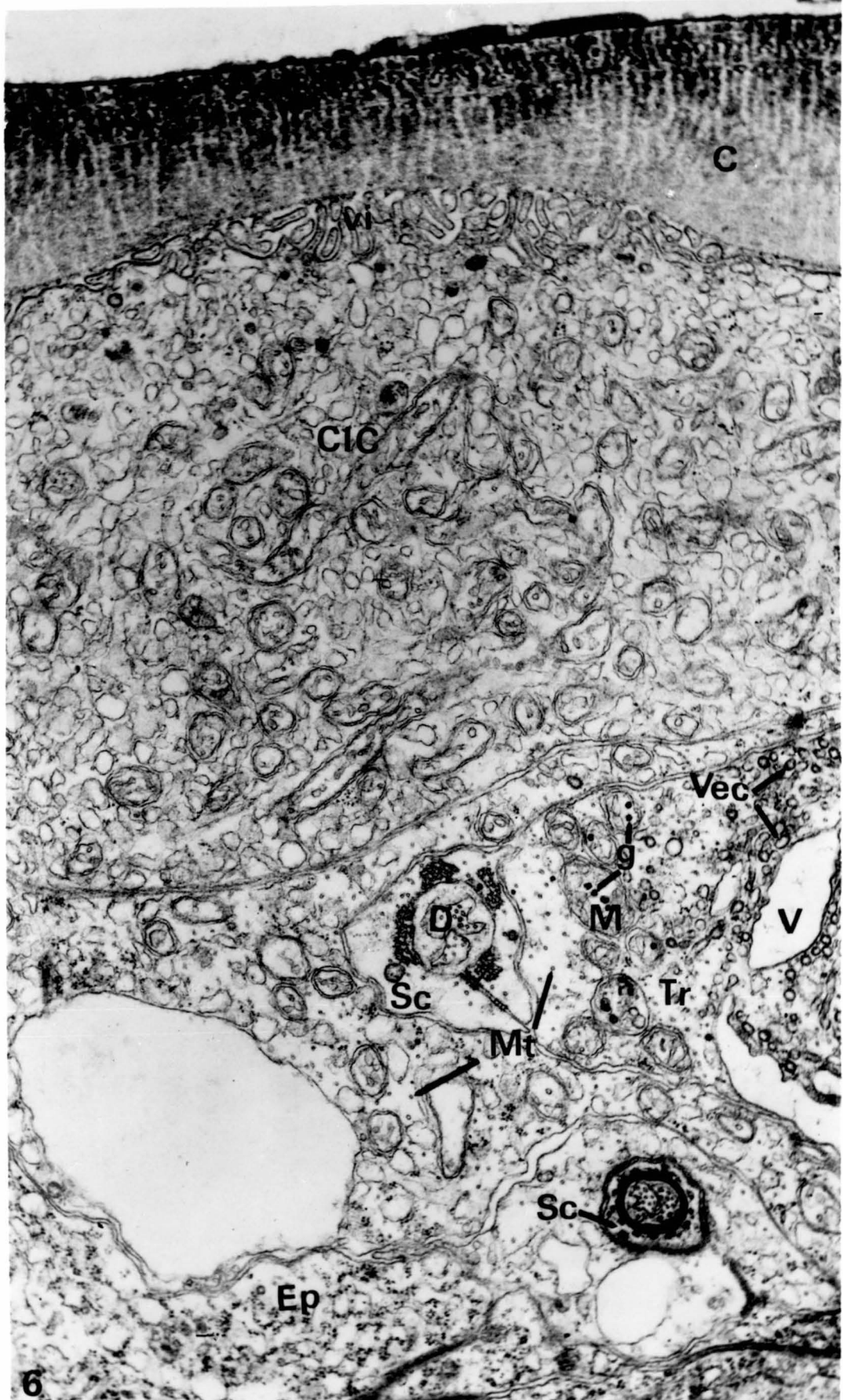
of a fibrous material.   The scolopale rods probably act
as supporting elements of the cell which in turn support
the dendrite of the sensory neurones.   Also present in
the scolopale cell are numerous microtubules, mitochondria
and vesicles.   The microtubules, like those in the other
cells bearing them in the mandible, are aligned with
their long axes parallel to the long axes of the dendrites
of the sensory neurones.

The "Trichogen Type" Cell   (Figs. 5 - 7).   As
used in the insects, the trichogen cell strictly speaking,
refers to the cell forming the hair or seta.   The structure
of this type of cell is complex.   Their function is not
known but they probably serve as supporting cells of the
dendrites of the sensory neurones and are also probably
involved in ionic or water transport.   The cell extends
the whole length of the mandible and terminates just in-
side the body of the animal, the nuclei being located at
the base of the cells.

The part of the cell towards the tip of the mandible
contains parallel strings of vesicles and constricted
tubular elements (Fig. 5).   Similar structures are found
in the trichogen cells of the pharynx of the cockroach
Blabera craniifer (Moulin, 1968), in the neck region of
the vestibular hair cells of the inner ear of the labora-
tory white rats (Hamilton, 1968) and in the oxyntic cells

Fig. 7.

Transverse section of a sensillum (blow-
up from Fig. 6) at the level of the scolo-
pale cell (Sc). The dendrites (D) con-
tain neurotubules (Nt). The scolopale
rods (Sr) appear to be aggregations
of microtubules (Mt) and microtubule
elements. The scolopale cell (Sc) is
held together by desmosomes (Des). The
cell on the left is a "trichogen type"
cell (Tr) and contains mitochondria
(M) with electron dense granules (g).
The cell on the right is a "chloride"
cell (ClC). Magn. X62,500.

Fig. 8.

Transverse section of the terminal part
of a sensillum, embedded in the cuticle (C).
The sensillum contains three dendrites
(D) surrounded by the cuticular sheath
(CS) which itself is surrounded by the
tormogen cell. Numerous microtubules
(Mt) and neurotubules (Nt) can be seen.
Magn. X141,500.

7

8
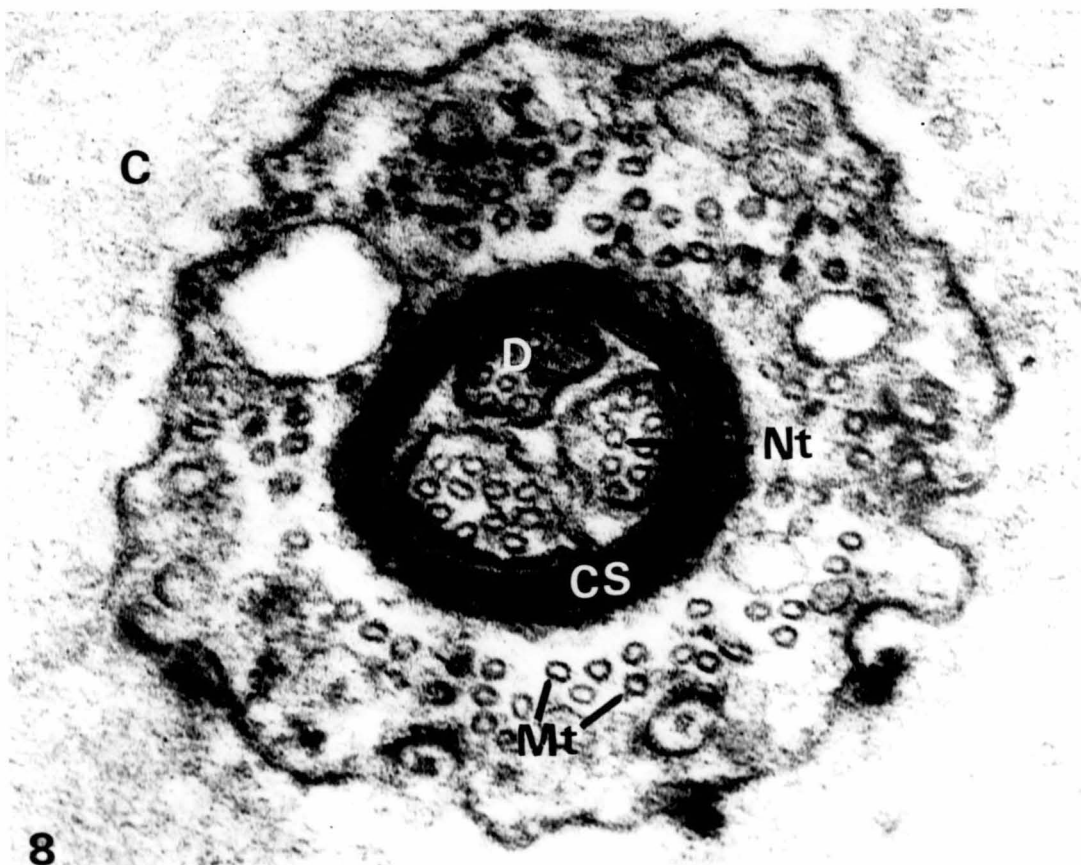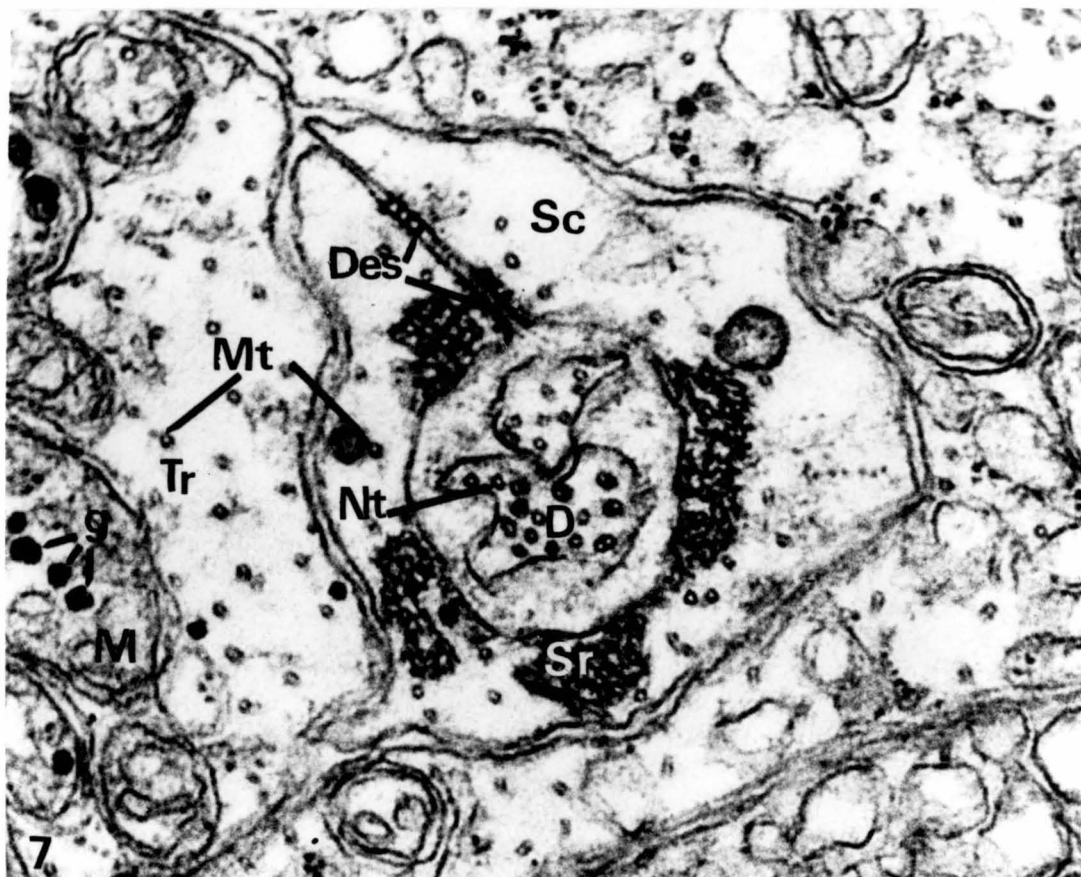
from the stomach of the frog <u>Rana pipiens</u> (Lillibridge, 1968). Lillibridge showed that these structures assumed a different morphology when different fixatives were used and termed the structures vesicotubules. He suggested that these vesicotubules have the potential for communicating with the cell exterior.

The tip of the cell lies below very thin cuticle; this cuticle is however itself below a rather thick layer of electron dense material which is apparently very brittle. The nature of this electron dense material is not known but it could well be a calcified layer.

The middle portion of the cell is situated adjacent to the dendrites of the perikarya of the sensory neurones. This region of the cell contains numerous vesicles, large irregularly shaped vacuoles, numerous microtubules, numerous mitochondria and what appear to be elements of smooth endoplasmic reticulum. The vesicles appear to lie in strings and numerous vesicles have coats of dense material. It is of interest to note that coated vesicles were also reported by Hamilton (1968) in the vestibular hair cells from the inner ear of white rats. The function of these vesicles is not known. Many of the mitochondria in this region of the cell contain extremely electron dense granules suggesting that these cells might be involved in ionic or water transport. Microtubules are extremely numerous in the cells. Details of their structure

are described in the following section.

The Sensory Neurones.

Two types of sensory neurones are discernible in the mandibles. These are the ciliary type and the non-ciliary type sensory neurones. These are basically identical except for the differences in the dendrites. The ciliary type sensory neurones have their perikarya situated in the body of the animal and form the mandibular ganglia. The perikarya of the non-ciliary type sensory neurones are situated at the base of and within the mandible.

The Non-Ciliary Type Sensory Neurone (Figs. 3, 4, 6,7,8). Like the ciliary type neurone, the non-ciliary type neurone is a bipolar structure. The dendrite is long (about 100/um) and extends nearly the length of the mandible. The perikaryon bodies, mitochondria, smooth endoplasmic reticulum and numerous vesicles. The axons of these sensory neurones contain a few mitochondria and scattered neurotubules.

The dendrites of the non-ciliary type sensory neurons terminate in a slightly bulbous structure at the cuticular opening (Fig. 4). This very terminal portion of the dendrite contains vesicles but lacks the microtubules more characteristic of the lower part of the dendrite. Most of the length of the dendrite, except the portion just distal to the perikaryon, contains only microtubules

(neurotubules). The portion just distal to the peri-
karyon contains vesicles and mitochondria as well as the
microtubules.

These microtubules have an external diameter of
21 nm and a wall thickness of about 6 mn. These structures
are present in all the cell types of the mandible except
the epithelial cells. It is just possible to discern
the subunits of the microtubules; each microtubule being
made up of between 11 and 14 sub-units (the exact number
cannot be properly discerned here). These structures have
been described in detail from plant material by Ledbetter
and Porter (1963 and 1964). These workers were able to
show (Ledbetter and Porter, 1964) that the microtubule
consists of 13 filamentous sub-units, with the sub-unit
having a centre to centre spacing of 4.5 nm and a wall
thickness of 7 nm. They observed in some of their
plant material a "natural 'negative' staining" effect.
Their material was fixed in glutaraldehyde and post fixed
in OsO_4 and lightly stained in uranyl acetate and basic
lead citrate. The present material has been fixed in
OsO_4 only but stained for a longer period in the same
heavy metals. It is quite evident that the "natural
'negative' staining" effect is not manifested here. The
fixation of the material here has been excellent, considering
that the material has not been prefixed in glutaraldehyde.
Ledbetter and Porter (1964) observed that the microtubules

Fig. 9.

The sensillum on the left of the electron
micrograph contains both the ciliary
type neurone (Dc) and the non-ciliary
type neurone (Dn).   The ciliary
structure is of the 9+0 type and in this
section the satellite fibres can be seen.
The structure to the top of the two scolo-
pale cells (Sc) are the expanded parts
of the dendrites (D) just distal to the
perikarya of the non-ciliary type
sensory neurones.   The surrounding
cells are epithelial cells (Ep).
Magn.   X24,400.

Fig. 10.

Transverse sections of the perikarya of
the ciliary type sensory neurones in
the mandibular ganglion.   Note the
well formed Golgi bodies (G).   N
nucleus, ER endoplasmic reticulum, M
mitochondria.   Magn. X7650
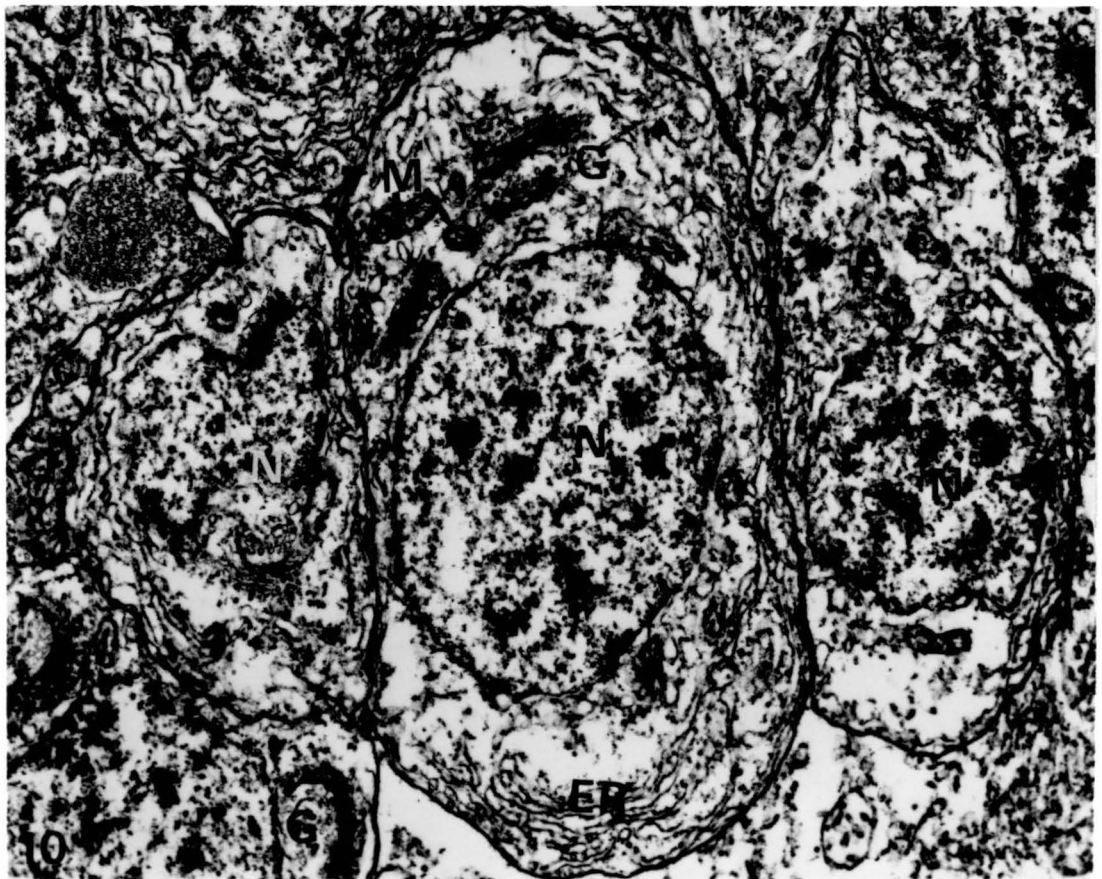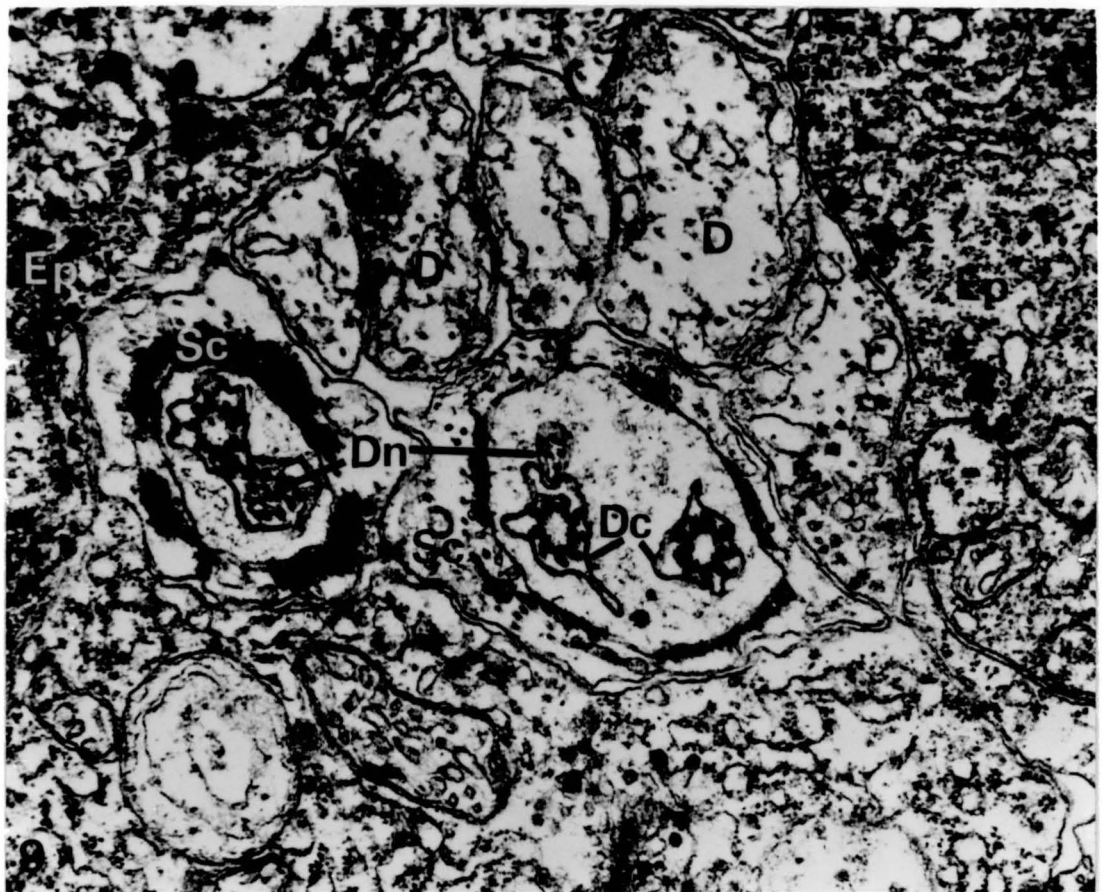
were associated with portions of cytoplasm which moves
and that the microtubules endow the cell with rigidity and
elasticity.   The cells in the mandible, because of the
articulation of the mandibles are no doubt subject to
considerable movement.

The Ciliary Type Sensory Neurone (Figs. 9 and 12).
The dendrite of this neurone extends the entire length
of the mandible to the perikaryon in the body of the
animal, dorsal to the base of the animal and apart from
the dendritic portion of the neurone it is identical to
the non-ciliary type sensory neurone.   The ciliary por-
tion of the dendrite is that portion enclosed by the
scolopale cell.   It is probable that only this portion
of the dendrite bears the ciliary structure: the present
author has been unable to obtain sections of the ciliary
type sensory neurones distal to the region of the scolopale
cell showing ciliary structures due mainly to orientation
difficulties.   The ciliary structures found in the sensory
neurones are simpler than any of the ciliary structures so
far reported in arthropod sensory receptors.   They consist
of the 9 + 0 arrangement of doublets on the more terminal
portions with the basal portion of the ciliary structures
having the same arrangement but there are what may be termed
outer fibres.   The whole ciliary structure is probably
very similar to the structure of the ciliated secretory

Fig. 11.

Longitudinal section of the perikaryon of
a non-ciliary type sensory neurone. One
of the mitochondria (M) in the perikaryon
contains aggregations of electron dense
granules (g). The "chloride" cell at
the bottom of the electron micrograph
contains lysosomes (Ly). Unstained.
Magn. X11,250.

Fig. 12.

Longitudinal section of parts of the den-
drites of the ciliary type sensory
neurones (D). The cell on the top of
the electron micrograph, under the
cuticle (c) is an epithelial cell (Ep)
bearing irregular nucleus (N), mito-
chondria (m) rough endoplasmic reticulum
(RER), ribosomes and vesicles (Ve).
Some of the vesicles contain electron
dense granules. ClC "chloride" cell
Tr, "trichogen type" cell. Magn.
X23,400.

11

12
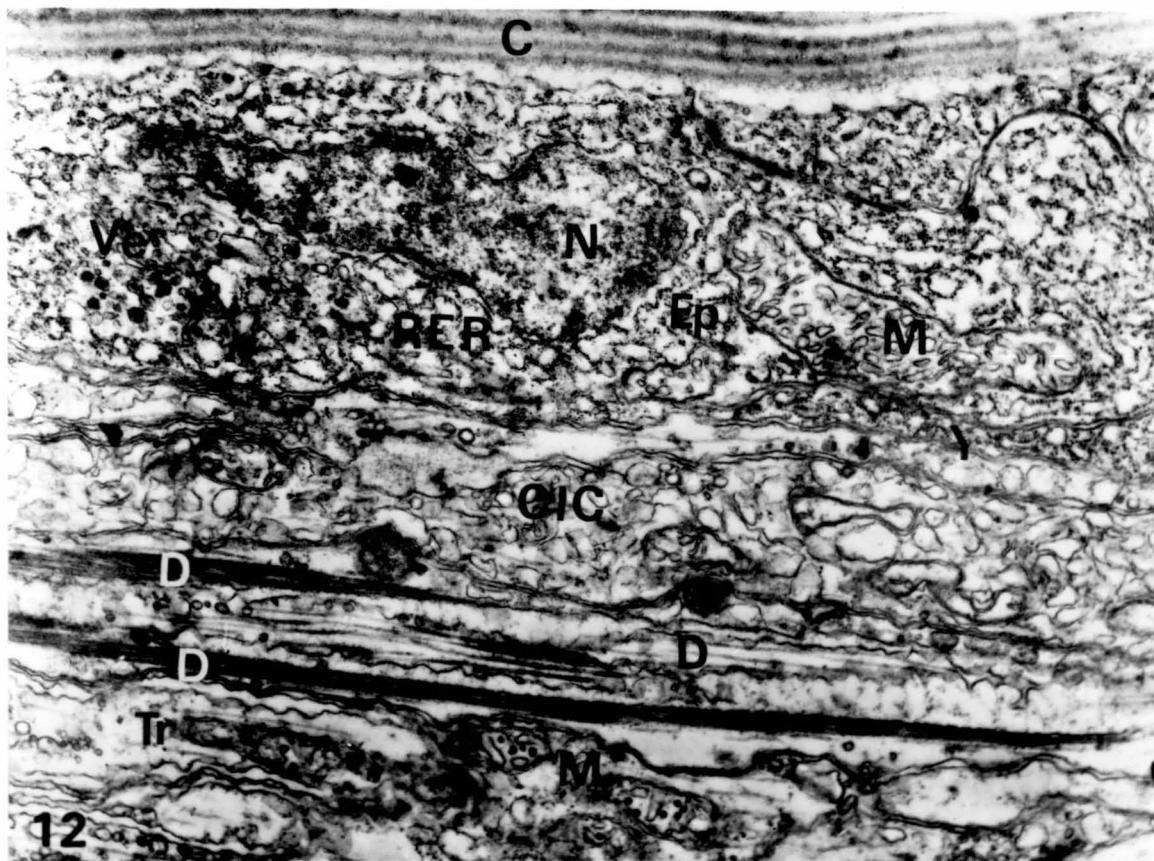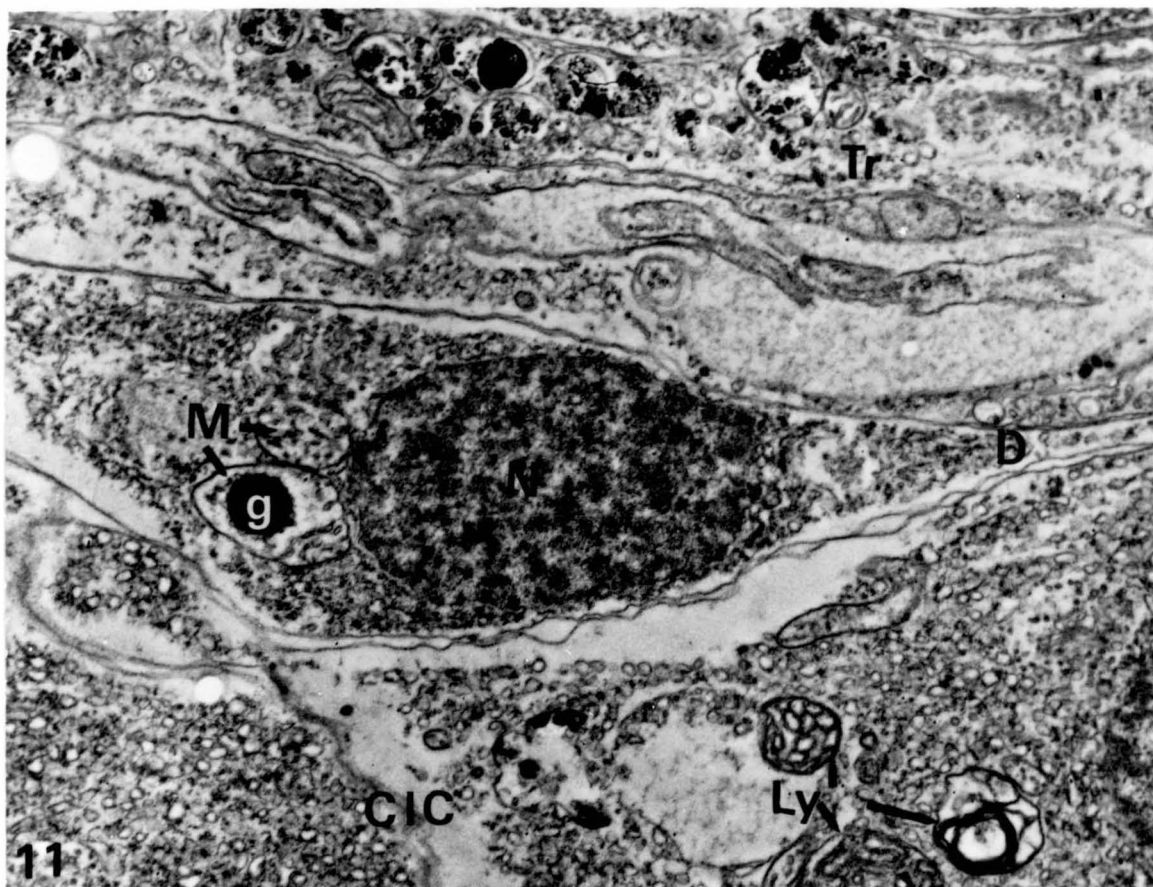
cells in the pars distalis of the mouse hypophysis described by Barnes (1961). The ciliary structure here is thus simple compared to the ciliary structure of the auditory organ of locust (Gray, 1960) where ciliary bodies, roots and rootlets are present. The heterodynal scolopidia in the chordotonal organs of Carcinus maenas (Whitear, 1962) and the sensory receptor of the statocysts of the crayfish Astacus fluviatilis (Schone and Steinbrecht, 1968) both have ciliary rootlets or axial filaments (Whitear, 1962) in the ciliary structure. Although ciliary bodies have not been found in the ciliary type of neurones in the mandibles these structures have been located in the ciliary type neurones of the antennae of this animal. Barnes (1961) has noted that cilia of the 9 + 0 type are usually sensory in contrast to the 9 + 2 type which are associated with motile structures.

DISCUSSION.

There are very few fine structural studies of the sensory receptors of crustaceans. Perhaps the only two works relevant to the following discussion are those of Whitear (1961) on the proprioreceptors in the shore crab Carcinus maenas and the recent work of Schone and Steinbrecht (1968) on the sensory receptors of the statocysts of the crayfish Astacus fluviatilis. A review on other works on the study of crustacean and other arthropod sensory receptors can be found in Bullock and Horridge (1965).

These authors have noted a number of works on the sensory structures of the mandibles of various arthropods but I have been unable to locate any work on the fine structure of the sensory receptors in the mandible of arthropods. Perhaps the closest is the recent work of Moulin (1968) on the hypopharyngeal organ of the cockroach <u>Blabera craniifer</u>. The fine structure and related work on the behaviour and electrophysiology of some of the sensilla of the other appendages of insects have been well documentated. These include the works of Gray (1960), Slifer (1954, 1955 and 1961), Slifer et al. (1957 and 1959), Dethier (1955) and Hodgson (1964). Since, perhaps with the exception of Hodgson (1958), Case et al. (1960) and Laverack (1962a and b), no electrophysiological data on chemoreceptors of crustacea are available, the above works on insects will be used here for comparative purposes.

<u>Function of the Sensilla of the Mandibles</u>. Because of the extremely small size of <u>Gladioferens</u> it is at present technically impossible to obtain electrophysiological data on the nature of the sensilla. The function of these sensilla can only be deduced from a comparative study of structural homology and less specifically from behavioural studies. The only data available for such a comparative study, as stated above, are the studies on insects. From these it would seem, as Slifer (1961) has inferred (although she asked the question as to whether cilia occur in sense

organs which are not mechanoreceptors) that the non-ciliary types of neurones are probably chemoreceptors. The non-ciliary type sensory neurones here have naked exposed terminations and Slifer (1961) has shown this to be the case with insect chemoreceptors. Moulin (1968) found the ciliary type neurones in the rather complex sensilla of the hypopharynx of the cockroach Blabera craniifer and suggested that these sensilla act as contact chemoreceptors. Some of the sensilla here contain both the ciliary and the non-ciliary type sensory neurones and these probably have the same function as the sensilla of the hypopharynx of the cockroach Blabera craniifer.

It appears, from available data, that the sensory neurones with ciliary structures in other arthropods innervate mechanoreceptors and until contrary data becomes available it can only be inferred that the ciliary type sensory neurones function as mechanoreceptors.

As described earlier, at least one of the cell types in the mandibles appear to be involved in water or ionic transport. It is thus probable that a third type of sensory receptor exists; an osmoreceptor. Unfortunately, Gladioferens is too small to lend itself to electrophysiological studies but behavioural work on salinity preferences is now being undertaken to determine their ability to detect changes in salinity.

The Sensilla and Selective Filter Feeding. From
the analysis of the gut content it seems that this animal
feeds almost, if not exclusively, on phytoplankton. The
actual feeding mechanism has never been observed but there
is no reason why it should not be basically similar to
that described for Eurytemora velox by Lowndes (1935).
Currents are set up by the oral appendages, the main current
flowing towards the anterior of the animal and impinging
on the labrum. Food particles carried by the current
are diverted into the mouth where they hit the labrum. It
is generally claimed that the maxillae are used in filtering
the food particles. However, the labral glands appear to
secrete mucus which aids in the filter feeding process by
concentrating the food particles into pellets just prior
to ingestion.

Since the sensilla are located mainly in the labrum
and the mandibles the animal could possible differentiate
between food and detritus and thus be able to reject
pellets containing a high proportion of dedritus. Analysis
of gut content shows that it is relatively free from detritus
and since the estuarine environment contains quite a high
concentration of detritus it seems that the animal is able
to select the food it ingests. The ability to differentiate
between food and detritus and selectively ingest only the
food could be an important factor of adaptation in estuarine
species of filter feeders.

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Electron micrograph through part of a
neurosecretory cell in the brain of
<u>Sulcanus conflictus</u> showing the numerous
electron opaque neurosecretory vesicles,
part of a Golgi complex (towards bottom
right of electron micrograph), mitochondria
and rough endoplasmic reticulum (bottom of
electron micrograph).   X69,000.
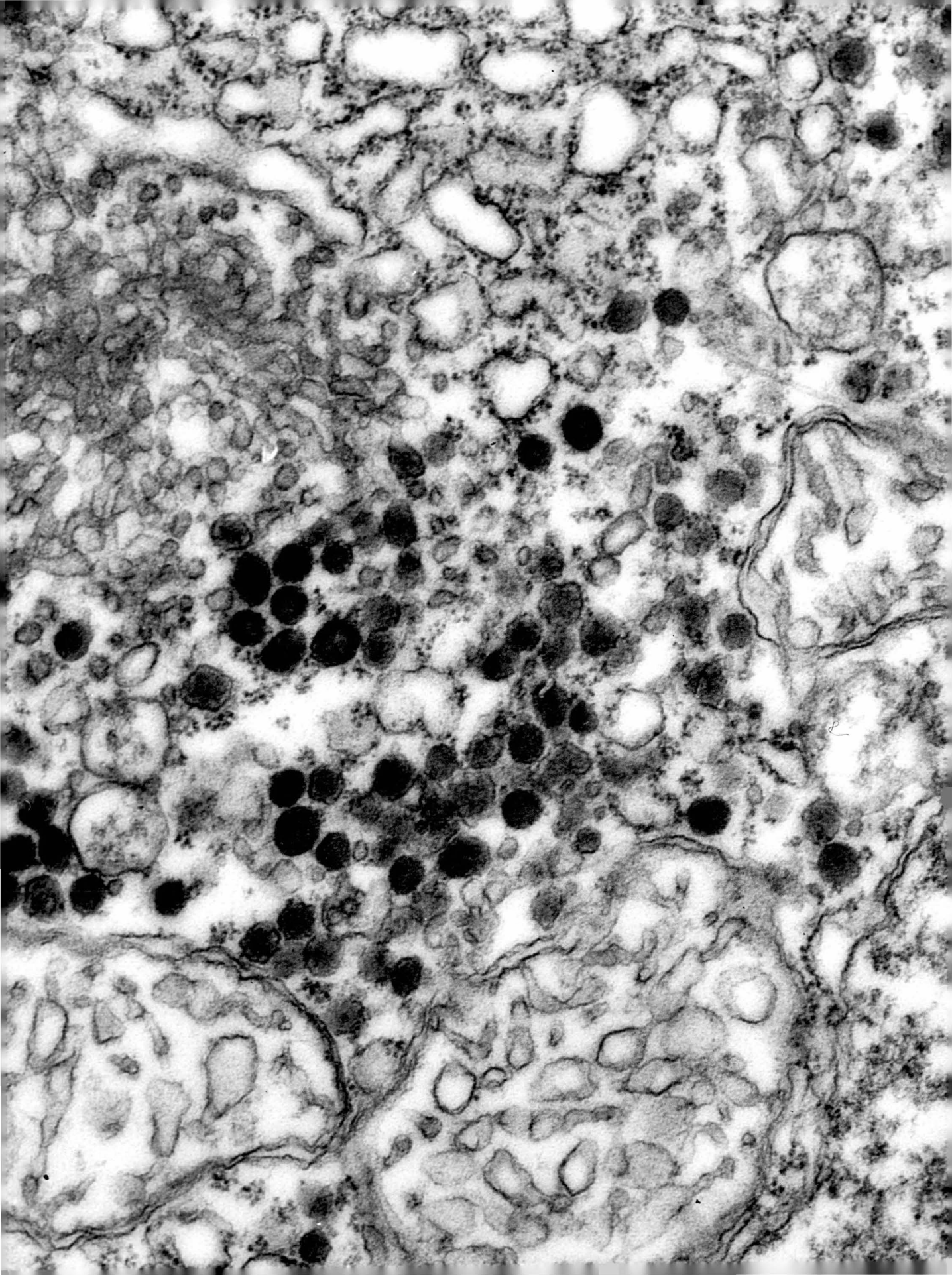
THE NEUROSECRETORY SYSTEM

INTRODUCTION

The term neurosecretion has been interpreted in different ways by different investigators. For this reason, the concept of neurosecretion and the diagnosis of neurosecretory cells will be briefly reviewed and discussed here. Neurosecretory cells are modified neurons which produce secretory materials that are secreted into the blood where they may effect a target organ or directly onto the cells of the target organ. In some systems the neurosecretory material is stored in swollen axonal endings, forming neurohaemal organs (Knowles and Carlisle, 1956), prior to discharge into the vascular system. In most systems neurosecretory cells form the link between non-secretory neurons and endocrine organs but neurosecretory fibres have also been shown to form synaptoid contacts with non-endocrine cells such as muscular elements (Bowers and Johnson, 1966), other neurosecretory cell axons, and non-neurosecretory axons (Scharrer, 1969).

Three main definitions of neurosecretory cells have been proposed. The first of these confines neurosecretory cells to those neurons that produce hormones (Bern, 1962 and 1966; Bern and Hagadorn, 1965).

A broader definition was proposed by Scharrer (1969) who included as neurosecretory cells not only those cells that secrete hormones but also all those cells that secrete "certain non-hormonal mediators that bridge the gap between neurotransmitters and neuro-hormones".   The third definition, an all embracing one, was that proposed by De Robertis (1964) who argued that all neurones are capable of secreting substances and may all be termed neurosecretory.   On this last definition, Scharrer (1969) has pointed out that the term neurosecretory is now firmly established and should be restricted to those neurons in which the production of chemical mediators has become the dominant feature.

The secretory material of neurons can be visualized under the electron microscope as vesicles of various sizes with contents of various electron opacity. Two main types of vesicles are found in neurosecretory cells: the first which are mainly polypeptides, are usually over 100 nm in diameter, spherical in shape and completely filled with electron opaque material; the second type, which are mainly biogenic amines, are usually less than 100 nm diameter, less regular in shape and the vesicles are not completely filled with electron opaque material leaving a clear halo between the dense contents and the membrane (Knowles, 1965).   A third type,

of vesicle is sometimes found: these are electron lucent
(Maddrell, 1967 and Scharrer, 1969). Neurohormones are
mainly peptides and appear as electron opaque vesicles
of between 100 nm and 300 nm. Neurohumors are neuro-
transmitters. These are mainly biogenic amines and
appear as clear or "halo" vesicles of under 100 nm
under the electron microscope. The presence of neuro-
hormones is localized, generally in axonic terminals
(Scharrer, 1969).

There appears to be one important property of
neurosecretory cells that is generally accepted by
most investigators in this field: Welsh (1961),
Knowles and Bern (1966) and Scharrer (1969) have all
pointed out that a striking difference between neuro-
secretory neurons and non-neurosecretory neurons is
the ability of the neurosecretory neurons to sustain
their effect on the target organ. Non-neurosecretory
neurons on the other hand have a short effect on their
target organs in that they can only secrete small
ammounts in short bursts and they have a relatively
long recovery cycle. It is thus possible to dif-
ferentiate micromorhologically, neurosecretory neurons
from non-neurosecretory ones because neurosecretory
neurones contain an abundance of secretory vesicles.

The demonstration of an abundance of granules
does not necessarily mean that the cell is continually

discharging the vesicles (Bern and Hagadorn, 1965).
Apart from having a very short period of discharge,
the neurotransmitters of non-neurosecretory neurons
are highly localized and at least in the case of
noradrenaline, there is a "shuttle service" type of
operation where the secretion is recycled (Scharrer,
1969).   This means that synaptic vesicles are normally
confined to axonic terminals in contrast to neurosecretory
vesicles which occur throughout the neuron.   Thus on
the above characters, a neuron may be termed a neuro-
secretory cell (as defined by Scharrer, 1969) if it con-
tains an abundance of vesicles which may range in size
from 30 to 400 nm in diameter (and containing material
of any electron opacity), extensive endoplasmic reticulum
and numerous Golgi complexes.   The latter two features
when present together are evidence of active synthesis
of proteinaceous material for extracellular transport
(Beams and Kessel, 1968).   If, as well as the above
characters, it can be demonstrated functionally that the
nerve cell has a sustained action on its target organ then
neurosecretion in that cell is established.   In this study
the main diagnostic characters used are the micromorphological
ones mentioned above.

While the literature on the neurosecretion of
Crustacea is reasonably extensive (see Bern and Hagadorn, 1965 and

Gabe, 1966) the bulk of it deals with the Decapoda. The
literature on neurosecretion of the class Copepoda,
like the other classes in the Entomostraca, is very
sparse. Carlisle and Pitman (1961), in a short paper,
reported a pair of lateral groups of cells in the
anterior of the brain of the calanoid copepod, <u>Calanus</u>.
They showed seasonal changes in the quantity of stain-
able material in these cells and demonstrated that
extracts of the brain and frontal organs (= X-organ?,
see following paragraph) had chromactivating effects
on the decapod, <u>Leander</u>. They thus established the
presence of a neurosecretory system in the cephalosome
of the Copepoda.

In a histological study of the calanoid
copepod, <u>Heliodiaptomus</u>, Govindarajulu (1968) briefly
reported the presence of three pairs of lateral groups
of neurosecretory cells (based mainly on staining
properties). The studies of Carlisle and Pitman
(1961) and Govindarajulu (1968) are the only published
works of which I am aware dealing directly with the
neurosecretory system of the Copepoda although there
have been papers dealing with the X-organ. Dahl (1963)
and Elofsson (1966) have shown that the structures
which had previously been designated as frontal organs
are not frontal organs but are more likely to be the
X-organ (also see section of this thesis on the X-organ

complex). It is evident from Elofsson's (1966) study
that the structure of the X-organ is very complex
and beyond the resolution of the light microscope.

There is a pair of organs situated in the an-
terior edge of the brain whose function is not clearly
understood. These organs were first described by
Claus (1863) in the calanoid copepod, _Eucalanus_
attenuatus and the organ was named after him. Claus
(1863) considered them to be statocysts. Esterly
(1908) regarded Claus's organs in _Eucalanus elongatus_
to be inverted eyes, but Lowe (1935) suggested that
Claus's organs in _Calanus finmarchicus_ were statocysts.
Elofsson (1966) was unable to locate Claus's organ in
a number of copepods (including _Calanus_) which he
studied. The anterior pair of neurosecretory cells
figured by Govindarajulu (1968) coincides with the
position of Claus's organs and could possibly be
identical with these. Carlisle and Pitman (1961)
did not figure the neurosecretory cells at the anterior
edge of the brain of _Calanus_ but from their description
of their position it is possible that these are also
identical with Claus's organs.

The following is a description of the ultra-
structural morphology of the neurosecretory cells of
the brain, Claus's organs and the X-organs of the cal-
anoid copepods, _Calanus helgolandicus_ and _Sulcanus_
conflictus with a discussion of their possible functions.

Comparison between the X-organ complex of these cope-
pods is made with those in the other Crustacea groups.

OBSERVATIONS

NEUROSECRETORY CELLS OF THE BRAIN

The position of the neurosecretory cells in the brain as well as Claus's organs and the X-organ complex is shown diagramatically in Fig. 1. Under the light microscope, there appear to be three pairs of neurosecretory cells, including Claus's organs.

The neuronal cell of Claus's organ contains elementary neurosecretory vesicles of about 120 nm in diameter (Fig. 5). There are also electron opaque, non-membrane-bounded granules of about 50 nm diameter amongst the ciliary coil of Claus's organ. (Fig.12).

The second (anterior lateral) pair of neuro-secretory cells are situated close to the nerves to the first antennae. There is only one cell on each side of the brain. The perikarya of these cells contain numerous electron opaque granules of about 250 nm in diameter, extensive rough endoplasmic reticulum and Golgi complexes (Figs. 2 and 3). The axons of these cells appear to curve around the periphery of the brain and extend towards the X-organ complex. I have not found their termination.

The posterior lateral pair of neurosecretory cells also consist of a single cell on each side of the brain
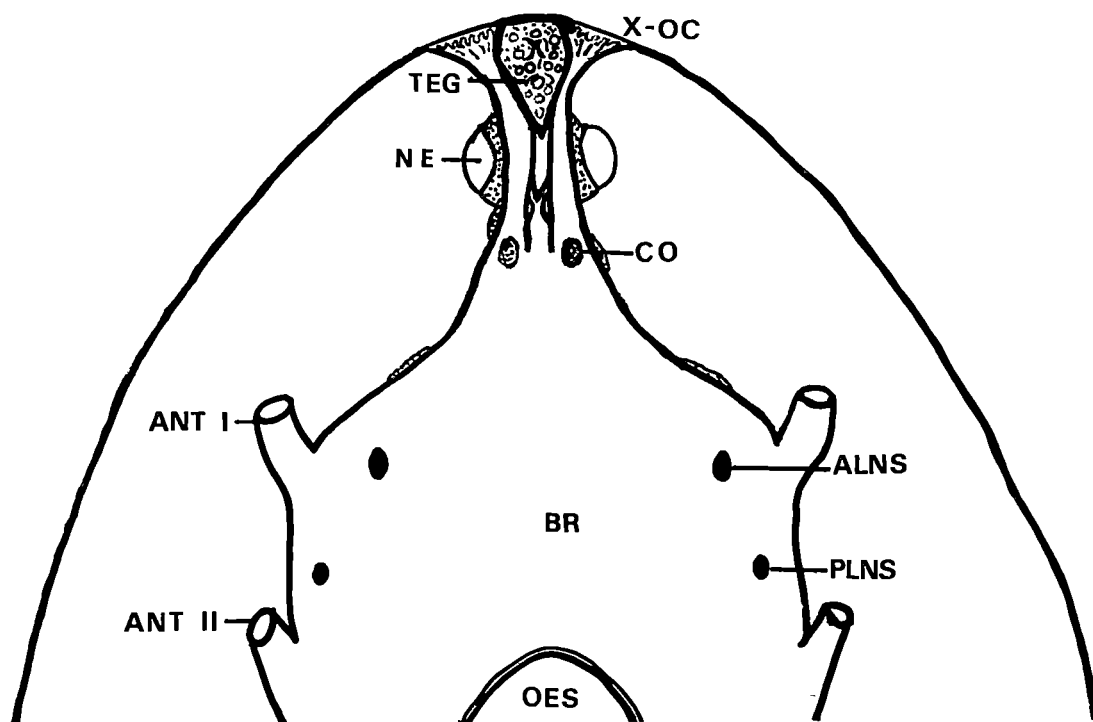
Fig. 1.

Schematic digram showing the main neuro-
secretory cells and organs of a general-
ized calanoid copepod.   ANT I = first
antenna;   ANT II = nerve to second antenna;
ALNS = anterior-lateral neurosecretory
cell;   BR = brain;   CO = Claus's organ;
NE = nauplius eye;   OES = oesophagus;
PLNS = posterio-lateral neurosecretory
cell;   TEG = tegumental gland and
X-OC = sensory pore X-organ.

Fig. 2.

Electron micrograph of a frontal section through
part of the brain of Sulcanus showing the
perikaryon of the anterior lateral neuro-
secretory cell with its numerous electron
opaque neurosecretory vesicles.   X9,000.

Fig. 3.

Electron micrograph of a section similar
to that in Fig. 2 but at a higher magni-
fication showing electron opaque neuro-
secretory vesicles,rough endoplasmic re-
ticulum (RER), part of a Golgi complex
(G); nucleus (N) and mitochondria (M).
X27,600.
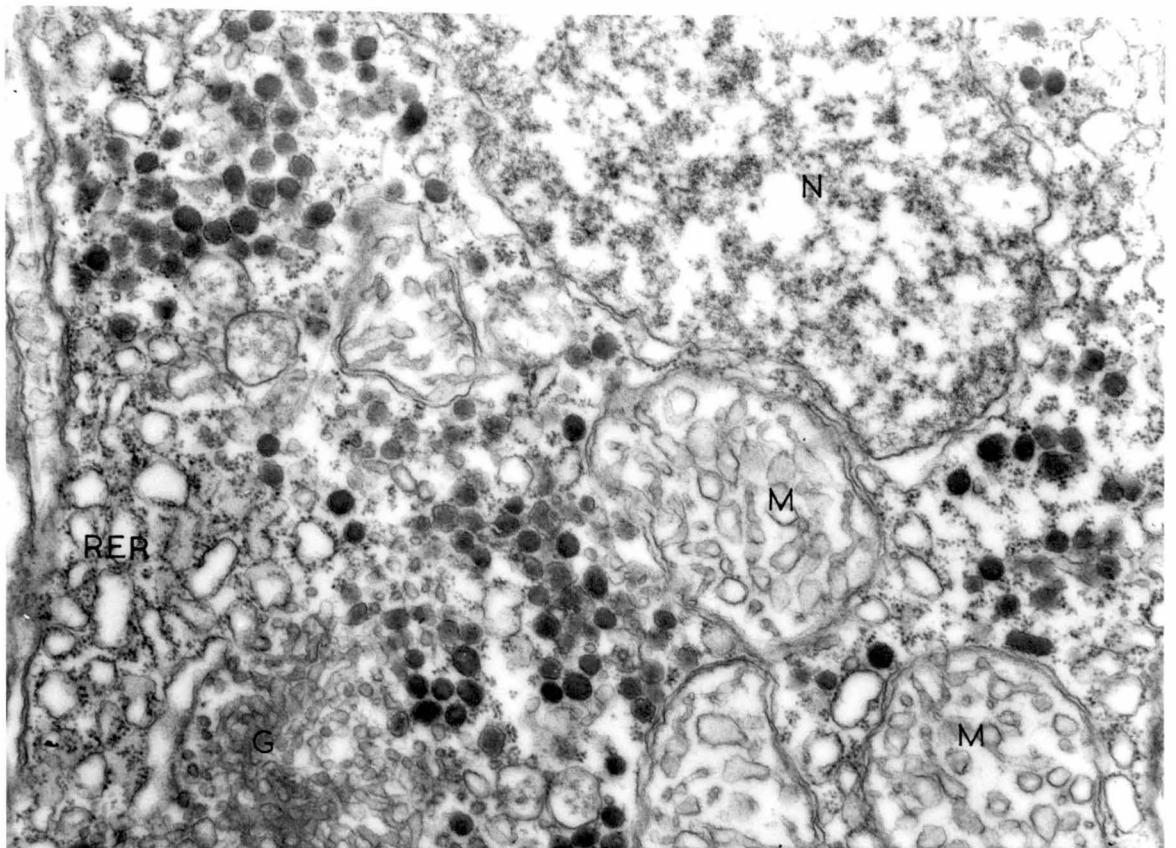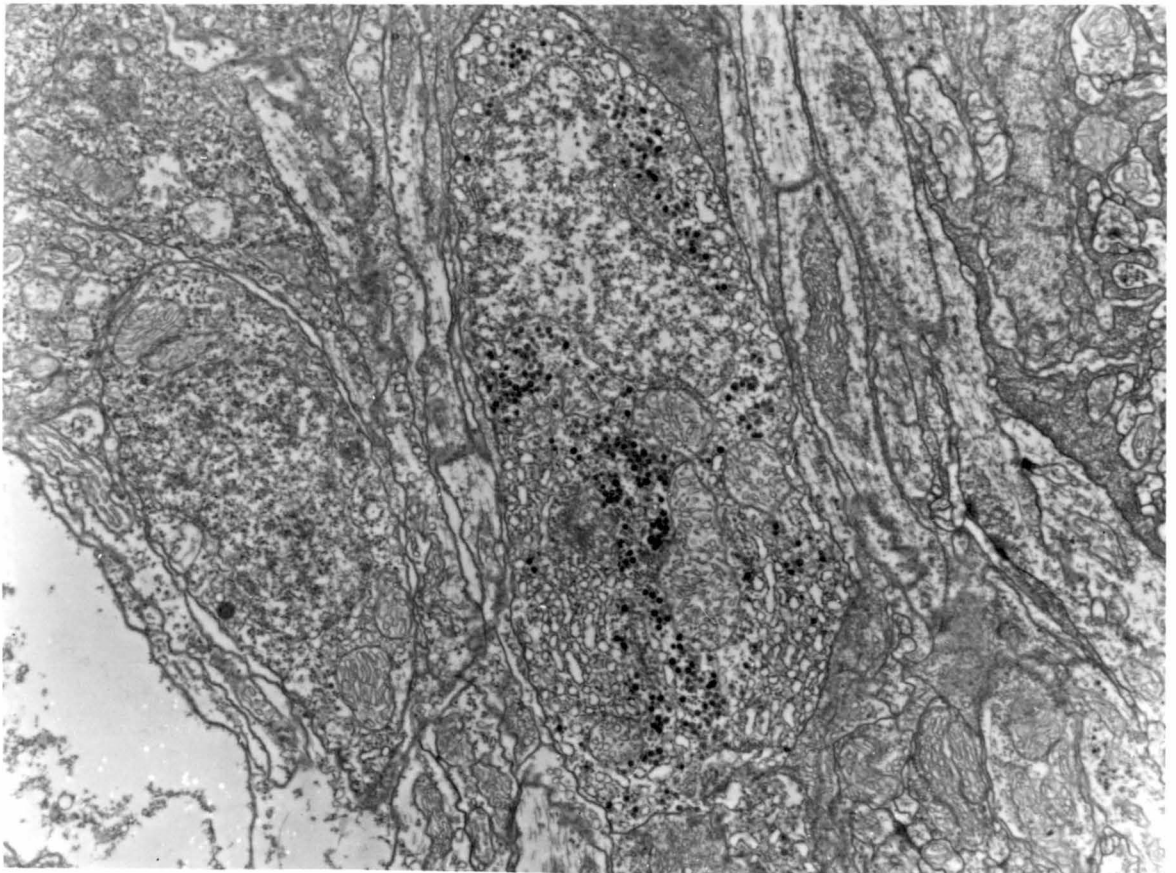
with the axons directed anteriorly. I have not been able to trace the axons far from the perikarya. The cells appear morphologically identical to those of the anterior lateral pair of neurosecretory cells.

CLAUS'S ORGAN

Calanus helgolandicus

There is a pair of Claus's organs, situated laterally and embedded in the extreme anterior region of the brain (Fig. 4). The organ consists of two cells: a ciliated neuron and a companion cell (Fig. 6). A schematic reconstruction of Claus's organ is shown in Fig. 7.

The ciliated neuron is a bipolar cell with the ciliated structure towards the anterior end and the axon extending posteriorly. The cilia show a 9 + 0 arrangement of fibrils characteristic of non-motile or sensory cilia. The 9 + 0 arrangement of fibrils extend only a short distance (about 0.6 um) from the base of the cilia (Fig. 5). The ciliary body is not well developed and there are no ciliary rootlets. The more distal parts of the cilia contain only a few fibrils (usually one or two). The extremely long cilia (possibly up to or over 200 um) are thrown into a compact oval-shaped ciliary coil, measuring about 14 um by 20 um. The periphery of the ciliary coil is surrounded by the "companion cell" and by the neuron itself, where the cilia arise. The perikaryon of the neuron contains an almost spherical nucleus measuring about 15 um in diameter.
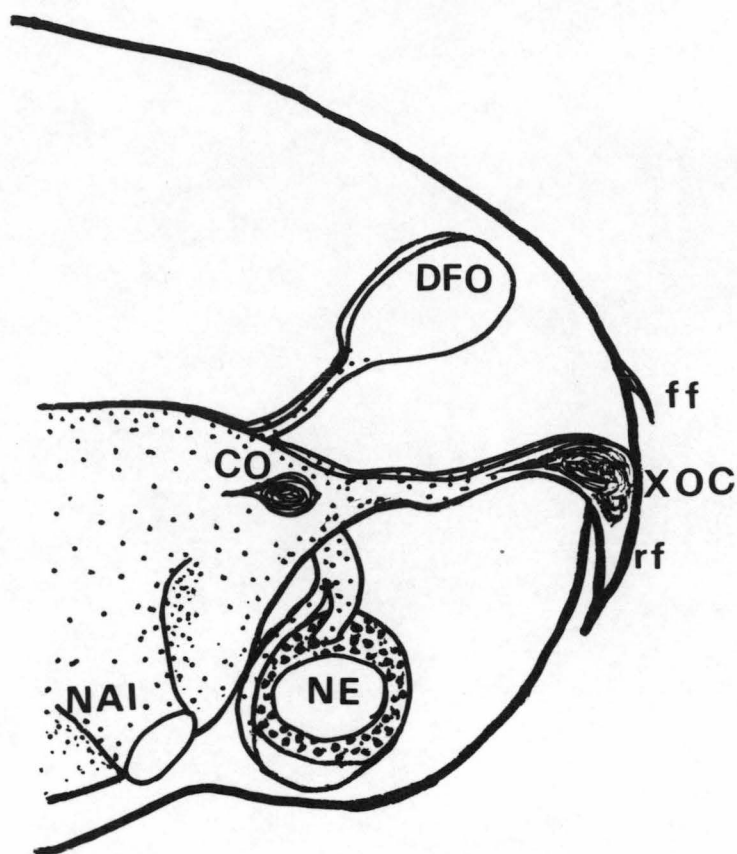
Fig. 4.

Diagram showing the brain and the sensory
and neurosecretory organs in Calanus.
CO = Claus's organ; DFO = dorsal frontal
organ; ff = frontal filaments; NAI =
nerve to first antenna; NE = nauplius eye;
rf = rostral filament and XOC = sensory
pore X-organ.

Fig. 5.

Electron micrograph of a section through
part of Claus's organ of Calanus showing
parts of the sensory neuron (SN) and the
"companion cell" (CP).   It is seen
that the 9 + 0 arrangement of fibrils
appear to extend only a short distance
from the basal body of the cilia.
Note the nucleus (N), mitochondria (M)
and the numerous electron opaque vesicles
in the sensory neuron.   The companion
cell contains microbodies (B) and
numerous microvesicles.   X27,600.

Fig. 6.

Electron micrograph of a section similar
to that shown in Fig. 5 but at a lower
magnification.   Note the numerous
electron opaque neurosecretory vesicles in
the sensory neuron (SN) and the micro-
bodies (B) in the "companion cell" (CP).
X7,500.
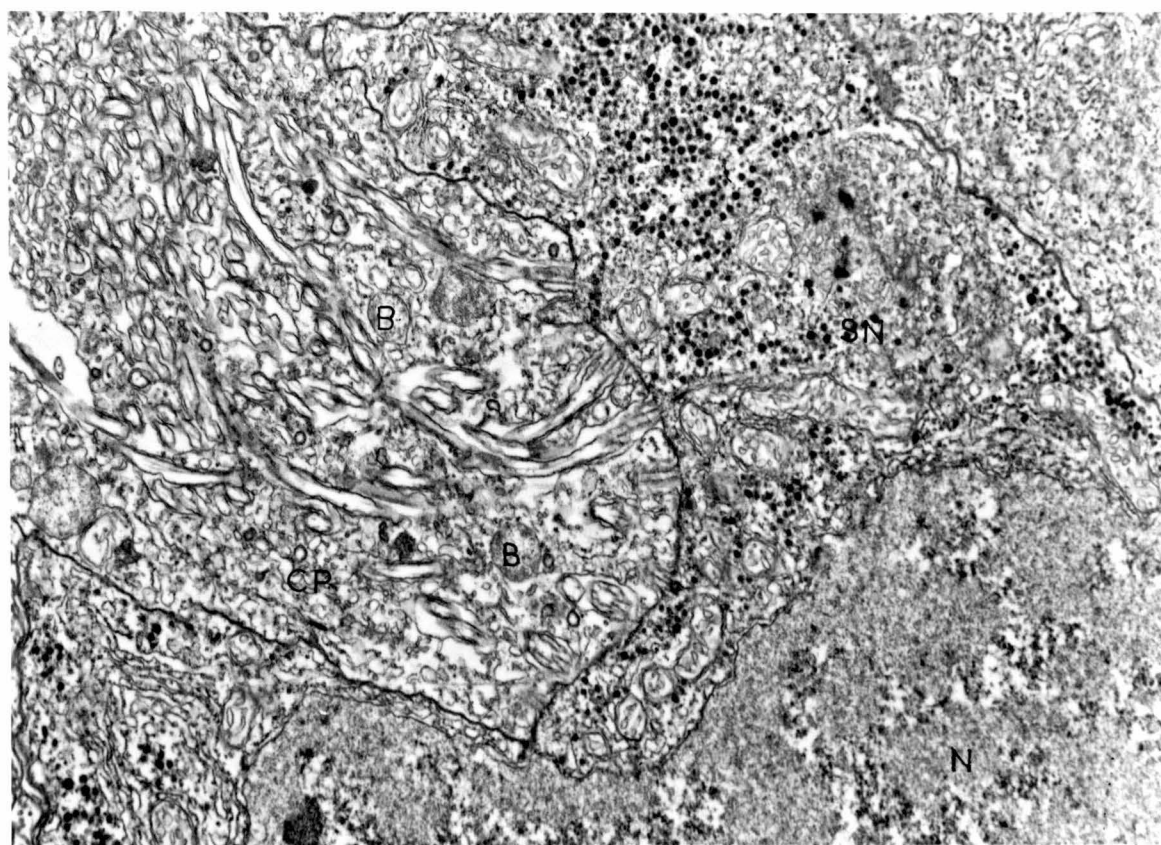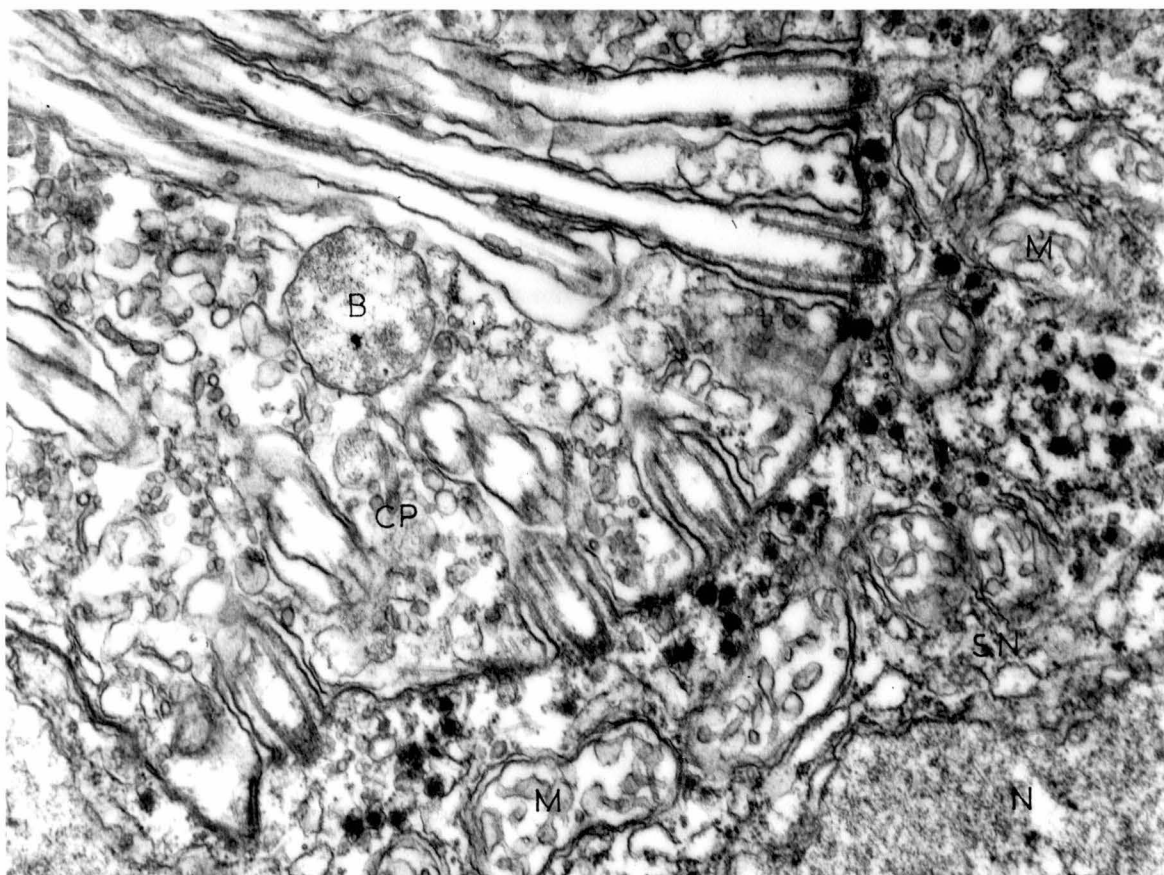
There are also numerous mitochondria, microvesicles, membrane bounded electron opaque granules measuring about 120 nm in diameter, extensive rough endoplasmic reticulum, neurofibrils, Golgi complexes, micro-bodies and free ribosomes (Fig. 8). The axon of the ciliated neuron also contains numerous 120 nm diameter membrane-bounded, electron opaque vesicles, neurofib-rils and scattered mitochondria. I have been unable to trace the axon to its termination nor have sites for the discharge of the electron opaque neurosecretory granules been detected.

The "companion cell" surrounds the ciliary coil of the ciliated neuron and processes of this cell "interdigitate" with the cilia on the periphery of the ciliary coil (Fig. 5). The "companion cell" con-tains numerous microvesicles, mitochondria, microbodies and endosplasmic reticulum. The "companion cell" forms the wall of the chamber containing the ciliary coil and this close association suggests that the "companion cell" may be involved in the maintenance of the unusually long cilia of the ciliated neuron.

Sulcanus conflictus

Unlike that in Calanus, Claus's organ in Sulcanus is not situated within the brain but in an anterior projection of the brain and dorsal to the nerves from the nauplius eye (Fig. 9). The nerves of the X-organ complex

Fig. 7.

Schematic reconstruction of Claus's organ,
showing the sensory neuron (SN), ciliary
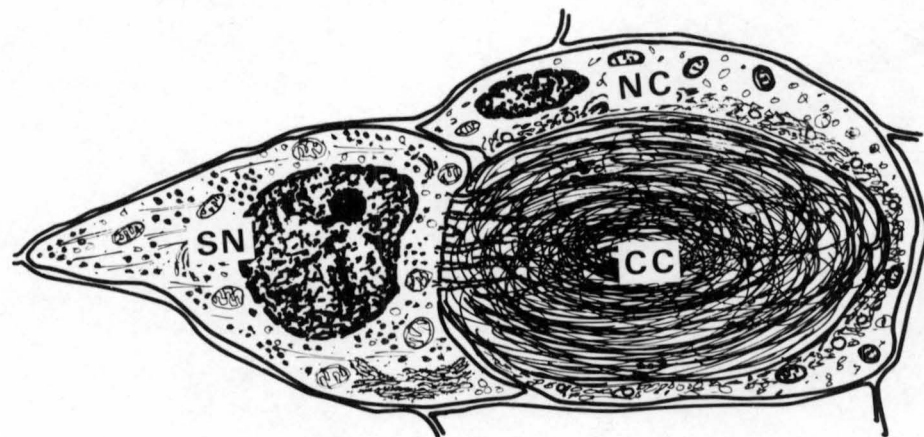coil (CC) and the companion cell (NC).

Fig. 8.

Electron micrograph of a section through
part of the sensory neuron of Claus's
organ in <u>Calanus</u> showing the nucleus (N),
rough endoplasmic reticulum (RER), Golgi
complexes (G), electron opaque neuro-
secretory vesicles, mitochondria (M)
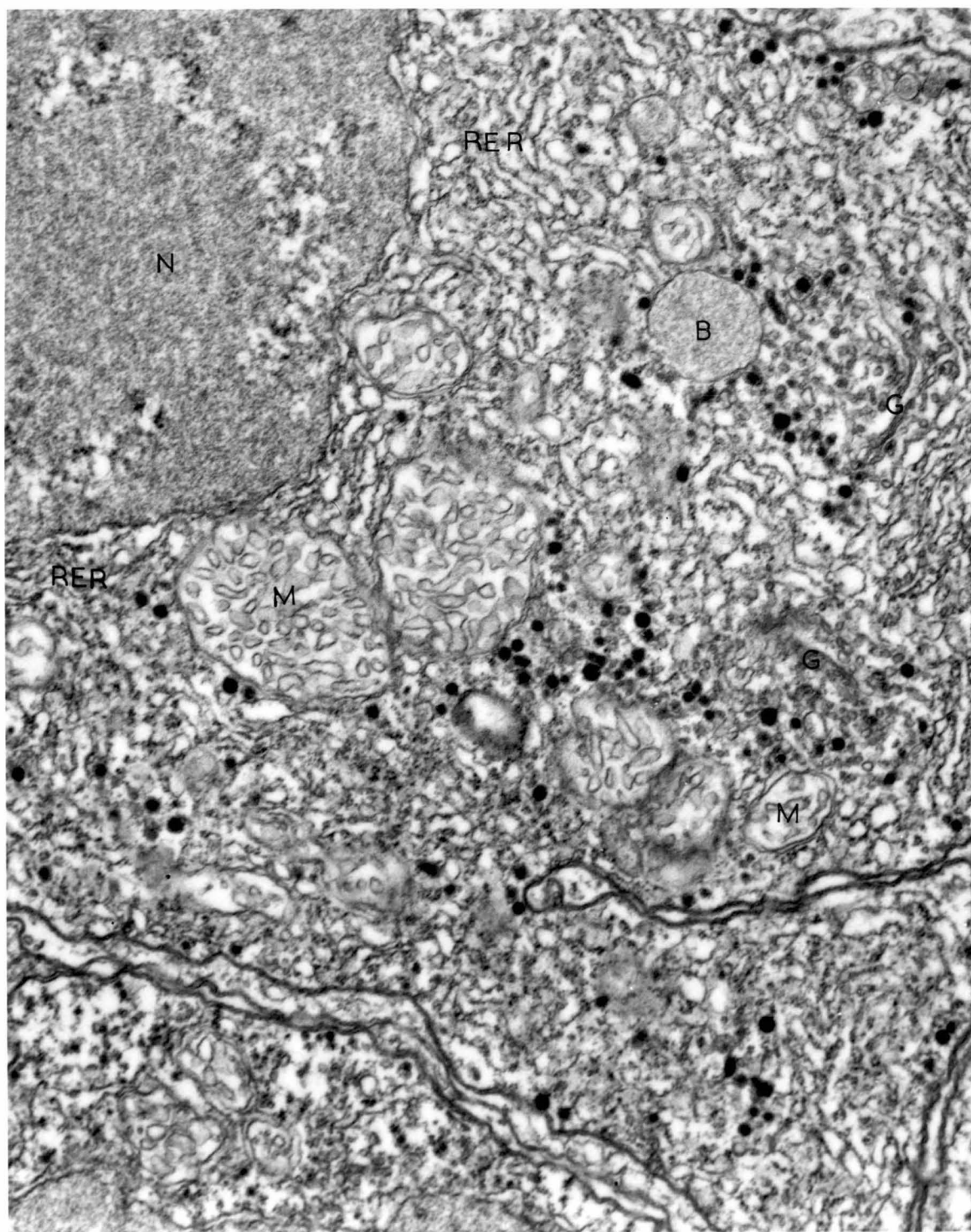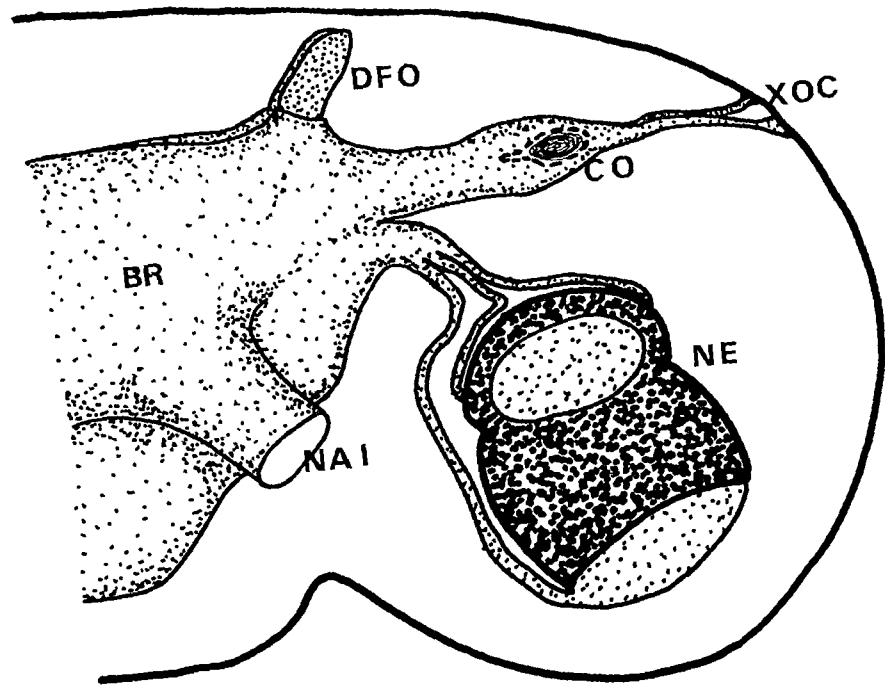and microbodies (B).   X18,000.

Fig. 9.

Diagram showing the brain and the sensory
and neurosecretory organs in Sulcanus. BR =
brain; CO = Claus's organ, DFO = dorsal
frontal organs; NAI = nerve to first
antenna; NE = nauplius eye and XOC =
sensory pore X-organ.

179.

skirt Claus's organ laterally Fig. 10), turn laterally anterior to that organ and terminate under the cuticle. In the "V" between the nerves of the paired X-organ complex are the conspicuous multinucleate tegumental cells (Fig. 11).

There is a pair of Claus's organ on each side of the extreme projection of the anterior part of the brain. The pairs of organs are in contact with each other (Fig. 10). In contrast to those in _Calanus_, where there is only one on each side of the brain and where they are separated from each other by some distance. The structure of the organ in _Sulcanus_ is nonetheless basically similar to that of _Calanus_ although there are some differences:

i) scattered amongst the cilia (not within the cilia), in the "haemocoelic space" of the ciliary coil are numerous electron opaque granules measuring about 50 nm in diameter (Figs. 12 and 13). These granules do not appear to be membrane bounded nor do they show the halo effect characteristic of monogenic amines.

ii) the "companion cell" appears to be highly developed compared to those seen in _Calanus_. The "companion cell" contain extensive smooth endoplasmic reticulum around the periphery of the ciliary coil (Figs. 10 and 13).

Fig. 10.

Electron micrograph of an oblique frontal
section through the ciliary coil (CC) of
Claus's organ of <u>Sulcanus</u> showing some of
the axons (a) and perikarya of the X-organ
skirting Claus's organ.   The smooth tubular
endoplasmic reticulum (STER) of the "com-
panion cell" of Claus's organ is quite
conspicuous. S_n = neurosecretory peri-
karyon of the secretory sensory neuron
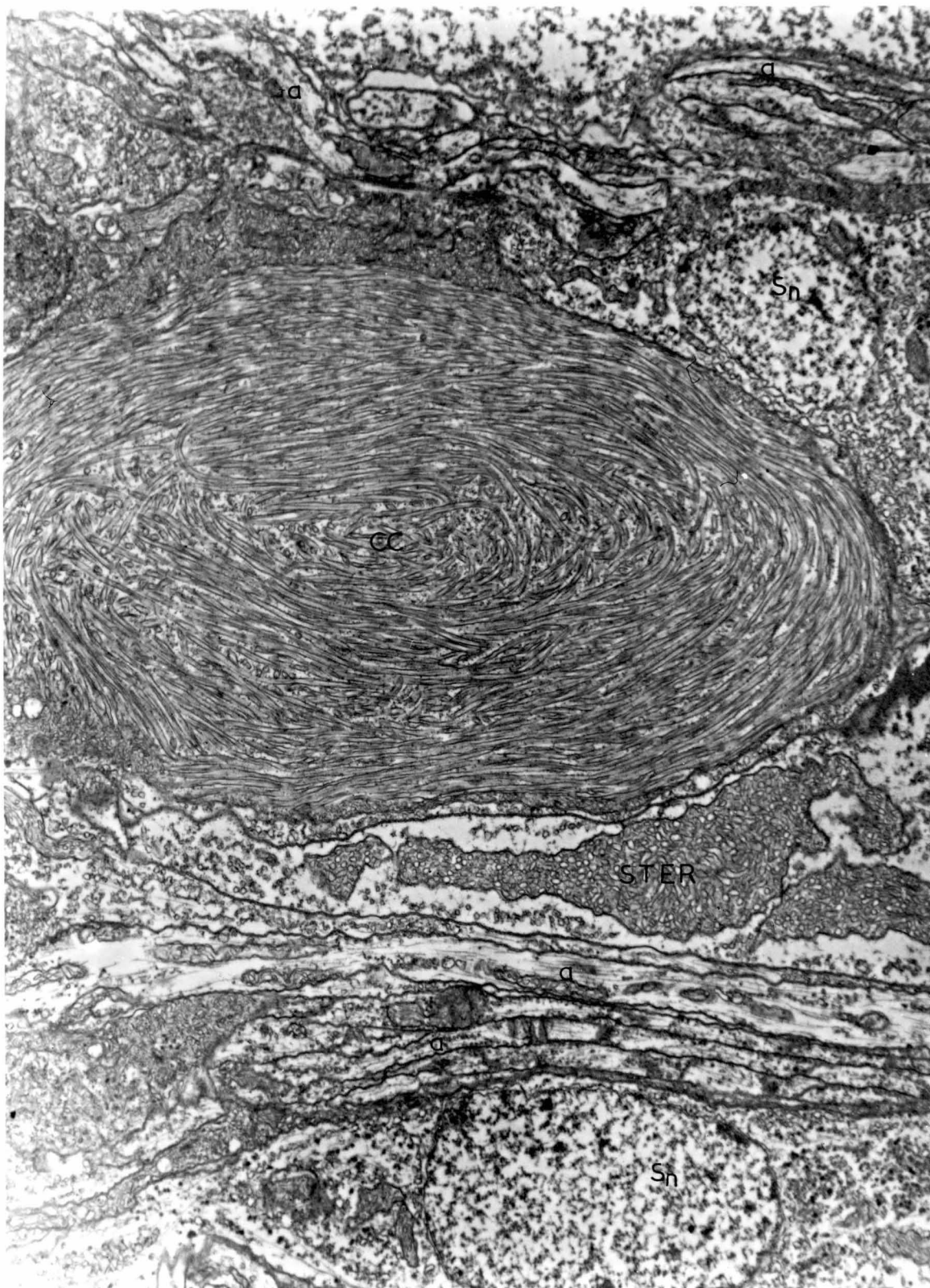of the sensory pore X-organ.   X7,500.

<u>Fig. 11.</u>

Electron micrograph of a frontal section through
the anterior region of Claus's organs of
<u>Sulcanus</u> showing the contact of Claus's organs
with the tegumental glands (TG) as well as
with the perikaryon of a sensory secretory
neuron of the X-organ.   Part of a neuro-
secretory axon can be seen on the top left
hand corner of the electron micrograph.   The
smooth tubular endoplasmic reticulum (STER) of
the "companion cell" of Claus's organs is
seen to surround the ciliary coil (CC) of Claus's
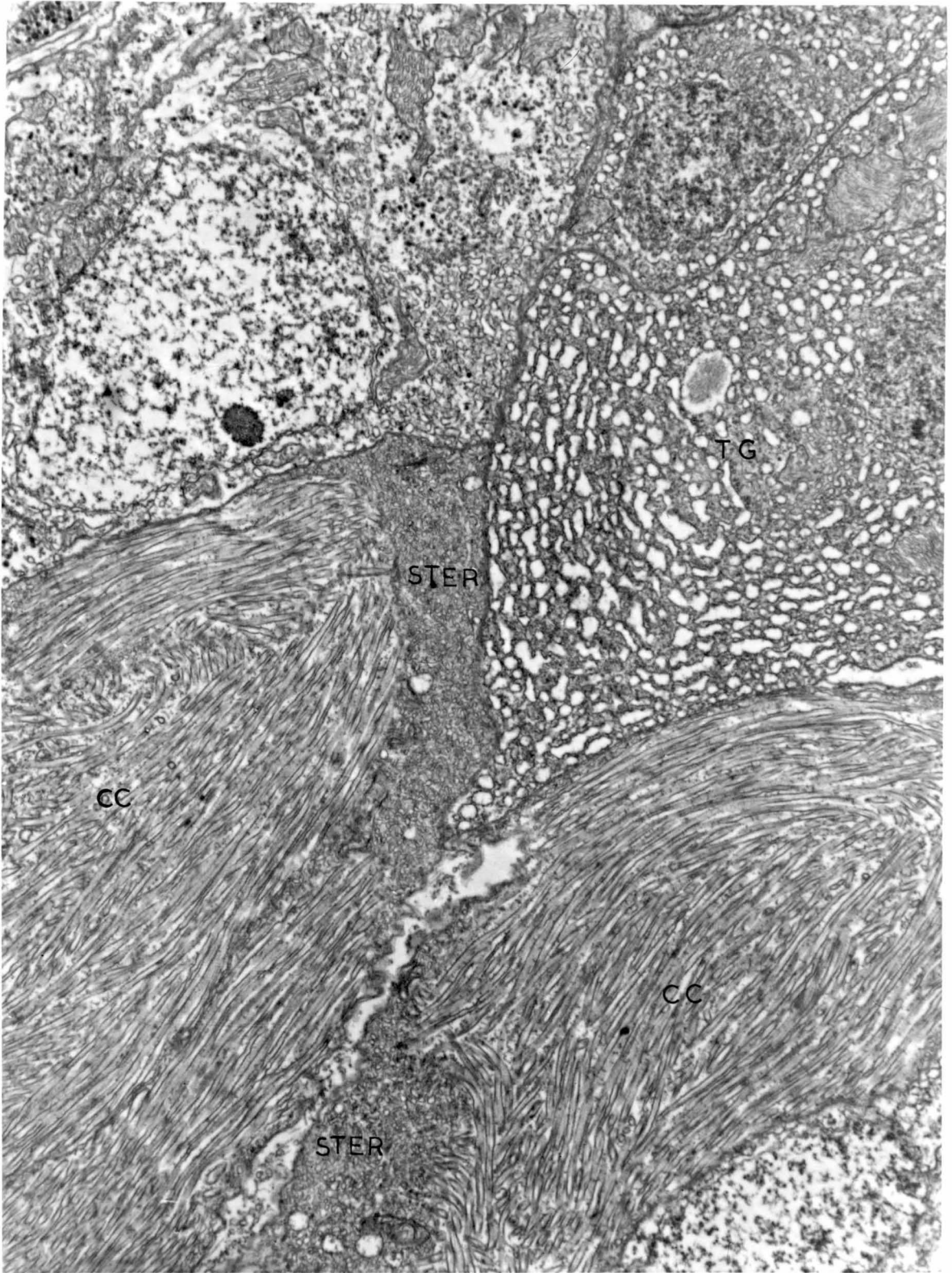organs.   X6,500.

<u>Fig. 12.</u>

Electron micrograph of part of the ciliary
coil of Claus's organ of <u>Sulcanus</u>.   Note
that there is one and sometimes two micro-
tubules (arrows) in each cilia.   The
electron opaque granules do not appear to
be membrane-bounded and are scattered
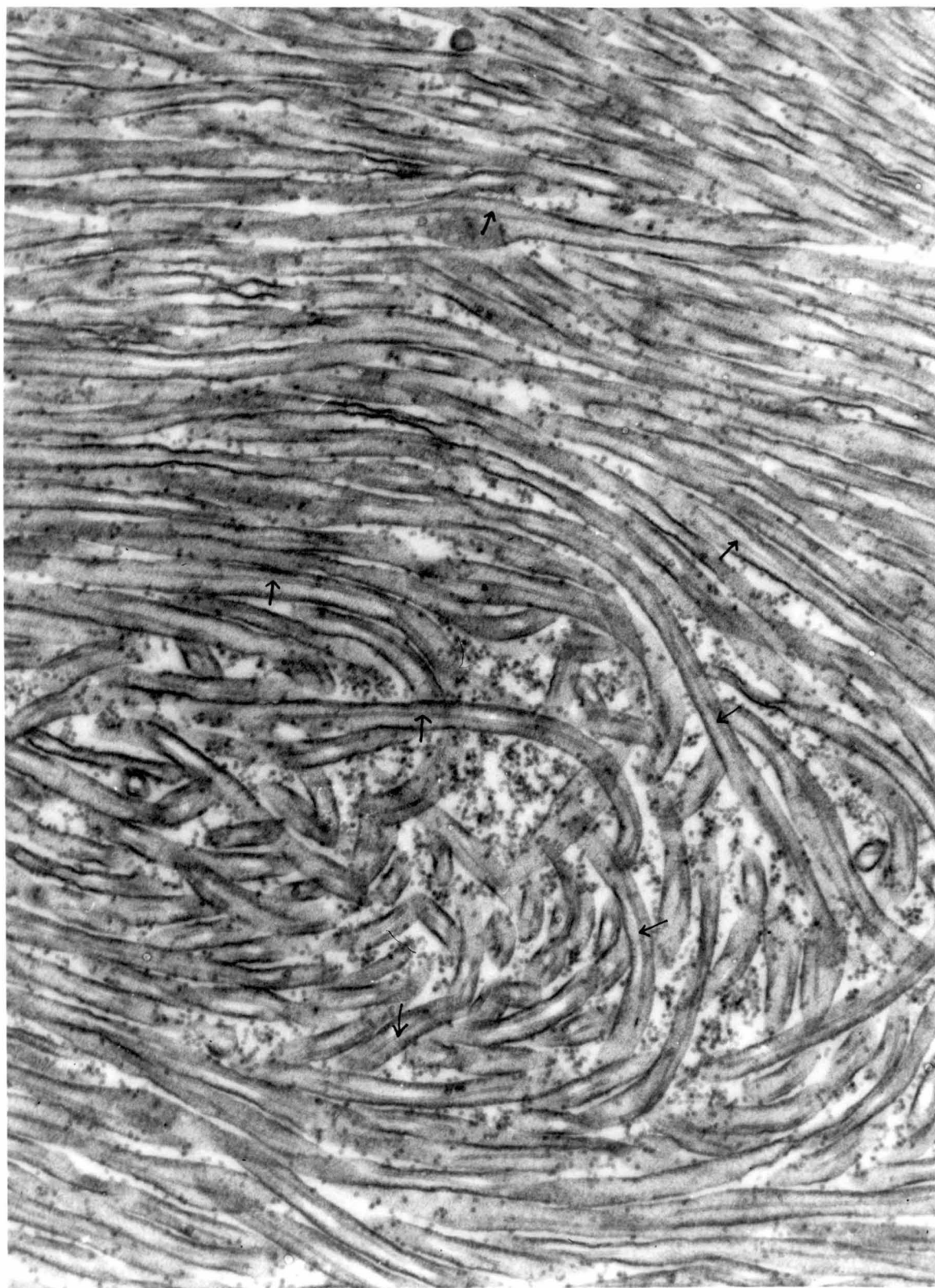amongst the ciliary coil.   X31,200.

Fig. 13.

Electron micrograph through part of Claus's
organ of Sulcanus showing parts of the ciliary
coil (CC) and part of the "companion cell"
(CP).   Part of the tegumental gland can be
seen on the bottom left of the electron micro-
graph.   Note the smooth tubular endoplasmic
reticulum (STER) in the "companion cell" and
the microtubules in the "companion cell" and in
the cilia (arrows).   X31,200

Fig. 14.

Electron micrograph of a parasaggital section
through part of Claus's organ of Sulcanus
showing that in this species each organ is
a paired structure.   The cilia with its
basal bodies can be seen to arise from the sen-
sory neuron (SN).   It can be seen that the
cilia contains from one to nine fibrils;
there is usually only a single fibril in the
more distal part of the cilia.   N = nucleus
of "companion cell" and CC = ciliary coils.
X10,000.
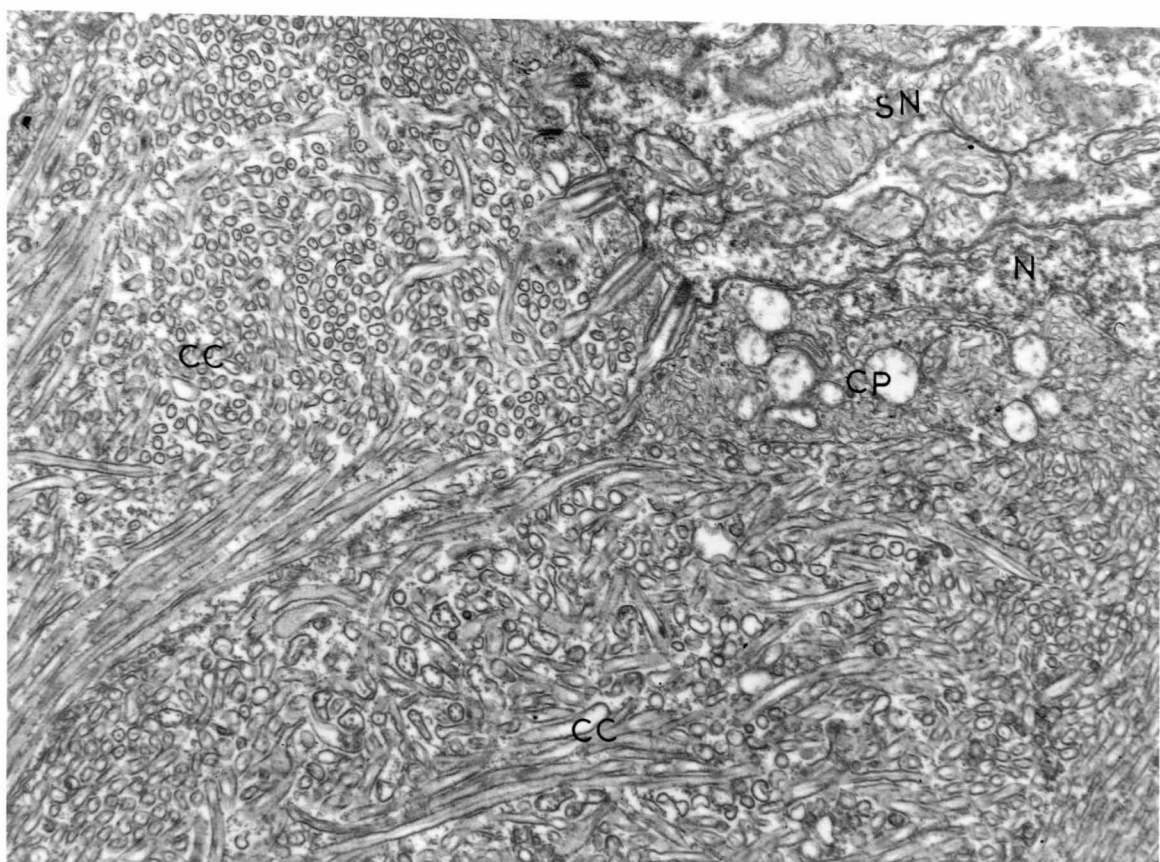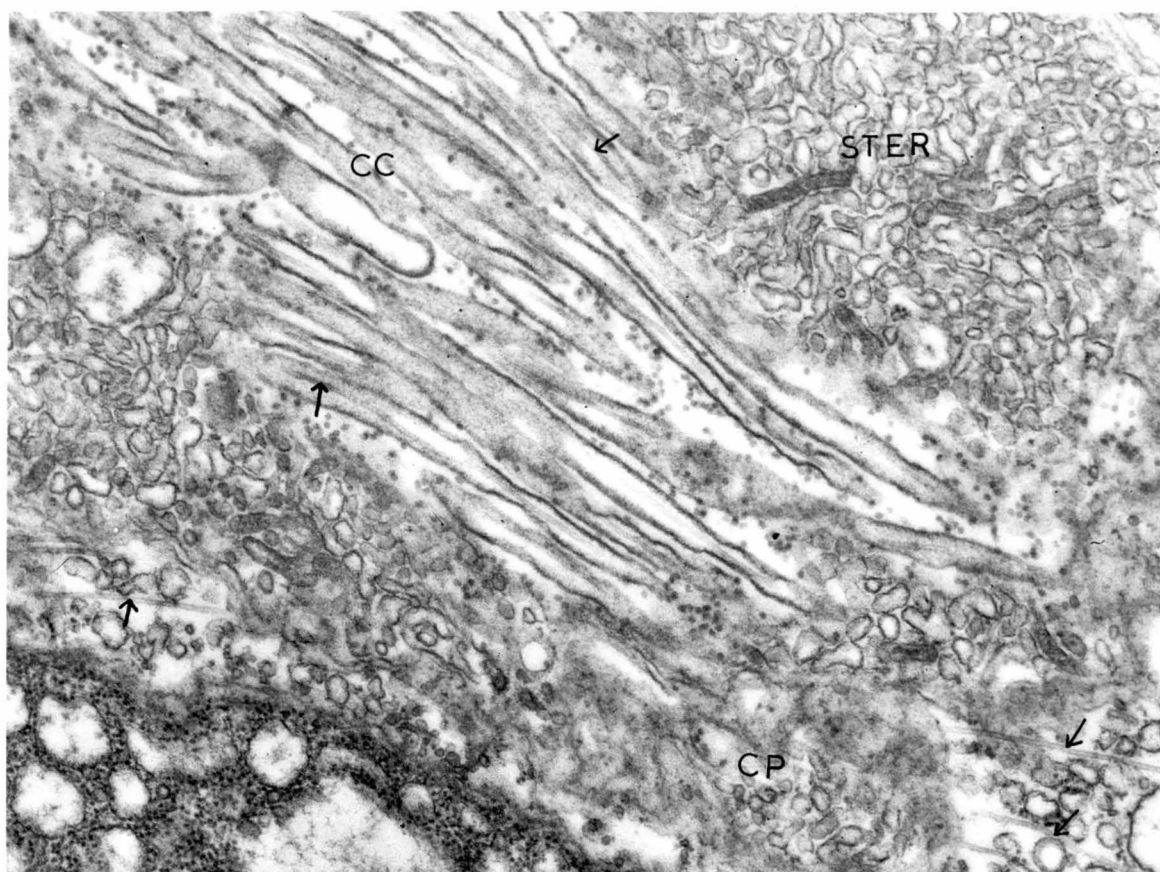
There are a few nerve fibres close to Claus's organ and some of these fibres contain membrane bounded electron opaque vesicles of about 250 nm in diameter (Fig. 11), others contain 120 nm vesicles (Figs. 10 and 11). I have been able to trace these neurosecretory axons only as far as the antero-lateral region of the brain but not directly to their perikarya which are possibly situated near the nerve to the antenna (the anterior lateral group of neurosecretory cells, described earlier). It is not known if these fibres are functionally related to either Claus's organ or the X-organ complex.

THE X-ORGAN COMPLEX

(Sensory pore :X-organ)

<u>Calanus helgolandicus</u>

This complex, associated with the frontal fila-
ments and the rostral filaments (Figs. 4 and 5), consists
of at least two types of sensory cells as well as other
"supporting" cells. One type of sensory cell is simi-
lar in organization to that of Claus's organ.   The
terminal section of its dendrite is a ciliated structure
and is thrown into coils.   The ciliary coil here is
however not as compact as that seen in Claus's organ.
The perikaryon, as in Claus's organ, contains numerous
mitochondria, microvesicles, membrane bounded 120 nm
diameter electron opaque vesicles, rough endoplasmic
reticulum, Golgi complexes, microbodies and neurotubules
Fig. 16).   The "companion cell" is also similar to that
seen in Claus's organ.   The axon of the neuron proceeds
towards the brain but I have not been able to locate
its termination (according to Lowe, 1935, the nerve
terminates near the nauplius eye centre).

Sensory cells of the other type do not contain
any electron opaque granules.   I have not been able
to trace the axons or dendrites of these cells to their
terminations but in <u>Sulcanus</u> the dendrites of this cell

Fig. 15.

Electron micrograph of a parasaggital section
through the rostral filament of Calanus
showing the ciliary coil (CC) of the sensory
pore X-organ and numerous dendrites of sensory
cells of the rostral filament.   A cilium
with the 9 + 0 arrangement of fibrils can
be seen (arrow).   E = epithelial cell,
C = cuticle and M = mitochondria.
X7,000.

Fig. 16.

Electron micrograph of part of the sensory
pore X-organ of Calanus showing the cil-
iated secretory neuron (SN), ciliary coil
(CC) and the "companion cell" (CP).   Note
the electron opaque vesicles and neuro-
tubules in the sensory neuron and the 9 + 0
arrangement of fibrils in the cilia (arrows).
X7,000.
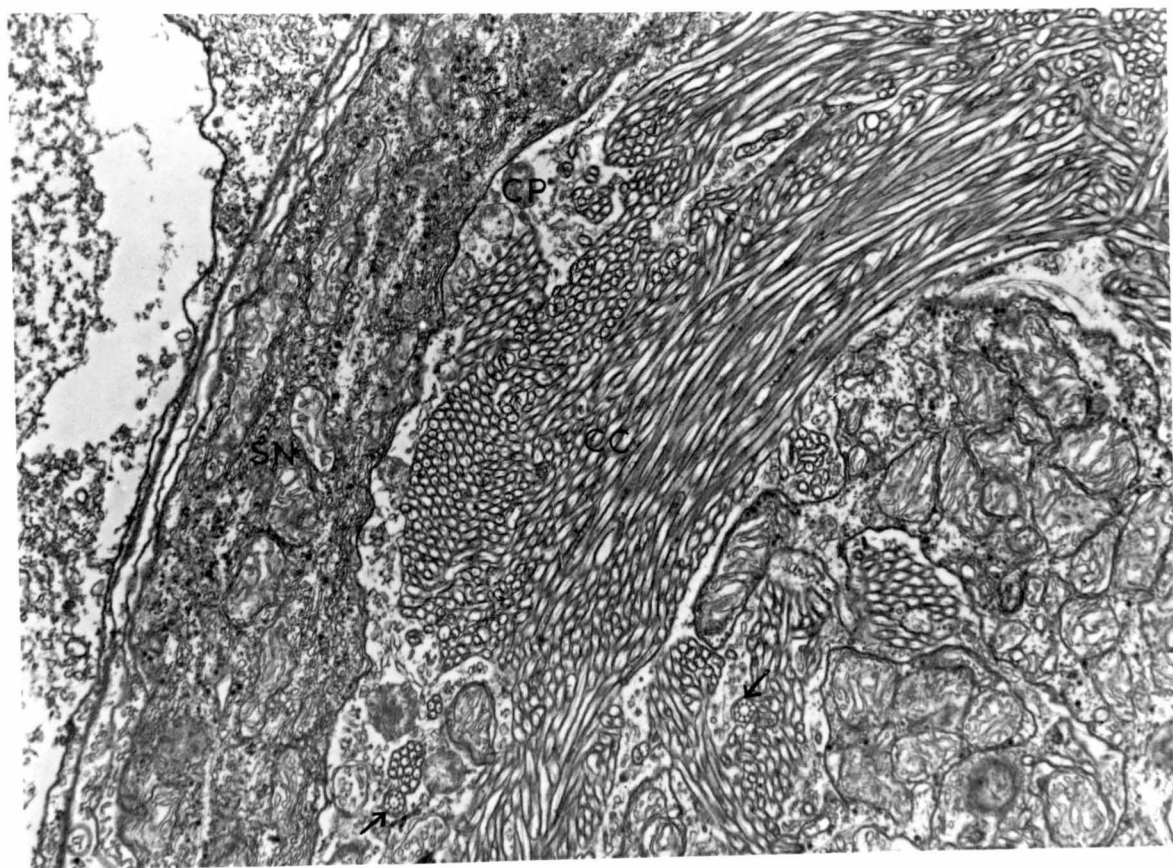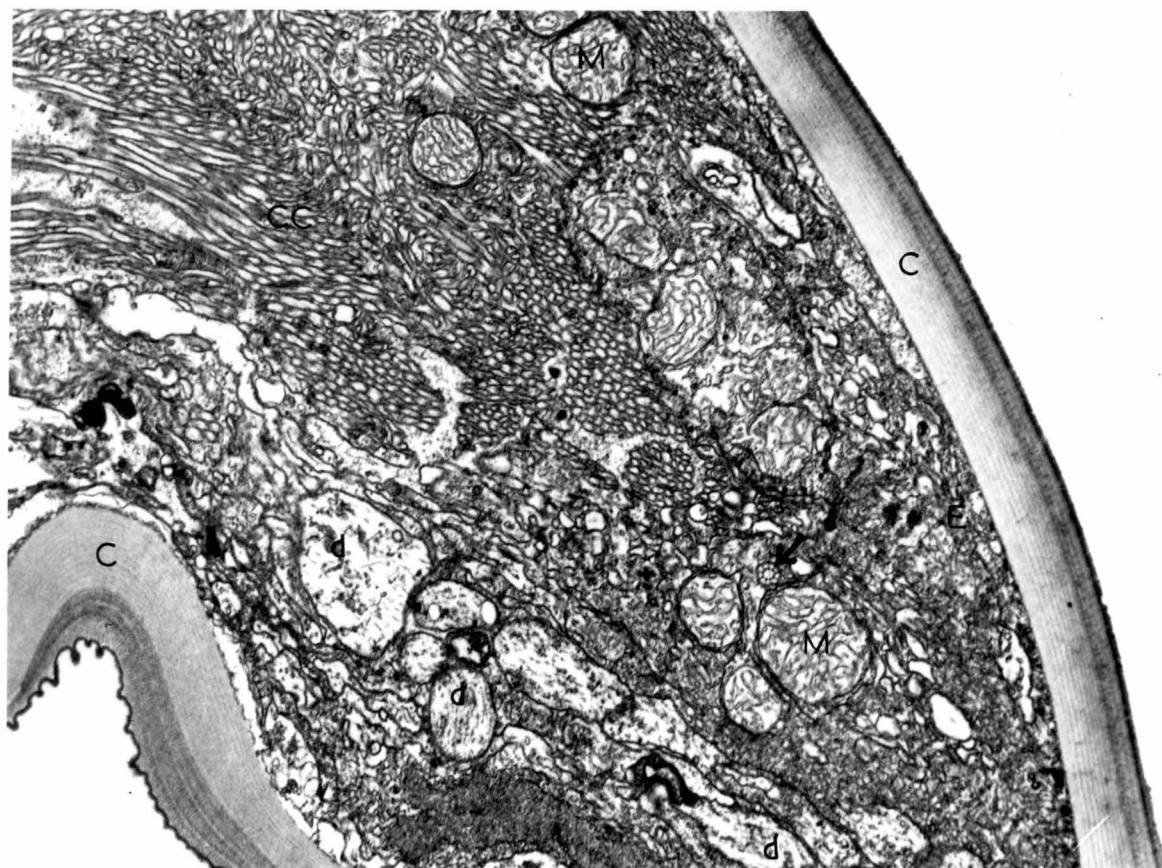
type terminate as ciliary structures. It is possible that in _Calanus_ these sensory cells are associated with the tiny frontal filaments.

There are various other cells in the region of the base of the rostral filament associated with the X-organ complex (Fig. 15). These are probably connective tissue cells and modified epithelial cells, associated with the support of the sensory cells.

Sulcanus conflictus

The complex is structurally very complicated, containing a multitude of cell types. A schematic reconstruction of the possible nature of the X-organ complex in _Sulcanus conflictus_ is shown in Fig. 17. There are two types of sensory neurones: a "secretory" sensory neuron and a "non-secretory" sensory neuron. The difference between these are seen in the types of vesicles present as well as the structure of their ciliary termination. As well as these sensory neurons there are other cell types in the complex. These are mainly epithelial cells and modified epithelial cells, possibly involved in the mechanical and nutritive support of the sensory cells.

The "secretory" sensory neurons have their perikarya located just lateral to the anterior end of Claus's organ (Figs. 10 and 11). The dendrites

Fig. 17

Schematic reconstruction of the sensory pore
X-organ complex of <u>Sulcanus</u> (in frontal
section).   C = cuticle; C_t = thin cuticle;
CC = ciliary coil of Claus's organ;   E =
epidermal cells;   NC = companion cell of
Claus's organ;   NS = neurosecretory axon;
pSx = perikaryon of "secretory" sensory
neuron of the sensory pore X-organ;   S =
"non-secretory" sensory neuron of the sen-
sory pore X-organ;   Sx = "secretory"
sensory neuron of the sensory pore X-organ;
TC = tegumental cell and TCc = tegumental
cell of the anterio-dorsal region of the
head.
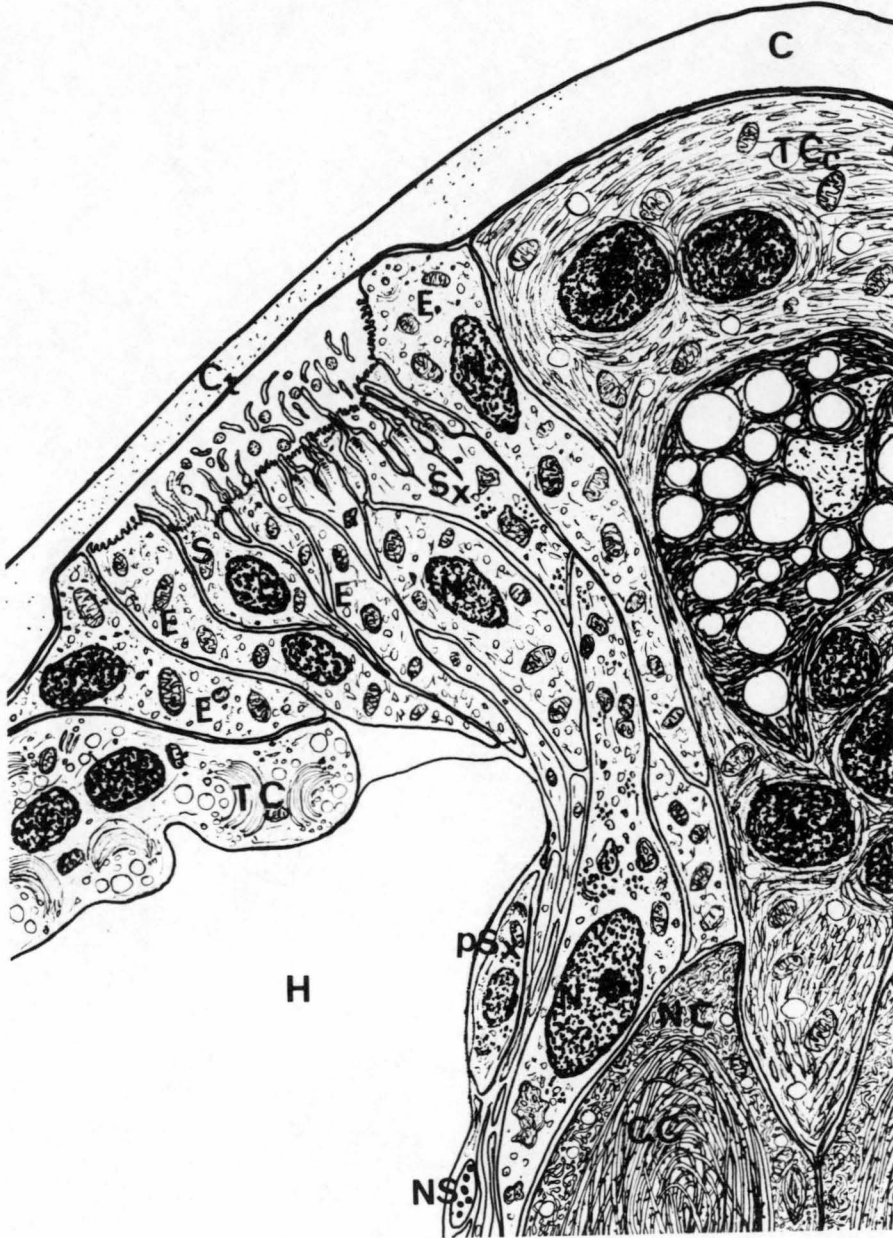
extend towards the cuticle of the anterior region of the head. The dendrite branches and terminate as cilia directly under the cuticle in the anterior region of the head (Fig. 18). The cilia are short and have a 9 + 0 arrangement of fibrils. These cilia have a rather well developed ciliary rootlet system (Fig. 19). The dendrites and the basal parts of the cilia are surrounded by modified epithelial cells and these possibly provide support for the dendrites and cilia. The perikarya as well as the dendrites and axon of the "secretory" sensory neurons contain electron dense, membrane bounded vesicles of about 120 nm diameter (Figs. 10 and 11). These are possibly neurosecretory granules. The neuron also contains numerous mitochondria, microvesicles, neurotubules and rough endoplasmic reticulum. These neurons may be homologous to the neurons in the X-organ complex of <u>Calanus</u> which also contain the 120 nm electron opaque vesicles.

Like the "secretory" sensory neuron, the "nonsecretory" sensory neuron is a bipolar structure (Fig. 20). The perikaryon however is situated closer to the cuticle and the dendrite is comparatively short. The cilia at the end of the dendrite is comparatively short. The cilia at the end of the dendrite also show a 9 + 0 arrangement of fibrils but there are no ciliary rootlets

Fig. 18.

Electron micrograph of part of the sensory
pore X-organ of _Sulcanus_ showing the term-
inations of the sensory cells under the
thin cuticle (C).   Pores (obliquely
sectioned) may be seen in the thin cuticle.
TC = tegumental cells.   X5,000.

Fig. 19.

Electron micrograph showing the ciliary rootlets
of the secretory neuron of the sensory pore
X-organ of _Sulcanus._   X20,800.
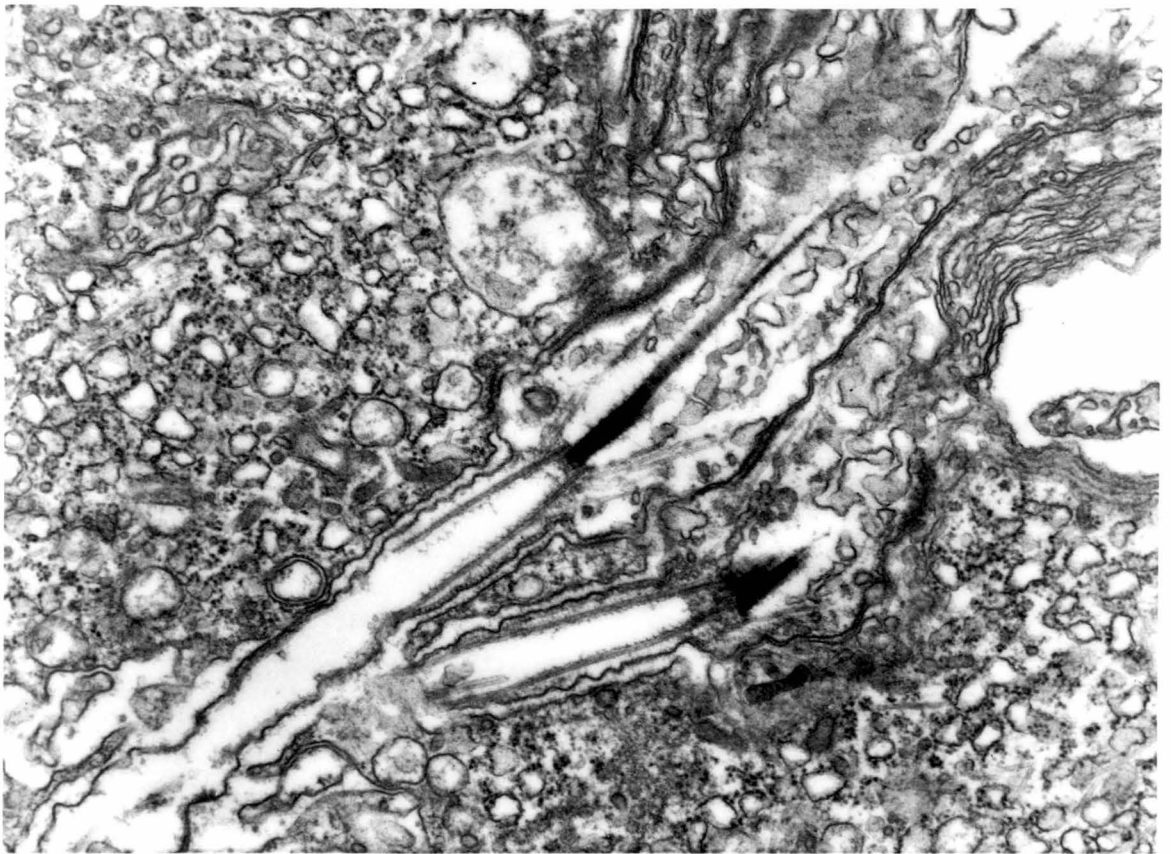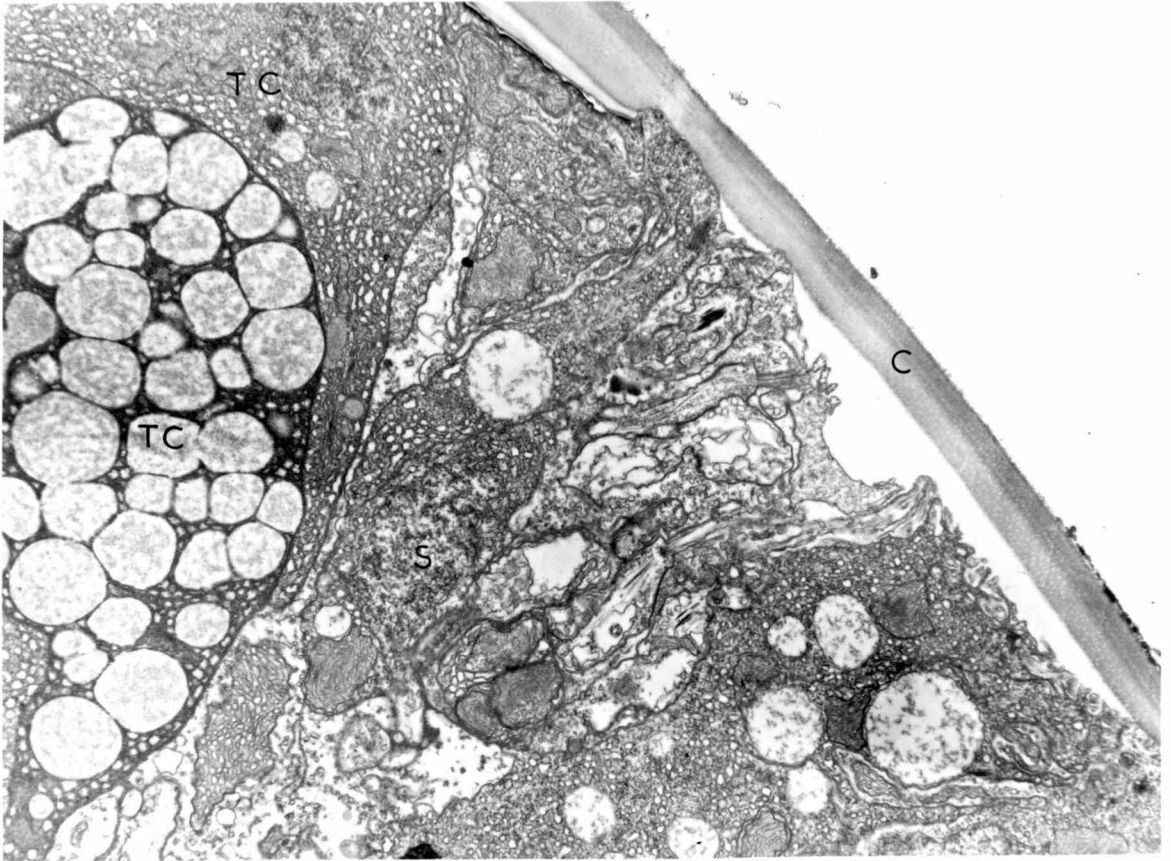
Fig. 20.

Electron micrograph of the perikarya of the
"non-secretory sensory neurons" of the sensory
pore X-organ of Sulcanus.   Note that the
cuticle (C) is thinner (arrow) where the term-
inations of the neurons occur.   E = modified
epithelial cell.   X5,000.

Fig. 21.

Electron micrograph of the terminations of
the "non-secretory neurons" (but note the
"clear" vesicles: arrows) of the sensory
pore X-organ of Sulcanus.   Note the pores
in the cuticle (C) and the absence of ciliary
rootlets in the cilia.   X27,600.
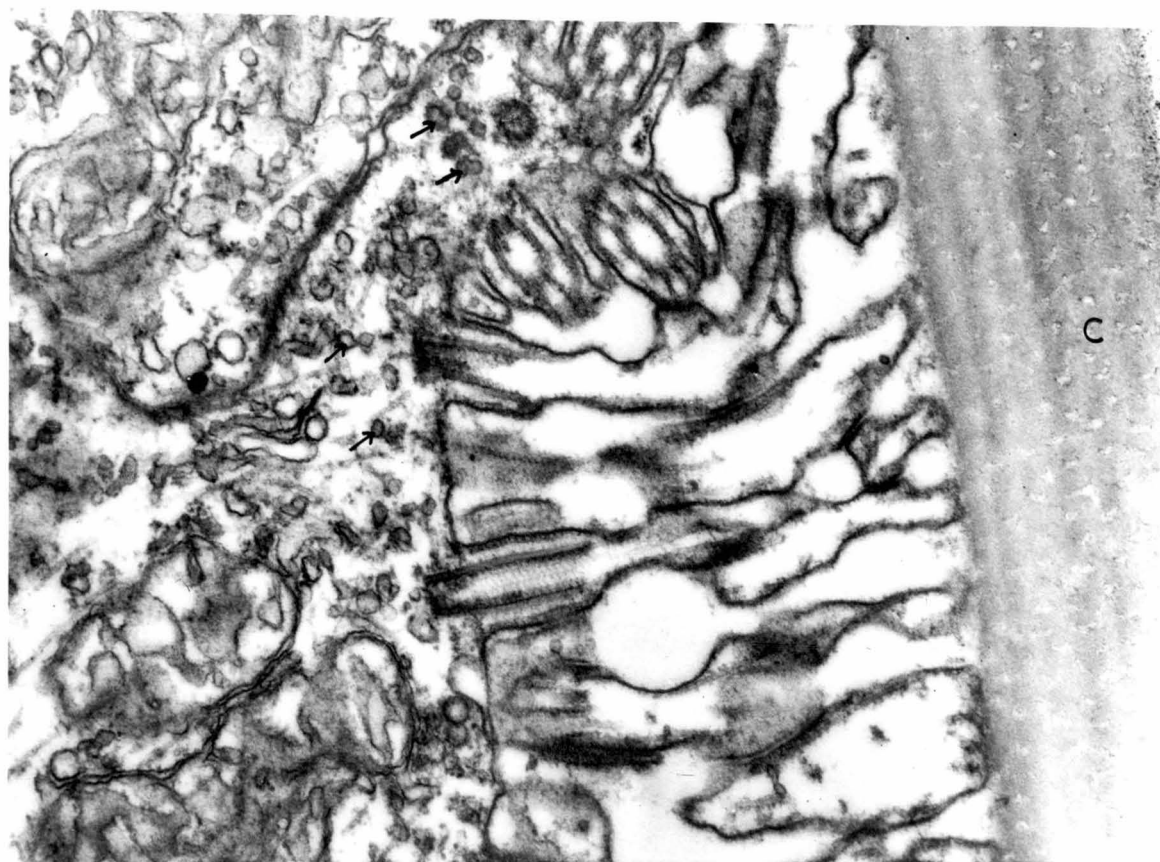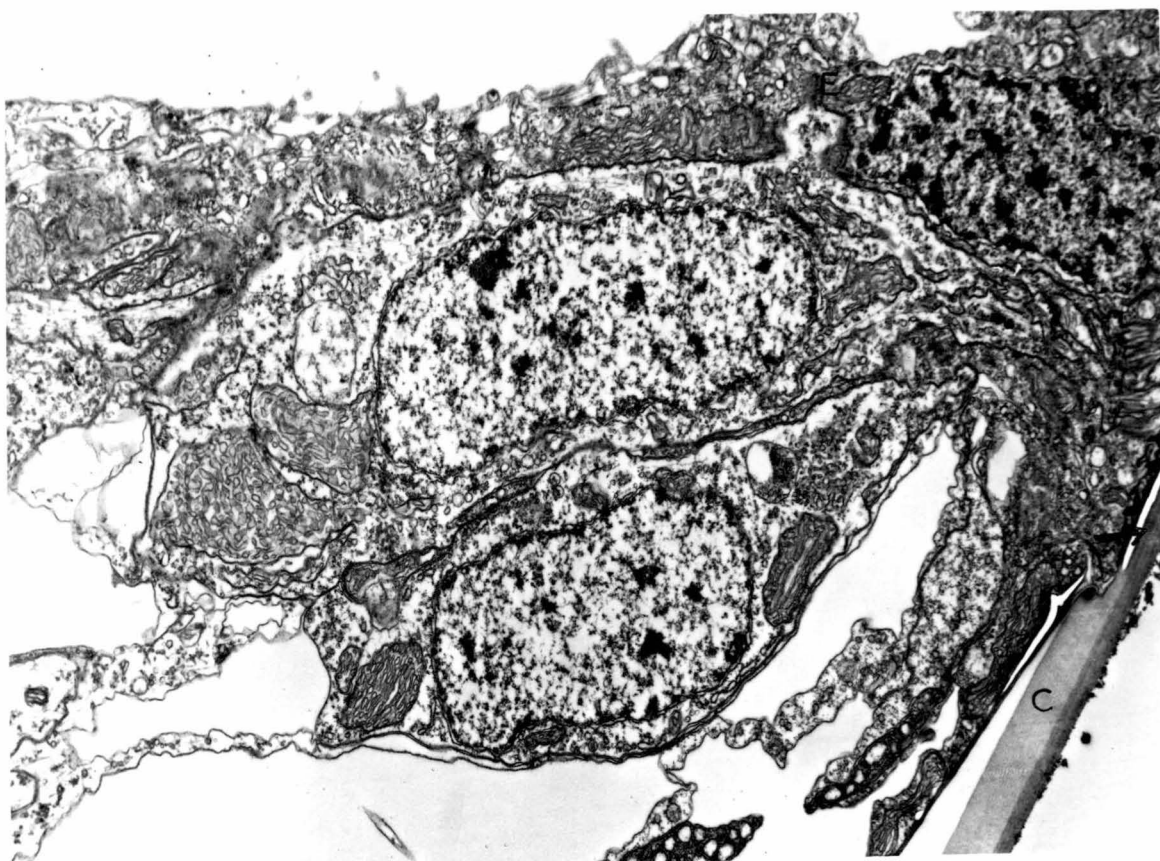
associated with the cilia (Fig. 21). The dendrites are also surrounded by the supporting modified epithelial cells. The cytoplasmic organelles and inclusions of the "non-secretory" neurons are similar to those of the "secretory" sensory neurons except that instead of the 120 nm electron opaque vesicles seen in the latter the "non-secretory" sensory neuron contain electron lucent vesicles of about 80 nm in diameter (Fig. 21).

The cuticle under which the two types of sensory neurons terminate is thinner than the surrounding cuticle and also contains numerous pores (Figs. 18 and 21). These cuticular pores are confined to the thin cuticle and a small area of the thick cuticle immediately surrounding the thin cuticle. This suggests that the cuticle above the X-organ complex is more permeable than the other parts of the body and that the sensory neurons may be involved in chemoreception (see Slifer, 1967).

DISCUSSION

The family Calanidae to which Calanus belongs is probably the most primitive family of the Calanoida. Sulcanus on the other hand is not far removed from the family Pontellidae which probably represents the most advanced family of the Calanoida. That Sulcanus is a specialized form is seen in the structure of the nauplius eye (see earlier section of this thesis).

The anterior region of the brain of Calanus is compact; the eye is small and situated close to the brain and Claus's organ is located within the brain. An expansion of the anterior region of the brain and the outward "migration" of the sensory and neurosecretory organs associated with that part of the brain is seen in Sulcanus; the head is very spacious, the large and elaborate nauplius eye is situated some distance from the brain and Claus's organs are situated on an anterior projection of the brain. The "diffusion" of the sensory and neurosecretory organs of the anterior region of the brain is probably at its zenith in the family Pontellidae, as seen for example, in the Epilabidocera amphirites where the nauplius eye occurs as two entities and the frontal filaments (= supra frontal sensilla) are quite removed from the X-organ

(Park, 1966).   Park (1966) made no mention of Claus's organ and without actually examining the species myself I am not able to say if Claus's organ has migrated and fused with the X-organ (the resolution of this question may be vital in determining if Claus's organ and the X-organ are closely related).   The structure described as the "end structure" by Park (1966) is most probably structurally similar to Claus's organ of both Sulcanus and Calanus as well as to the X-organ of Calanus.   This suggests that Claus's organ and the secretory part of the X-organ form into a single structure or that one of these is lost in the more advanced Calanoida.   This point no doubt requires a more thorough comparative investigation.

Claus's organ: There are no statoliths in Claus's organ of either Calanus or Sulcanus.   The statoliths reported by Claus (1863) in Eucalanus attenuatus and Lowe (1935) in Calanus finmarchicus were probably artefacts.   Esterly (1908) described Claus's organ in Eucalanus elongatus as "inverted eyes" but from his description it is quite probable that the "inverted eyes" were as he suggested, photoreceptors, and may thus be the dorsal frontal organs rather than Claus's organ.   It seems unlikely that these "inverted eyes" are Claus's organ but I cannot be certain without examining the animals myself.   I have little doubt

however, that the structure described here as Claus's
organ in <u>Calanus</u> is identical to those described by
Lowe. (1935) in <u>Calanus finmarchicus.</u>

The most conspicuous structure of Claus's organ
is the ciliary coil. The presence of cilia with a
9 + 0 arrangement of fibrils suggests that Claus's
organ may be a sense organ. Its sensory function at
this stage can only be speculated upon. Ciliated
structures with a 9 + 0 arrangement of fibrils may be
present in photoreceptors (e.g. see Eakin, 1965 and Horridge,
1964) but the fact that the organ is embedded in the brain
(as in <u>Calanus</u>) or surrounded by other tissues (as in
<u>Sulcanus</u>) reduces this possibility. Also, the long
cilia of Claus's organ each contain· one or two micro-
fibrils (Fig. 12) and as far as I am aware this has
never been reported from photoreceptor microvilli.
It is equally unlikely that the organ is a chemoreceptor,
except possibly for the rather unlikely function of
localized chemoreception of fluids around that organ.
The extremely well developed and long cilia suggest
that the structure, may perceive stimuli from every
direction and it may therefore function as a baro-
receptor; any small change in hydrostatic pressure
could easily cause a deformation of the ciliary coil
which could trigger it.

Since there is no convincing evidence of any
special organ capable of detection of hydrostatic
pressure changes (see Knight-Jones and Morgan, 1966)
evidence to support the speculation that Claus's organ
is a baroreceptor through structural complementarity
can only be suggestive.   However, the ciliary
structure of the coronet cells of the saccus vasculosus
of certain fishes show a basic similarity to the
ciliary structure of Claus's organ (e.g. Bargmann and
Knoop, 1961 and Jansen and Flight, 1969).   The function
or functions of the coronet cells of the saccus vas-
culosus is not clear.   They have been implied to
be sensory and even linked with pressure reception (e.g.
Dammerman, 1910, Scharrer, 1948 and Harrach, 1970).
Jansen and Flight (1969) considered it unlikely that
the coronet cells of the saccus vasculosus of the rain-
bow trout are sensory receptors in that they do not have
axonic processes nor make synaptic contact with axons.
Since the saccus vasculosus is probably involved in
secretion and/or absorption (Jansen and Flight, 1969)
(in this respect Claus's organ shows a functional sim-
ilarity in that it is probably secretory as well as
sensory) it is possible to visualize a highly autonomous
cell regulating its own secretion or absorption through
its own sensory receptor system i.e. an absence of a

synapse need not necessarily exclude its possible sensory function.

Calanus has been shown to respond to hydrostatic pressure changes (e.g. Hardy and Paton, 1947 and Rice, 1962) but the organ responsible for the detection of these changes is not known. Digby (1961) found that the potentials across the surfaces of decapod Crustacea varied with pressure changes and suggested that this may be brought about by a change in the thickness of a hypothetical film of hydrogen on the cuticle of the crustacea. This theory for detection of changes in hydrostatic pressures is in doubt since Enright (1963) has produced evidence which is contrary to Digby's theory.

In Sulcanus electron opque granules (non-membrane bounded) of about 50 nm diameter are present in the space between the cilia in the ciliary coil. I am unable to determine the origin of these granules. The ciliary coil of Claus's organ stain a very faint purple with paraldehyde fuchsin. An almost identical type of granule was described by Dahl and Mecklenberg (1969) in the vesicle of the sensory papilla X-organ of the mysid, Boreomysis artica. These granules show a positive staining reaction to Gomori's chrome Haematoxylin and these authors termed themneurosecretory

granules.   While these tiny non-membrane bounded
electron opaque granules may not be common in known
neurosecretory cells, they have been reported in the
sinus glands of the crayfish, Cambarellus by Fingerman
and Aoto (1959) and in the corpora cardiaca of the
stick insect, Carausius by Meyer and Pflugfelder (1958).
It is thus possible that the 50 nm diameter non-
membrane bounded electron opaque granules in Claus's
organ of Sulcanus may be neurosecretory granules.   The
absence of these granules in Calanus may be because the
animals were at different stages of moult or diapause
since the animals were obtained at different times
of the year;   Calanus were taken in winter (when the
animals were possibly in diapause) and Sulcanus, in autumn.
As previously stated, Carlisle and Pitman (1961) have
reported that the neurosecretory cells on the anterior
edge of the brain of Calanus showed a depletion or ab-
sence of stainable neurosecretory material in winter.

The perikaryon and axon of the ciliated neuron
of Claus's organ contains conspicuous membrane bounded
120 nm diameter electron opaque vesicles.   As well
as the electron opaque vesicles the perikaryon contains
an extensively developed rough endoplasmic reticulum,
Golgi complexes, mitochondria and free ribosomes.
This may be a suggestion that the electron opaque gran-
ules are being actively produced and possibly continuously

secreted. It seems probable that the 120 nm diameter granules are neurosecretory granules. These appear to be more plentiful in _Calanus_ than in _Sulcanus_. It is not known if these 120 nm granules give rise to the 50 nm granules found between the cilia of the ciliary coil.

The "companion cell" of Claus's organ appears to be more active in _Sulcanus_ than in _Calanus_. The striking feature of the "companion cell" in _Sulcanus_ is the extensive smooth endoplasmic reticulum. This type of endoplasmic reticulum has been linked with steroid synthesis, wax metabolism, glycogenolysis and detoxication. The lutein cell of mammalian ovaries (Blanchette, 1966), the interstitial cells of mammalian testes (Fawcett, 1966) and the prothoracic glands of insects (Beaulaton, 1968) contain extensive smooth endoplasmic reticulum which has been regarded as being associated with steroid synthesis. Locke (1969) described smooth endoplasmic reticulum in the oenocytes of insects and suggested that these organelles may be involved in wax metabolism or even the production of ecdysone (i.e. steroid synthesis). Orrenius and Ericsson (1966) showed that rats injected with phenobarbital were induced to form smooth endoplasmic reticulum and enzymes for oxidative demethylation in the liver. There is no evidence of either lipids or glycogen

in the "companion cell" so that it is unlikely that the cell is at a stage of steroid synthesis or glycogenolysis. The "companion cell" is however in contact with the antero-medio-dorsal tegumental gland of the cephalosome and there is a possibility that the "companion cell" and the tegumental gland together may be involved in steroid synthesis or glycogenolysis. Lake and Ong (in press) have also hinted at the possibility that the extensive "endoplasmic reticulum" in the sensory pore X-organ of the decapod, _Paratya tasmaniensis_ may possibly be involved in steroid synthesis.

The X-organ complex: Little is known of the neurosecretory system of the Copepoda apart from the short notes of Carlisle and Pitman (1961) on _Calanus_ and that of Govindarajulu (1968) on another calanoid, _Heleodiaptomus_. Elofsson (1966.) described the X-organ of the calanoid _Pareuchaeta norvegica_ and other copepods but made only a passing mention of their neurosecretory role.

The present findings are in general agreement with the above-mentioned works. It appears likely that the pair of neurosecretory cells in _Calanus_ described by Carlisle and Pitman (1961) correspond to Claus's organ.

It is apparent that Claus's organ and the X-organ complex are closely related and the question arises as

to whether they should be considered as one neuro-
secretory complex. If one considers these organs in
Calanus, Sulcanus and Epilabidocera a certain trend may
be seen: in Calanus the "secretory" cell of the
X-organ complex is structurally very similar to that
of Claus's organ, in Sulcanus the "secretory" sensory
neuron shows very little resemblance to Claus's organ
but Claus's organ in Sulcanus consists of a pair of
ciliated neurons, whereas in Calanus Claus's organ con-
sists of only a single neuron (it is not possible to
determine if this is due to fusion of Claus's organ and
part of the X-organ complex) and finally in Epilabidocera
(as described by Park, 1966), Claus's organ is
apparently absent but the X-organ contains what is
probably a well developed ciliary coil, similar to those
of Claus's organ of both Sulcanus and Calanus. On
this basis I would tentatively consider Claus's organ
as being closely related to and perhaps part of the X-
organ complex. It is apparent that more extensive
comparative work is needed.

It has been suggested earlier that the sensory
structures of the X-organ are possibly chemoreceptors
in that the cuticle covering the endings of their
dendrites are pierced by numerous pores. On this
basis the X-organ complex may be regarded as a sensory
pore X-organ. Kauri (1966) has shown that the organs

associated with the frontal filaments of the larvae
of the cirriped, _Balanus_ are sensory papilla X-organ,
containing both sensory and secretory neurons. The
SPX-organ is of the Peracarida type and it is likely
that the SPX-organ of the Copepoda may be homologous
to these in the cirriped larvae.

Little is known of the sensory pore X-organ (or
the organ of Bellonci) of the non-decapod Crustacea.
The recent works of Dahl and Mecklenburg (1969)
on the SPX-organ of the mysid, _Boreomysis artica_ and
that of Chaigneau (1969) on the organ of Bellonci of
the isopod, _Sphaeroma serratum_ and that of Lake and
Ong (in press) on the SPX-organ of the decapod,
Paratya tasmaniensis are the only electron microscope
studies on the SPX-organ that I am aware of. These
however provide examples representing two basic types
of SPX-organs: the sac-like fibrous type as seen in
the Peracarida (and possibly also in the larval decapods,
Hubschman, 1963) and the "onion body" type as seen in
the Eucarida and Hoplocarida.

The ciliary coil of Claus's organ shows a strik-
ing similarity to the SPX-vesicle of _Boreomysis_ except
that the tubules of the SPX-vesicles are considered by
Dahl and Mecklenburg (1969) to be nerve endings and the
endings of the SPX-cells. These authors did not
report any ciliary structure in the SPX-organ of

<u>Boreomysis</u>.   Chaigneau (1969) was able to demonstrate
the presence of long cilia in the organ of Bellonci
(=SPX-organ) of the isopod, <u>Sphaeroma</u> and in this
respect shows its resemblance to the SPX-organ of the
Copepoda. It thus appears that the SPX-organ of the
Copepoda is of the fibrous or Peracarida type.

     This study is centred mainly on the sensory and
neurosecretory systems of the cephalosome of calanoid
copepods.   The two systems are intimately connected
and form links with the environment on the sensory side
and with the endocrine and effector systems on the
neurosecretory side.   The sensory system acts as a
transducer of environmental cues and the neurosecre-
tory system acts as a mediator between the sensory
system and the endocrine or effector systems (Fig. 22).
The pathway from the environment to the effector system
via the sensory, neurosecretory and endocrine systems
is not completely understood and is a challenging
field.

     The sensory pore X-organ complex (i.e. including
Claus's organs) appears to exhibit the sensory-neurosecretory
linkage mentioned above.   In Claus's organ, it appears
that the sensory and neurosecretory systems are located
within the same cell (left-hand side of Fig. 22).   The
chain linking the environmental cue to the effector system
is apparently shortened and this appears to be a simple

Fig. 22:

Schematic diagram illustrating the main pathways of
information from the environment through the neuro-
secretory system, to the final target organ in a
simple generalized arthropod.*   The brain, which in
many instances forms the link between the sensory
system and the neurosecretory system and where in-
formation is encoded and redirected is not shown in
this simple scheme.   The broken lines represent
short-term or reflex-type reactions (interneuronal
connections are not shown).

1A.ENV.CUE.-- SENS.-- N.S.-- VAS.-- ENDO.-- VAS.-- F.T.O.
1B.ENV.CUE.-- SENS.-- N.S.-- VAS.------------------- F.T.O.
2 .ENV.CUE.-- SENS.-- N.S.---------- ENDO.-- VAS.-- F.T.O.
3 .ENV.CUE.-- SENS.-- N.S.------------------------- F.T.O.

The diagram on the left of the figure is that of Claus's
organ.   Here the sensory and neurosecretory components
are located within the same cell.

* Data for this diagram are obtained from various sources
(references in Scharrer, 1969).
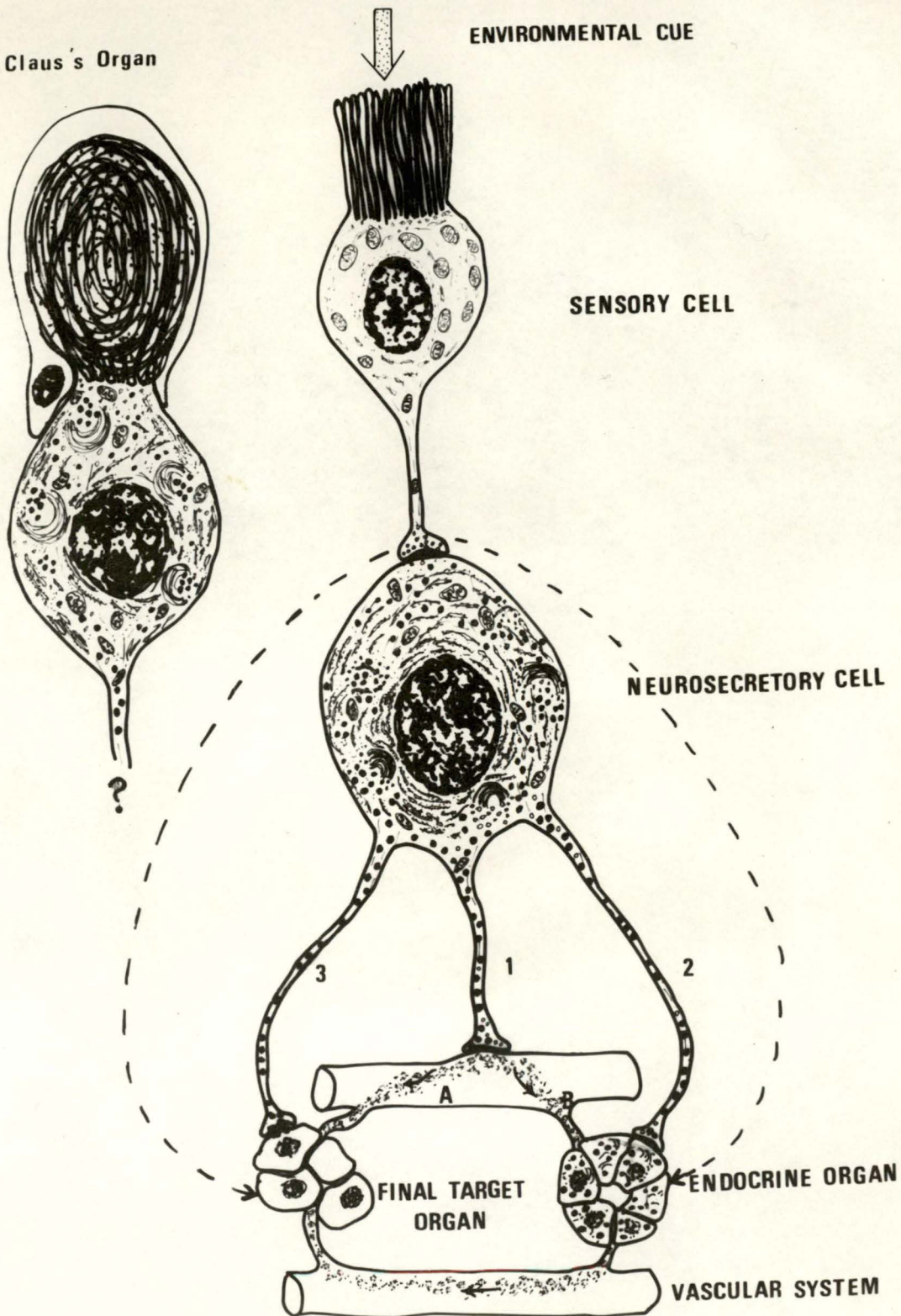
Claus's Organ

ENVIRONMENTAL CUE

SENSORY CELL

NEUROSECRETORY CELL

?

3   1   2

A   B

FINAL TARGET
ORGAN

ENDOCRINE ORGAN

VASCULAR SYSTEM

system.   Two main questions remained unanswered:
the function of the sensory system, i.e. the nature of
the environmental cue is not known;   hydrostatic
pressure being suspected and it is not known where
the neurosecretory products of the cell are discharged
and what organ or organ system they effect.

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Electron micrograph of parts of the microvilli
in the epithelial cell of the mid-gut divert-
iculum of Calanus helgolandicus.   X176,100.
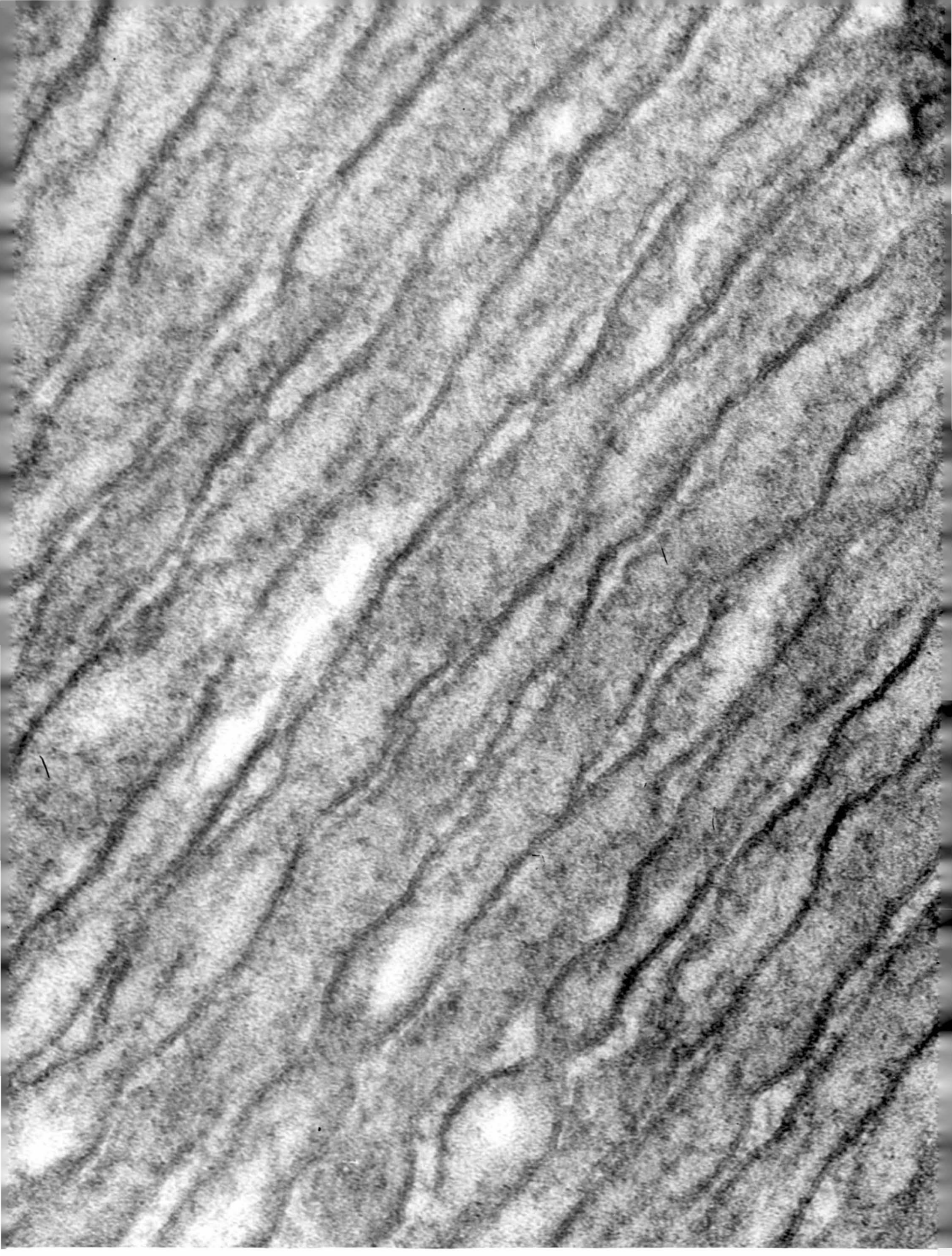
THE DIGESTIVE SYSTEM

INTRODUCTION

Copepods form the bulk of the second trophic level in the marine environment so that knowledge of their nutritional value to members of the higher trophic levels and their efficiency in utilizing primary producers is relevant to both marine biologists and fisheries research. Marshall and Orr (1955a) have shown that some copepods are able to digest and absorb as much as 90% of the carbon they ingest. It is not known how the animals achieve this remarkable efficiency in assimilation as little is known of the physiology and ultrastructural morphology of the digestive system of the Copepoda.

Most copepods are filter feeders and the filter feeding mechanism has been observed and described in a number of copepods by Lowndes (1935). The oral appendages, with their elaborate arrangement of setae, set up a current and mechanically sieve the food particles out of the water. Dahl (1963) has hinted that mucus may be involved in this filter feeding process.

THE LABRAL GLANDS

INTRODUCTION

The labral glands of some fresh water copepods were described by Richard (1891) as unicellular glands comparable to unicellular glands associated with certain of the limbs of these animals. Lowe (1935) gave an excellent description of the labral glands of Calanus finmarchicus. She showed that each gland is a syncitium or multinucleate cell and opens on the postero-ventral surface of the labrum. Lowe did not suggest the function of the glands apart from their possible secretory role. The labral glands of Calanus finmarchicus, an exclusively filter feeder, have been shown to secrete PAS-positive material (Dahl, 1963). In the Crustacea, the involvement of mucus in filter feeding is known only in the Branchiopoda and some of the Mysidacea (Dahl, 1963). In the Branchiopoda, the mucus is secreted into the filter chamber (Cannon, 1928 and 1933) but this is apparently not the case in the Mysidacea (Dahl, 1963).

The following is a description of the ultrastructural morphology of the labral glands of the calanoid copepods Gladioferens pectinatus and Sulcanus conflictus, with a discussion as to their possible function in the filter feeding mechanism.

OBSERVATIONS

In both <u>Gladioferens</u> and <u>Sulcanus</u> there are four glands on each side of the labrum. The general architecture of the labral glands is shown in Fig. 1. Each labral gland may be divided into two components: the labral gland cell and the cuticular valve.

There is a slight variation in the ultrastructure of the labral gland cells, some cells being less electron opaque than others (Fig. 2). This variation in opacity is due mainly to the presence of more free ribosomes in the cytoplasm of the more electron opaque cells. As well as the variation in electron opacity, some cells contain more Golgi complexes than others. These differences are possibly due to the different stage of development of the cells.

For ease of description, the labral gland cell may be divided into the basal portion (the part of the cell next to the brain) and the apical portion (the part of the cell next to the cuticular valve). The basal portion of the cell contains the nuclei (the cell being a syncitium). These are more or less oval in shape, measuring about 3/um by 2/um and each containing a single small nucleolus. The rest of the basal portion of the cell contains secretory vacuoles, an extensive system of rough endoplasmic reticulum, Golgi complexes,

Fig. 1.

Diagram of a reconstruction of the labral
gland showing a parasaggital section through
the labrum.   Each glandular cell is
associated with a cuticular valve (V).
Br = brain; C = cuticle; E = epidermal
cells; G = Golgi complexes; N = nucleus
and S = secretory vacuoles.
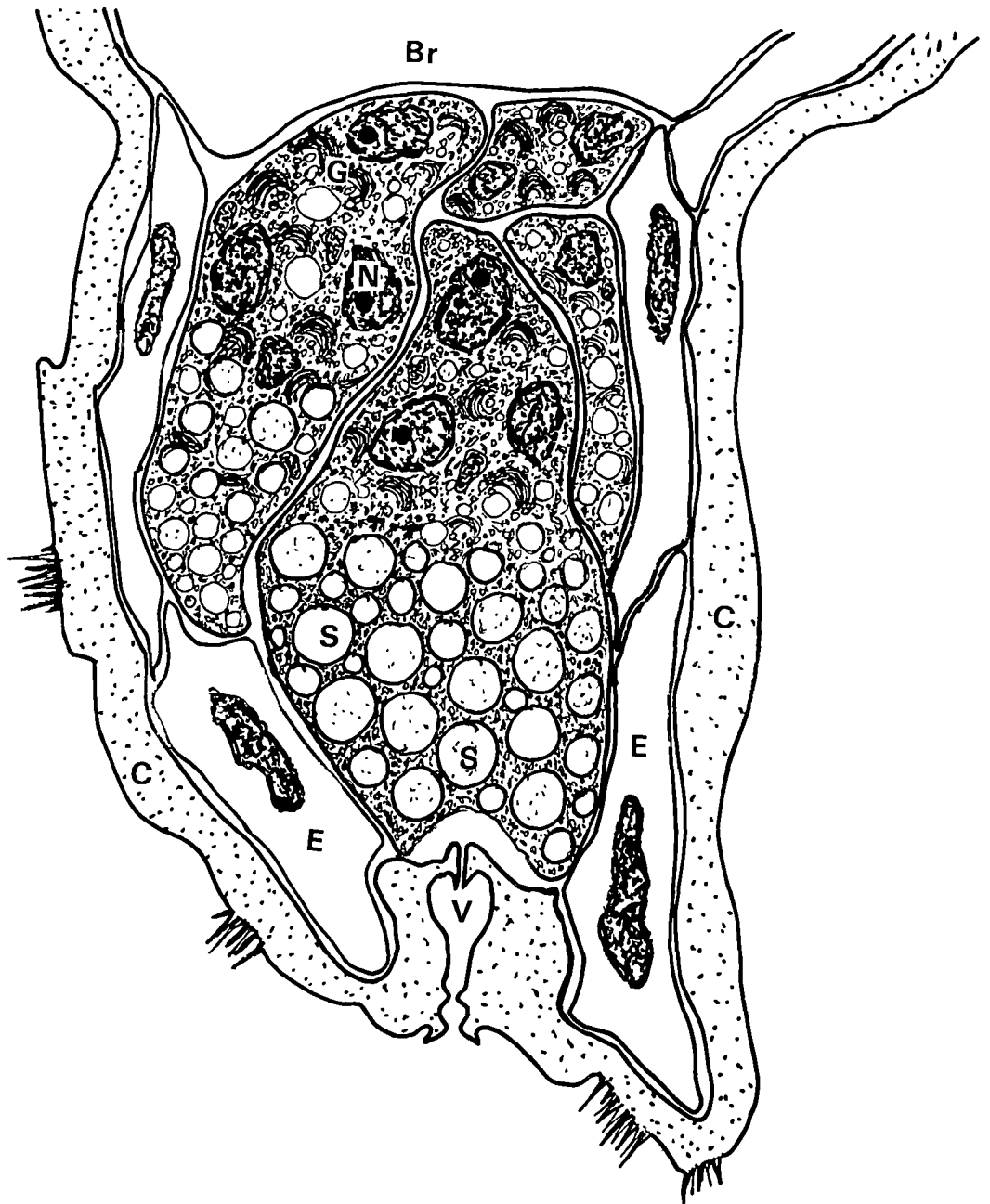
a few mitochondria and free ribosomes (Figs. 2 and 3).
The apical portion of the cell contains numerous large
secretory vacuoles of about 7 /um in diameter which
are surrounded by cytoplasm containing extensive rough
endoplasmic reticulum (Fig. 4).   The vacuoles contain
moderately electron opaque, almost finely granular and
slightly agglutinated material similar to the mucus
droplets of mammalian goblet cells (Fawcett, 1966).

The valve of the labral gland is a modified
part of the cuticle of the labrum.   The valve may be
divided into two parts for ease of description: the
tubular entrance and the valve proper.   The valve
proper is concave when viewed from the inside (labral
gland cell side) and is convex (conical) when viewed
from the outside.   The material forming the valve is
electron lucent and is similar to the innermost layer
of the labral cuticle (Fig. 5).   The whole valve struc-
ture is lined on the outside by a fine layer of electron
opaque material, probably cuticulin.

From its structure it appears that the valve is
pressure operated.   The flow of the valve is from the
labral gland cell side out.   Pressure acting on the
valve from the inside will cause the valve to open
because of the concavity on this side.   Pressure applied
on the conical part of the valve on the other hand will

Fig. 2.

Electron micrograph showing parts of four
labral gland cells of Sulcanus. The cell
on the right appears much darker than the
others. The light cells and dark cells
contain an extensively developed rough
endoplasmic reticulum.   There appear
to be more free ribosomes in the dark cell.
S = secretory vacuoles; M = mitochondria
and RER = rough endoplasmic reticulum.
X 6,250

Fig. 3.

Electron micrograph showing the Golgi
complexes (G) and secretory vacuoles
(S) in the basal part of the labral
gland cell of Sulcanus.   X 17,000
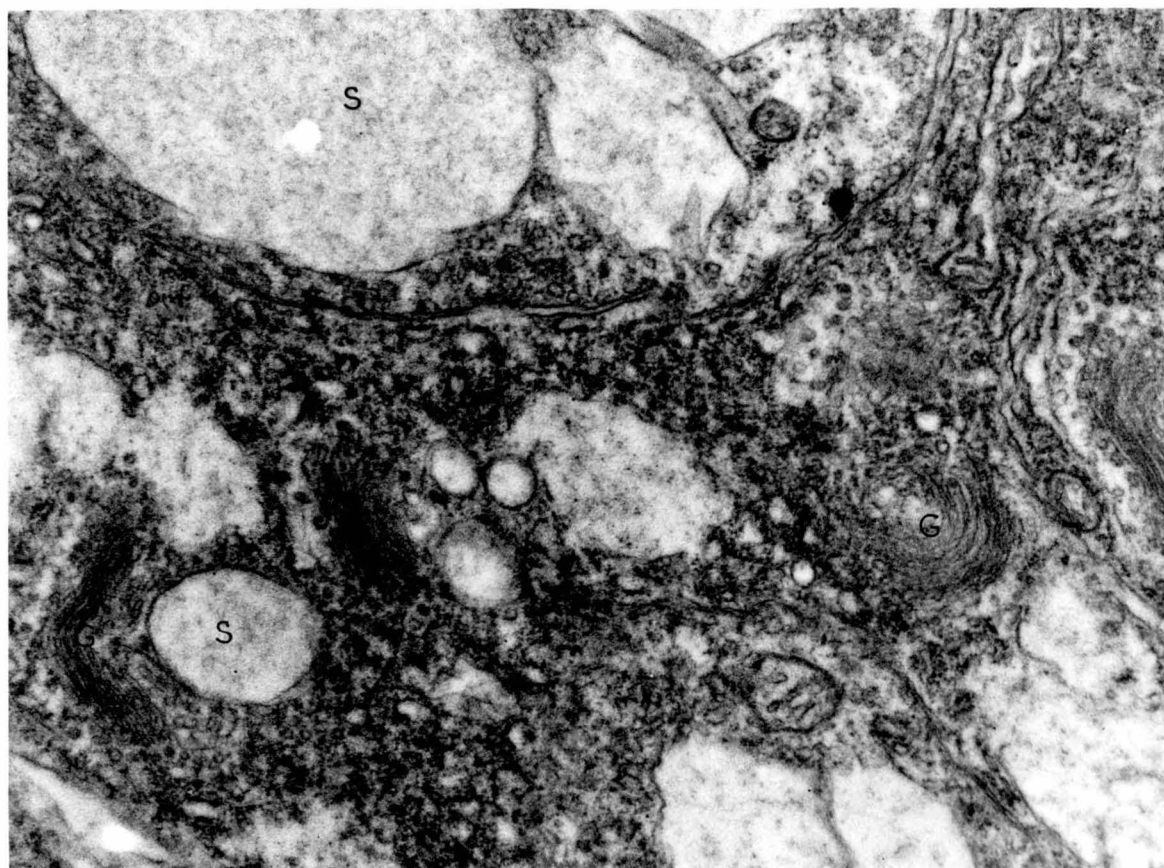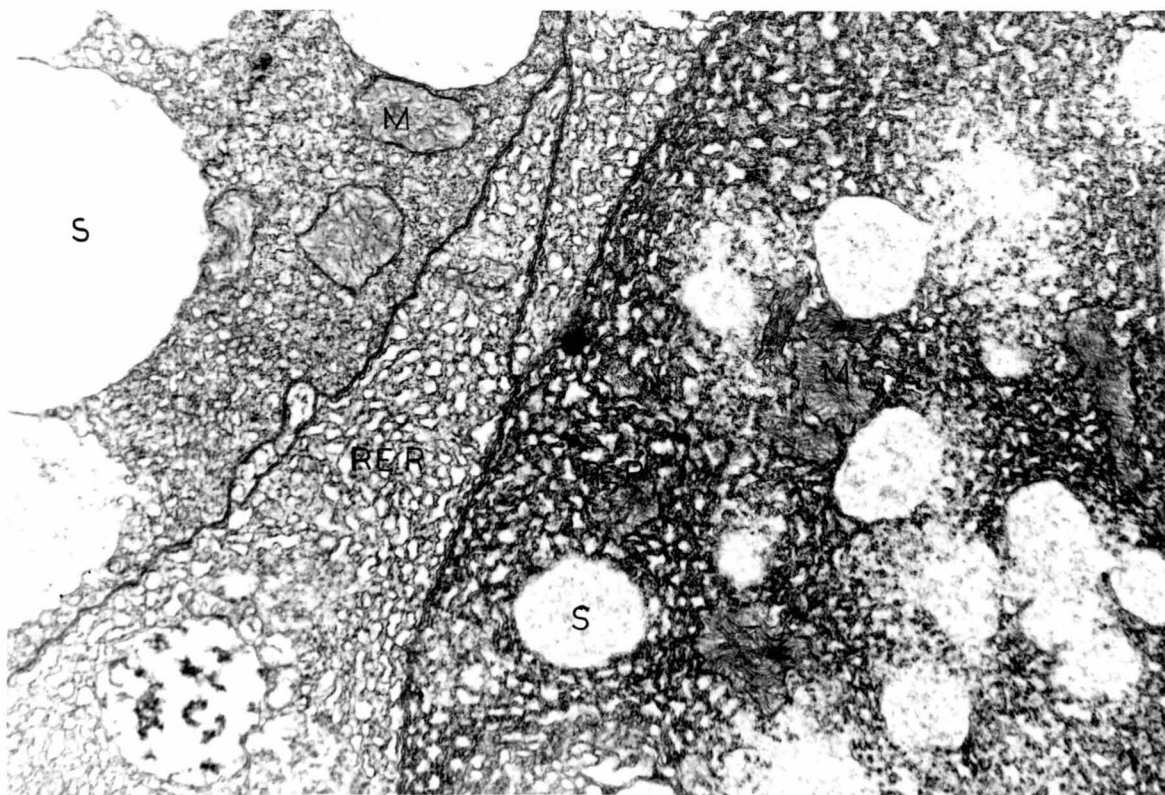
<u>Fig. 4.</u>

Electron micrograph of part of three labral
gland cells of <u>Sulcanus</u> showing the num-
erous secretory vacuoles (S) and the rough
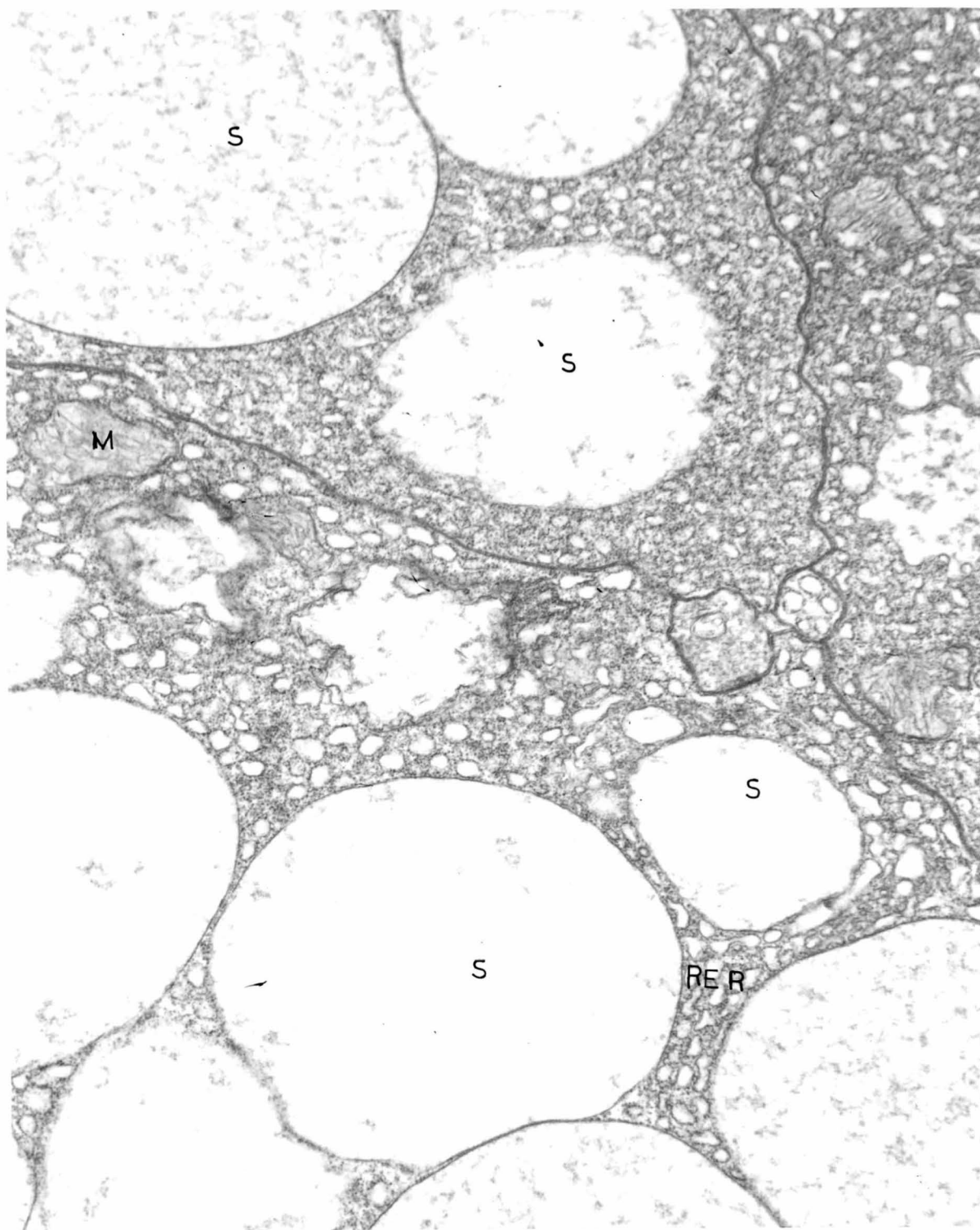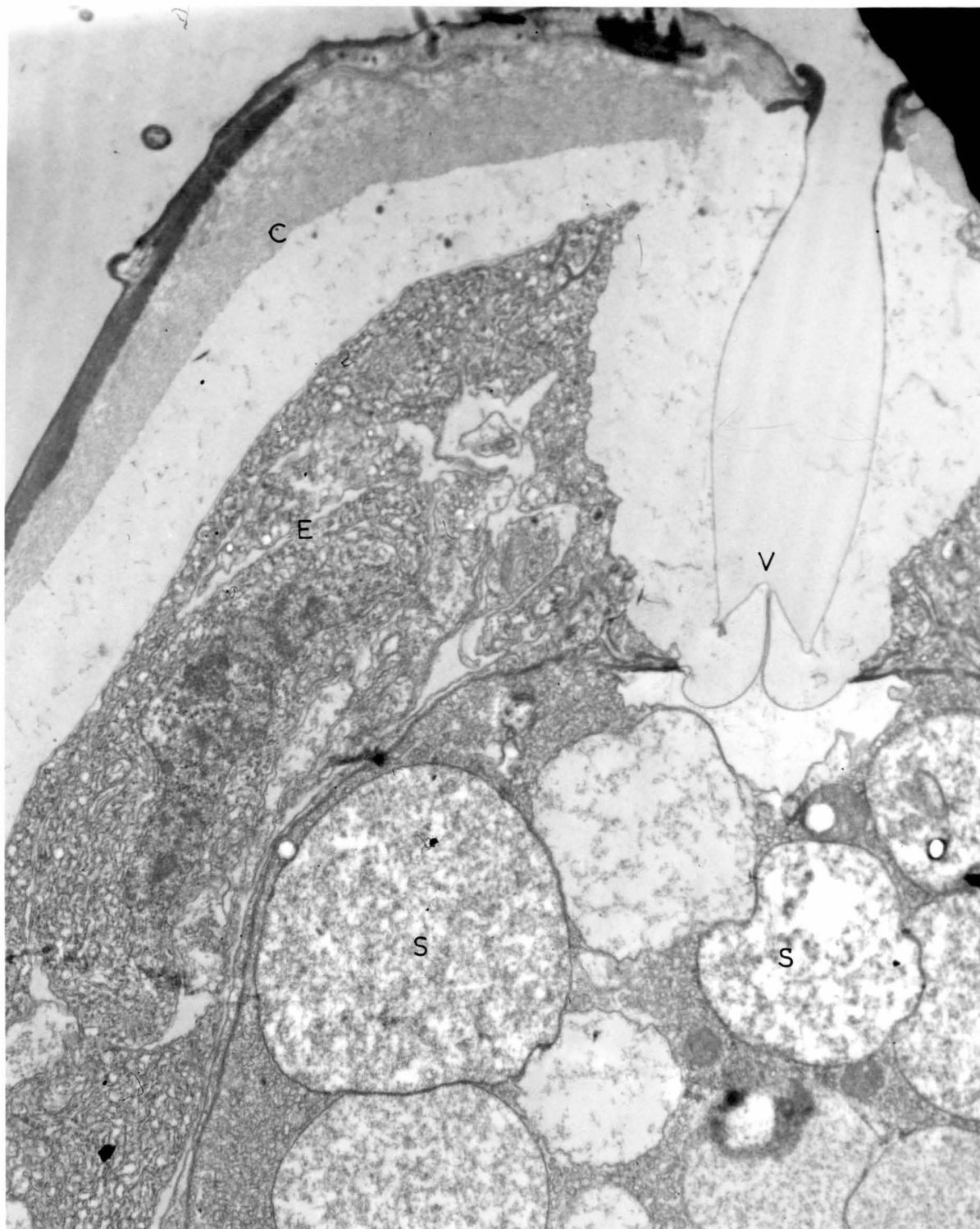endoplasmic reticulum (RER).   X13,750

Electron micrograph of a parasaggital
section through part of the labral
gland of <u>Gladioferens</u> showing the
cuticular valve (V), the epidermal cell
(E) and the large secretory vacuoles (S)
of the labral gland cell.   The secretory
vacuoles contain different amounts of
electron opaque material.   X 7,500.

cause the valve to close but since hydrostatic pressure is non-directional, pressure will also be exerted on the walls of the valve entrance and this would cause the valve to open, but only if the wall is a rigid structure. It is more probable that the wall of the valve is quite flexible since there is no indication that the cuticle is in any way mineralized. This being the case, hydrostatic pressure from the outside will cause the space bounded by the conical part of the valve and the wall of the valve entrace to assume a shape that tends towards the sphere. Thus as the external hydrostatic pressure is increased the concavity of the wall of the valve entrace would increase. It would thus be possible for secretions of the gland cell to be extruded but impossible for water from the external environment to enter the labrum. Such a structure is probably important especially to animals living in deep water.

DISCUSSION

The positive reaction of the labral glands to paraldehyde fuchsin suggests that some form of mucin is produced (Humason, 1962). The presence of rough endoplasmic reticulum and Golgi complexes in the labral gland cells is further support that the labral glands secrete mucus (Beams and Kessel, 1968). And as stated

previously, it has been shown that the labral glands
secretion is PAS-positive (Dahl, 1963). The presence
of valves is a strong suggestion that the glands secrete
into the exterior.

The labral glands appear to be highly organized,
with the structurally simple but apparently efficient
valve. Since there are no muscles associated with
the valve and there is no extensive muscle system
in the labrum, the mechanism of secretion by the labral
glands is unlikely to be through muscular contraction.
Pressure of the haemolymph is a possible mechanism for
the extrusion of the secretion of the labral gland but
this appears highly unlikely. The most probable mech-
anism of secretion by the labral glands is one caused
by the pressure built up within the labral gland cells.
The secretory material is thus extruded as the amount
of secretory material increases. The question of
what controls the secretory activity of the labral glands
cannot yet be answered.

It is not known with any certainty whether
filter feeding copepods can regulate their filter
feeding mechanism or whether filter feeding is con-
tinuous. The filtration rate appears to be largely in-
dependant of the concentration of food in the environment
(Gauld, 1951) indicating that the rate of filtration
is constant. Marshall and Orr (1955b) have shown that

Calanus kept in cultures of food organisms on which
they are known to feed do not produce faecal pellets.
When no faecal pellets were produced it was observed
that there were agglutinations of food particles,
possibly held together by the mucus secretion associated
with filter feeding.   It thus appears that while fil-
tration of food may be continuous, ingestion of food
by the animals is not necessarily continuous and is
possibly selective (the latter has been considered
earlier in this thesis).   It thus appears that the
filtration of food particles, in Calanus at least, is
continuous.

The secretion of mucus may also be continuous.
If this is true it seems unlikely that discharge of
secretory material is under reflex or neurosecretory
control, as it may be if there is a cyclical or erratic
filtration rate.

It appears that mucus secretion by the labral
glands is an integral part of the filter feeding mech-
anism.   Filtration rate appears to be constant and
filtration, a continuous process.   Animals are able
to selectively ingest or reject agglutinated particles.
Thus while ingestion may not be continuous the production
of mucus probably is.   When agglutinated particles are
not ingested some mucus is lost into the environment by

the animals.   Mucus that is ingested with the agglu-
tinated particles may be resorbed in the gut of the
animal, but energy is expended in the production and
resorption of the mucus.   It was stated earlier that
some copepods have been shown to be remarkably effic-
ient at digestion and absorbtion.   The gross production
of the animals is extremely high, but if mucus is being
constantly secreted the net productivity of these animals
would be considerably lower than the gross productivity.
It would thus be useful to determine the amount of
energy the animal expends in mucus secretion.

It appears that mucus secreted by the labral
glands may increase the filtration efficiency, especially
when smaller particles are involved.   It is unlikely
that mucus will improve filtration efficiency when larger
particles are involved.   While mucus secretion may in-
crease the filtration efficiency when small particles
are being filtered the process may be a wasteful one
when larger particles are involved.   However, the
possible added filtration efficiency with mucus may be
a vital factor at certain times of the year when only
tiny food organisms are present or in environments where
only very small food organisms are present.

MID-GUT DIVERTICULUM *

INTRODUCTION.

The ubiquitous marine copepod <u>Calanus finmarchicus</u> (Gunnerus) has been reported to be able to absorb organic material (from the digestion of phytoplankton) with the remarkable efficiency of between 50 and 80% and often over 90% (Marshall and Orr 1955a). This indicates a highly efficient digestive system. The alimentary canal in <u>Calanus</u> is simple, and does not have distinct digestive organs such as hepatopancreas. The anatomy of the gut at the light microscope level has been described by Dakin (1908), Lowe (1935), and Marshall and Orr (1955b). I am unable to locate any published work on the ultra-structure of the gut of copepods. The following is a description of the ultrastructural morphology of the midgut diverticulum of the calanoid copepod <u>C. helgo-landicus</u> (Claus) - a species closely related to <u>C. finmarchicus</u> - with a discussion on its possible functions.

OBSERVATIONS.

(a) <u>Gross Structure</u>

A longitudinal section of the midgut diverticulum is shown in Figure 1. The anatomy is in agreement with Lowe's (1935) description of <u>C. finmarchicus</u> and
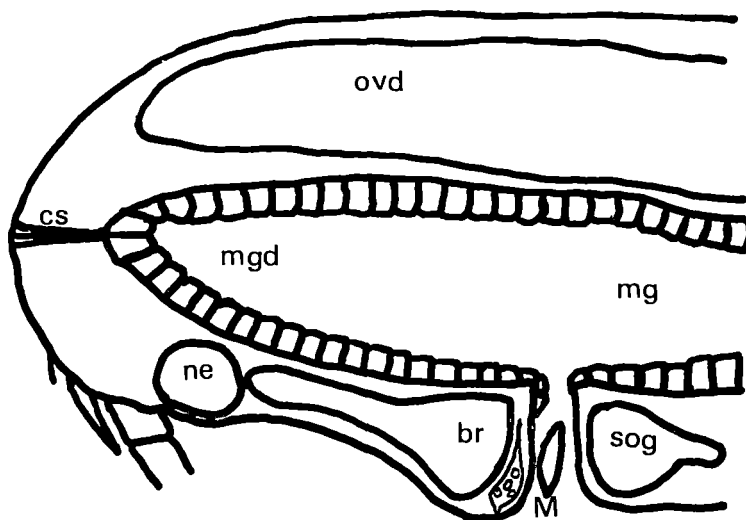
* Please see page 243.

Fig. 1.

Diagram of a longitudinal section
through the anterior-region of
C. helgolandicus showing the position
of the midgut diverticulum (mgd) in
relation to the other structures in the
cephalosome.   Abbreviations used:
br, brain;   cs, connective strands;
M, mandible;   mg, midgut;   ne, nauplius
eye;   ovd, oviduct diverticulum,
sog, suboesophageal ganglion.

for further gross description of the gut, reference
may be made to her excellent work. The midgut divert-
iculum is a conical extension of the midgut and extends
from the front of the ᴄoesophagus into the cephalic
region where it is attached to the cuticle by a strand
of connective tissue. All the epithelial cells of the
midgut diverticulum appear to be morphologically similar.
As described by previous workers they appear as cuboidal
cells with large centrally situated nuclei, each con-
taining a single nucleolus. The epithelium is lined
on the basal side (haemocoel side) by patches of flat
myoepithelial cells. This layer corresponds to the
muscular layer described by Lowe (1935). A thick
well-developed basement membrane is present between the
base of the epithelial cells and the myoepithelial cells,
(Fig. 10).

(b) Histochemistry

The methyl blue-positive layer of the apical surface
of the midgut (facing the lumen) described by Lowe (1935)
proved to be positive for the alcian blue technique for
acid mucopolysaccharides. This layer corresponds to
the microvilli layer of the midgut. The alcian blue
also stains, to a lesser extent, the peritrophic membrane
particularly in the more posterior section of the midgut.
The labral glands showed a negative reaction to alcian
blue.

The contents of the gut stain purple with para-
ldehyde fuchsin and this is a suggestion of some form of
mucin (Humason 1962). It appears that the layer from
which the microvilli arise also showed a positive reaction
to paraldehyde fuchsin; the resolution of the light
microscope prevents a definite interpretation. The
epithelial cells of the more posterior part of the midgut
showed a positive reaction. The labral glands stain
a distinct purple and it is probable that the secretion of
these glands contributes to the purple staining of the
gut content.

(c) <u>Ultrastructure</u>

    (i) <u>The Epithelial Cell</u>

The cell is columnar and measures about 45/um long
and 25/um in diameter although at the light microscope
level it appears to be more or less cuboidal in shape.
The centrally situated nucleus is about 20 by 8/um and
contains a single nucleolus. For ease of description
the cell may be arbitrarily divided into two parts: the
apical part (lumen side) and the basal part (haemocoel
side)(Fig. 2).

In the apical half of the cell the plasma membrane
forming the side walls is more or less flat (Fig. 2).
The plasma membrane of the cell facing the lumen of the
gut consits of tightly packed microvilli. Beneath these
is an electron-dense layer approximately 0.2/um thick (Figs.

Fig. 2:

Electronmicromontage showing a longitudinal
section of the midgut diverticulum of
C. helgolandicus. The apical portion bears
microvilli (v), numerous mitochondria (m),
numerous microvesicles (mv) of varying
electron opacity and size, and Golgi-like
bodies (arrowed).   The nucleus (n) is in
the centre of the epithelial cell (Ep). The
plasma membrane of the basal portion of the
epithelial cell is extremely folded and con-
tains numerous mitochondria (m) and micro-
vesicles (mv), In this section the basement
membrane (bn) and the myoepithelial cell
(Me) are not highly developed.   An
axon containing what appear to be elem-
entary neurosecretory granules is seen in
the haemocoel (h) and almost in contact with
the myoepithelial cell. X 3240.
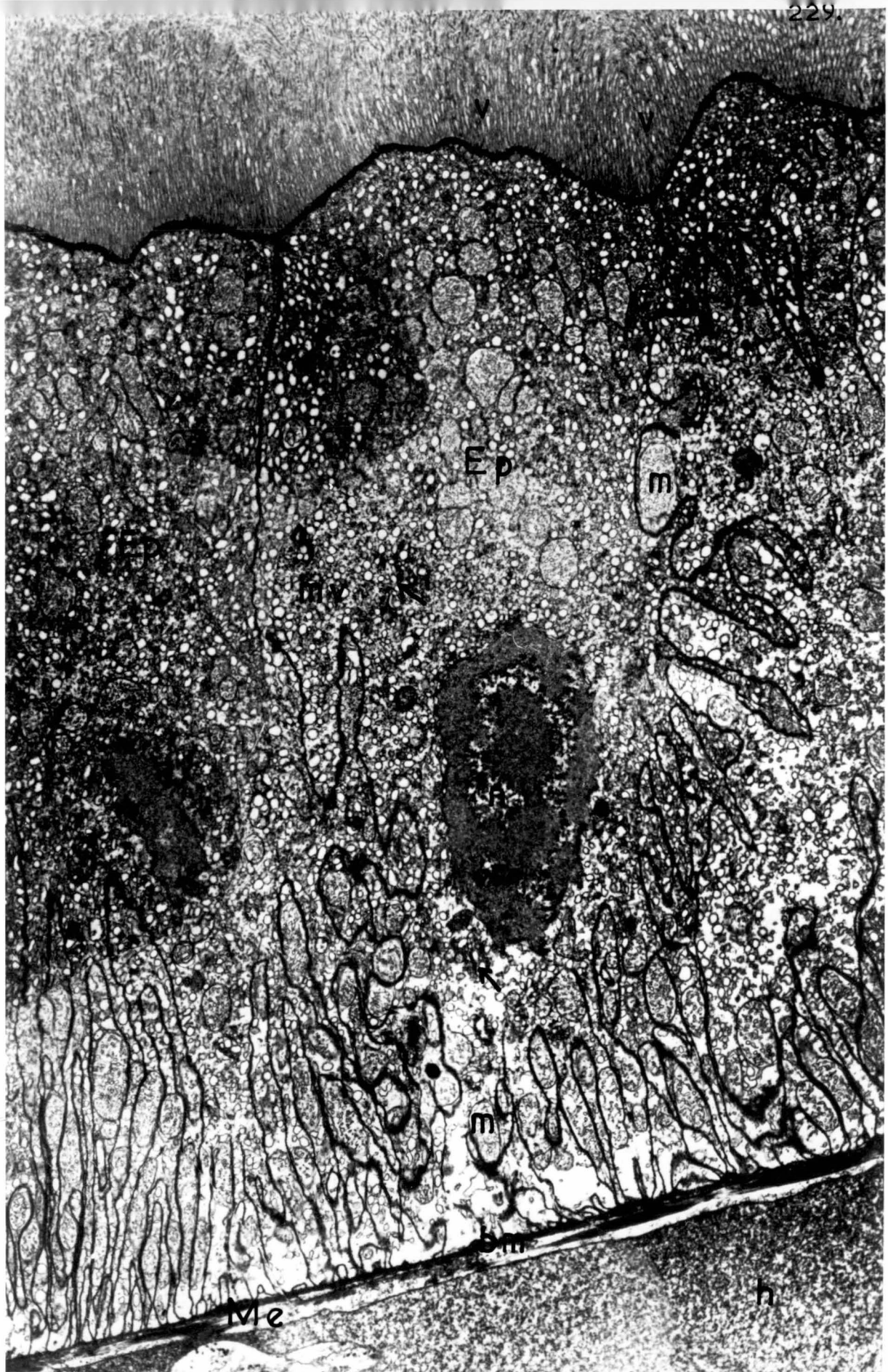
3 and 4). There is a pronounced constriction at the base of the microvilli. The microvilli are between 2.5 and 4.5/um long and about 92 nm in diameter; the constricted region being about 36 nm in diameter. The tips of the microvilli are slightly club-shaped and the walls here are more electron-dense than at any other part of the microvilli. The microvilli do not appear to contain any distinct microtubules or fibrils. There is a homogeneous layer of medium electron-dense material at the tips of the microvilli and this is especially noticeable at the apex of the midgut diverticulum where the lumen is narrow and the tips of the microvilli from the cells opposite are in close proximity. This homogeneous layer may represent the beginning of a peritrophic membrane.

The cytoplasm of the apical portion of the epithelial cell as well as the cytoplasm of the basal portion of the cell around the nucleus contains numerous mitochondria with tubular cisternae (Figs. 2, 5, and 6), numerous electron-lucent microvesicles ranging in size from 0.2 to 0.5/um in diameter (Figs. 3 and 4), groups of medium electron-dense small microvesicles of about 90 nm in diameter, scattered ribosomes, and Golgi-like bodies (Fig. 9). The epithelial cells appear to be held together by tight junctions near the microvillous border (Fig. 4). It is seen from Figure 4 that micro-

Fig. 3:

Electronmicrograph of the apical portion of
an epithelial cell (Ep) showing the electron-
dense layer (dc) from which the microvilli
(v) arise.   It is seen that the tips of the
microvilli are more or less club-shaped
and their plasma membrane is more electron-
dense than the plasma membrane in the prox-
imal parts of the microvilli.   A layer
of medium electron density (p) is seen
outside the tips of the microvilli.
X 41,000.

Fig. 4:

Electronmicrograph of the epithelial cells
at the microvillous border (v) showing the
tight junction (tj) between two epithelial
cells.   Note the microvesicles which in.
places appear to be associated with the
electron-dense layer (dc) at the base of
the microvilli.   In places it is possible
to see the "unit membrane" of the micro-
villi.   Free ribosomes (r) and electron-
lucent microvesicles (mv) can also be
seen. X 58,500.
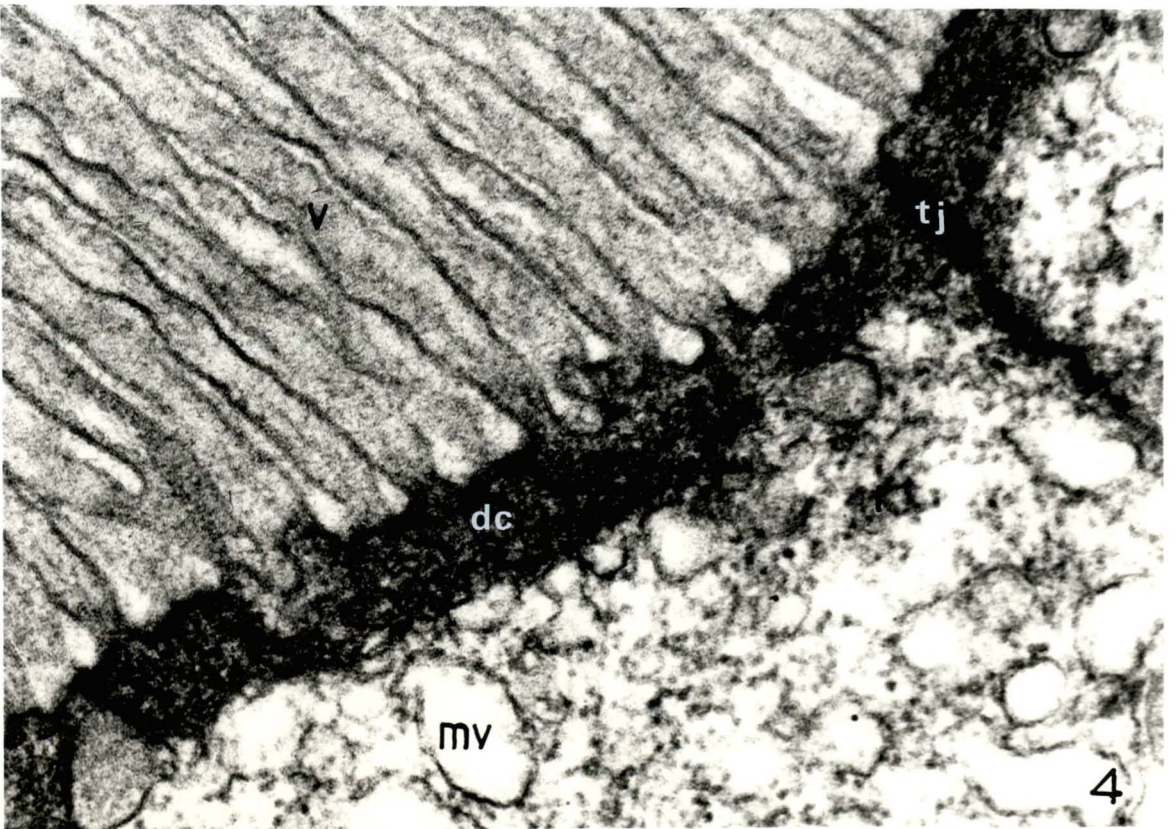
vesicles of medium electron density are present next
to the microvilli.   It is not known if these are mat-
erials absorbed from the gut.   The plasma membrane of
the basal portion of the epithelial cell is extremely
interdigitated with the plasma membrane of adjacent
cells.   The plasma membrane forming the base of the
cell is thrown into extensive convolutions which contain
microvesicles and numerous elongated mitochondria with
tubular cisternae (Figs. 2, 5, and 6).   There appears to
be a lack of rough endoplasmic reticulum (Figs. 5 and 6).
This tends to suggest an absence of synthesis of protein
material for external secretion.

(ii) The Basement Membrane

Below the convoluted plasma membrane is a well-
developed basement membrane.   This basement membrane
does not appear to ensheath the midgut completely so
that there are uncommon intermittent breaks where the
epithelial cells are in direct contact with either the
myoepithelial cell (Fig. 8) or the haemocoel.   The
basement membrane is about 0.2/um thick, being made
up of what appears to be a number of layers of electron-
dense material, each layer being about 10 nm thick with
intervening electron-lucent layers of about the same
thickness (Fig. 10).

(iii) The Myoepithelial Cells

These cells are situated below the epithelial
layer.   Like the basement membrane these cells do not

Fig. 5:

Electronmicrograph of a transverse section
near the middle region of an epithelial
cell showing the extensively folded plasma
membrane of the side walls.   The nucleus
(n) is centrally situated and the cyto-
plasm contains numerous mitochondria (m),
microvesicles of varying sizes and
densities (mv), as well as tiny Golgi-
like bodies (g).   X4750.

Fig. 6:

Electronmicrograph of a longitudinal section
of the basal part (haemocoel, h, side) of
the midgut diverticulum showing an epithelial
cell with its extensively folded plasma mem-
brane and the numerous elongated mitochon-
dria (m) between the folds.   A well
developed basement membrane (bm) lies
between the epithelial cell (Ep) and the
myoepithelial cell (Me).   The myoepithelial
cell contains a flattened nucleus (n),
mitochondria and myofibrils.   X8600.

5

6
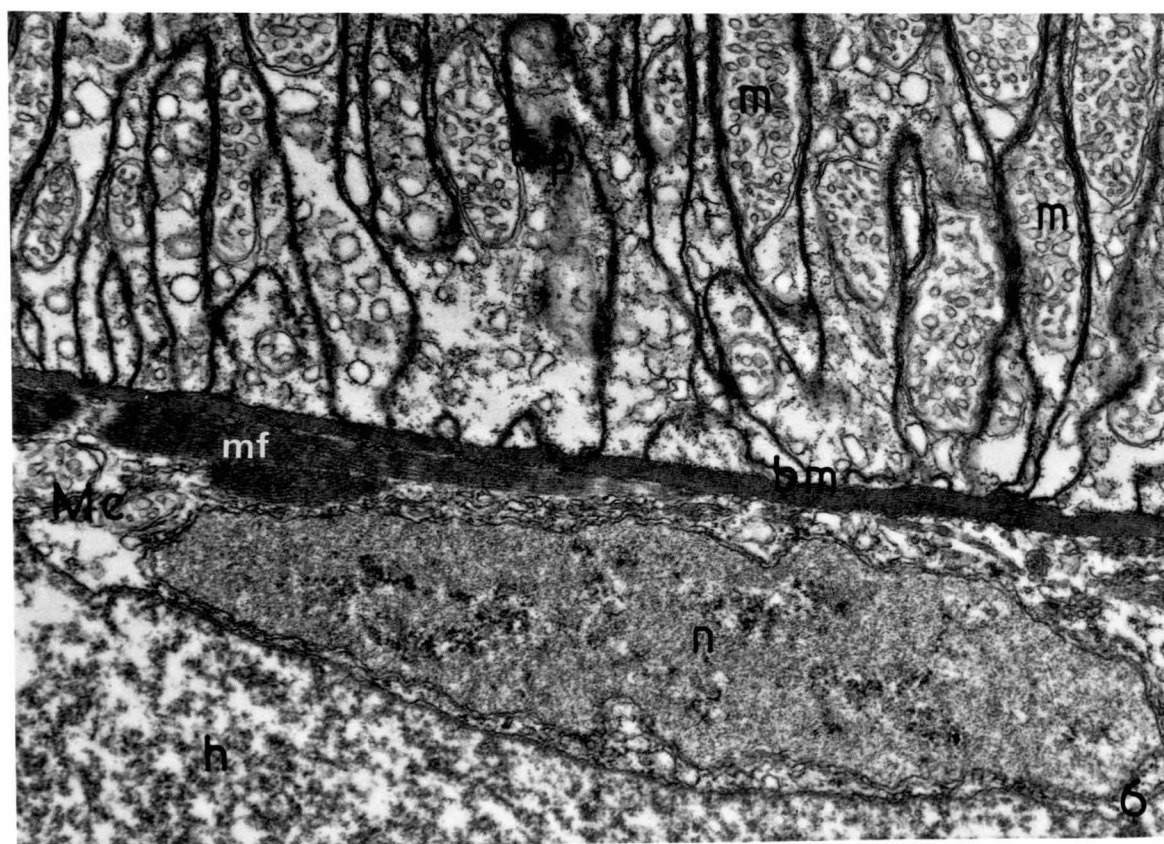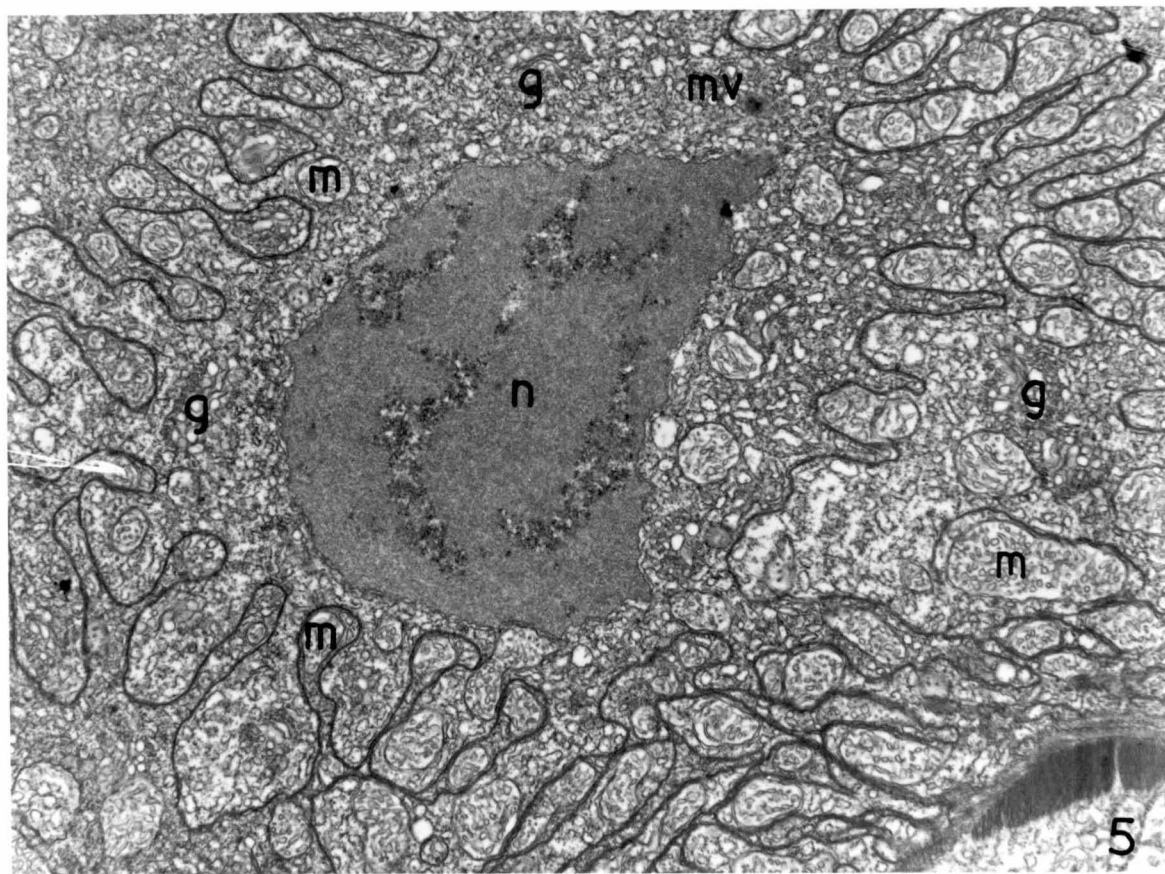
completely ensheath the gut. The cells are flattened
and have flattened nuclei (Figs. 5 and 6). As well as the
nuclei, the cells contain conspicuous bundles of both
circular and longitudinal myofibrils in the same cell, a
few mitochondria with tubular cisternae, electron-lucent
microvesicles ranging in size from 0.02 to 0.2/um in
diameter, and occasionally groups of small (about 100 nm
diameter) electron-dense microvesicles. The myofibrils
consist of thin electron-dense strands (of about 10 nm
diameter) embedded in a matrix of medium electron-dense
material (Figs. 7 and 8).

DISCUSSION.

The most striking feature of the midgut diverticulum
of C. helgolandicus is the presence of an abundance of
mitochondria in the epithelial cells. This is indicative
of a high metabolic activity. The cells appear to have
two morphological parts; the apical portion which is
possibly involved in the secretion of mucopolysaccharides,
and the basal portion which is probably involved in active
transport of some form.

The absence of rough endoplasmic reticulum in the
epithelial cells indicates, perhaps, the absence of any
synthesis of proteinaceous material which may be formed
for extracellular secretion. Thus it appears, unlikely
that these cells secrete enzymes. The presence of Golgi
bodies and clear vesicles that are budded off from them,

Fig. 7:

Electronmicrograph showing the base of an
epithelial cell (Ep) and part of a myo-
epithelial cell (Me) next to it. The
myoepithelial cell contains highly developed
myofibrils (mf), mitochondria (m), and
both electron-lucent (mv) as well as electron-
dense (dmv) microvesicles. The basement
membrane is between the epithelial cell and
the myoepithelial cell. X 12,350.

Fig. 8:

Electronmicrograph showing a similar situation
as in Figure 7. Both longitudinally arranged
(mf) and circularly arranged (cmf) myofibrils
can be seen in the myoepithelial cell. It
is seen here that the basement membrane (bm)
does not completely separate the epithelial
layer from the myoepithelial layer. X 7150.

7

8
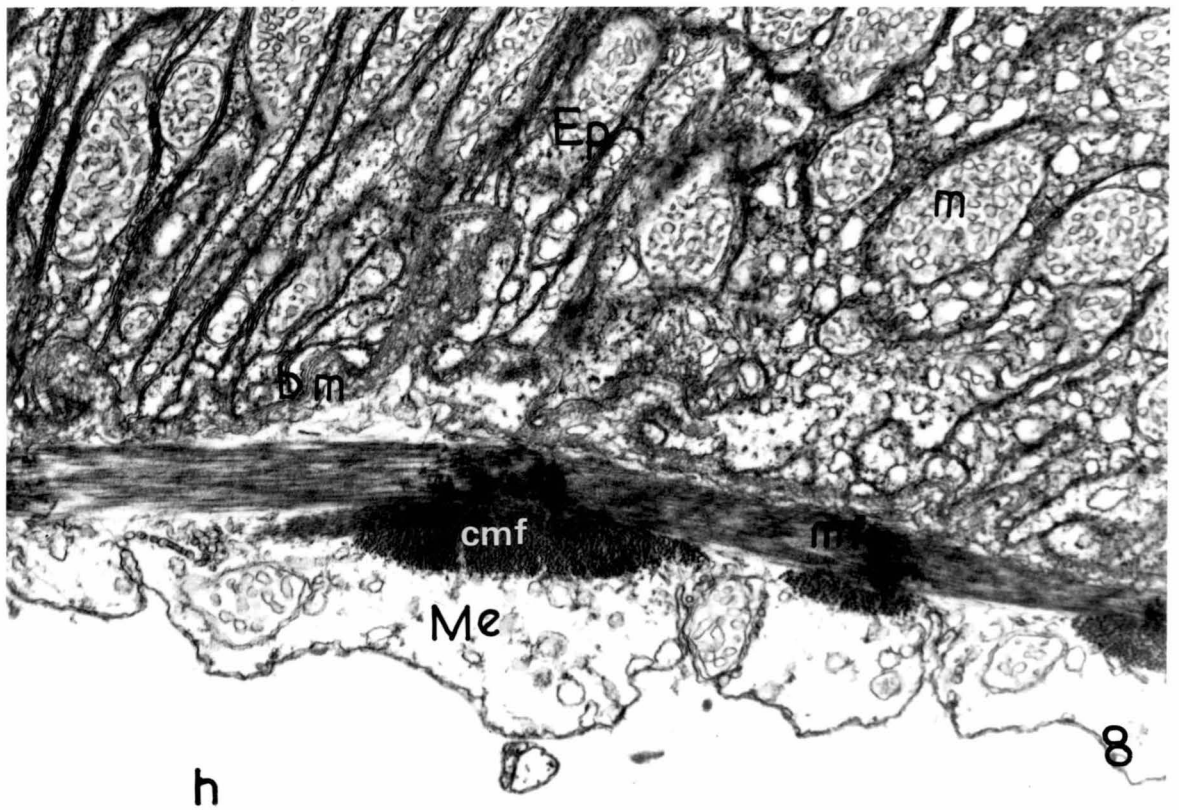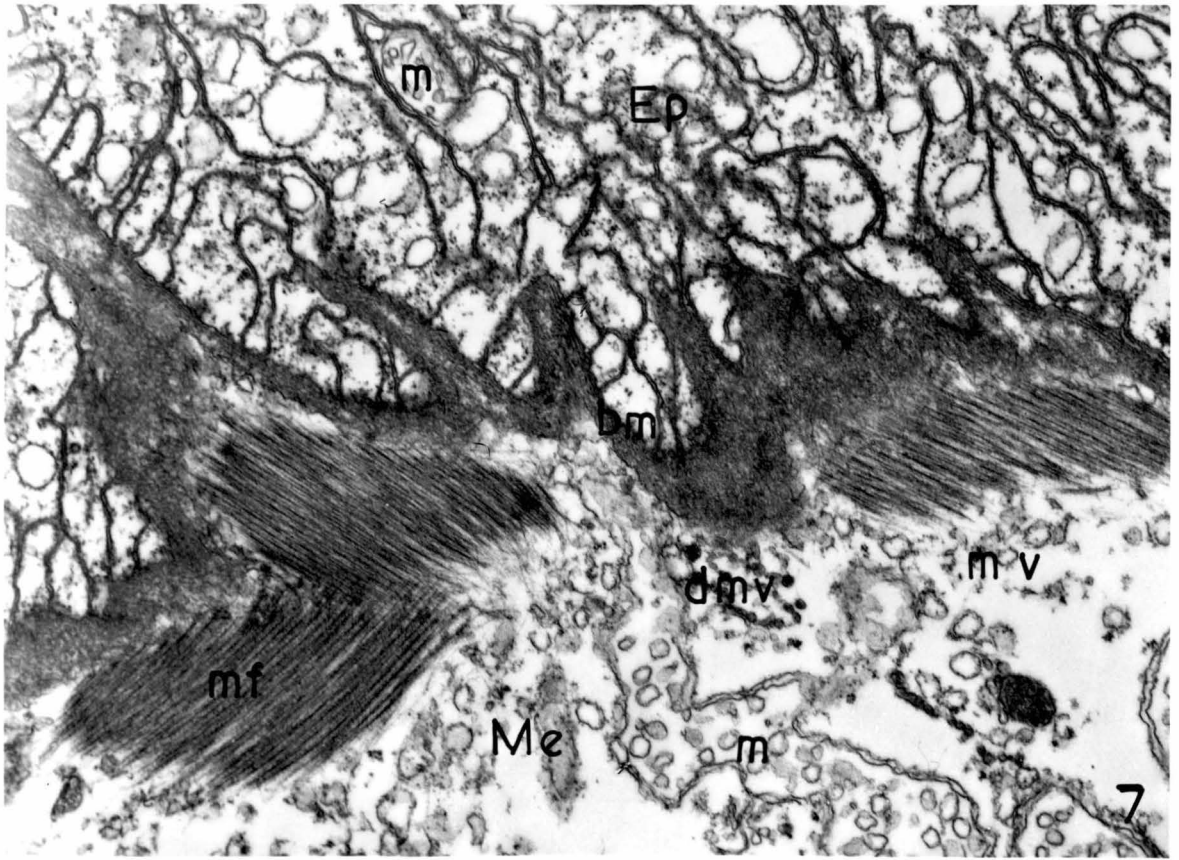
as well as the positive reaction of the microvilli to alcian blue, suggest that acid mucopolysaccharides may be secreted by the epithelial cells (vide Beams and Kessel 1968). The alcian blue-positive material is probably involved in the formation of the peritrophic membrane, the membrane being properly formed only in the more posterior part of the midgut (Gauld 1957). Gauld (1957) observed that food is churned in the midgut diverticulum of Calanus and suggested that the midgut diverticulum acts as a stomach. Park (1966) working on another calanoid, Epilabidocera amphitrites, suggested that the midgut diverticulum in that animal was highly secretory and that it, as well as the more anterior parts of the midgut, secretes digestive enzymes. The present study indicates that it is improbable that enzymes are secreted by the midgut diverticulum. From observations on live animals we agree with Gauld (1957) that the midgut diverticulum acts as a stomach only in as much as food is mechanically churned in this region. It is possible that digestive enzymes are present in the lumen of the midgut diverticulum, but their origin is the anterior part of the midgut proper. The contents of the lumen stain purple with paraldehyde fuchsin and this suggests that some form of mucin is present (Humanson 1962). Mucin has been shown to be secreted by the labral glands by Dahl (1963) and Ong (see section on labral glands).

<u>Fig. 9</u>:

Electronmicrograph showing the Golgi-like
bodies of the epithelial cell. The Golgi-
like bodies are situated around the nucleus
(n) and consist of two to three cisternae
(c) surrounded with microvesicles of dif-
ferent sizes and electron densities.   In
section, the cisternae are seen to have
bulbous ends (b).   X 51,500.

<u>Fig. 10</u>:

Electronmicrograph showing the lamellated
basement membrane (bm) between the epithelial
cell (Ep) and the myoepithelial cell (Me).
Part of the nucleus (n) of the myoepithelial
cell can be seen at the bottom of the electron-
micrograph.   X 28,200.

237.
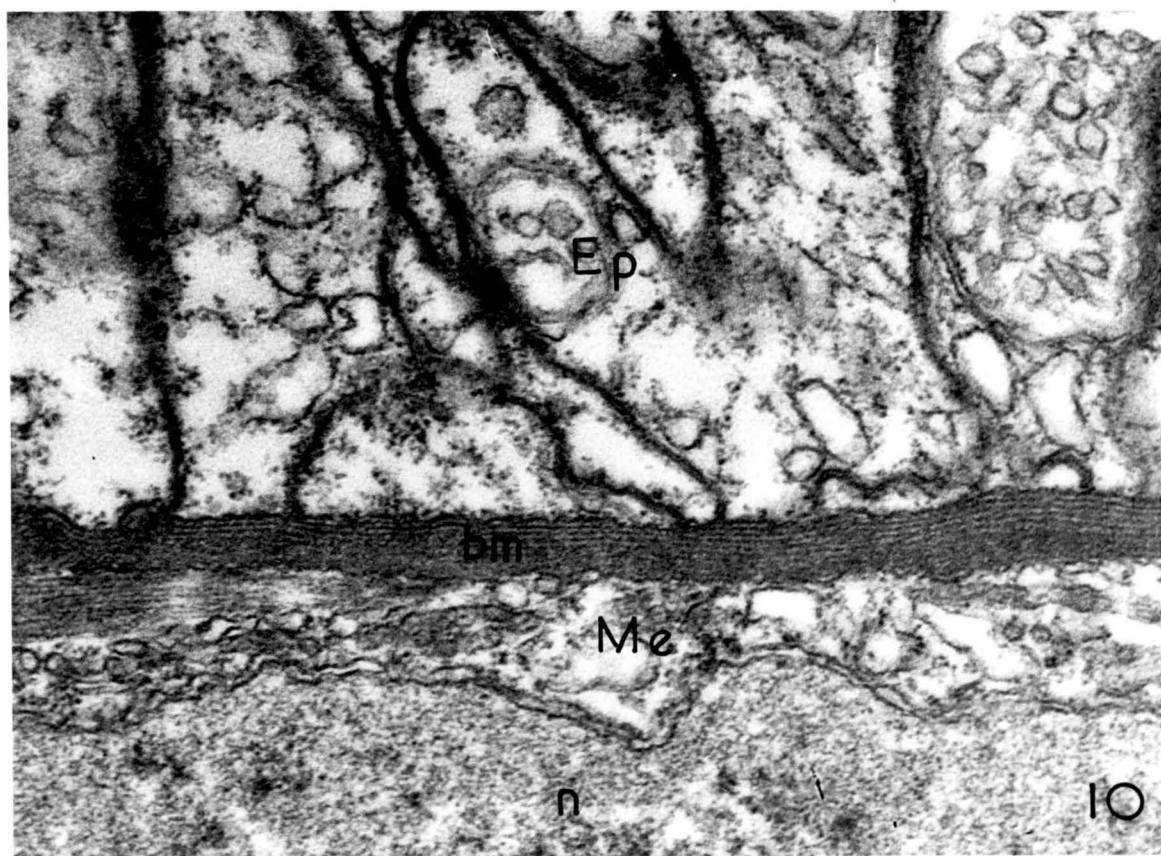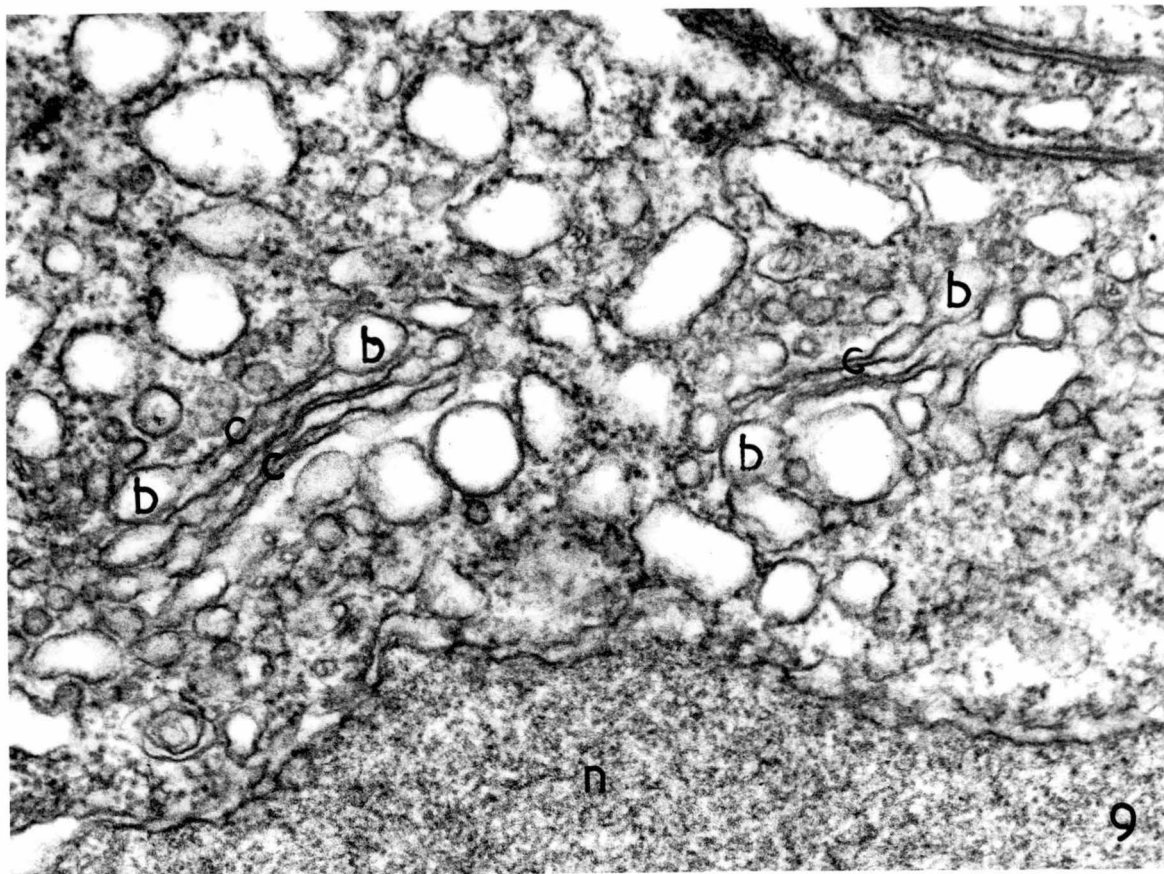
This mucus is possibly responsible for the protection of the gut from its own enzymes. The secretion of the labral glands is mixed with the food as it passes into the oesophagus. This is particularly important in the midgut diverticulum where the peritrophic membrane is not present.

In insects there is no mucus present in the digestive tract, but the chito-protein of the peritrophic membrane of insects has mucopolysaccharides (Wigglesworth 1966, p. 43). Since mucus is a variable mixture of mucoprotein and muco-polysaccharides there does not appear to be any great chemical difference in this respect between insects and crustaceans. It seems that, because the midgut divert-iculum is not protected by a peritrophic membrane, the mucus secreted by the labral glands plays an important part in the digestive physiology of the copepods.

The basal portion of the epithelial cell is com-posed of an extensively convoluted plasma membrane and resembles the "mitochondrial pump" of the gills of the land-crab, Gecarcinus lateralis (Copeland 1968). There is a strong suggestion of active transport but neither the components being transported nor the direction of transport can be known without further study. Calanus is known to be able to tolerate reduced salinity (Marshall and Orr 1955b) although it is an oceanic form and Cowey and

Corner (1963) have suggested that it is able to do so by regulation of the amino acid content of its body fluids. No data on the osmotic pressure of the tissue fluids of Calanus are available but in another oceanic genus, Centropages, the osmotic pressure is nearly isotonic with sea water (Lance 1960, 1965). It thus seems that the transport system operating across the basal portion of the epithelial cell involves amino acids, probably absorbed from the gut through the microvilli. This would help account for the remarkable efficiency of absorption of the alimentary canal of this animal (Marshall and Orr 1955a).

The myofibrils of the myoepithelium of the midgut diverticulum are extremely well developed and observers like Lowe (1935) have even been able to detect this layer under the light microscope. The longitudinal muscle described by Lowe (1935) as nonstriated and forming the fine fibrillae has been termed myoepithelial cells in the present study. This is in accordance with the terminology of Stanier, Woodhouse, and Griffin (1968). These do not have their fibrils aligned in any specific direction and substantially differ, in this respect, from normal muscle cells. Lowe (1935) could not detect the circular fibrillae, like the ones shown in Figure 8. Lowe (1935) observed that the myofibrils of the midgut diverticulum are not as well developed as they are in the hinder

parts of the midgut. The development of the myofibrils of the midgut diverticulum seen here appears to be similar to those reported in the hepatopancreas of crabs (e.g. Stanier, Woodhouse, and Griffin 1968), the gastric caeca of mosquito larvae (e.g. Jones and Zeve 1968), and the midgut diverticula of Chirocephalus (Lake, unpublished data).

There appears to be one fundamental difference of the midgut diverticulum of Calanus compared to those of other arthropods: the epithelial cells do not appear to contain any globules or granules of stored material (e.g. fats) or any mineral concretions (e.g. calcium). The midgut diverticulum thus probably does not act as a storage organ.

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* This section was carried out in collaboration with
  Dr. P. S. Lake. Dr. Lake performed the alcian blue
  staining and collaborated on the sections on histo-
  chemistry and the discussion.

GENERAL DISCUSSION

This study has been essentially morphological and was primarily intended to provide a basis for studies of a more experimental nature. A serious restriction to using copepods as experimental animals is their minute size. Because of this, for example they cannot be used for electrophysiological studies. Nonetheless, many other types of experimental study on copepods are possible.

Electron micrographs are two-dimensional representations. Through serial sectioning, three-dimensional reconstructions of cells and tissues are possible and are indeed almost routine in many morphological studies. The fourth dimension, time, is often neglected although it is sometimes essential to and almost always useful in the proper interpretation of structures. Possibly the simplest experimental approach in electron microscope studies is through the monitoring of ultrastructural changes or possible ultrastructural changes in animals through the seasons of the year as well as through the various stages of the animals' life cycle.

Many of the structures treated in this thesis are apparently related to one another either directly or through feed-back links. It is possible that a single

environmental cue may trigger off a sequence of events
involving a series of related tissues, from sensory cells
to peripheral target organ.   In monitoring the struc-
tural changes through the seasons and through the
various stages of the animals' life cycle it is thus
desirable to monitor the possible structural changes
not only in a single tissue-type but in all the tissues
that may possibly be involved in a particular sequence
of processes.   An example of such a possible inter-
related system is the sensory pore X-organ complex (i.e.
including Claus's organs) and the integument.   By
monitoring the ultrastructural changes of these tissues
throughout the moulting cycle it may be possible to
trace the sequence of events from one tissue type to
the next.   In this way it may be possible to locate
the effector tissue and the affected tissue.   In this
particular hypothetical example the sequence of events
starts with the perception of environmental cues, internal
or external, by either the sensory portion of Claus's
organs or by the chemoreceptor sensilla of the sensory
pore X-organs.   This may lead to changes in the secre-
tory activity of the neurosecretory cells of the sensory
pore X-organ complex which in turn might effect the
amount of the 50 nm diameter electron opaque granules
present amongst the cilia of Claus's organs.   The content

of the 50 nm granules in the Claus's organs may lead to structural changes in the "companion cells" of Claus's organs which would result in the secretion of a factor or factors that trigger changes in the epidermal or tegumental cells of the integument;   the whole process resulting in the animals going into moult.

Physiological changes in cells or tissues are not always manifested as structural changes so that some physiological events may be missed when only structural changes are monitored.   The adaptation and refinement of light microscope histochemical methods for electron microscope studies in the past few years, though many of these methods are still not very reliable, have provided the electron microscopists with a further useful tool. Thus if histochemical monitoring is used concurrently with ultrastructural monitoring then some of the physiological changes in cells and tissues that do not manifest themselves ultrastructurally may be detected histochemically.   A combined histochemical and ultra-structural monitoring programme will provide a better but not necessarily complete picture.

The ultrastructure and histochemistry of many organelles and cellular membranes undergo detectable and significant changes when they are subjected to certain environmental factors to which they are normally sensitive. For example, the rhabdomeric microvillii of photoreceptors

have been shown to undergo ultrastructural changes when
subjected to prolonged periods of light and darkness (e.g.
Rohlich, 1967). It is thus sometimes possible to induce
ultrastructural and histochemical changes in sensory
tissues by subjecting these tissues to the environmental
factor or factors to which they are normally sensitive.
Thus as the electrophysiologist by subjecting tissues to
various environmental factors, such as light or gravity,
and recording the electrical responses of the tissues is
able to determine the ability of the tissues to respond
to certain environmental factors, the electron microscopist
can do likewise by monitoring ultrastructural or histo-
chemical changes. In this particular example the
electrophysiologist is at an advantage in that he is able
to greatly amplify the electrical impulses generated by
his tissues so that he has a vastly more sensitive method.
The above method, though not as sensitive as the electro-
physiological method is nonetheless quite useful.

The above technique can be used in the study of the
function of the phaosomes of the copepod nauplius eye. By
subjecting some animals to a prolonged period of light, others
to a prolonged period of darkness and a third group to normal
light/dark conditions (as controls) the effect of prolonged
periods of light and darkness on the phaosomes can be de-
termined. If the phaosomes are shown to respond to either

prolonged light or darkness it may then be possible to
determine to which colours these structures are sensitive
by subjecting groups of animals to prolonged periods in
light of different colours and studying changes in the
phaosomes. It may also be possible to determine thres-
holds of sensitivity but it is unlikely that this method
would be as accurate as electrophysiological methods.
This same method may be applied to the study of the rhab-
domeres.

Non-sensory cells such as the "chloride cells"
in the mandibles of Gladioferens pectinatus may be studied
using a similar method. Different individuals of
Gladioferens can be adapted to different salinities
and their "chloride cells" examined for ultrastructural and
histochemical (e.g. ATPase) changes. Cells that have
been subject to harsher conditions i.e. those from very
low and very high salinities, may exhibit hyper- or
hyphotrophy or show a variation in the numbers of organelles
as mitochondria.

Behavioural work have often been found useful in
the study of sensory organs of insects, crustaceans and
many other groups of animals. The copepods are no exception.
It has been suggested earlier in this thesis that there may
be osmoreceptors in the mandibles of Gladioferens. It
should be possible from behavioural studies to determine
if Gladioferens are able to detect osmotic changes.

Gladioferens appear to show a preference to water with a salinity of about 14% when put in a salinity gradient. Thus by observing Gladioferens in an osmotic gradient, using sucrose, glucose, manitol, sodium chloride and other like chemicals to build the osmotic gradient it is possible to determine if the animals are able to detect osmotic pressure differences.

There are a variety of means for the further study of some of the organs described in this study and it is hoped this work has provided enough of the groundwork for some of the possible studies mentioned in this discussion.

* RÖHLICH, P. 1967. Fine structural changes induced in photoreceptors by light and prolonged darkness. Symposium on Neurobiology of Invertebrates 1967 (Hungarian Academy of Science). p. 95 - 109.

APPENDIX

This section contains electron micrographs, with appropriate legends, on the various structures described in the main part of this thesis but from species of copepods other than those treated there. This is intended for comparative purposes.

<u>Fig. A 1.</u>

Electron micrograph of a section through part
of a pigment cup of the estuarine calanoid
copepod, <u>Gladioferens pectinatus</u>.   The pig-
ment granules are packed in membrane bounded
sheets (bottom of electron micrograph).   The
pigment granules are very tightly packed as
seen in the hexagonal configuration assumed
by the granules.   X27,500.
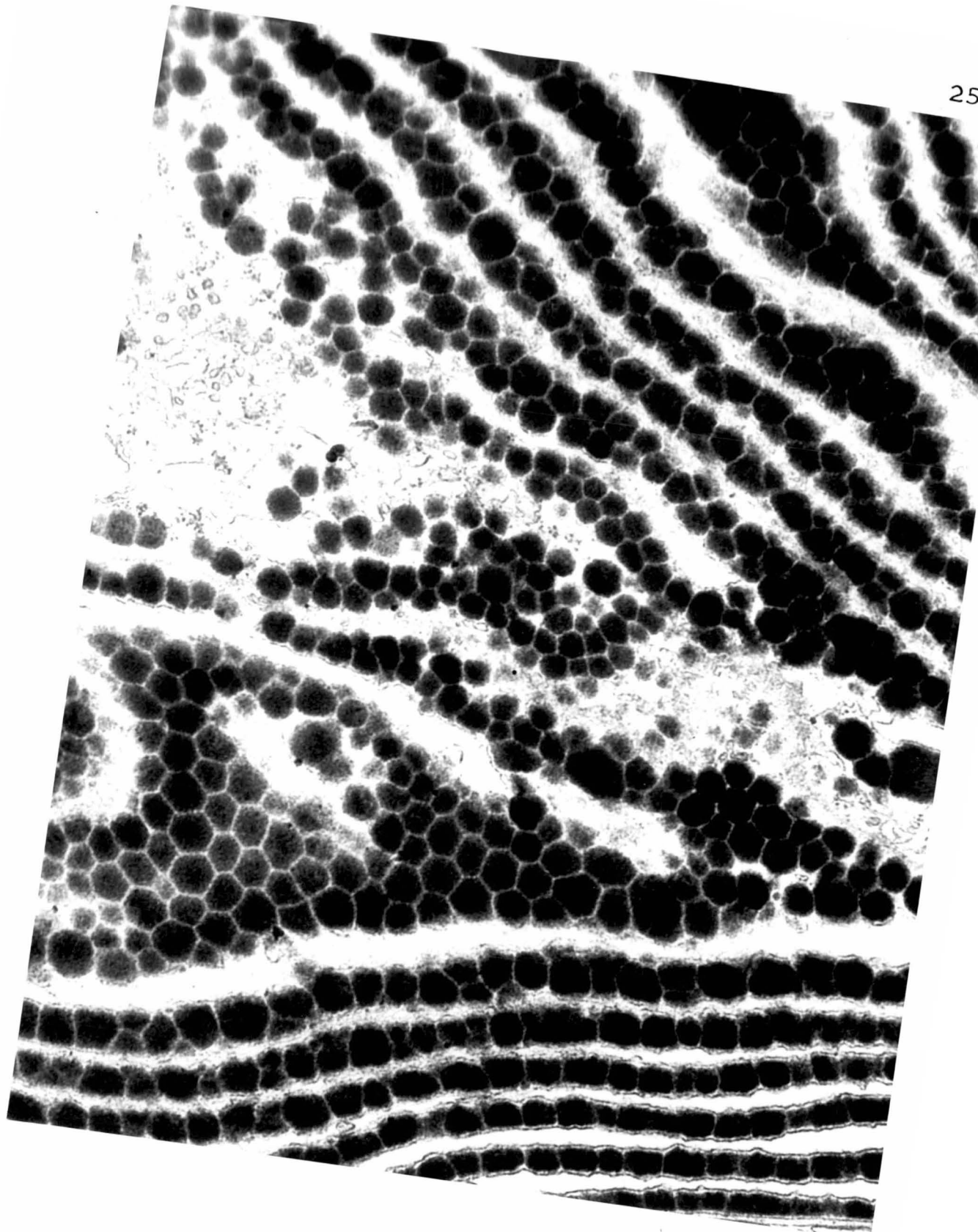
Fig. A. 2.

Electron micrograph of a section through the
nauplius eye of the marine calanoid copepod,
Calanus helgolandicus.   The eye is immediat-
ely anterior to the brain (Br).   Note the
well developed phaosomes (P) in some of the
retinular cells.   The pigment cup is not
as well developed or as highly organized as
that in Sulcanus but both the small dense
granules and the larger lipid pigments are
present.   X3,000.

<u>Fig. A 3.</u>

Electron micrograph of a section through
part of the nauplius eye of <u>Calanus</u>
<u>Helgolandicus</u> showing the tightly packed
microvilli of the rhabdomeres (Rh) of two
adjacent retinular cells (S).   The re-
tinular cells contain numerous small
mitochondria (**M**) and cisternal stacks (C)
similar to miniature phaosomes (P):
part of the pigment cup can be seen on
the bottom right of the electron micro-
graph.   The reflectile plates of the
tapetum can also be seen.   Note the
two types of pigment.   X7,500.
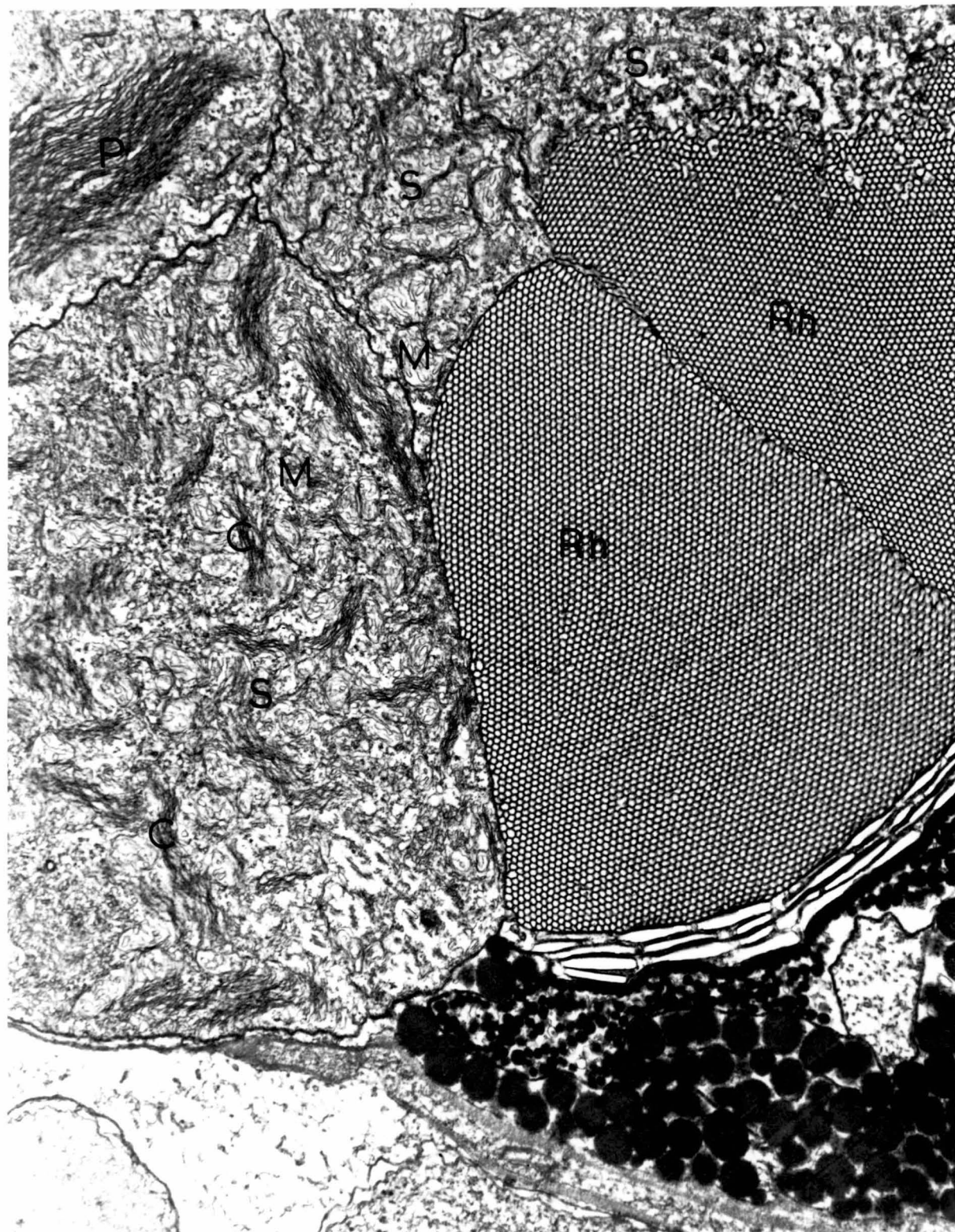
Fig. A 4.

Electron micrograph of a section through
part of a lateral ocellus of the nauplius
eye of the marine calanoid copepod,
Labidocera cervi.   There is a separation
of the reflectile plates of the tapetum (T)
from the rhabdomeric microvilli (Rh) by an
amorphous layer (A).   This is not seen in
any other of the copepods studied.   There are
numerous small mitochondria (compared to
those present in the nauplius eye of Sulcanus),
in the retinular cells and this is similar
to those seen in Calanus.   The space be-
tween the tapetum and the pigment cup (top
right of electron micrograph) is possibly
caused by dehydration artefact.   The
dark myelin-like structure (arrow) is pos-
sibly broken down rhabdomeric microvilli.
X9,900.

Fig. A. 5.

Electron micrograph of a section through the
median (ventral) ocellus of the marine calanoid
copepod, Labidocera cervi showing the extensively
developed "double-walled tubular elements".
These are possibly the result of inpushing
microvilli and provides greater areas of cell
contact.   There are also numerous mito-
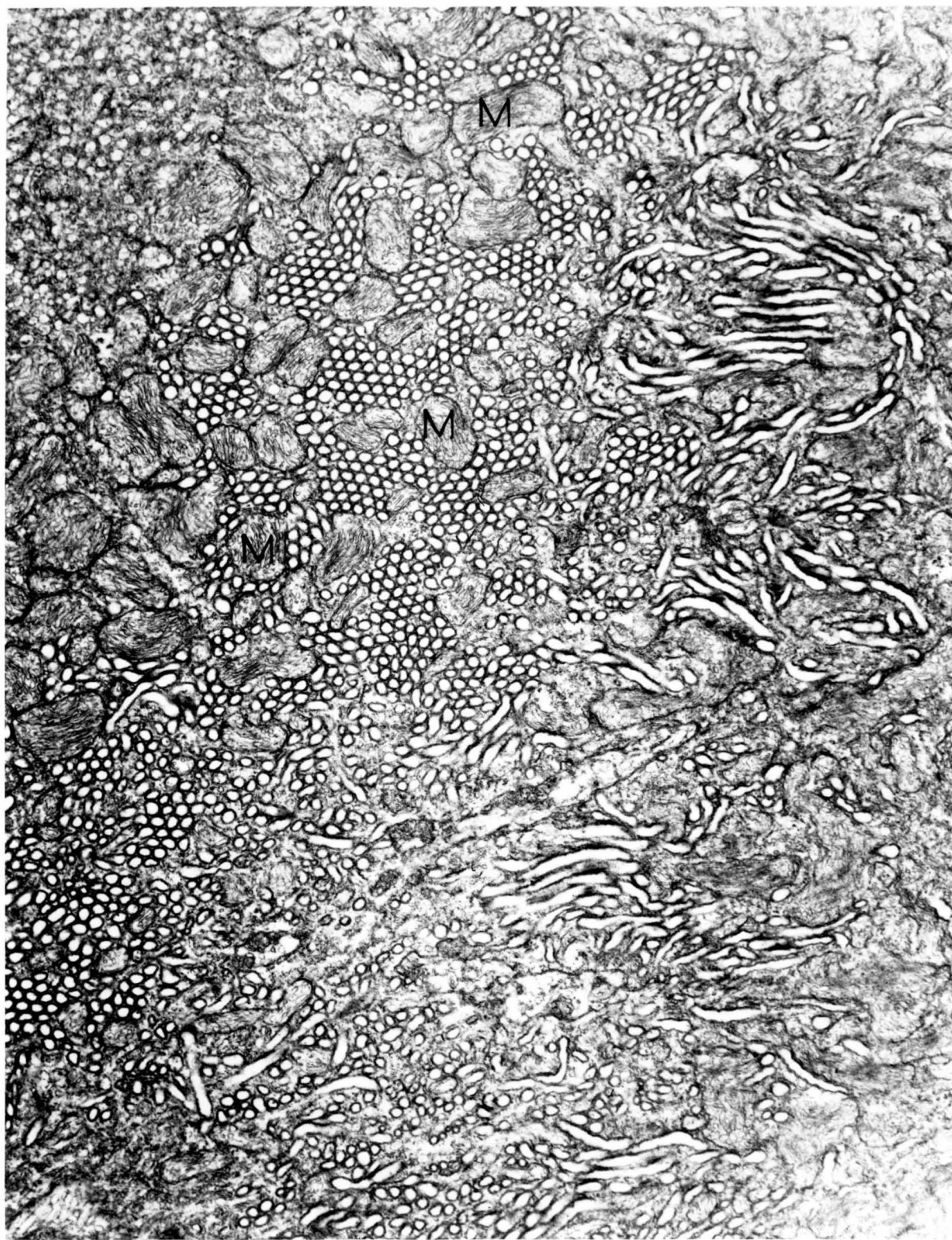chondria (M) in this area.   X29,400.

Fig. A 6.

Electron micrograph showing a transverse
section through the regularly packed array
of "double-walled tubular elements" of
the ventral ocellus of the nauplius eye of
Labidocera cervi.   Note that the
tubules are double walled (and not "unit
membrane") and that in some tubules there
are "granular" structures (arrows).
X82,800.
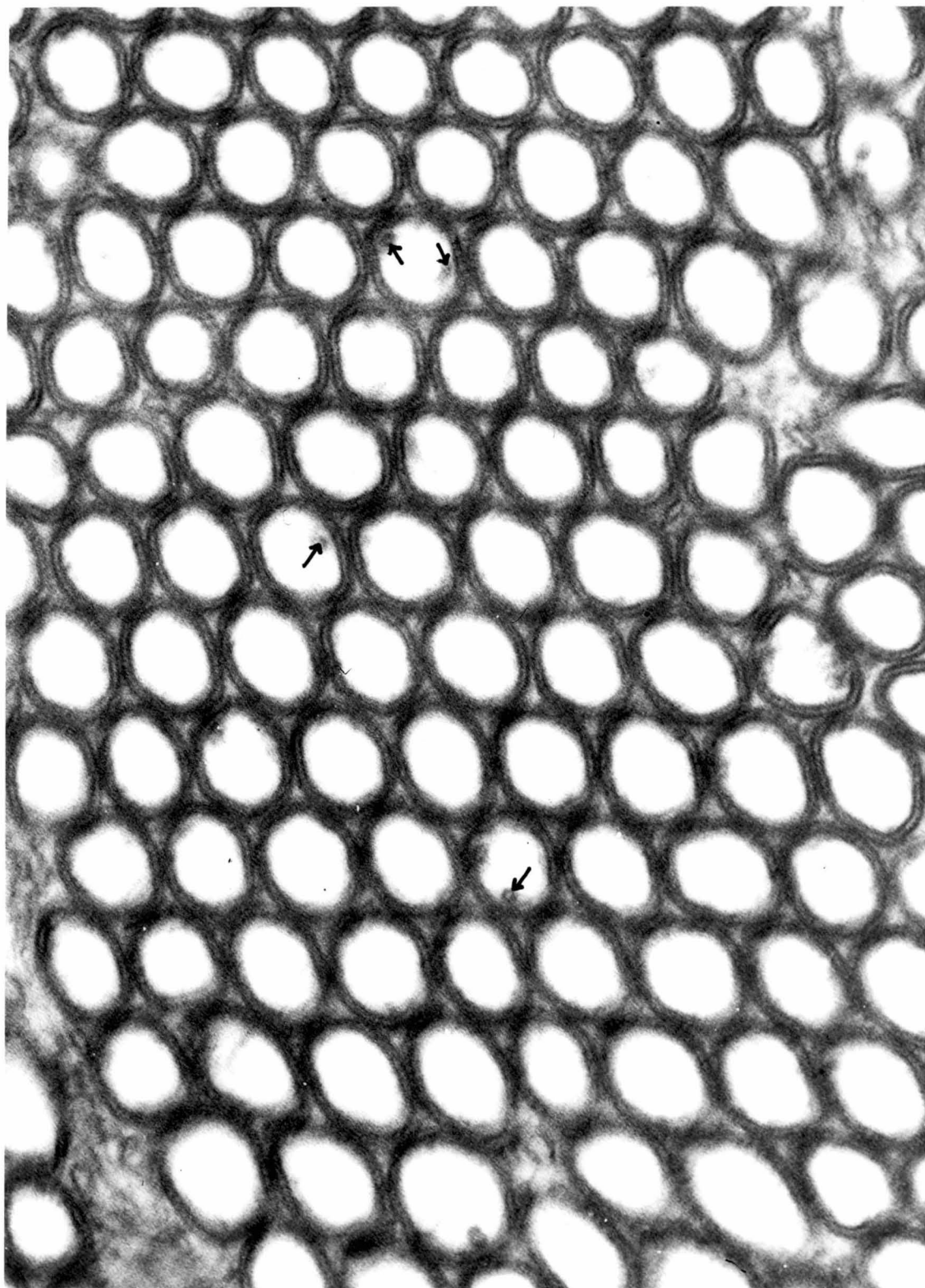
<u>Fig. A 7.</u>

Electron micrograph of a section through
part of a lateral ocellus of the nauplius eye
of <u>Labidocera cervi</u>.   Part of the pigment
cup (PC) and retinular cell (S) is towards
the right side of the electron micrograph.
The structure on the left side of the
electron micrograph, is outside the pig-
ment cup and the "cell" is almost spherical
in shape.   The nature of this strange
structure is not known but similar struc-
tures, arising from cilia have been reported in
the <u>Pecten</u> (Mollusca) eye (Barber, V.C.,
E.M. Evans and M.F. Land. 1967 Z. Zellforsch
76: 295-312.   X10,000.
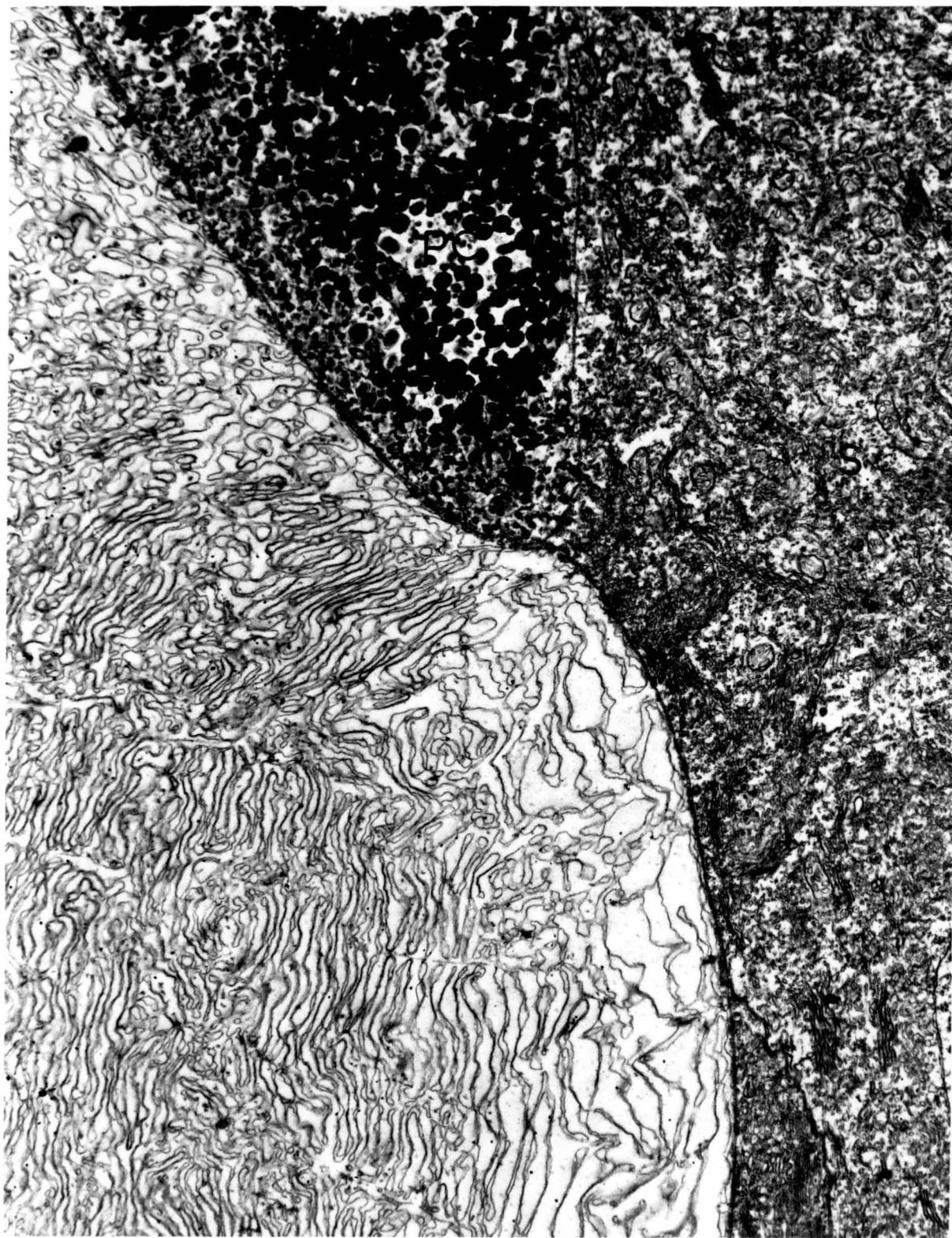
Fig. A 8.

Electron micrograph of a section similar to that in Fig. A 7 showing the membranous struc- ture at a higher magnification.   The membranes are possibly photoreceptor membranes. X31,000.
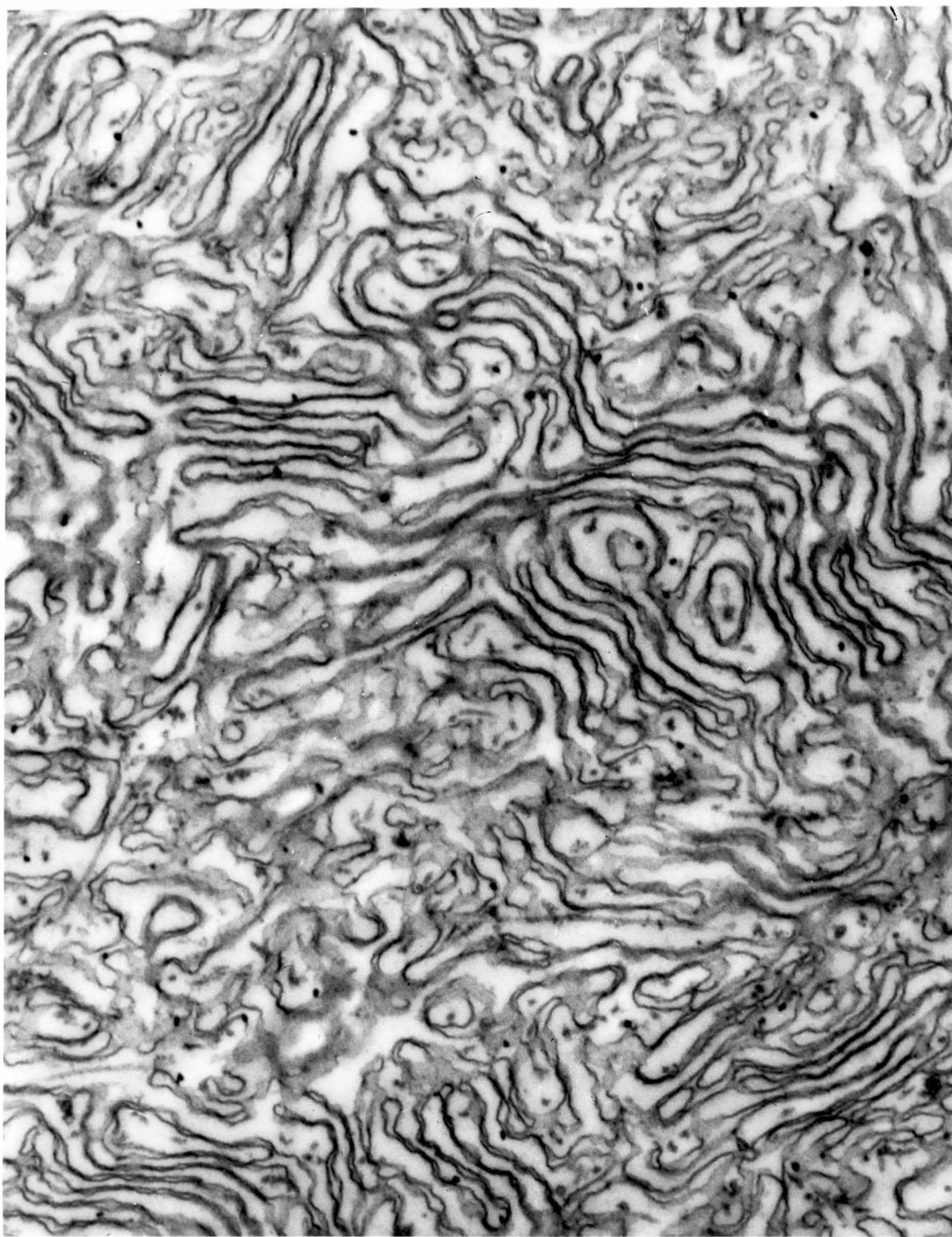
Fig. A 9.

Electron micrograph of an oblique parasaggital
section through the dorsal frontal organ of
the marine calanoid copepod, Calanus helgo-
landicus.   The phaosomes (P) and the
"tubular bundles" (T) are the most conspicuous
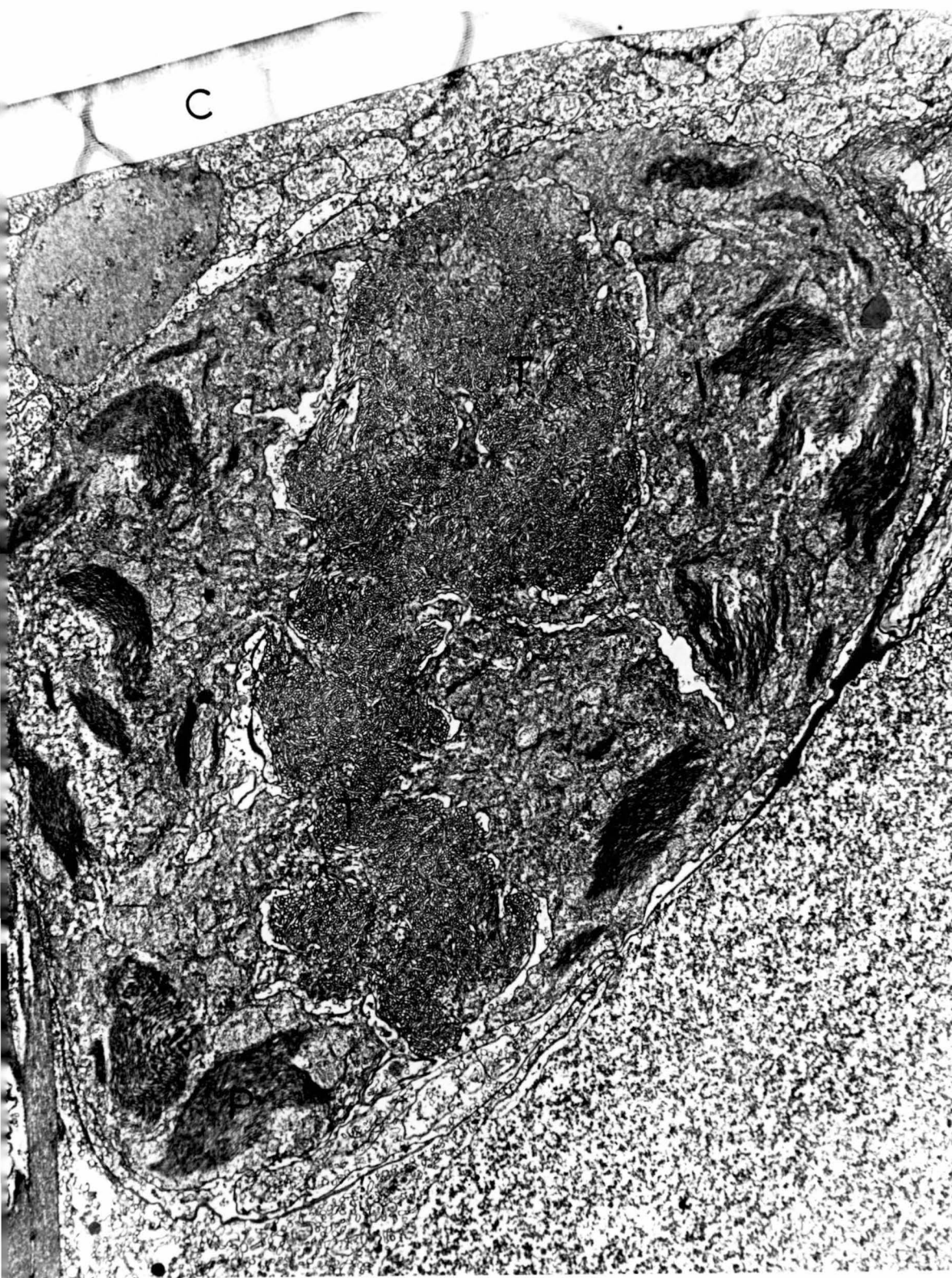organelles of the organ.   C = Cuticle.
X 4,500.

C

Fig. A 10.

Electron micrograph of the "tubular bundle"
of the dorsal frontal organ of Calanus
helgolandicus.   These are possibly a type
of rhabdomeric microvilli.   X115,800.

Fig. A 11.

Electron micrograph showing the phaosomes (P)
in the dorsal frontal organ of Calanus
helgolandicus.   N = nucleus.   The
membranes of the phaosome cisternae are
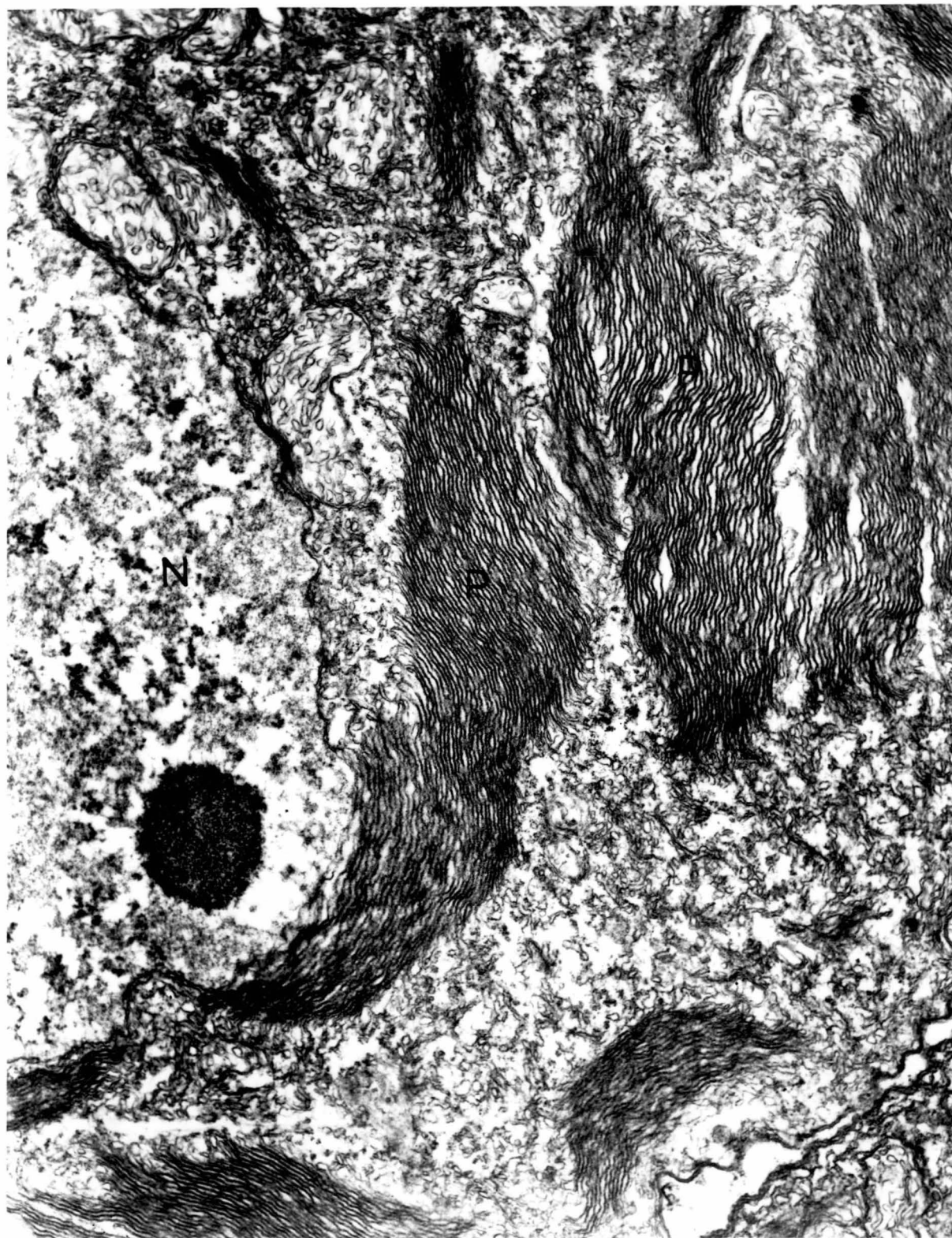smooth and resemble Golgi cisternae.
X13,500.

Fig. A 12.

Electron micrograph of part of the axons of
the dorsal frontal organ of Calanus
helgolandicus.   Note the abundance
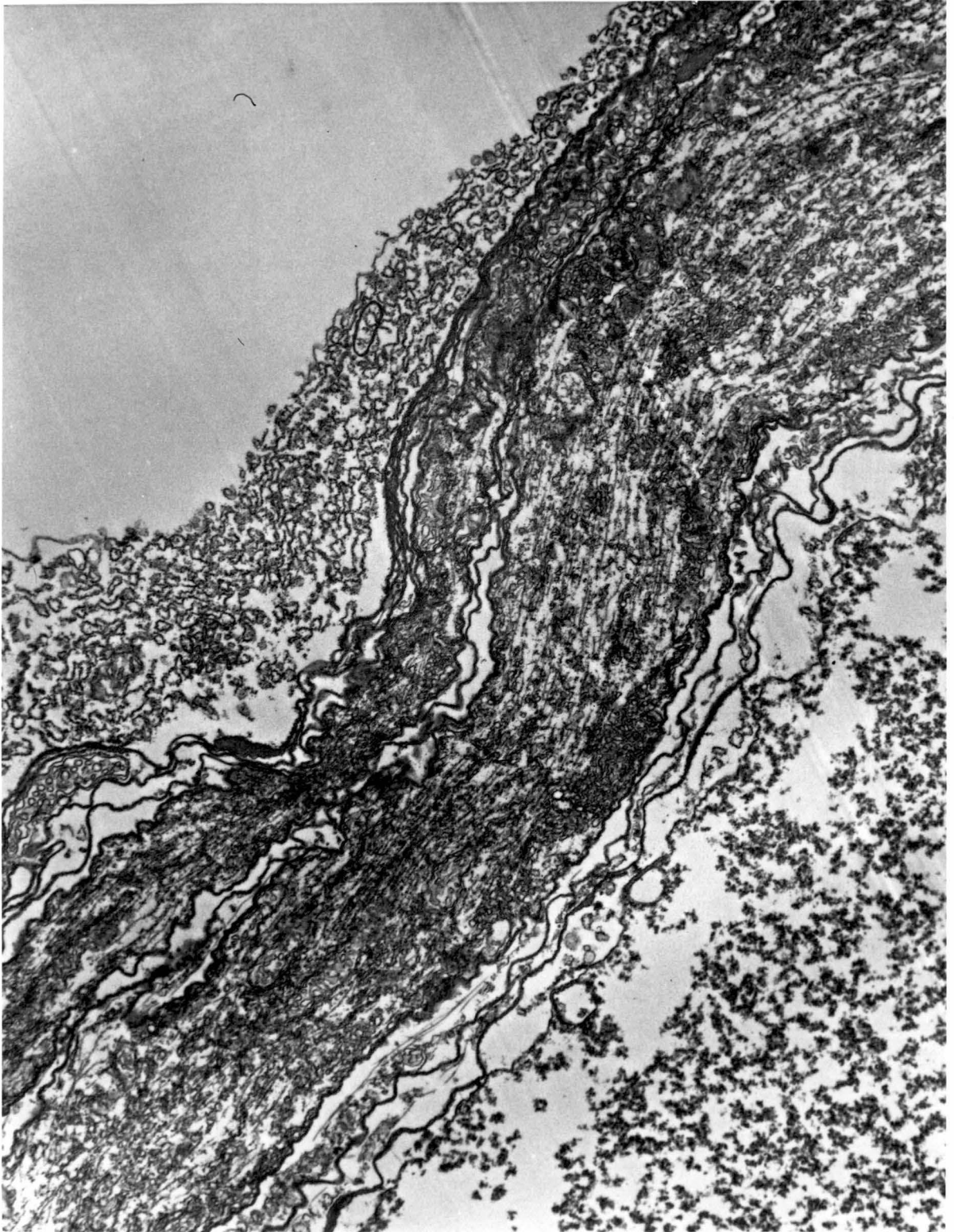of neurotubules.   X7,500.

Fig. A 13.

Electron micrograph of a section of part of
the mid-gut diverticulum of Gladioferens
pectinatus showing the microvilli of the
gut epithelium and the formation of the
peritrophic membrane (PM).   The fine
structure of peritrophic membranes of
various Crustacea has recently been
studied by R. Georgi (1969): Z. Morph.
Tiere 65, 225-273 and Z. Zellforsch. 99,
570-607.   The electron opaque granules
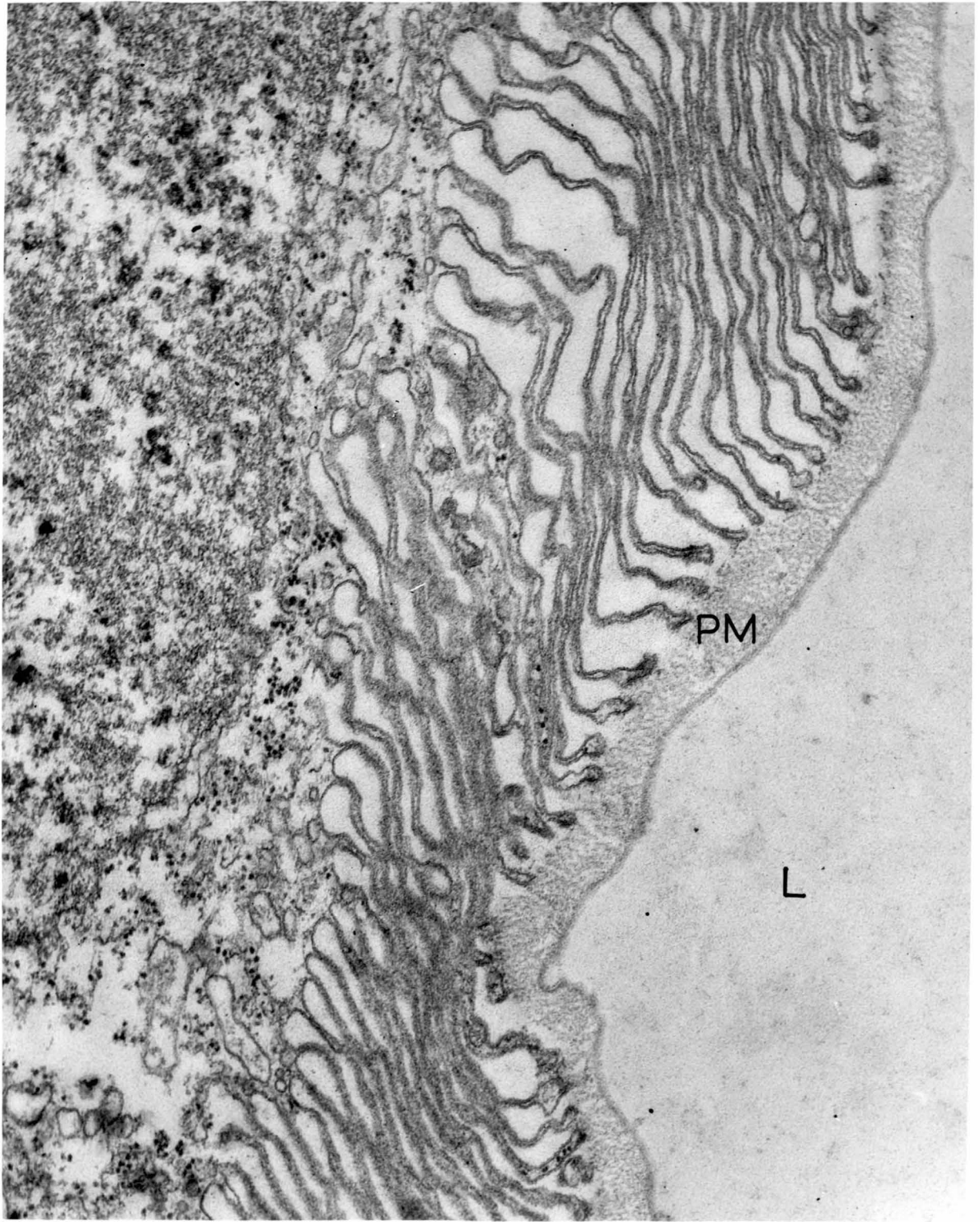are possibly glycogen granules.
X31,200.

PM

L

Fig. A 14.

Electron micrograph of a section similar to
that in Fig. A 14 but at a higher magnification
showing the microvilli of the gut epithelium
and the peritrophic membrane (PM).   Note that
the peritrophic membrane has an outer layer
morphologically comparable to the cuticulin
layer of cuticle.   The exact function of
peritrophic membranes is not known but they
are thought to afford both mechanical and
enzymatic protection for the gut epithelium.
X113,000.

PM
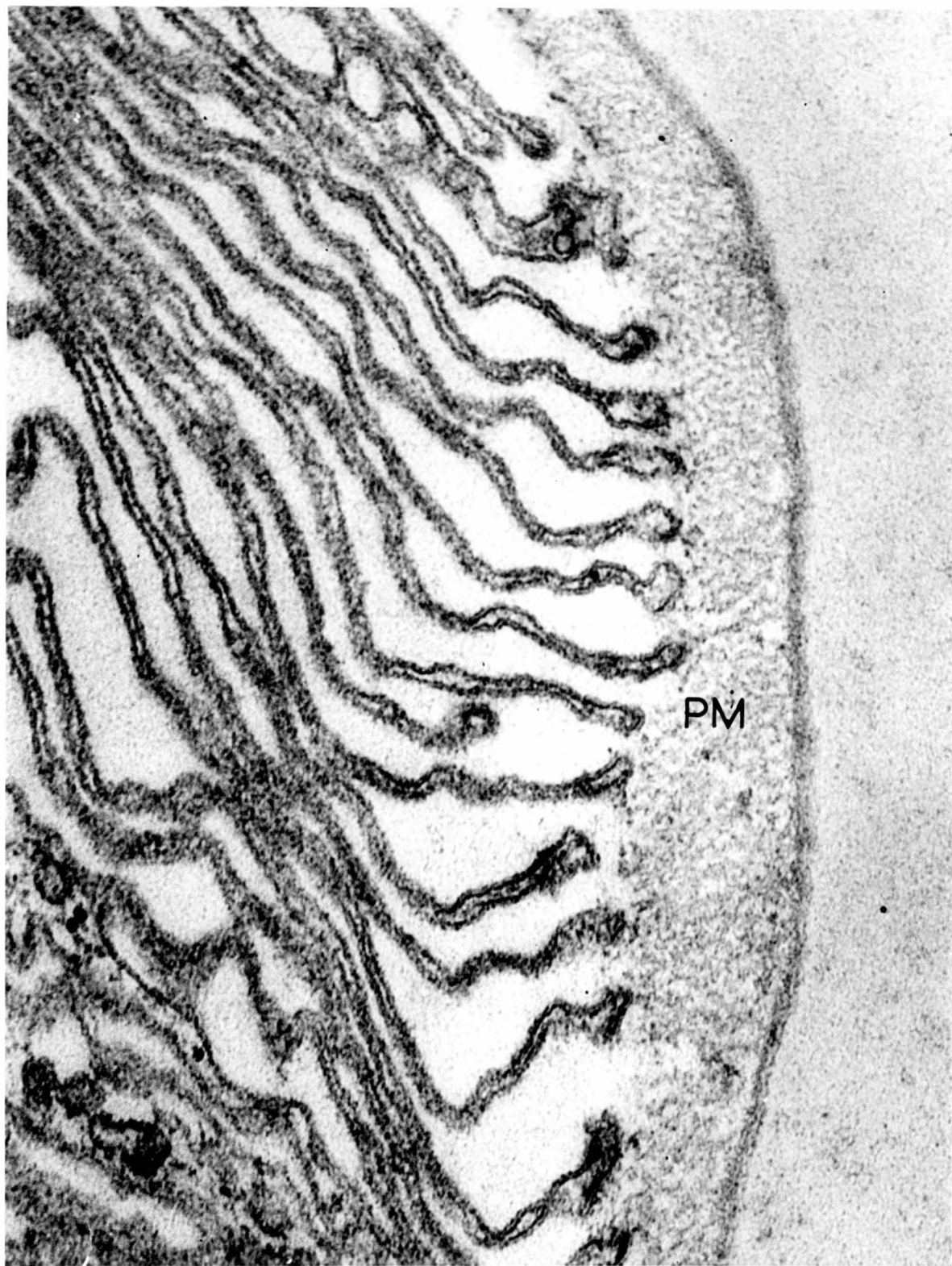
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Electron micrograph of a section through part of the
rhabdomere of the nauplius eye of <u>Calanus helgolandicus</u>.
The rhabdomeric microvilli are cut longitudinally.
X 69,000.