DEVELOPMENTAL MORPHOLOGY OF THE OPTIC CUSHION AND OCELLI OF THE SEA STAR LEPTASTERIAS POLARIS (MULLER & TROSCHEL)

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Developmental Morphology of the Optic Cushion and Ocelli of the Sea Star Leptasterias polaris (Müller & Troschel)

by

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ABSTRACT

A study of the morphology of the optic cushion and ocelli of the sea star Leptasterias polaris (Müller and Troschel) was undertaken to provide a description of their fully differentiated condition, and as a comparison with similar work on the ocelli of other species of asteroids and on the photoreceptors of other animals. In addition, the development of the optic cushion and its ocelli through embryogenesis, and the regeneration of adult tissues were investigated. For these studies, larvae of L. polaris, and the optic region of the fully formed adult, and of adult regenerating rays, were examined by light, scanning electron, and transmission electron microscopy.

The ocelli consisted of numerous pigmented and sensory cells that lined a lumen. The designation of the sensory cells as ciliary photosensory receptors was confirmed by observations of the prominence and microtubular irregularities of the numerous cilia present in the lumen, and by the origin of villi from the base of their shafts, as well as from the cell membrane of the expanded apical portions of the sensory cells. The presence of coated cell membrane invaginations, coated vesicles, and multivesicular bodies, suggested a mechanism of endocytosis of villous membrane, such as has been documented as a photoreceptoral membrane recycling pathway in rhabdomeric photoreceptors.

The pigmented cells possessed small pigment granules, and larger polymorphic bodies proposed to be stages in autophagocytosis. Corneal cells subserved a supportive function, surrounding the ocellus and

projecting processes containing vesicular profiles and a microfilamentous bundle, over the lumen.

In both larval and regenerative development, a region of epidermal cells at the base of the terminal tentacle, commenced differentiation into the three classes of ocellar cells. This region then invaginated and the corneal components extended processes over the lumen, which gradually became filled with the apices of the sensory cells and their cilia and villi. Endocytosis of villous material was noted in the sensory cells in newly formed ocelli, and the polymorphic profiles of autophagocytosis were present in very early stages of pigmented cell differentiation. The small pigment granules appeared to arise from a granular material present in the cytoplasm, and apparently transported from the Golgi apparatus by vesicles.

The optic region thickened to become a pad of tissue. Establishment of this cushion and numerous ocelli occurred rapidly through regeneration, while the larvae, which developed optic pigmentation and an ocellus after the establishment of their third pairs of tube feet, maintained the single ocellar condition for an extended period of time.

"It takes a membrane to make sense out of disorder in biology . . . . . To stay alive, you have to be able to hold out against equilibrium, maintain imbalance, bank against entropy, and you can only transact this business with membranes in our kind of world".

Lewis Thomas
in "The Lives of a Cell"
Viking Press, New York, 1974.
153 pp.

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INTRODUCTION

A. PRELIMINARY STATEMENT

Most animals perceive and are behaviourally affected by light. To detect and analyze the light stimulus, numerous species in most animal phyla have evolved localized and organized photoreceptive regions described by such terms as 'eyes' or 'ocelli'. In addition to behavioural and physiological investigations of photoreception in animals, numerous studies of the fine structure of these organized photoreceptors have led to some understanding of the functions of these organs.

Several reviews of the structure of invertebrate photoreceptors are available, one of the most comprehensive being that by Eakin (1972). In this article, emphasis was placed on the form of the receptoral surfaces thought to house the photopigments responsible for the reception of the incident light energy. Organelles common to photoreceptoral cells were described with reference to their function, and the effects of light intensity on their structure and on the structure of the photoreceptive surfaces were also briefly discussed. The functional morphology of pigmented supportive cells, typically interspersed among the photosensitive cells, was also described.

One reason for the particular interest in the structure of photoreceptors concerns the evolutionary relationships between phyla of animals. With some exceptions, evidence from the structure of photoreceptors corroborates observations of a developmental and biochemical nature, upon which was based the concept that there are two main animal

lineages, the protostomes and the deuterostomes, which branched from a common ancestry. The chordates, protochordates, echinoderms, chaetognaths, ctenophores, and coelenterates, generally have photoreceptor membranes modified from cilia, whereas the molluscs, arthropods, and annelids form the rhabdomeric line in which the photoreceptive surfaces are extensions of the cell membrane (Eakin 1963, 1965, 1968).

Since the difference between ciliary and rhabdomeric types of photoreceptors is an expression of different routes of development, as well as describing the morphology of fully elaborated photoreceptors, many studies also discuss the embryological development of such receptors (Eakin 1968). A recent publication by Barnes (1974) on the ultrastructure of developing ascidian tadpole photoreceptors, also lists such studies and points out that the work on ciliated photoreceptor development has been restricted to those of vertebrates. Several studies of the elaboration of invertebrate rhabdomeric receptors have been published, however, (see Barnes 1974), and a description of the eyespots of a hemichordate larva, with both ciliary and rhabdomeric characteristics, included a few observations of ocellar development (Brandenburger et al. 1973).

Several studies have resulted in asteroid photoreceptors being described as ciliary (Eakin 1963, 1968; Eakin and Westfall 1962b, 1964). However, no information is available on their development. In this study, therefore, it is proposed to investigate, primarily at the ultrastructural level, the structure of the optic cushion and its constituent ocelli in adult specimens of the sea star, Leptasterias polaris, (Echinodermata: Asteroidea), and to describe the development of the

optic region through normal embryology, and regeneration experiments.

B. STRUCTURE OF THE ASTEROID OPTIC CUSHION AND OCELLI

While numerous echinoderms have been described as being responsive to light, detection of the stimulus in the majority of these cases is through dermal light sensitivity (Millott 1968). Only certain members of the Asteroidea possess ocelli, which are localized at the tips of their rays.

The radial canal of the water vascular system is situated along the oral surface of each ray of sea stars. Numerous paired, and usually suckered, tube feet are arranged on either side of it. The radial water canal ends distally as a single, extensible, suckerless, modified tube foot, variously referred to as the terminal, azygous, or sensory tentacle.

In most species of asteroids, on the terminal tentacle's oral surface immediately distal to the last pair of tube feet, is an elliptical pad, the optic cushion (Fig. 1A). This cushion, also termed the eyespot, is very brightly pigmented with an orange-red colouration, and consists of numerous cup-shaped ocelli, or optic cups.

Vahl (1780) is credited as being the first worker to note the presence of optic cushions in sea stars. Since that time, the histological structure of these eyespots has been described in more than a dozen asteroid species by numerous investigators. Pfeffer (1901) cited 16 studies of the asteroid optic cushion in the last half of the 19th century, including the more extensive works of Hoffmann (1873), Hamann (1883), and Cuénot (1887). Cuénot (1887) summarized that most of these

Figure 1. Diagrams of asteroid optic cushion and ocellus.

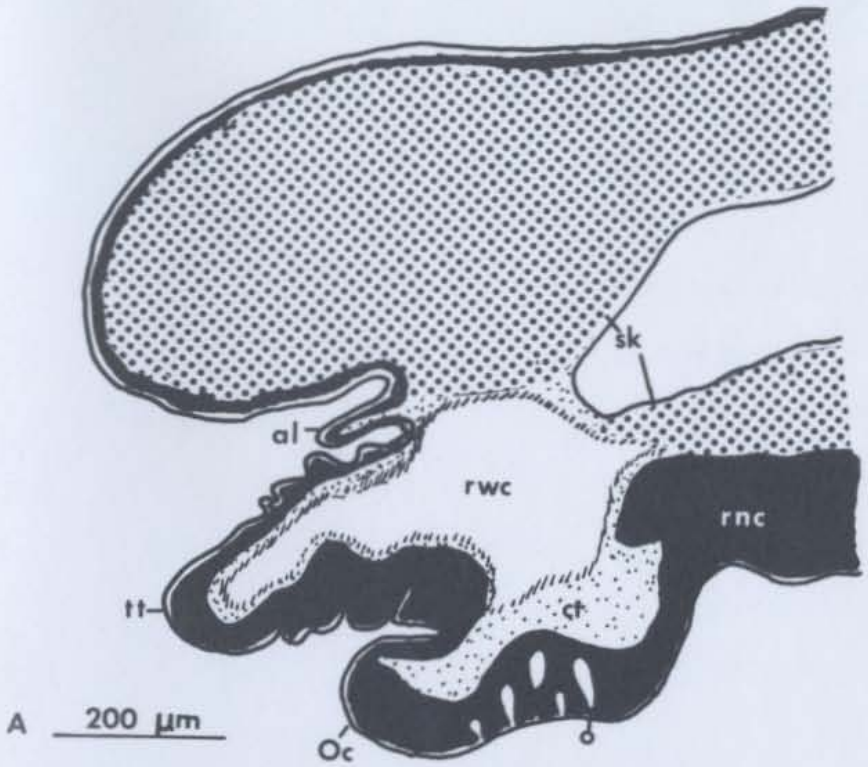
A. Diagram of sagittal section through ray tip of Marthasterias glacialis. Epithelium and subepithelial plexus are figured in black. From Smith (1937).

al - aboral lappet
ct - connective tissue
o - ocellus
Oc - optic cushion
rnc - radial nerve cord
rwc - radial water canal
sk - skeletal tissue
tt - terminal tentacle

B. Diagram of longitudinal section through an ocellus of Asterias rubens. Modified from von Harnack (1963).

Corneal cells with numerous vesicles ('lenticular structure', 'lens') arch over the opening of the ocellus. The lumen is lined by pigmented and sensory cells. The pigmented cells contain small pigment granules and larger polymorphic inclusions. The sensory cells pass up between the pigmented cells and give rise to cilia and villi. An expanded portion below the pigmented cells houses the nucleus and Golgi apparatus. Vesicles and lamellar bodies are common in their cytoplasm. Epithelial (supporting) cells with fibre bundles surround the ocellus. A revestment ('cuticle') covers the surface.

c - cilium
CC - corneal cells
EC - epithelial cells
PC - pigmented cells
re - revestment
SC - sensory cells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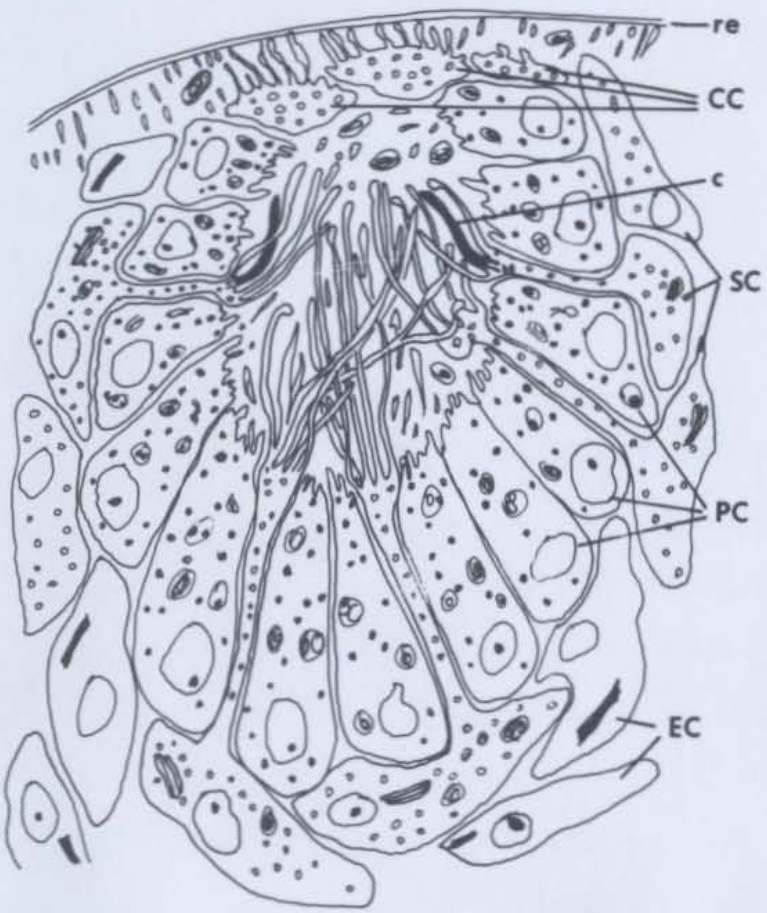

re
CC
c
SC
PC
EC
B

investigators described portions of the optic cups in terms of the classic elements of cornea, lens, and retina, and tied this morphology to the concept of the echinoderm nervous system prevailing at the time. In considering the nature of the cells lining the ocelli, some workers, e.g. Cuénot (1887) and Pfeffer (1901), maintained that one cell type served both sensory and pigmentary functions. Hamann (1883), however, had been the first to suggest that there were two distinct cell types comprising the optic cup lining: sensory cells whose basal extensions merged with the underlying nerve plexus, and supportive pigmented cells whose proximal ends were thought to abut on a connective tissue layer beneath the subepithelial plexus.

In 1937, Smith included in his extensive paper on the nervous system of the sea star Marthasterias glacialis, a detailed description of the optic cushion, based on observations utilizing various histological staining techniques. He described the opening of the optic cups as being overlain with 'cuticle' beneath which was what he termed a "hyaline lenticular structure". This structure was described as being penetrated by fibres similar to those in the epithelial supporting cells surrounding the optic cups, and so Smith suggested that the 'lens' was in fact composed of extensions of the epithelial cells themselves, rather than secretions from them, or derivatives of the 'cuticle'. Smith also described the pigmented cells, noting differences from typical epithelial cells, outlining the distribution of pigment granules within the cells, and inferring that these cells secreted fluid into the cup's lumen. He noted that the presumptive sensory cells contributed extensions into the lumen of the ocellus, housed their nucleus

in an expanded central portion, and tapered proximally into the sub-epithelial plexus.

Millott and Vevers (1955), in addition to their description of the carotenoids of the optic cups of M. glacialis, included observations of the appearance of pigment-containing cells in fresh preparations. They found these cells to be larger than those described by Smith (1937) and to have their pigment scattered throughout the cytoplasm.

More recent descriptions of asteroid ocelli have been at the ultrastructural level. Philpott and Chaet were the first to describe such electron microscopical observations on the eyespots of Asterias forbesi in an abstract published in 1960. They briefly described the external layer covering the individual ocelli, and two sizes of pigment granules, and made reference to a cellular lining of the cup contributing microvilli into the lumen.

Since that brief report, several papers have more comprehensively described the fine structure of asteroid ocelli. Von Harnack (1963) affirmed at the ultrastructural level, the presence of two types of cells lining the eye cup of Asterias rubens. Pigmented cells possessed large and small pigment granules and short microvilli that projected into the ocellar lumen. Occasional multilamellar whorls and ciliary roots were also found in these cells. The perikaryon of the sensory cells possessed a variety of inclusions such as vesicles, ciliary roots, lamellar whorls, multivesicular bodies, and isolated pigment granules. The neck portions of the sensory cells 'squeezed up' between the pigmented cells and from their distal ends sent cilia and long, twisted, and sometimes bifurcated villi into the lumen of the optic cup. Isolated lamellar whorls were also

found in the lumen. Von Harnack also described fibre-containing supporting cells around the ocelli, and vesicle-filled, ciliated cells lying over the optic cups (Fig. 1B).

Eakin (1963, 1968) and Eakin and Westfall (1962b, 1964) have provided additional information on ocellar fine structure in the asteroids, Leptasterias pusilla, Patiria miniata, and Henricia leviuscula. Their work indicated that some of the long, tangled villi, which fill the ocellar lumen, emerged from the base of the cilia of the sensory cells, and in H. leviuscula they observed that some of these villous extensions originated from the ciliary shafts distal to the level of the basal body (Eakin 1968). The cilia displayed a microtubular complement of '8 + 1' for L. pusilla, and in the case of H. leviuscula, either '9 + 0' (Eakin 1963) or '9 + 2' (Eakin 1968). Vesicles were also shown to be numerous in the broad ciliary bases. A single type of membrane-less granule was described in the pigmented cells of the sea stars examined (Eakin 1972).

Other ocelli that possess ciliary photoreceptors modified by the presence of long villi, have been described in several species of coelenterates (Eakin and Westfall 1962b, Singla 1974, Bouillon and Nielsen 1974).

Eakin and Westfall (1964) confirmed Smith's (1937) contention that the structures overlying the ocellar openings were arched extensions of surrounding epithelial cells, and termed this layer a cornea, suggesting that these extensions correspond to a cornea rather than to a lens in shape, position, and probable function. The corneal cells possessed microvilli on their exterior surface and these microvilli, like those of other epithelial cells, extended through the 'cuticle'.

Because this external covering has been noted to differ from the arthropod cuticle in thickness and chemical composition, and undergoes changes effected by the metabolic state of the animal, a number of workers (Eakin and Westfall 1964, Huet 1972a, Holland and Kubota 1975) have abandoned the term 'cutucle' for the echinoderm case. The term 'revestment', initially mentioned in reference to the external covering of asteroid tube foot epithelium by Souza Santos and Silva Sasso (1970), will be utilized in this study.

C. PHYSIOLOGICAL AND BEHAVIOURAL EVIDENCE ON THE FUNCTION OF ASTEROID OCELLI

Ultrastructural observations lend support to the hypothesis of a photoreceptive function for the optic cushions of asteroids. To date, however, there has been only one report of a direct electrophysiological response of eyespots to light, that of Hartline et al (1952) on an unidentified species of Asterias.

The bulk of the evidence concerning the possible role of ocelli in asteroids has been behavioural. Hyman (1955) and Yoshida and Ohtsuki (1968) reviewed many of these investigations, some of which discount the importance of eyespots in photoresponses. However, a particularly elegant study by Yoshida and Ohtsuki (1968) concluded that for Asterias amurensis, the ocelli function as primary photoreceptors. Light perception by the ocelli results in positive phototaxis and these sea stars move away from shadows perceived by the eyespots. Secondary photoreception by the general body surface operates concurrently with, and in the absence of, the eyespots.

Other behavioural studies have included examination of pigments extracted from ocelli to more clearly establish the chemical basis for light reception in these echinoderms. Rockstein (1956) illustrated that

the presence of the optic cushions was not essential for the positive phototaxis displayed by Asterias forbesi under experimental conditions. He and his co-workers (Rockstein 1956, 1962; Rockstein and Rubenstein 1957; Rockstein et al. 1958; Rockstein and Finkel 1960) by biochemical extraction and analysis, isolated 'stellarin' which was found to be a photosensitive substance common to both the dorsal surface and the eyespots of this species. They suggested that the ocelli might display higher or differential sensitivity to specific wavelengths of light.

Yoshida and Ohtsuki (1966) determined that removal of the optic cushion from Asterias amurensis raised the threshold for a light-induced response by a factor of ten and shifted the wavelength eliciting the most effective response. In addition, a carotenoid extracted from the eyespots exhibited an absorption spectrum maximum approximating the peak wavelength of the action spectrum for this light response in rays with intact ocelli.

Peskin (1951) and Millott and Vevers (1955) have also investigated carotenoid pigments of eyespots, the latter authors having isolated B-carotene and esterified astaxanthin.

D. EMBRYOLOGICAL DEVELOPMENT OF ASTEROID OPTIC CUSHIONS

Cuénot (1887) commented that earlier echinoderm biologists had noted that the optic cushion was present in very young stages of developing sea stars. A search of the literature, however, has not revealed any detailed study specifically of the embryological development of asteroid ocelli. Eakin (1968) noted that he attempted to locate and examine ocelli in the bipinnaria larva of the sea star, Pisaster ochraceus, but without success.

Although there are no accounts of the embryological development of Leptasterias polaris, there is information available on this aspect of the biology of other species of Leptasterias. Osterud (1918) made preliminary observations on the embryological development of the Pacific species, L. hexactis, but did not make reference to the formation of the optic cushion. Kubo (1951) described the spawning behaviour of adult L. ochotensis from Japan and the development of larvae to post-metamorphosis, and mentioned the appearance of the eyespot rudiment after the development of several pairs of tube feet.

The most detailed information concerning the reproductive biology of a species of Leptasterias is available in the doctoral thesis (1964) and two later publications of Chia (1966, 1968). Chia (1964, 1966) discussed the brooding behaviour of L. hexactis and included observations on the maintenance of embryos. Further, he described, principally from histological work, the internal and external development of this species from the unfertilized egg to post-metamorphosed larva. Thus, for this Pacific species, observations on maintenance and a chronology of embryological development have been documented.

Metamorphosis commences about 25 days after fertilization and the young L. hexactis are ready to lead an independent existence after three months. The terminal tentacles are developed after 32 days and the eyespots are obvious on five of the six rays at day 40, these rays developing their fourth pair of tube feet at this stage. The optic cushion on ray six is noticeable five days later.

E. REGENERATION OF ASTEROID OPTIC CUSHIONS AND OCELLI

Eyespots also develop through regeneration, and sea stars are very suitable experimental animals for studies of this type, the regenerative powers of asteroids having long been noted. King (1898, 1900), for example, studied various aspects of regeneration in Asterias forbesi, and among her findings noted that when a portion of a ray is removed, development of a new tip rapidly commences, and a new eyespot is visible within a week. Pfeffer (1901) illustrated a shallow ocellus in the regenerating optic cushion of another species of sea star.

Although no specific study of the regeneration of asteroid eyespots has appeared in the literature, the work of Huet (1966, 1967, 1972a, 1972b, 1975) provides important information of the redevelopment of damaged ray tissues of Asterina gibbosa with incidental observations on the optic cushion. Sectioned rays underwent wound healing and subsequent regeneration of healthy tissues, the appearance of pigmented ocelli being noted within a month. Huet (1972b) elaborated that in the course of regeneration of ray tips, small cups that later develop as photoreceptoral ocelli, differentiate near the ray terminus where the neuroepithelial cells are in contact with fibres of the radial nerve cord. He also illustrated that although coelomocytes may phagocytize damaged cells in the wound area, new tissues regenerate from the same tissue type, i.e., new epidermis differentiates from previously existing epidermal cells.

Huet (1967, 1972b, 1975) also investigated the role of the radial nerve cord in the regeneration of damaged tissues. He found that the

radial nerve cord must be connected to the circumoral nerve ring, and be present at the wound site, for the tube feet and optic cushion to regenerate. Another publication (Huet 1972a) illustrated ultrastructurally the dedifferentiation, followed by the subsequent activation and differentiation of neuroepithelial cells in regenerating rays of A. gibbosa.

F. RATIONALE AND DETAILS OF PRESENT STUDY

The six-armed sea star, Leptasterias polaris, is an abundant littoral species that can be easily collected by divers from rocky bottoms in Newfoundland waters. These sea stars are readily maintained in the laboratory, their preferred food item being the common blue mussel, Mytilus edulis (Emerson 1973).

Regions of the ray tips of adult specimens including the optic cushion, were excised and prepared for light, transmission electron, and scanning electron microscopy to provide information on the morphology of the optic cushion and its ocelli.

Leptasterias polaris was specifically selected because of the proposal to investigate aspects of embryological development. L. polaris is the larger of the two species known to brood their young in Newfoundland waters and it is more readily obtained in large numbers (Emerson 1973). The phenomenon of brooding facilitates the collection of larvae in the field, thus eliminating many of the problems associated with obtaining and maintaining the planktotrophic larvae of other asteroid species.

Larvae of L. polaris obtained from females in the laboratory and from the field were prepared for light and electron microscopy at intervals to provide information on the embryological development of the

optic region.

Because sea stars also readily regenerate their rays, a comparative study of the elaboration of the optic cushion and ocelli by this second mechanism was possible. The terminal tentacles including the optic cushion were removed from the rays of adult specimens, and regenerative portions were prepared for examination by light and electron microscopy. Information was also noted on the rate of ocellar regeneration.

In addition to contributing information in the specific area of asteroid biology, these studies provide some interesting comparisons with the published literature on other photoreceptors. Emphases include the possible significance of the ciliary nature of the asteroid photoreceptors, details of the functional morphology of the sensory, pigmented, and corneal cells, and the development of the ocelli and their specialized cells.

G. CLASSIFICATION OF EXPERIMENTAL ANIMAL

	from Hyman (1955)	from Fell and Pawson (1966)
Phylum	Echinodermata	Echinodermata
Subphylum	Eleutherozoa	Asterozoa
Class	Asteroidea	Stelleroidea
Subclass		Asteroidea
Order	Forcipulata	Forcipulatida
Family	Asteriidae	
Subfamily	Asteriinae	

Species: Leptasterias polaris (Müller and Troschel)

Synonymy: *Asteracanthion polaris*

A. polare

Leptasterias polaris

L. polaris acervata (from Grainger 1966)

Taxonomic characteristics for the species are given in Grainger, among them, an R:r ratio of 3.5:1 to 6.3:1. The adult specimens used in this study gave R:r ratios of 3.4 to 6.0:1 (mean 4.6:1, n=32) (Appendix II). R is the distance from the centre of the central disc to the tip of the longest ray, and r is the radius of the central disc.

MATERIALS AND METHODS

A. ADULT *LEPTASTERIAS POLARIS* STUDY

1. COLLECTION AND MAINTENANCE

Adult male and female specimens of *Leptasterias polaris* were collected by SCUBA divers in depths of less than ten metres from 'Bread and Cheese Cove', Bay Bulls, Newfoundland (Fig. 2), on May 30, 1973, August 10, 1973, and May 2, 1974. Sea stars were readily maintained in the Marine Sciences Research Laboratory (M.S.R.L.) of Memorial University of Newfoundland, in A-frame tanks supplied with a continuous flow of sea water. Over the two and one-half year holding span for specimens, the temperature of the circulating sea water in the laboratory varied less than 1°C from the source of the sea water in Logy Bay, and ranged from -1.5°C to 15.0°C (see Appendix I).

Adult *L. polaris* individuals were fed primarily upon common blue mussels (*Mytilus edulis*), with very occasional additions of chopped caplin (*Mallotus villosis*), squid (*Illex illecebrosus*), or herring (*Clupea harengus*), when mussels were not available.

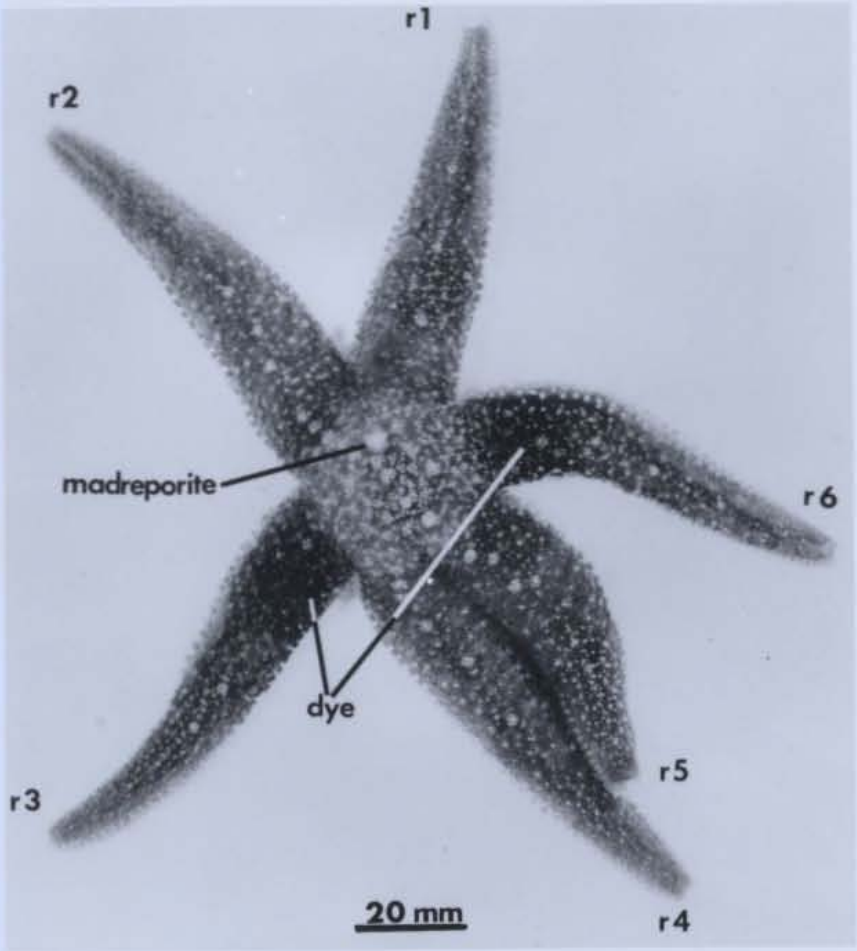
For purposes of identification, specifically for the regeneration experiments, individual adult sea stars were marked with dye (Fig.3). Specimens were removed from the sea water, the aboral surface of their rays was blotted with tissue, and a solution of 1% Nile Blue A (Fisher Scientific Co.) in distilled water was dabbed with a cotton swab onto the appropriate rays near their attachment to the central disc. Dye markings of single rays or of combinations of the six rays permitted a numerically

Figure 2. Map of Newfoundland with expanded section to show collection and maintenance sites.


N
100 km
Newfoundland
MSRL
Logy Bay
St. John's
Bay
Bulls
Bread &
Cheese Cove

Figure 3. Photograph of an adult, dye-coded sea star. (X0.7)

r - ray


r1
r2
madreporite
r6
dye
r5
r3
20 mm
r4

coded identification of individual animals. Nile Blue A proved to be a suitable stain for sea stars (see Loosanoff 1937, and Kvalvågnaes 1972, for a discussion of tagging sea stars), with markings still easily distinguishable after periods of ten months. Specimens were routinely re-stained, however, every four to eight weeks.

Specimens were measured with vernier calipers to determine the radius (R), the distance from the centre of the central disc to the tip of the longest ray (Appendix II). Measurement of the radius of the central disc (r) permitted calculation of the ratio (R:r), used as a descriptive feature of sea star species.

2. PREPARATION FOR LIGHT MICROSCOPY

(a) Whole Tissues

Photographs of optic cushions dissected out from excised ray tips were recorded on Kodak High Speed Ektachrome (Tungsten 3200K) film with an Asahi Pentax camera mounted on a Wild binocular microscope.

(b) Histological Preparation

The distal three to four millimetres of adult rays were excised with a clean, sharp razor blade and prepared for histological examination following the routine fixation and embedding techniques described in Humason (1967). The ray tips were fixed in Bouin's solution for one to four days, rinsed well with several changes of 50% ethanol, dehydrated in a graded ethanol series, cleared in cedarwood oil, and embedded for transverse or sagittal sectioning in 'Paraplast' (Sherwood Medical Industries) tissue embedding medium.

Seven micrometre thick sections cut on an American Optical Spencer

'820' rotary microtome, were stained with Mallory-Heidenhain's Stain: Rapid One-Step Method, and with Delafield's Haematoxylin and Eosin by the progressive staining method, as outlined in Humason (1967). All paraffin sections were examined and photographed with a Zeiss Photomicroscope II using Kodak Panatomic-X film.

3. PREPARATION FOR TRANSMISSION AND SCANNING ELECTRON MICROSCOPY (TEM AND SEM)

(a) Fixation

The terminal few millimetres excised from adult ray tips were immediately immersed for 1 1/2 to 3 hours at 4°C in one of the following fixatives:

3% glutaraldehyde in Millonig's phosphate buffer (pH 7.2 - 7.4) (Pease 1964)

3% paraformaldehyde - 3% glutaraldehyde in Millonig's phosphate buffer (pH 7.2 - 7.3)(Karnovsky 1965)

3% paraformaldehyde - 3% glutaraldehyde in filtered sea water (pH 7.9)(Bal et al. 1968)

After primary fixation, the tissue pieces were rinsed with several changes of Millonig's phosphate buffer (pH 7.3 - 7.4) over a period of two to eighteen hours.

For TEM, the terminal tentacle with its optic cushion was carefully dissected away from the hard tissues of the ray tip and then post-fixed in 1% osmium tetroxide in Millonig's phosphate buffer (pH 7.2)(Millonig 1961) for 1 1/2 to 2 hours at 4°C. Some specimens for SEM were trimmed to remove the spines from one side of the ray tip to permit easier viewing of the optic cushion. The remaining tissue was then post-fixed as for TEM.

Examination of material prepared with a variety of fixatives determined that fixation in 3% glutaraldehyde in Millonig's phosphate buffer followed by 1% osmium tetroxide in Millonig's phosphate buffer, was as suitable as any of the others tried, and was thereafter primarily utilized. Micrographs illustrate material fixed in this manner unless otherwise indicated on the facing page.

Previous workers (e.g. Cobb 1970) have tried numerous fixatives and variations in fixation and embedding techniques, and have found many echinoderm tissues difficult to preserve.

(b) Transmission Electron Microscopy

The tissue was rinsed well with Millonig's phosphate buffer, washed briefly with doubly distilled water, dehydrated in a graded series of ethanol, passed through several changes of propylene oxide, and then infiltrated with Epon 812 (Luft 1961).

Sections were cut with glass or diamond knives on a Reichert Om U2 Ultramicrotome. Thick (1 μm) sections were mounted on glass slides, stained with 1% toluidine blue in a 1% borax solution (Mercer and Birbeck 1972), and examined and photographed as for histological sections.

Thin sections were picked up on uncoated copper grids, and stained with a saturated solution of uranyl acetate in 50% ethanol (Watson 1958), followed by staining in lead citrate (Venable and Coggeshall 1965). Grids were examined with a Zeiss EM 9A electron microscope operating at an accelerating voltage of 60 kV, and micrographs were recorded on Kodak Electron Microscope Plate Film (Estar Thick Base).

(c) Scanning Electron Microscopy

Post-fixed material was rinsed in several changes of Millonig's phosphate buffer, and stored in buffer until processed for freeze drying or critical point drying.

For freeze drying, the material was rinsed with doubly distilled water and rapidly frozen by plunging the tissue into the quenching fluid Freon 12 (dichlorodifluoromethane), which was maintained just above its melting point of -158°C over liquid nitrogen. The frozen blocks of ice containing the specimens were transferred to an Edwards Speedivac-Pearse Tissue Dryer Model 1 and freeze dried at a probe temperature of -60°C for six hours (Boyde and Barber 1969).

For critical point drying, specimens were passed from buffer through a graded series of ethanol with three changes of absolute ethanol for a total of one hour. The tissue was carried through 2:1, 1:1, and 1:2, solutions of ethanol:Freon 113 (trichlorotrifluoroethane) for 20 minutes each, and then into two changes of Freon 113 for 20 minutes each. The specimens were then critical point dried from Freon 13 (chlorotrifluoromethane) in a Bomar SPC-900/EX Critical Point Drying Apparatus (Cohen et al. 1968).

Freeze dried and critical point dried material was attached to aluminum stubs with epoxy cement or silver conducting paint (Ladd Research Industries) and coated with a layer of gold, approximately 20 to 30 nm thick, in an Edwards Vacuum Coating Unit Model E12E with continual rotation using a planetary rotation stage. The specimens were examined with a Cambridge Scientific Instruments 'Stereoscan' Mark 2A scanning electron microscope operating at an accelerating voltage of 10kV.

Micrographs were recorded on Kodak Tri-X Pan film with 40 or 100 second scans.

One adult L. polaris optic cushion embedded in epoxy resin and sectioned for TEM was subsequently de-embedded by the method described by Erlandsen et al., (1973). Excess resin was trimmed from the block which was then supported on a TEM copper grid and placed in a flow (approximately 50 ml per hour) of filtered 1.5% NaOH in absolute ethanol that had been aged for several days. After three hours, the de-embedded optic cushion was rinsed well in absolute ethanol and then critical point dried, mounted, gold coated, and examined with the SEM as described above.

B. REGENERATION STUDY

Under a dissecting microscope, terminal tentacles containing the optic cushion were removed with fine forceps and micro-scalpel from healthy, marked, adult sea stars. In most cases, some of the terminal spines, which partially obscure the optic cushion, were clipped with scissors to facilitate terminal tentacle removal. Typically, four of the six rays of each individual were operated upon and the specimens returned to their holding tanks. Four groups of sea stars, indicated in Appendix II, underwent terminal tentacle removal. As confirmation of completeness of optic cushion removal, several ray tips so treated were immediately severed and fixed for histological sectioning.

Operated sea stars were then periodically examined under a dissecting microscope to note wound healing, terminal tentacle regeneration, initial appearance of pigmentation, and the further elaboration of the mature pigmentation pattern of the optic cushion. Arm tips were excised

after various time intervals and prepared for light and transmission microscopy as described above. Observations on those animals whose ray tips had been removed for LM or TEM, were also continued, to note subsequent regeneration.

C. STUDY OF LARVAE OF LEPTASTERIAS POLARIS

1. COLLECTION

Leptasterias polaris spawns during the months of January through March and the females then brood the young, in some cases, until late July. In 1973, 1974, and 1975, adults spawned in the laboratory and some females brooded fertilized eggs. For some reason(s), perhaps because of disturbance by the experimenter, interaction with other co-existing invertebrates, the light regime in the laboratory, or the unsuitability of the substrate, the females eventually abandoned their embryos, which then, if left undisturbed, accumulated debris on their fertilization membranes, and either decayed, or were consumed by small crustacea, sea urchins, and whelks.

Young abandoned embryos were transferred to glass vials and plastic bottles with sea water changed every day or so, or to floating nylon mesh containers. They continued their development for several weeks, but decay and scavenging by protozoa eventually eliminated them. Thus a concerted effort was not made to raise larvae from eggs spawned in the laboratory. The accessibility to divers of a large aggregation of brooding female L. polaris on an underwater cliff face in 'Bread and Cheese Cove', Bay Bulls (Emerson 1973), permitted large numbers of larvae to be collected on May 30, June 7, and June 16, 1973 and on May 2, 1974. Subsequent

examination of larvae collected from brooding females later in the reproductive season, on August 10, 1973 from Bay Bulls, and on July 23, 1974, from Logy Bay, allowed comparison of the stage of development of laboratory-maintained larvae with those in the field.

The initial method of collection by divers, by scraping the larvae off the rock substrate, proved inefficient. Subsequent samples were removed by drawing the larvae up into 30 cc plastic syringes whose tips were removed to produce a larger diameter (3 to 4 mm) bore. The larvae were then expelled underwater into a 275 cc wide-mouth plastic bottles through a slit in a rubber diaphragm secured over the mouth, and the bottles were returned to the M.S.R.L.

2. MAINTENANCE

Collected larvae were raised in the laboratory by various methods. In 1973, the initial system involved their retention in the 275 cc plastic collection bottles, with sea water, filtered through Whatman No. 1 filter paper, changed every day or so. Larvae were maintained in these containers for 50 days, at which time the running sea water was unexpectedly shut off causing the water temperature in the wet bench to rise. The specimens that survived were kept a further 50 days in a ring (30 mm high and 75 mm in diameter) of polyvinylchloride (PVC) pipe covered with 505 μm nylon mesh over one end, that was placed in an A-frame, allowing sea water to circulate through the nylon screen.

A later collection of metamorphosed larvae, which showed signs of disintegration after 18 days in the plastic collection bottles, even with daily changes of filtered sea water, was also transferred to a PVC ring

(150 mm in diameter). These specimens were maintained an additional 30 days, with the introduction of small pieces of squid mantle upon which the larvae appeared to feed.

In 1974, various systems of keeping larvae in the laboratory were employed in an attempt to eliminate the problems apparently arising from water stagnation, and from predation by protozoa, rotifers, and small crustaceans. One method, from Larsen (1937), involved the use of a wide-mouthed glass bowl with a capacity of 2.5 l. Filtered sea water which had been boiled and cooled, was exchanged in the bowl every three to eight days, and an air stone aerated the water with a continuous gentle stream of bubbles. After a period of two months, sea water, which continued to be changed every three to seven days, was still filtered but was no longer boiled and cooled. Larvae continued to develop for an additional three months.

Plastic aquaria were also used to maintain larvae collected in 1974. These aquaria were 180 mm long, 80 mm wide and 110 mm high, and had fine mesh nylon screening fixed over the exit in one end of the tanks. Sea water entered one aquarium (tank I) through a nylon mesh 'cage' attached to the end of the tank opposite the exit. Water flowed into tank II after passing through a 505 μm mesh nylon screen, and then a more finely meshed piece of nylon. These screens were periodically cleaned by flushing with fresh water.

The screen 'cage' of tank I was less efficient at filtering sea water than was the system for tank II, and debris accumulated on the bottom of the aquarium. After eleven days, the larvae from tank I were switched to a 2.5 l glass bowl in which cooled, boiled, filtered sea

water was exchanged every few days, and in which an air stone bubbled slightly. The larvae survived an additional 16 days. A later collection of metamorphosed young were retained in tank I for two months.

Tank II provided the better system of larval retention and after periodic removal of healthy specimens for microscopical preparation, and of unhealthy sea stars that were discarded, about a dozen larvae still remained after a period of over ten months. After the stomodaeum had broken through in the developing sea stars, a layer of algae and diatoms was allowed to accumulate on the walls of the aquarium, and small pieces of mussel tissue were introduced at very occasional intervals.

The larvae maintained by all of the above means were examined under a binocular microscope at intervals to make observations on their development and to remove any unhealthy ones. Healthy specimens were prepared periodically for whole mounts, paraffin sections, and transmission and scanning electron microscopy.

In 1975, although they were not prepared for microscopical examination, larvae were collected in the laboratory after they were abandoned by female adults, and were kept in nylon mesh cylinders which floated in the circulating water of the A-frame by a means of a styrofoam 'collar' affixed to the upper, open end. This apparatus proved to be the most satisfactory means of larval retention. In addition, in 1975, three females continued brooding for five months on the vertical walls of a wet-table, and larvae were carefully pipetted from under one for occasional viewing. Observations were made on all of these larvae to compare their development with that of previous years.

3. PREPARATION FOR LIGHT MICROSCOPY

(a) Live Material

Live specimens were photographed using Kodak High Speed Ektachrome (Tungsten 3200K) film with an Asahi Pentax camera mounted on a Wild binocular microscope at magnifications of 12 to 150 times.

(b) Whole Mounts

As a record of gross morphology, larvae were prepared at various times as whole mounts. Specimens were fixed in Bouin's solution for 24 hours, washed with many changes of 50% ethanol, and stained following Galigher's procedure for Grenacher's Borax Carmine (Humason 1967). After dehydration in ethanol, larvae were cleared in cedarwood oil and mounted on depression slides with 'Permount' (Fisher Scientific Co.).

(c) Histological Preparation

Specimens were fixed and embedded in 'Paraplast' for standard histological examination as outlined for the adult sea stars.

4. PREPARATION FOR TRANSMISSION AND SCANNING ELECTRON MICROSCOPY

Preparation of larval material for TEM and SEM involved the use of the variety of fixativeslisted in the 'adult' section.

Again, 3% glutaraldehyde in Millonig's phosphate buffer was the principle primary fixative (with fixation time 1 1/2 to 3 hours at 4°C) and can be assumed to have been used unless otherwise indicated. After thorough rinsing in buffer, larvae were post-fixed in 1% osmium tetroxide in Millonig's phosphate buffer (pH 7.2) for 1 1/2 to 2 hours at 4°C and rinsed again in phosphate buffer.

(a) Transmission Electron Microscopy

Fixed larvae were embedded, sectioned, stained, and examined as outlined in Section A 3(a, b). A small number of grids was examined with a Philips 300 electron microscope operating at an accelerating voltage of 60 kV, and micrographs were recorded on Kodak Fine Release Positive 35 mm film.

(b) Scanning Electron Microscopy

Fixed larvae were freeze dried or critical point dried for viewing in the scanning electron microscope as described in section A 3 (c).

RESULTS

A. ADULT OPTIC CUSHION AND OCELLI

1. GENERAL OBSERVATIONS

When moving, and often when stationary, Leptasterias polaris individuals raise the tips of their rays and expose the bright orange-red optic cushions situated between the adambulacral spines on the oral surface near the terminus of each ray. Even brooding sea stars often lift the distal portions of their ray tips from the substrate (Fig. 4A).

The optic cushion is positioned on the oral surface of the terminal tentacle near its base, immediately distal to the level at which the youngest, and smallest, pair of tube feet originate (Fig. 1A, 5A). The cushion, or eyespot, is an elliptical pad that has its long axis parallel to the longitudinal orientation of the ray. In adult animals, it measures approximately 500 μm in length and 300 μm in width.

The cushion appears as a single concentration of red pigment to the naked eye, but closer examination with a dissecting microscope reveals that the eyespot consists of numerous (100 to 200) 'dots' of pigment arranged in rows that radiate from the midline of the cushion Fig. 4B). A low power scanning electron micrograph illustrates raised areas, 20 to 30 μm across, on the optic cushion (Fig. 4C). Several flaps of tissue occur on the raised areas and a SEM micrograph of a de-embedded cushion previously sectioned for TEM, showed that these are corneal extensions that cover each ocellus (Fig. 4D).

Figure 4A. Photograph of a disrupted, brooding female showing the optic cushion at the ray tip, and eggs beneath the ray base. (X1.5)

Figure 4B. Colour photomicrograph of an optic cushion illustrating pigment distribution. (X175)

Figure 4C. Scanning electron micrograph (SEM) of an optic cushion showing sites of numerous ocelli. (X145)

Figure 4D. SEM of a de-embedded, sectioned, optic cushion, showing corneal processes over the ocellar lumen. (X700)

Cp - corneal cell process
o - ocellus
ol - ocellar lumen
Oc - optic cushion
tt - terminal tentacle

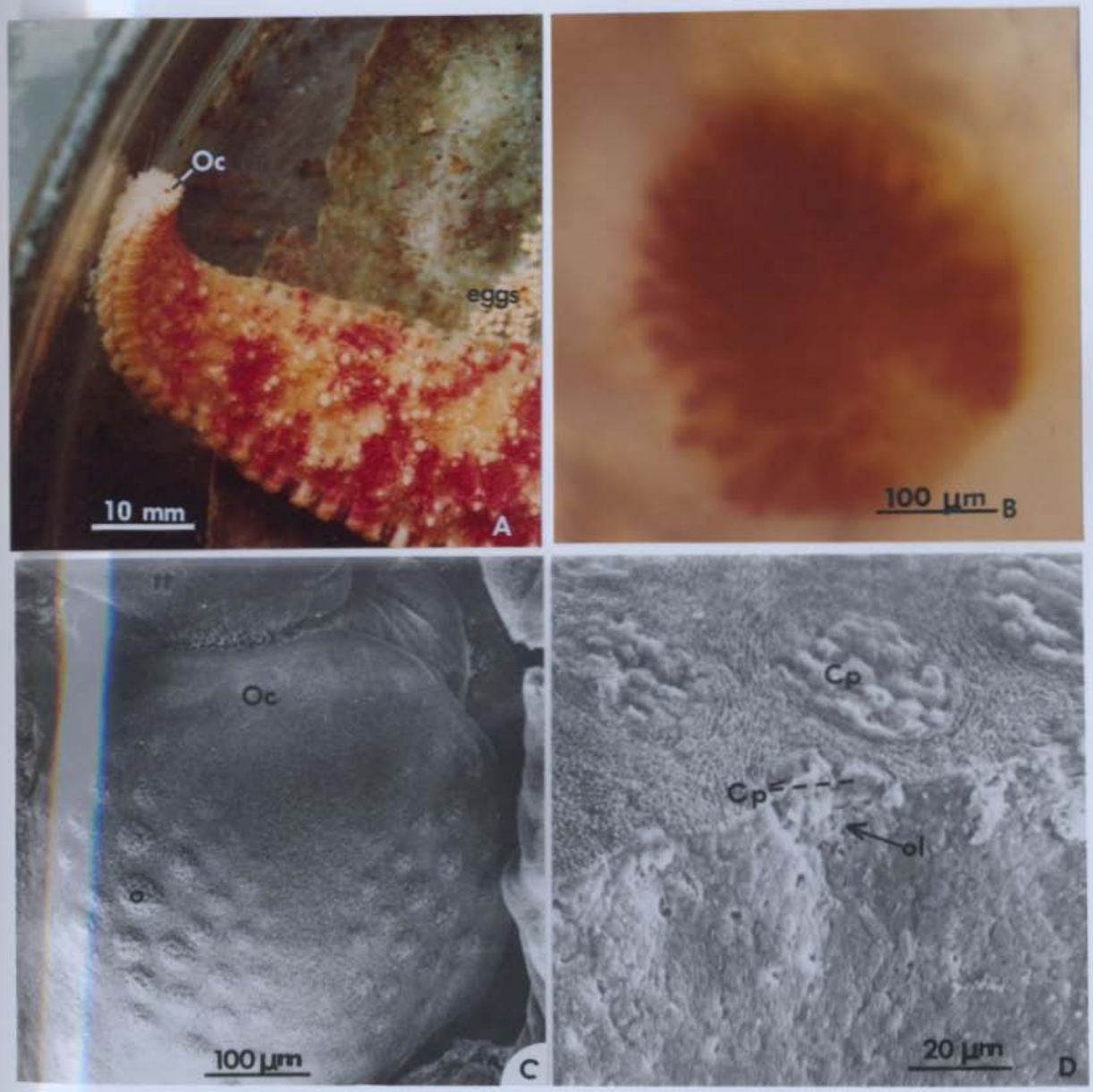
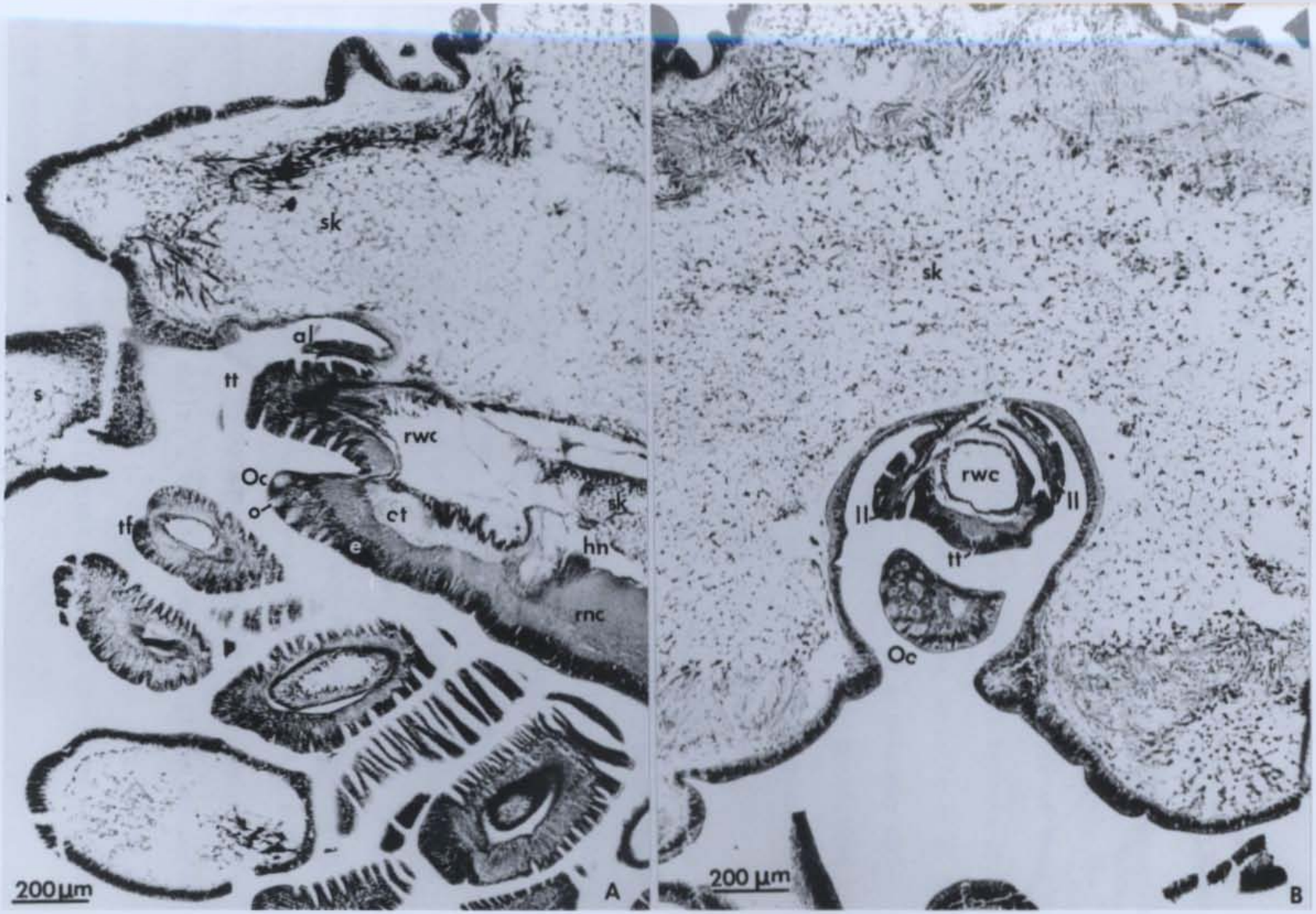

Oc
eggs
10 mm
A
100 µm
B
Oc
100 µm
C
Cp
Cp
ol
20 µm
D

Figure 5. Light micrographs (LM's) of sections through adult ray tip to show the optic cushion.

A. Sagittal section (S/S) through optic cushion at base of terminal tentacle. (X62) Mallory-Heidenhain's Stain.

B. Transverse section (T/S) through protruding optic cushion showing numerous ocelli. (X62) Delafield's Haematoxylin and Eosin.

al - aboral lappet
ct - connective tissue
e - epithelium
hn - hyponeural sinus
ll - lateral lappet
o - ocellus
Oc - optic cushion
rnc - radial nerve cord
rwc - radial water canal
s - spine
sk - skeletal tissue
tf - tube foot
tt - terminal tentacle


sk
al
tt
s
rwc
Oc
o
ct
sk
tf
e
hn
rnc
200 µm
A
sk
rwc
ll
ll
tt
Oc
200 µm
B

2. OBSERVATIONS OF HISTOLOGICAL SECTIONS

A sagittal section along the midline of a ray tip shows a tentacle extending as the distal terminus of the radial water canal beneath the calcified terminal ossicle (Fig. 5A). A small flap of tissue, the aboral lappet, is present over the aboral portion of the base of this terminal tentacle, and in a cross-sectional view, lateral lappets are seen to hang down around the sides of the base of the tentacle (Fig. 5B).

The wall of the terminal tentacle consists of a columnar epidermal layer beneath which lie a few nerve fibres, a thin connective tissue layer, longitudinal muscle fibres, and the cuboidal epithelium which delimits the lumen of the radial water vessel (Fig. 6A).

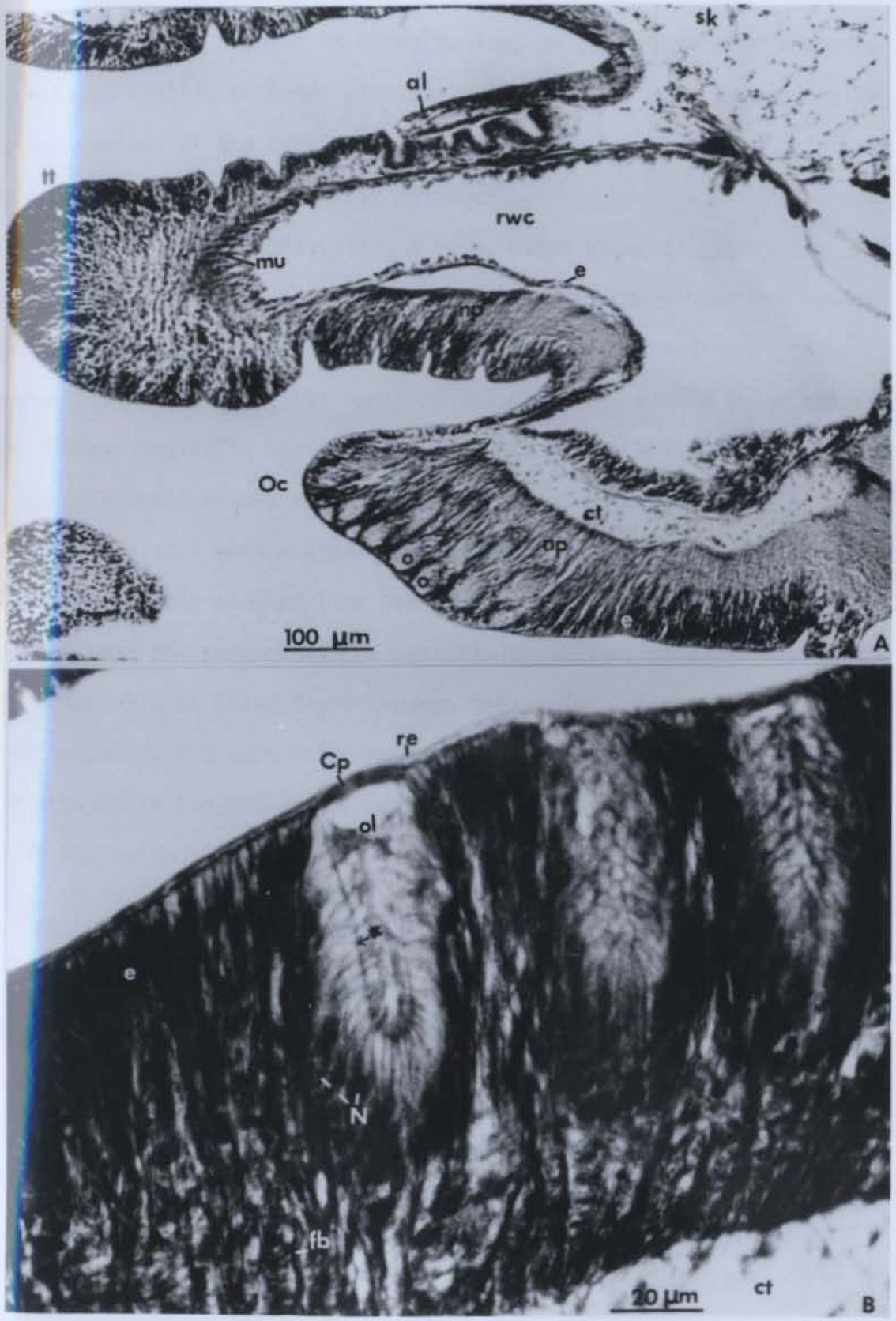
The radial nerve cord extends from the circumoral nerve ring down the midline of each ray. The nerve cord continues into the base of the terminal tentacle as part of a prominent expansion, the optic cushion (Fig. 6A). The cushion is also limited internally by a cuboidal epithelium which may be thrown into folds. This epithelium lines the hyponeural sinus for most of the length of the radial nerve cord. However, near the ray tip, the sinus terminates and the epithelium continues as the lining of the radial water vessel, which opens into the terminal tentacle (Fig. 5A). Beneath this epithelium lie a few longitudinal muscle cells, a layer of connective tissue containing collagen and some scattered cells, a 40 to 75 μm thick plexus of nerve fibres, and an epithelial layer, 50 to 75 μm thick (Fig. 6A). This layer includes fibre-containing cells that penetrate the nerve plexus to the connective tissue layer.

Figure 6. LM's of S/S through optic cushion.

A. Section shows tissue layers of terminal tentacle and optic cushion. (X145) Mallory-Heidenhain's Stain.

B. Longitudinal section through an ocellus illustrating the revestment, corneal cell processes, and form of the ocellus. * level of intercellular junctions. (X750) Mallory-Heidenhain's Stain.

al - aboral lappet
ct - connective tissue
Cp - corneal cell process
e - epithelium
fb - filamentous bundle
mu - muscle
np - nerve plexus
N - nucleus
o - ocellus
ol - ocellar lumen
Oc - optic cushion
re - revestment
rwc - radial water canal
sk - skeletal tissue
tt - terminal tentacle


sk
al
tt
rwc
mu
e
np
Oc
ct
np
e
100 µm
A
re
Cp
ol
e
N
fb
20 µm
ct
B

The optic cushion is characterized by the presence of numerous cup-shaped ocelli, oriented with their longitudinal axes perpendicular to the surface of the cushion (Fig. 6A). They measure approximately 70 to 100 μm in length and 25 μm in diameter.

At higher magnification, a thin, clear external layer, the extracellular revestment, forms a continuous structure over the epidermis and ocelli (Fig. 6B). Beneath this layer are corneal cell processes that extend over the lumen of the ocellus. Within these processes are fibres similar to those present in many of the epithelial cells.

The wall of the ocellus is composed of hundreds of cells that have their distal ends aligned approximately at right angles to the lumen. A densely stained line extending across these cells, and also evident near the surface of the general epithelium, probably represents the intercellular connections between them. Many of the ocellar cells send expanded, and apparently membranous apices into the lumen. Nuclei are present in the mid-region of the cells, while proximally, the cells taper and extend into the nerve plexus (Fig. 6A).

3. OBSERVATIONS OF THICK SECTIONS OF EPON-EMBEDDED TISSUE

Thick Epon sections stained with toluidine blue illustrate most of the features previously described, and some additional structures as well. The thin superficial revestment appears to be penetrated by numerous short projections from the epithelial cells and is continuous over the raised ocelli (Fig. 7A). Extensions of corneal cells containing fibre bundles arch over the distal end of the ocellus beneath this transparent layer.

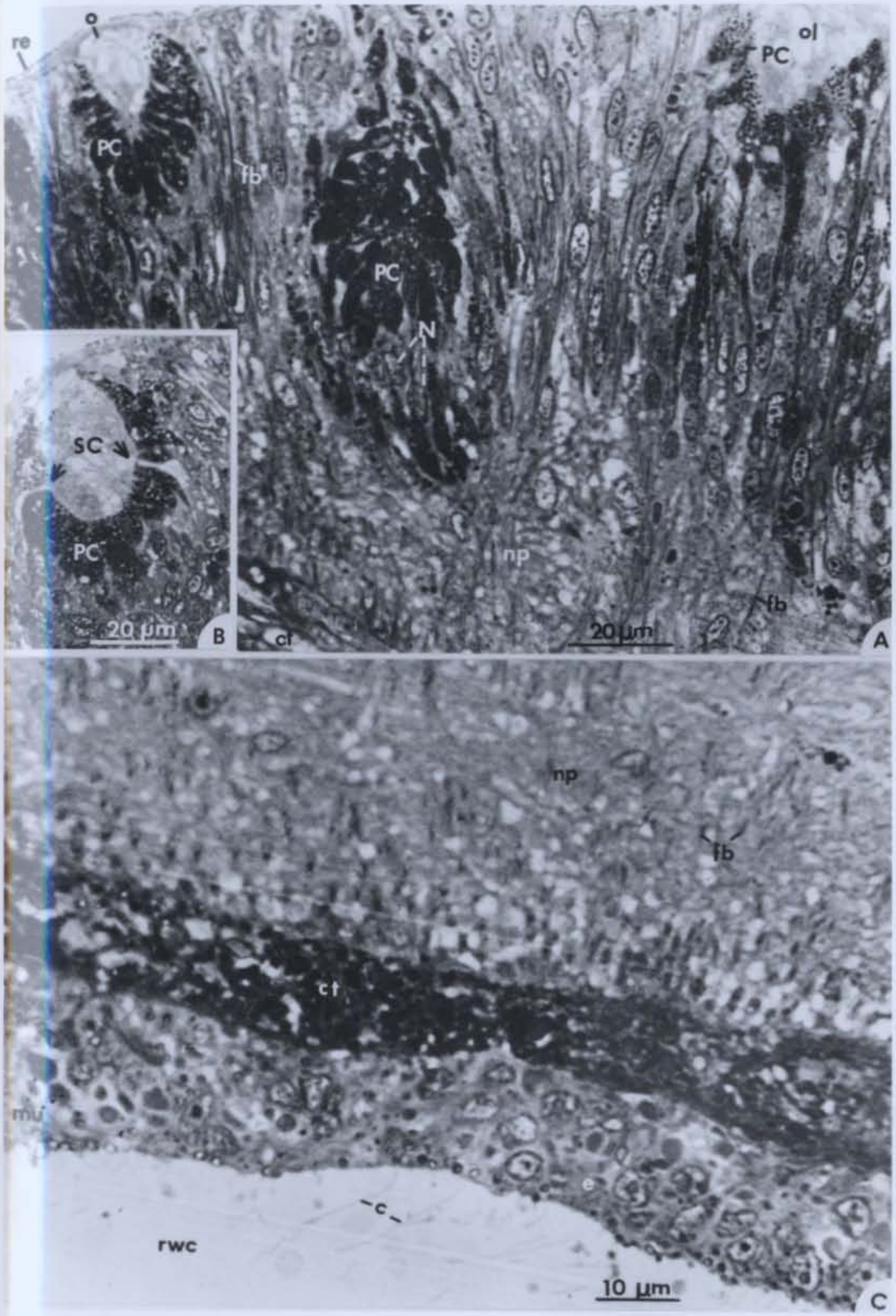
Figure 7. LM's of S/S of Epon-embedded optic tissues.

A. Section through optic cushion shows pigmented cells constituting the bulk of the ocellar wall. Epithelial cells with filamentous bundles traverse the nerve plexus. (X875)

B. Section through an ocellus shows sensory cells extending up between pigmented cells in the ocellar wall. (X750)

C. A layer of connective tissue separates the ectoneural plexus from the hyponeural, muscular, and ciliated epithelial tissues bordering the radial water canal. (X1400)

c - cilium
ct - connective tissue
e - epithelium
fb - filamentous bundle
mu - muscle
np - nerve plexus
N - nucleus
o - ocellus
ol - ocellar lumen
PC - pigmented cell
re - revestment
rwc - radial water canal
SC - sensory cell


re
o
PC
fb
PC
N
ol
PC
SC
PC
20 µm
B
np
fb
cf
20 µm
A
np
fb
cf
mu
c
rwc
10 µm
C

The wall of the ocellus is lined by pigmented cells that contain cytoplasmic granules with diameters up to one μm. These granules are also present in the more proximal parts of the cells where they taper and extend toward the subepithelial plexus. Also present in the ocellar wall between the granule-filled pigmented cells, are narrow (one to two μm) portions of the sensory cells (Fig. 7B). These cells stain very lightly as do the membranous contents of the lumen. The sensory cells curve proximally and pass into the layer of nervous tissue. The elongated nuclei of both cell classes form a cup-shaped layer around the sides and base of the ocellus, and many possess a nucleolus (Fig. 7A).

Surrounding the ocelli and also scattered throughout the optic cushion are epidermal cells that contain fibre bundles (Fig. 7A). These bundles extend from the distal end of the cells, pass down the proximal processes through the nerve plexus, and terminate at the basal lamina that delineates the connective tissue layer. The epidermal cell nuclei are scattered in a layer 75 μm in thickness.

Beneath this region is the ectoneural nerve plexus which has its nerve fibres oriented along the longitudinal axis of the ray (Fig. 7A, C). This plexus is penetrated by the fibre-filled processes of the epithelial supporting cells. Adjacent to the plexus is a connective tissue layer (Fig. 7C). A ciliated epithelium lines the radial water canal, and between this epithelium and the connective tissue layer are scattered muscle cells.

4. ULTRASTRUCTURAL OBSERVATIONS

(a) Epidermal Cells of the Optic Cushion

A double-layered extracellular revestment covers all of the epidermis (Fig. 8). The outer layer, which is approximately one to two μm thick, is composed of two fibrous strata, the outer stratum appearing denser than the inner one. Below the outer layer, separated by a space of 0.5 μm, lies a thinner, 0.5 μm thick, layer of loosely packed filaments lying parallel to the cell surface. The columnar epithelial cells give rise to numerous microvilli which are one to three μm long, and 0.1 μm in diameter, and project through the revestment (Fig. 9). The electron-dense termini of the microvilli extend slightly above the outer layer and are coated with short radiating filaments. The microvilli arise singly from the surface of the epidermal cells or branch from common trunks. There are no organelles present in these structures, nor are there elements interspersed between the cell membrane and the inner layer of the revestment.

A cilium, 0.25 μm in diameter, with a slightly ruffled shaft membrane, arises from each epithelial cell and extends for several micrometers beyond the surface layers (Fig. 9). The axoneme displays a '9 + 2' configuration of microtubules. The cilium sits in a depression, and the adjacent microvilli display an accumulation of electron-dense material in their cytoplasm facing the cilium. Each cilium also possesses an accessory centriole oriented at right angles to the basal body, and a root with a pattern of striations repeating every 60 nm.

The epithelial cells show specialized intercellular connections

Figure 8. Transmission electron micrograph (TEM) of epithelial cells adjacent to an ocellus. (X5,050)

The columnar epithelial cells project microvilli into the stratified revestment, and contain elongate nuclei and filamentous bundles.

Cp - corneal cell process
fb - filamentous bundle
n - nucleolus
N - nucleus

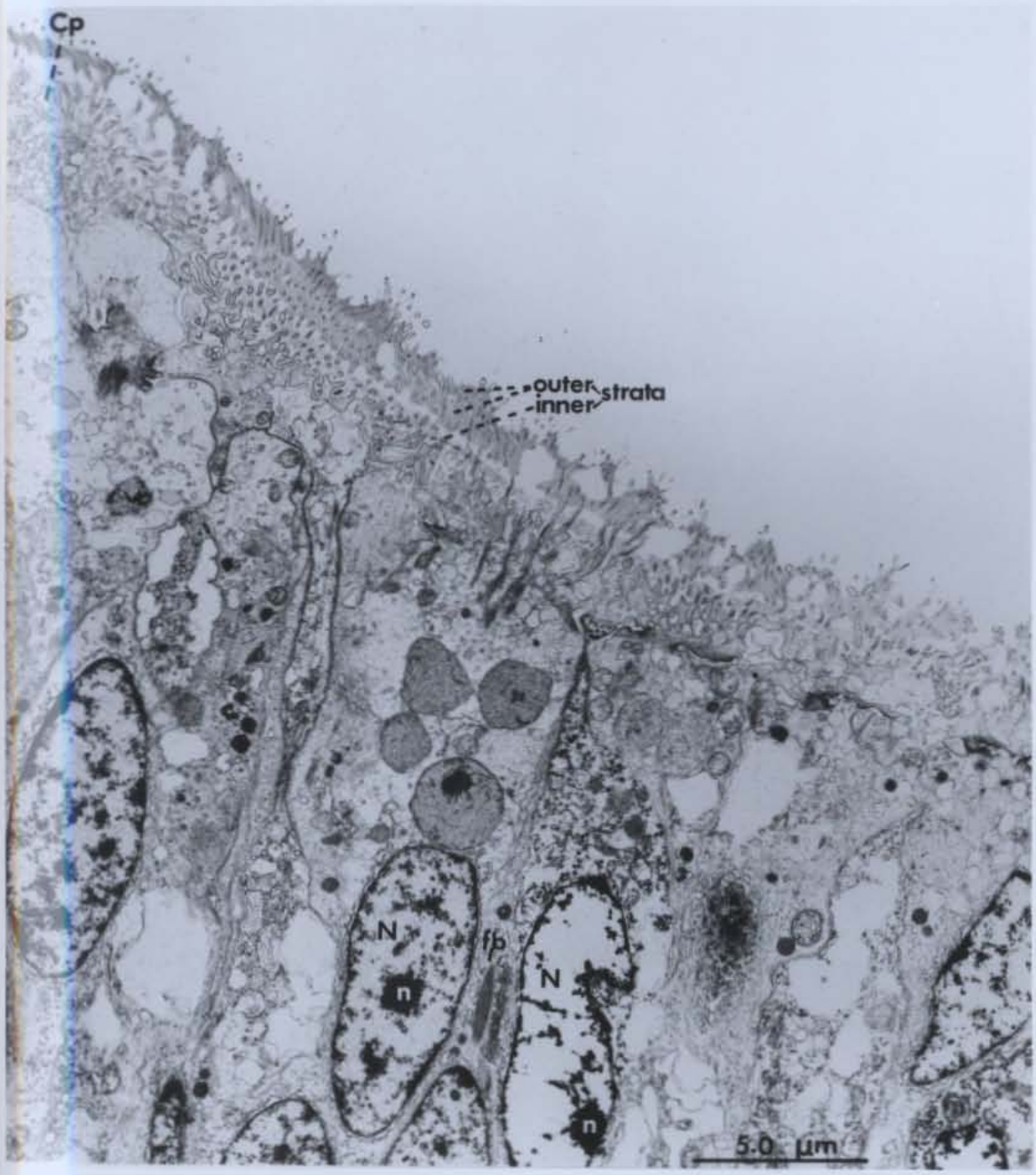
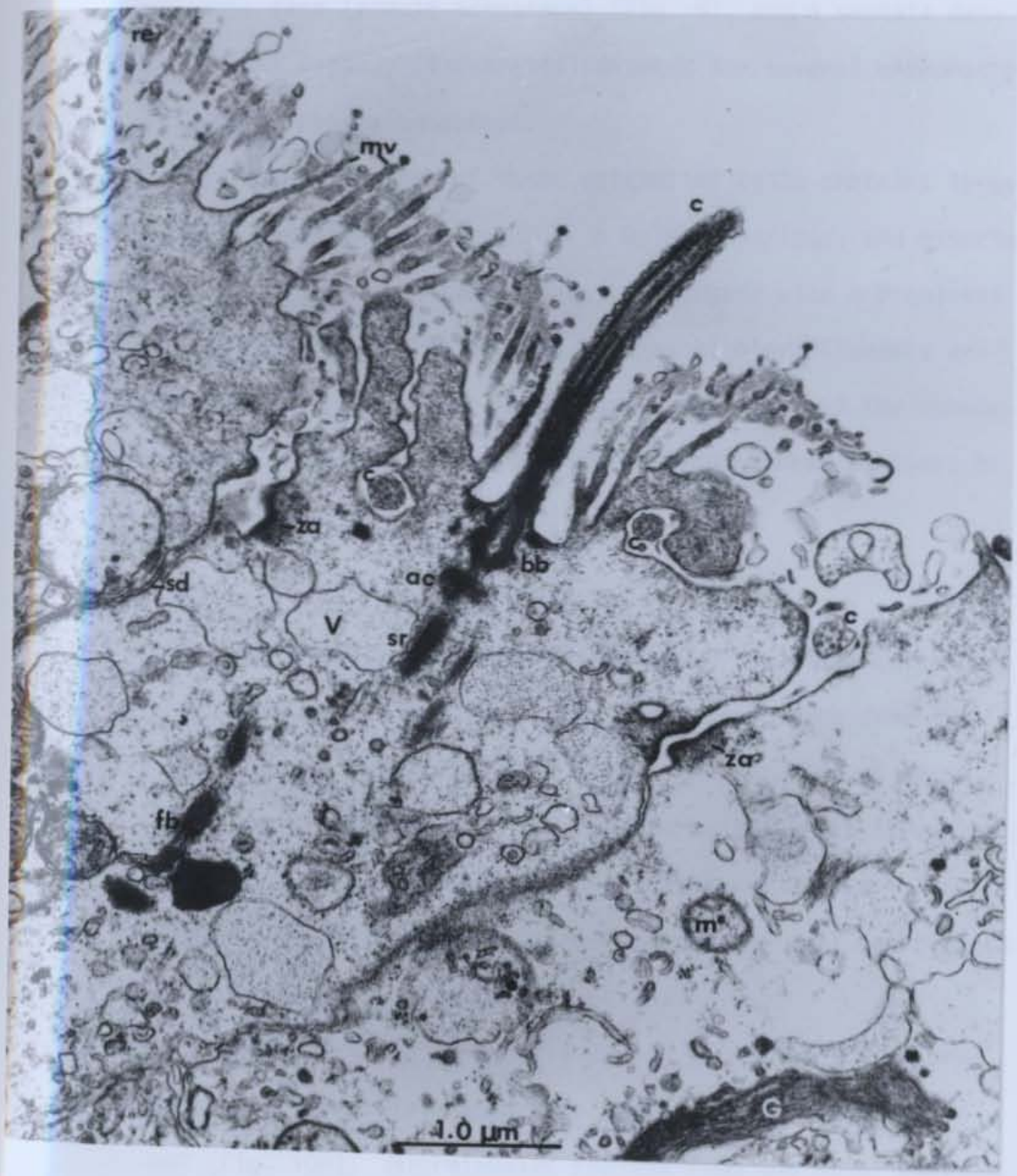

Cp
outer
inner
strata
N
n
fb
N
n
5.0 µm

Figure 9. TEM of longitudinal section (L/S) through ciliated epithelial cell of optic cushion. (X24,000)

Cilia are seen in T/S and L/S, and the epithelial cells display specialized junctions with each other.

ac - accesory centriole
bb - basal body
c - cilium
fb - filamentous bundle
G - Golgi apparatus
m - mitochondrion
mv - microvillus
re - revestment
sd - septate desmosome
sr - striated root
V - vacuole
za - zonula adhaerens


re
mv
c
c
za
bb
ac
sd
V
sr
c
za
fb
m
G
1.0 µm

at their adjacent distal borders. There is a distal gap where the plasma membranes display electron-dense accumulations of material along their cytoplasmic face (zonula adhaerens) (Fig. 9), and a septate desmosomal region (not clearly illustrated) extends for several micrometres proximally from the zonula adhaerens.

Briefly, the cytoplasm of these epithelial cells contains large, apically located vacuoles, mitochondria, a Golgi apparatus, and assorted vesicular profiles (Fig. 9). The nucleus is elongate with a prominent nucleolus (Fig. 8). Many cells possess bundles of microfilaments extending down the cells, and these terminate at the level of the connective tissue layer separating the ectoneural and hyponeural portions of the radial nerve cord (Figs. 6A,B, 28A).

(b) Corneal Cells

The cornea is composed of the extensions of specialized epidermal cells that surround the ocellus. These extensions arch over the opening of the ocellar lumen (Fig. 10A). From the exterior of these cells, as from other epidermal cells, arise microvilli which irregularly arboresce from their bases and narrow to approximately 0.1 μm in diameter. The microvilli extend one to three μm from the cell and project through the revestment.

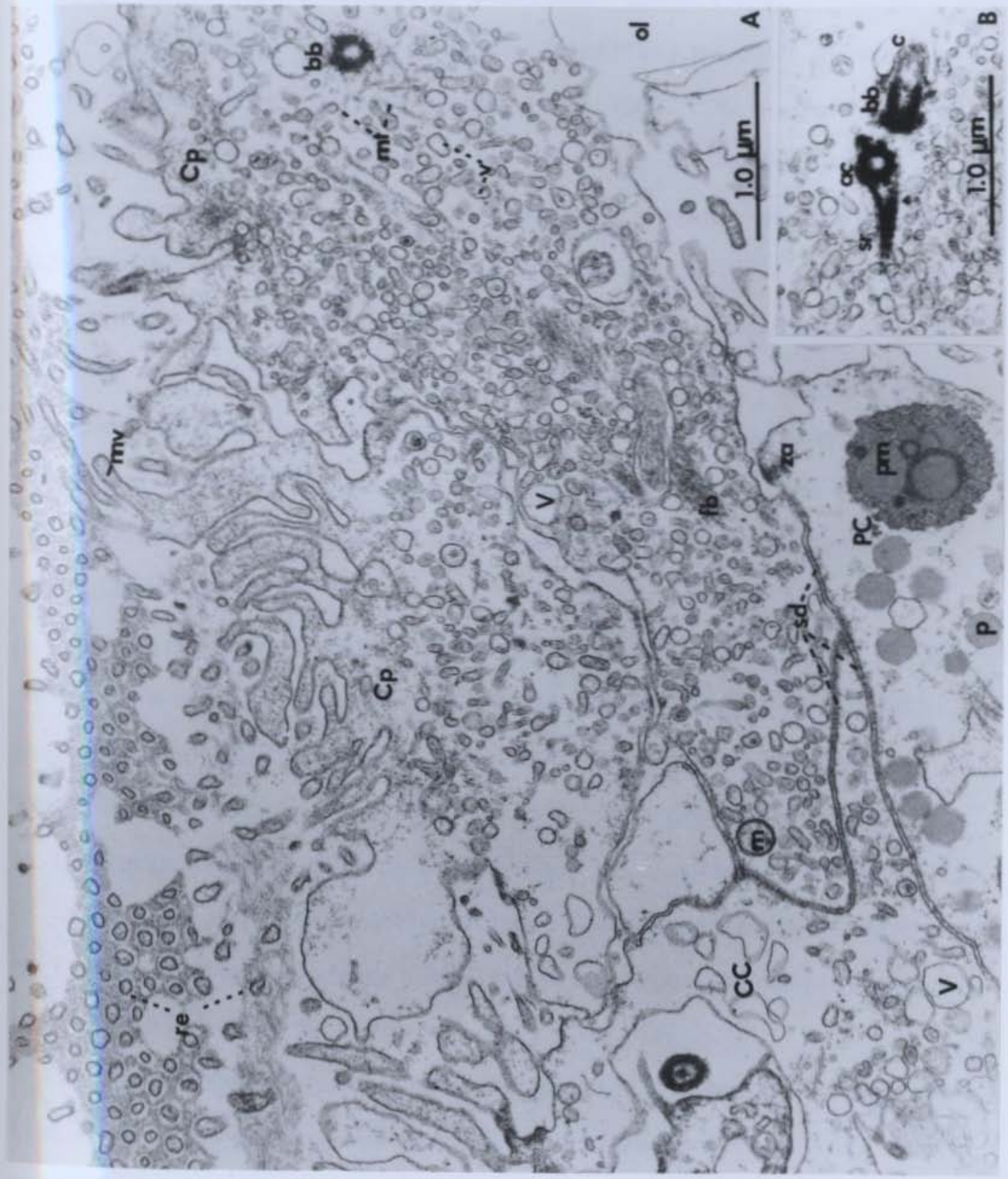
The corneal cells also possess a cilium with a '9 + 2' microtubular arrangement (not illustrated), an accessory centriole, and a striated root (Fig. 10B). Microtubules radiate from the basal body (Fig. 10A). The corneal cells are connected by septate desmosomes to each other and to neighbouring epidermal and pigmented cells. Their

Figure 10. TEM's of corneal cell processes.

A. Processes overhang the ocellar lumen and contain numerous low density vesicles and microtubules. The corneal cells display septate desmosomal juctions with neighbouring cells. (X25,100)

B. Section shows basal morphology of corneal cell cilium. (X24,000)

ac - accessory centriole
bb - basal body
Cp - corneal cell process
fb - filamentous bundle
m - mitochondrion
mv - microvillus
ol - ocellar lumen
p - pigment granule
pm - polymorphic body
PC - pigmented cell
re - revestment
sd - septate desmosome
sr - striated root
v - vesicle
za - zonula adhaerens
V - vacuole


A
1.0 µm
ol
bb
mi
Cp
mv
V
fb
za
pm
PC
P
sd
m
CC
re
B
1.0 µm
c
bb
ac
sr

extensions over the lumen may be closely apposed to each other, but are not seen to be associated by desmosomal connections.

The corneal cells are readily identified by their abundant vesicles and short tubular segments (Fig. 10A). These profiles are clear or slightly electron-dense, and are 80 to 150 nm in diameter. Larger vacuoles, 0.5 µm in diameter, are also present, as are scattered microtubules. In addition, large numbers of microfilaments surrounded by microtubules align to form fascia up to one µm in diameter, which traverse the extensions overlying the ocellar lumen, and then extend down the cells as they bend and pass down around the ocellus (Fig. 11). These bundles may divide around vesicles and vacuoles, and reunite, giving them the appearance, in cross-section, of having a central vesiculated core (Fig. 12).

Rounded to elongate mitochondria with tubular cristae are found in the main body of the cell and are frequently located at the cell periphery, but are not usually present in the cellular projections that extend over the lumen (Fig. 10, 11). Also present in these cells at the level at which they bend around and are juxtaposed to the cells of the ocellar wall, is a well-developed Golgi apparatus with numerous small (60 nm diameter) dense vesicles, with larger low density vesicles and tubular profiles in close proximity (Fig. 11).

The elongated nucleus (not illustrated) is housed in the portion of the cell that curves down and encapsulates the ocellus. Rough endoplasmic reticulum and scattered ribosomes are seen in this region of the cell. The corneal cells taper, and their proximal processes

Figure 11. TEM of mid region of corneal cell between two pigmented cells. (X25,100)

A filamentous bundle extends down the corneal cells past the mitochondria and well developed Golgi region with numerous vesicles. Pigmented cells possess small pigment granules, some of which are surrounded by low density vesicles→. Granular vesicles are also often seen near the pigment granules (*). A variety of large polymorphic bodies (B,C) also characterize these cells.

CC - corneal cell
fb - filamentous bundle
G - Golgi apparatus
hv - high density vesicle
lv - low density vesicle
m - mitochondrion
p - pigment granule
PC - pigmented cell
SC - sensory cell
V - vacuole

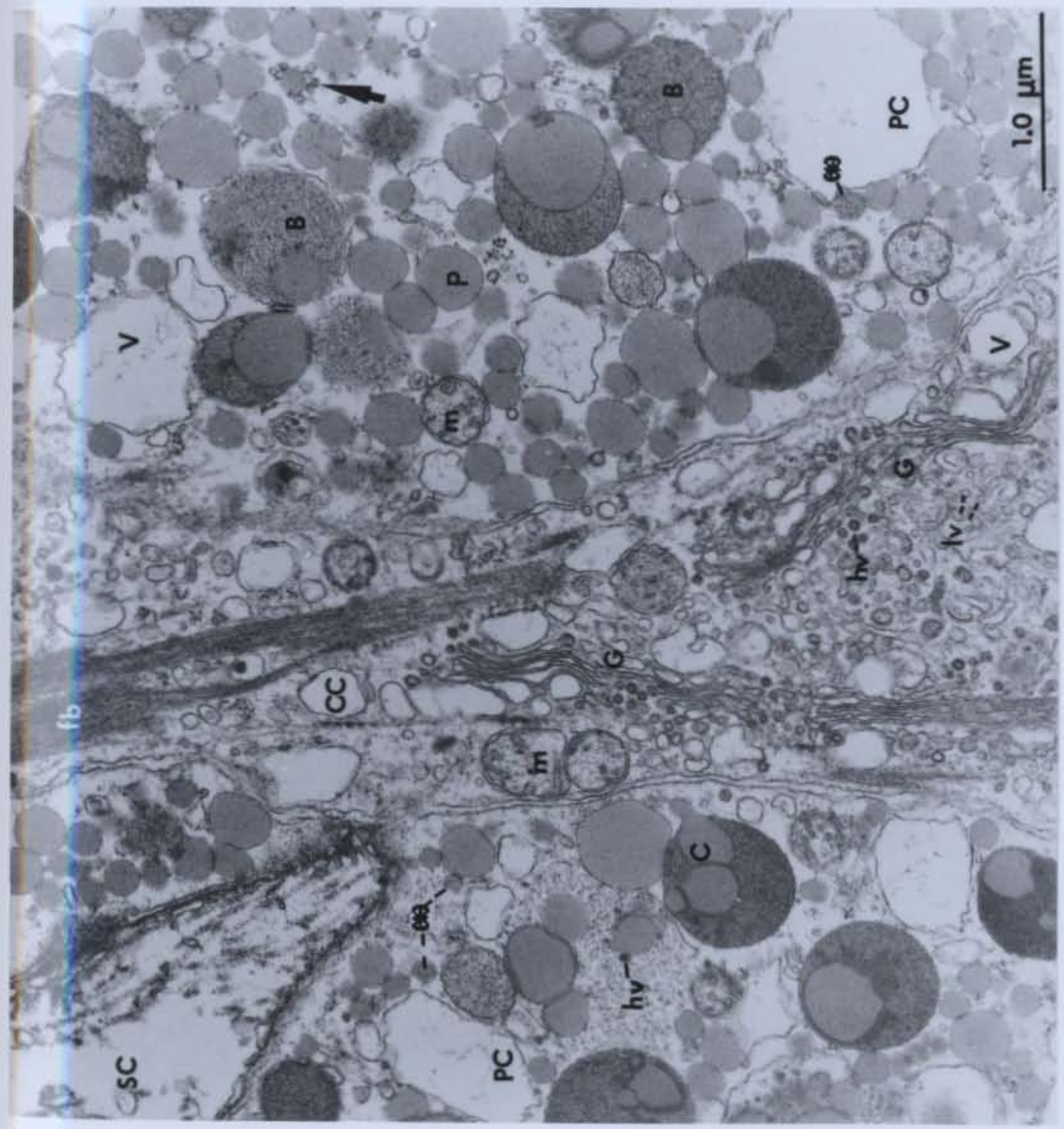
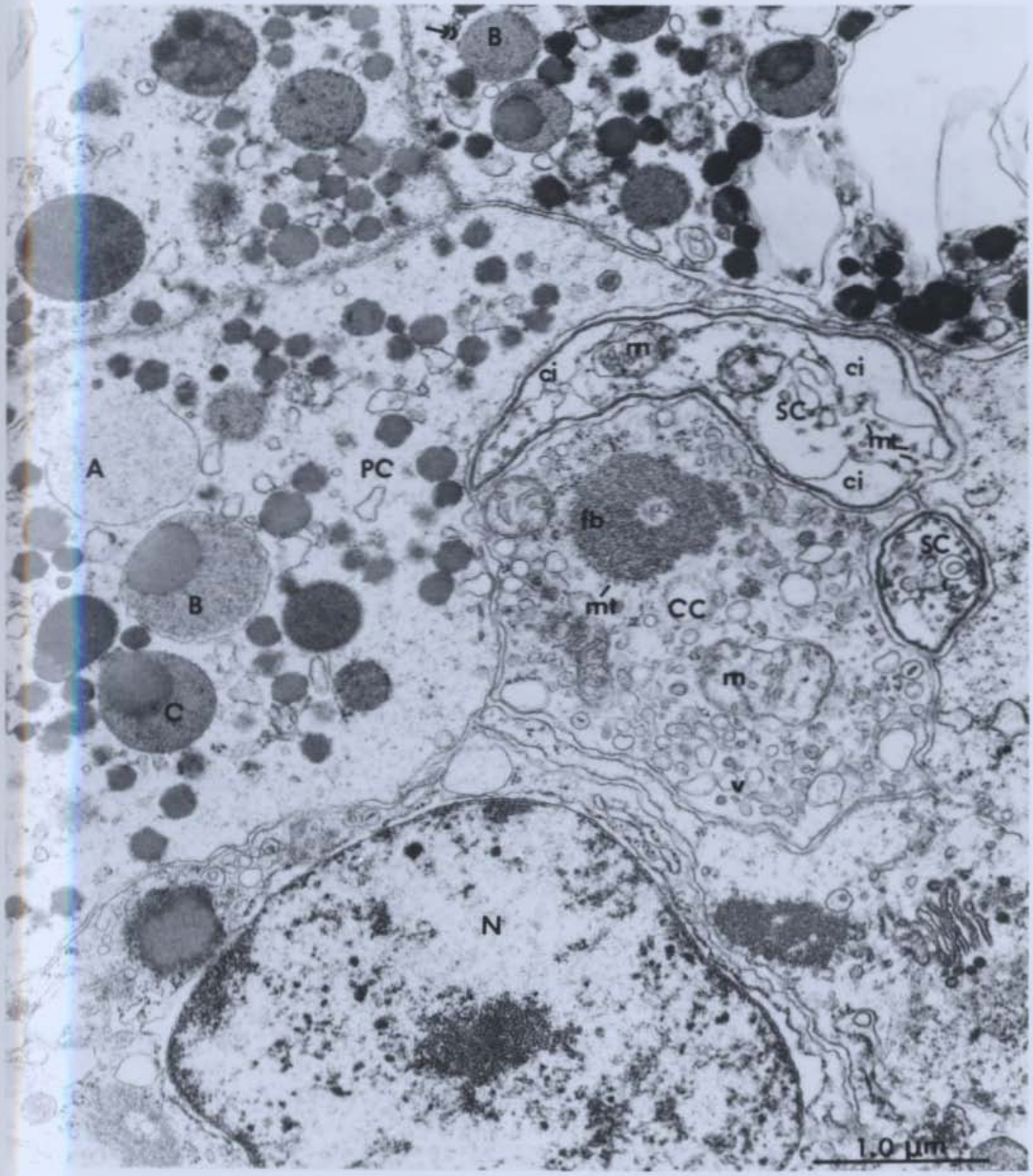

SC
fb
PC
hv
C
m
CC
G
B
P
V
PC
hv
G
V
1.0 µm

Figure 12. TEM of T/S of corneal, sensory, and pigmented cells. (X25,100)

Corneal cell shows T/S of filamentous bundle with surrounding microtubules, and also, numerous mitochondria and vesicles. T/S's through sensory cell necks illustrate subsurface cisternae, microtubules, and mitochondria. Pigmented cells show several types of polymorphic inclusion, with a double membrane surrounding a type B body ⟶» .

ci - subsurface cistern
CC - corneal cell
fb - filamentous bundle
m - mitochondrion
mt - microtubule
N - nucleus
PC - pigmented cell
SC - sensory cell
v - vesicle
A,B,C - polymorphic inclusions


B
ci
m
ci
SC
mt
ci
A
PC
fb
SC
B
mt
CC
m
C
v
N
1.0 µm

extend, as do those of epithelial supporting cells, to pass through the nerve plexus, and presumably also abut on the connective tissue layer.

(c) Sensory Cells

Two types of cells comprise the wall of the lumen itself (Fig. 13). One type, obvious because of the conspicuous inclusions in their cytoplasm, are termed the pigmented cells. The second type of cells lining the ocellar lumen, the sensory cells, extend up between the pigmented cells. Where they are aligned with the pigmented cells to comprise the ocellar wall, the sensory cells are narrow, this portion being termed the neck. The sensory cells expand into the lumen distal to the neck region and give rise to cilia and elongated villi. The cells bend from the neck region proximally, and are aligned perpendicularly to the epidermal surface of the optic cushion. The cells are expanded in the region of their nucleus, and then taper basally into processes that merge with the subepithelial nerve plexus. The ultrastructural description of the sensory cells will treat these regions separately.

(i) Distal Portion

Expanded portions of the sensory cells project beyond the distal margins of their neighbouring pigmented cells (Fig. 14). These distal expansions of most sensory cells extend for several micrometres into the ocellar lumen, while in others they are less extensive.

The cytoplasm of the distal portions, like that of the rest of the sensory cell, is less electron-dense than that of the pigmented cells (Fig. 13). Longitudinally oriented microtubules, 25 nm in diameter,

Figure 13. TEM of portion of an ocellus. (X9,050)

The ocellar lumen contains cilia and villi from the apical portions of sensory cells. The ocellar wall is composed of sensory and pigmented cells.

c - cilium
ol - ocellar lumen
PC - pigmented cell
SC - sensory cell
vi - villi

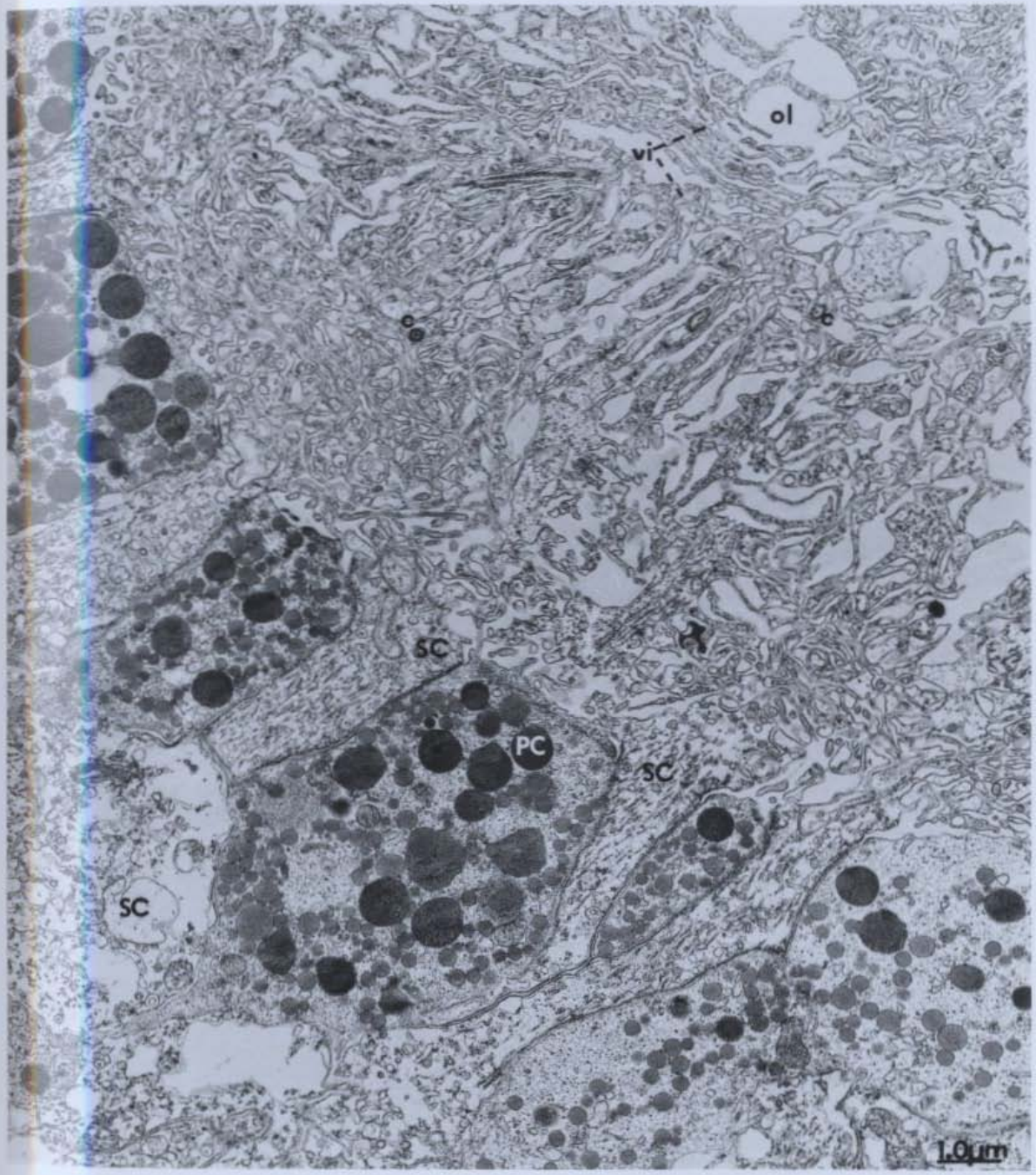
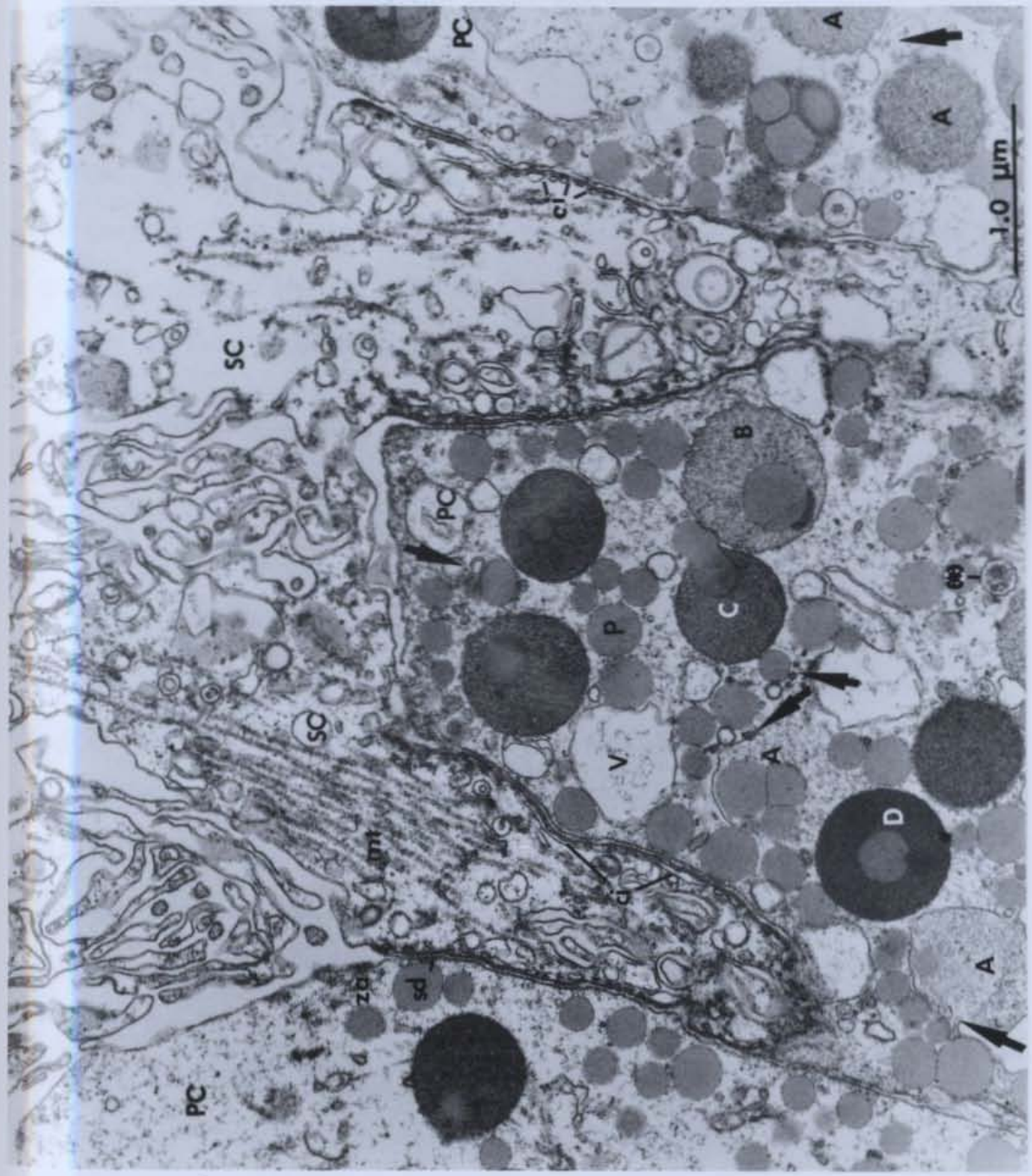

ol
vi
Cc
SC
PC
SC
SC
1.0 µm

Figure 14. TEM of several cells of the ocellar wall. (X25,100)

The sensory cell neck is lined by subsurface cisternae, and contains numerous microtubules and vesicles. The sensory cells form septate desmosomal junctions with adjoining pigmented cells. In the pigmented cells, low density vesicles ⟶ and granular vesicles (*) are occasionally found near the small pigment granules. Low density vesicles are also seen near type A polymorphic bodies ⟶.

ci - subsurface cistern
mt - microtubule
PC - pigmented cell
sd - septate desmosome
SC - sensory cell
V - vacuole
za - zonula adhaerens
p - pigment granule
A-D - polymorphic inclusions


PC
SC
SC
SC
PC
PC
PC
zb
sd
mi
cj
cj
V
P
C
B
A
A
A
A
D
1.0 µm

are continuous into the distal portions from the neck.

A variety of vesicles are numerous in the cytoplasm of these expansions. Some are large and clear and may in some instances represent sectioned indentations of the cell membrane (Fig. 15A). Other profiles are C-shaped with electron-lucent interiors. Numerous other vesicles, usually 100 to 200 nm in diameter, contain a single, often spherical, inclusion within them (Fig. 16). These vesicles and the C-shaped profiles display a 15 to 20 nm thick bristled coating on their external surface (Figs. 15A, 17A). A similar coating can be seen on the internal surface of the cells' plasma membrane in regions where it is invaginated and often apparently engulfing debris from the lumen (Figs. 15A, 17A).

The distal expansions of the sensory cells are very irregular in form, the plasma membrane of much of the surface being thrown into numerous villous extensions (Figs. 14, 15A). These extensions are readily distinguished from the microvilli of the pigmented cells, being longer and less consistent in width, with less electron-dense interiors (Fig. 18). Also, they never display the surface coating occasionally noted on pigmented cell microvilli (Fig. 17A). The cytoplasm of the sensory cell villi has an appearance similar to the cell proper, and contains few inclusions. The villous extensions, which often measure up to 0.1 μm in diameter, appear long and sinuous and generally present an irregular 'tangled' appearance in the lumen (Fig. 13).

A single cilium also extends into the ocellar lumen from the distal portion of each sensory cell (Fig. 17A). The cilium usually

Figure 15. TEM's of sensory cell distal expansions and cilia.

A. A villus extends from the ciliary shaft membrane, and other villi extend from the plasma membrane of the sensory cell expansion in the lumen. Numerous coated invaginations (≫) of the sensory cell membrane can be noted, apparently producing coated endocytotic vesicles. (X33,000)

B. T/S of cilium illustrates '9 + 2' microtubular pattern. (X42,000)

C. Base of sensory cell cilium shows nine transitional fibres extending to the plasma membrane. Associations of low density vesicles (→) and granular vesicles (*) with small pigment granules can be noted in an adjacent pigmented cell. (X32,000)

c - cilium
ci - subsurface cistern
cv - coated vesicle
i - indentation
mt - microtubule
vi - villus

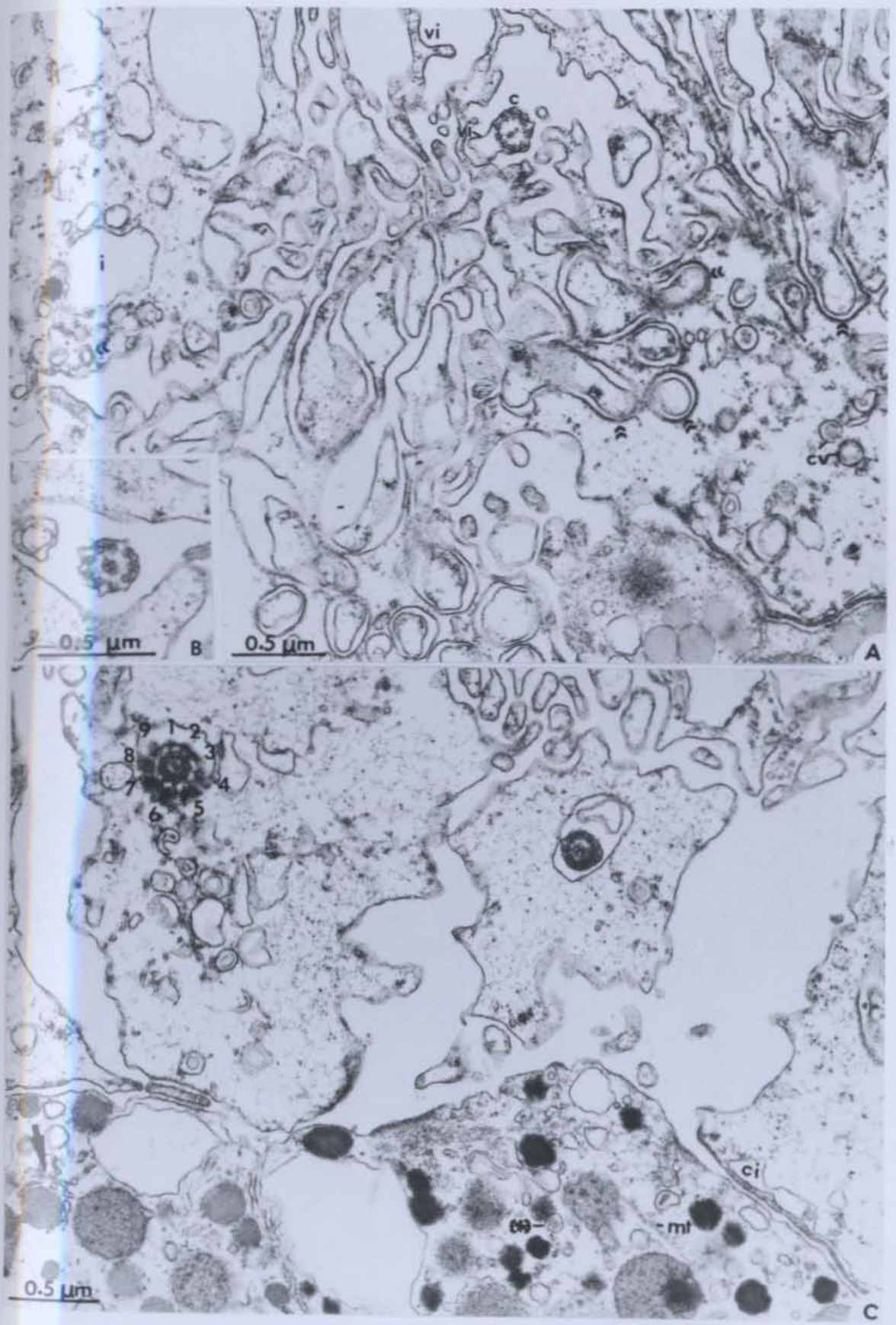

vi
c
i
cv
0.5 µm
B
0.5 µm
A
9
1
2
3
8
4
7
6
5
ci
mi
0.5 µm
C

Figure 16. TEM of T/S of sensory cell cilia showing a variety of microtubular complements. (X20,100)

mt - microtubule
mv - microvillus

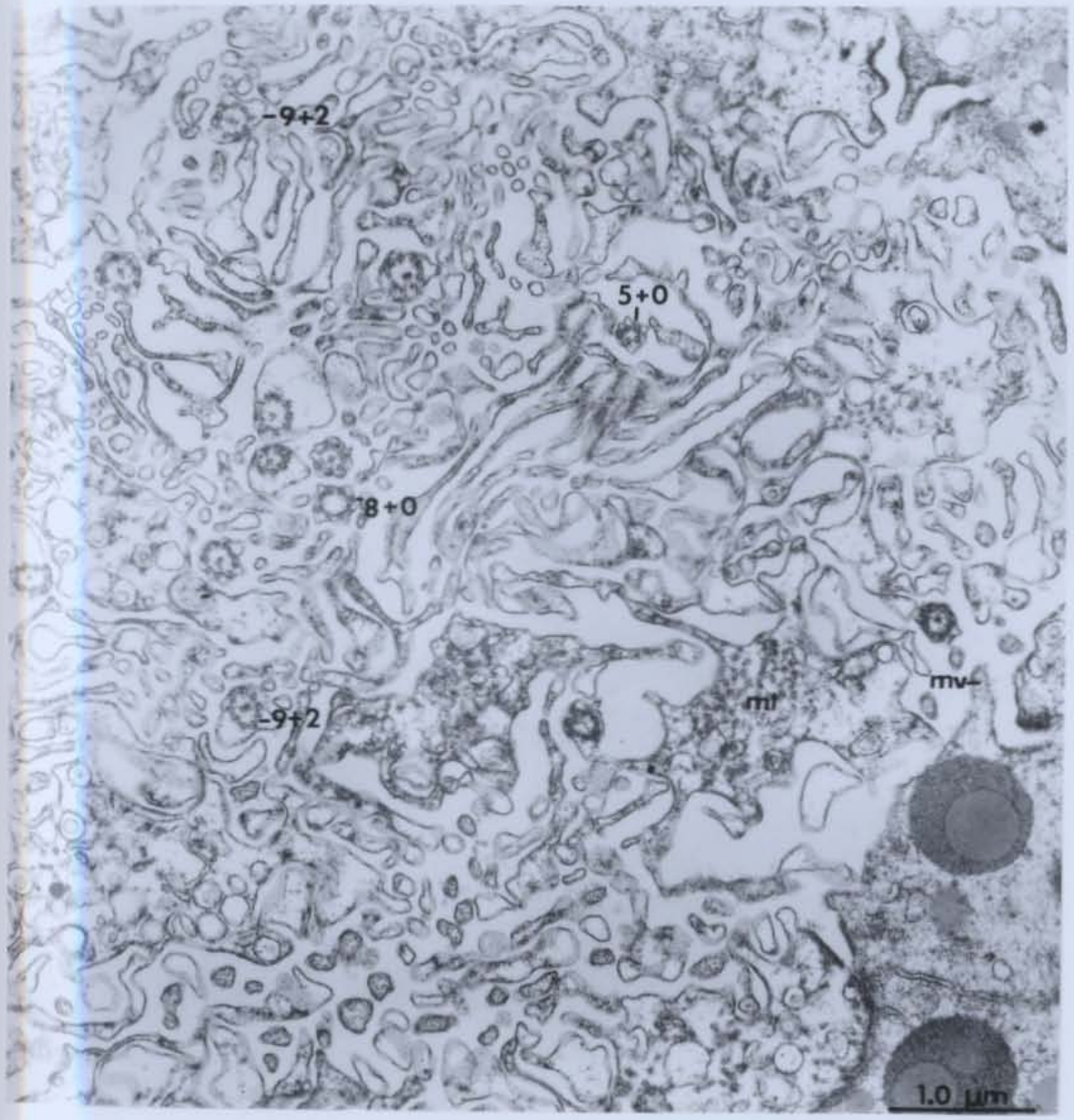

-9+2
5+0
8+0
-9+2
mf
mv
1.0 µm

Figure 17. TEM's of sensory cell cilia showing extensions of villi.

A. L/S of cilium shows villous extension from the shaft, and also the basal plate, basal body, and transitional fibres of the cilium. Sensory cell expansions possess coated invaginations (≫) of the plasma membrane, and numerous vesicles, some coated. Pigmented cells possess microvilli with a filamentous coating. (X25,100)

B,C. T/S's of sensory cell cilia show villi extending from the shaft. (X32,000)

bb - basal body
bp - basal plate
c - cilium
cv - coated vesicle
mv - microvillus of pigmented cell
t - transitional fibre
vi - villus

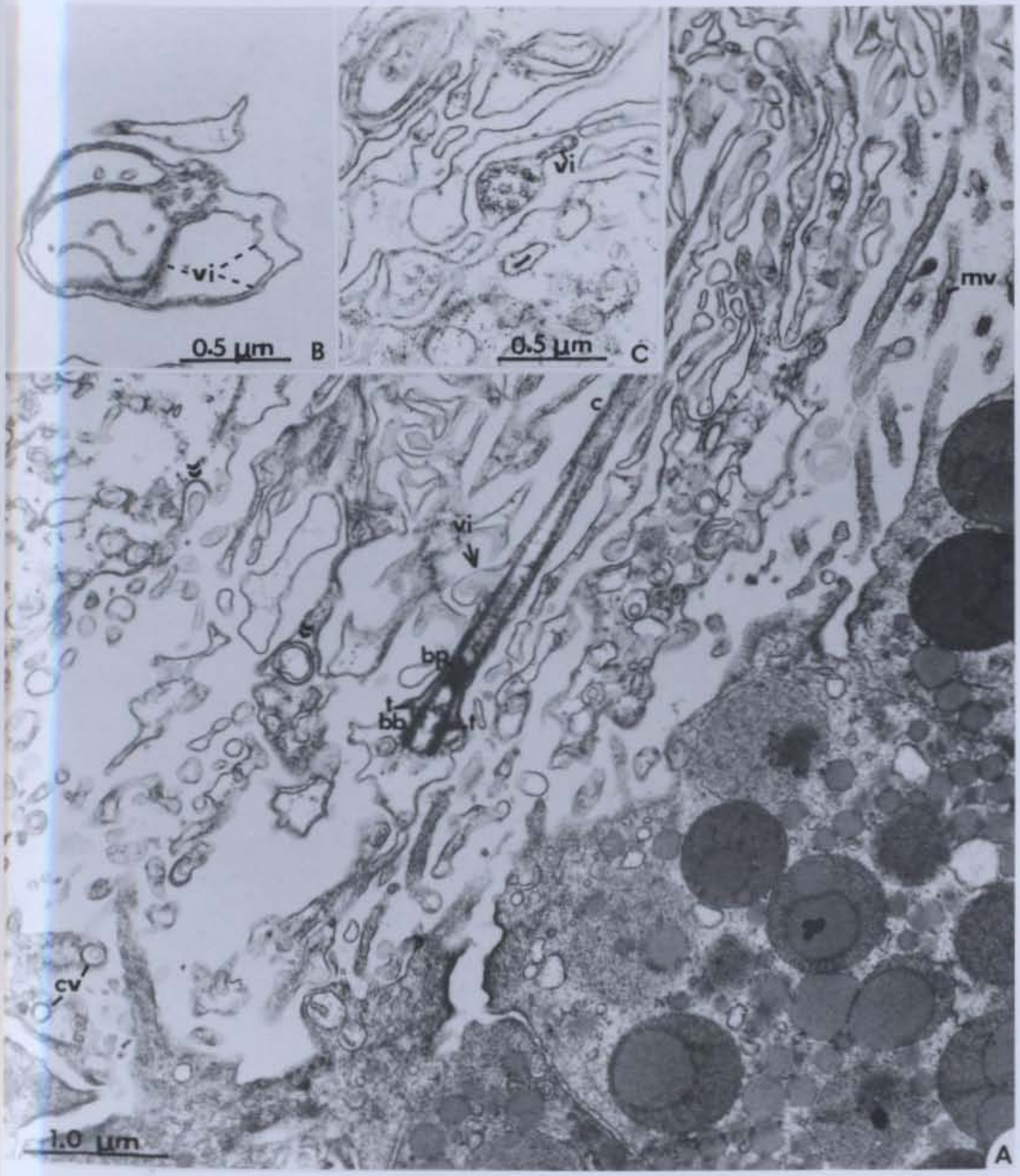
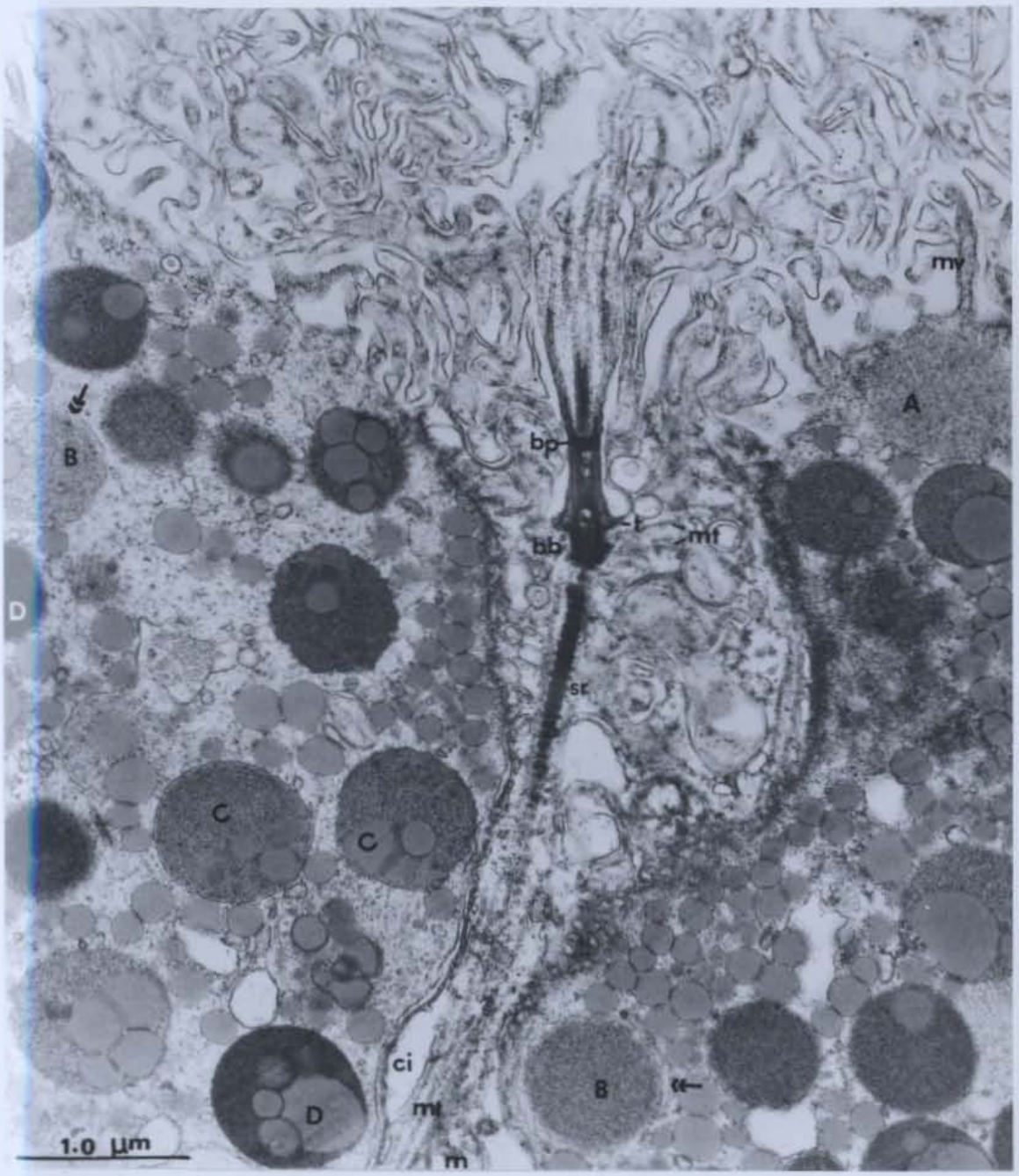

vi
0.5 µm
B
vi
0.5 µm
C
mv
c
vi
bp
bb
cv
1.0 µm
A

Figure 18. TEM of sensory and pigmented cells at the level of the lumen. (X25,100)

Basal morphology of the sensory cell cilium is shown. Type A to D polymorphic bodies are present in the pigmented cells, and two type B bodies display double membranes →» .

bb - basal body
bp - basal plate
ci - subsurface cistern
m - mitochondrion
mt - microtubule
mv - microvillus
sr - striated root
t - transitional fibre


my
A
B
bp
t
mf
bb
D
sf
C
C
ci
D
mt
B
1.0 µm
m

originates from the more elevated apex of the cells, but in those cells without an expanded portion, it arises at the level of the distal border of the pigmented cells (Fig. 18).

The axoneme of the sensory cell cilium is composed of nine pairs of peripheral, and one pair of central microtubules (Figs. 15B, 16). Microtubules of the cilia of sensory cells appear less distinct and regular than do those of the epidermal cells whose axonemal complement was more readily determined. It was not clear whether arms were present on the a-tubules of the peripheral pairs. The central and sometimes peripheral microtubules of the cilia of the sensory cells are often absent, thus producing a variety of axonemal configurations in some sections (Fig. 16).

The cilium is usually situated in a depression in the cell surface, and its electron-dense basal plate is located up to 0.5 μm above the level at which the ciliary membrane rises from the cell membrane (Fig. 18). The basal bodies measure approximately 0.3 μm in length and 0.2 μm in diameter. Nine projections (transitional fibres) extend from the distal ends of the basal body to the plasma membrane (Figs. 17A, 18), and are also readily noted in cross-sectional views (Fig. 15C). The area of the cell membrane contacted by these projections and continuing toward the basal plate appears thickened.

A prominent extension from the proximal end of the basal body is the root, 100 nm in width with a periodicity of striations of approximately 60 nm (Fig. 18). This root projects deep into the neck of the cell. Oriented at right angles to the basal body is an accessory

centriole (not illustrated), and radiating from the basal body and often extending along beside the striated root, are microtubules (Fig. 18).

As described above, the plasma membrane of the distal expansion of the sensory cell bearing the cilium, gives rise to numerous extensions. Such extensions may also occasionally be seen to originate from the lower portions of the ciliary shafts (Figs. 15A,17A,B,C). Many of the cilia in cross-section, however, present merely an irregular contour of the ciliary membrane (Fig. 16), suggesting that the villi do not arise from along the whole length of the shaft, or may in fact be few in number. Some longitudinal sections through portions of cilia present an appearance much like that of typical motile ones, with ciliary shafts extending for up to four μm, devoid of any extensions from their membrane.

(ii) Neck Portion

The portion of the sensory cell that extends down between the pigmented cells is narrow, being generally less than two μm in diameter (Fig. 13). Zonulae adhaerentes and septate desmosomes seen in some sections connect these cells with the adjacent pigmented cells (Fig. 14).

The lateral borders of the neck are further specialized. Below their most distal contact with pigmented cells, the sensory cells possess cisternae or flattened sacs that are applied against the internal face of the plasma membrane (Figs. 14, 15C). The space between the cell membrane and the adjacent cisternae is of a relatively constant width

of approximately 20 nm, and is filled with an electron-dense material. Cross-sections through the neck illustrate that virtually the total periphery of the sensory cells is lined in this manner (Fig. 12). Some cisternae are very flattened in profile, while others are expanded into the cell and may contain a small amount of finely filamentous material. Numerous microtubules running longitudinally down the neck may often be seen in close association with these cisternae. Mitochondria too may be closely applied to the cisternae (Fig. 12) and very occasionally, ribosomes are present nearby on the cytoplasmic side of the cisternal membrane. This cisternal lining of the cell membrane terminates distal to the nuclear region of the sensory cells.

The varied vesicles described in the distal expansions of the sensory cells are also very numerous in the neck (Fig. 14). These include large clear vacuoles (some perhaps glancing sections of the cisternae that line the cell's borders), clear vesicles 150 nm in diameter, C-shaped sacs, and vesicles with an inclusion, these latter two types of profiles often displaying coated external membranes.

The striated root originating from the cilium in the more distal region of the sensory cell, extends for some distance down the neck (Fig. 18). Mitochondria are usually found in the proximal portion of the neck region (Figs. 12, 18).

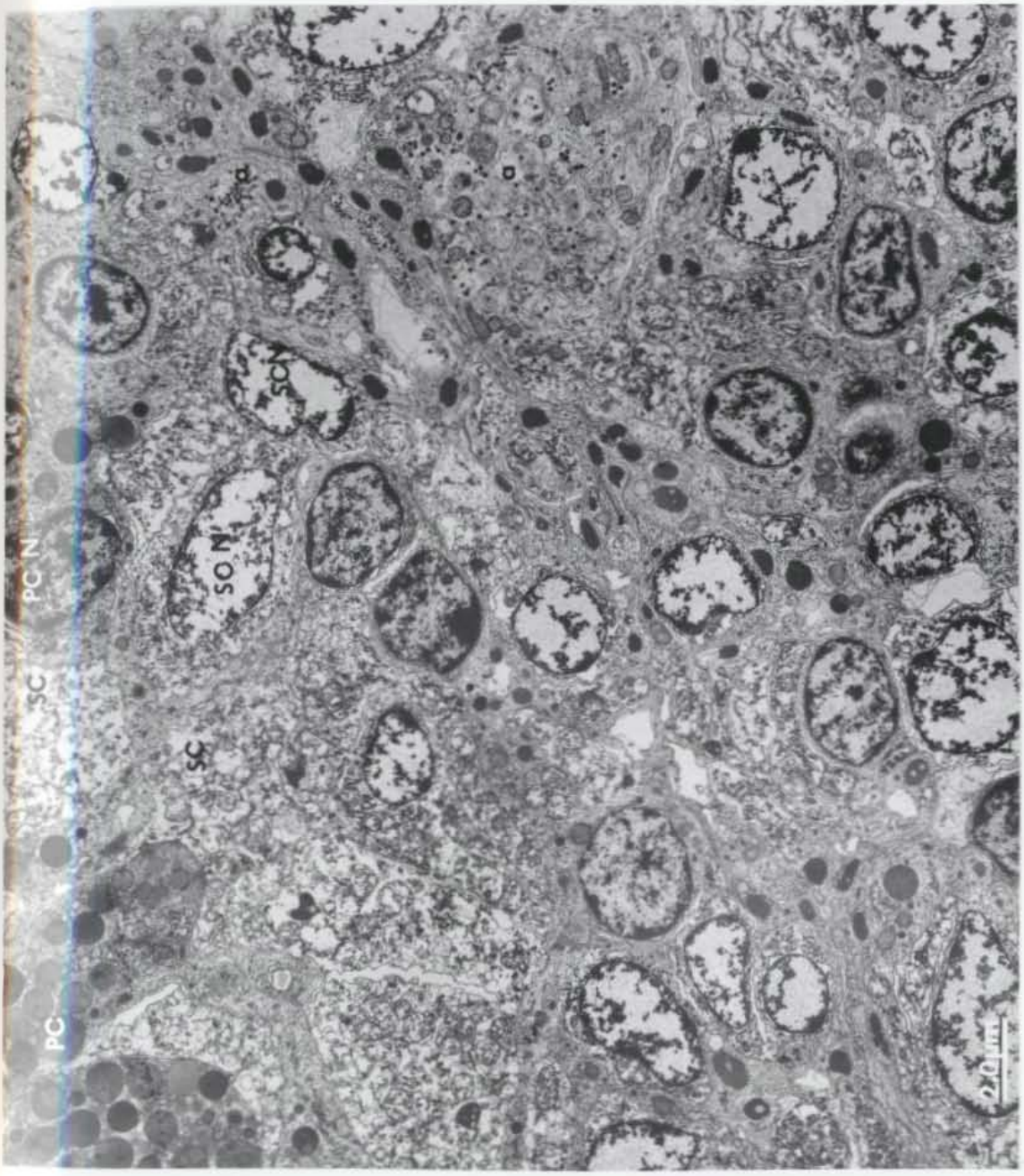
(iii) Nuclear Region

After passing between the pigmented cells, the sensory cells expand in diameter into a region that contains the nucleus and other organelles (Fig. 19). The cytoplasm of the sensory cell is readily

Figure 19. TEM of oblique section of nerve plexus below an ocellus. (X6050)

Sensory cells at upper left are readily distinguished from pigmented cells by the nature of their cytoplasmic contents and by the lighter staining characteristics of their nuclei. Axons, to the right, possess vesicles.

a - axon
N - nucleus
PC - pigmented cell
SC - sensory cell


PC
SC
PC N
SO N
SC
SO N
SC
D
D
1.0 μ

distinguished from that of the interspersed epidermal, and pigmented cells, by its electron-lucent ground substance.

Mitochondria are very numerous in this area, and they are broader (> 0.5 μm in diameter), more irregular in outline, and possess a less electron-dense matrix than those in the pigmented cells (Figs. 20, 21). The inner and outer mitochondrial membranes are often separated by an irregular gap, and tubular cristae, generally more extensive, though less clearly delineated than those of the pigmented cell mitochondria, project from the inner mitochondrial membrane. Rough endoplasmic reticulum cisternae are frequently found in close association with the mitochondria.

Rough endoplasmic reticulum also surrounds the nucleus and is generally prominent in the cytoplasm of this region (Fig. 21). Clumps of ribosomes are present as well. Microtubules continue through the mid portion of the cell and a few extend proximally past the nucleus (Fig. 23).

A well developed Golgi complex is situated distal to the nucleus (Fig. 22). It consists of low density outer saccules, and several, more flattened, inner cisternae with denser interiors. Some clear vesicles are associated with the extremities of some of the outer cisternae, while the inner ones appear to give rise to small, 60 nm diameter, dense vesicles, some of which appear coated (Fig. 22). Other, larger coated vesicles in the mid portions of the sensory cells include the C-shaped and other endocytotic vesicles noted in the distal and neck regions (Figs. 22, 23). Other vesicles do not possess an external coating.

Figure 20. TEM of supranuclear regions of sensory and pigmented cells. (X25,100)

High and low density vesicles are associated with the Golgi apparatus of the pigmented cells. The sensory cells contain vesicles (some coated), and prominent rough endoplasmic reticulum, some cisternae of which surround the numerous swollen mitochondria.

EC - epithelial cell
fb - filamentous bundle
G - Golgi apparatus
hv - high density vesicle
lv - low density vesicle
m - mitochondrion
PC - pigmented cell
rb - residual body
SC - sensory cell
pm - polymorphic body
rer - rough endoplasmic reticulum

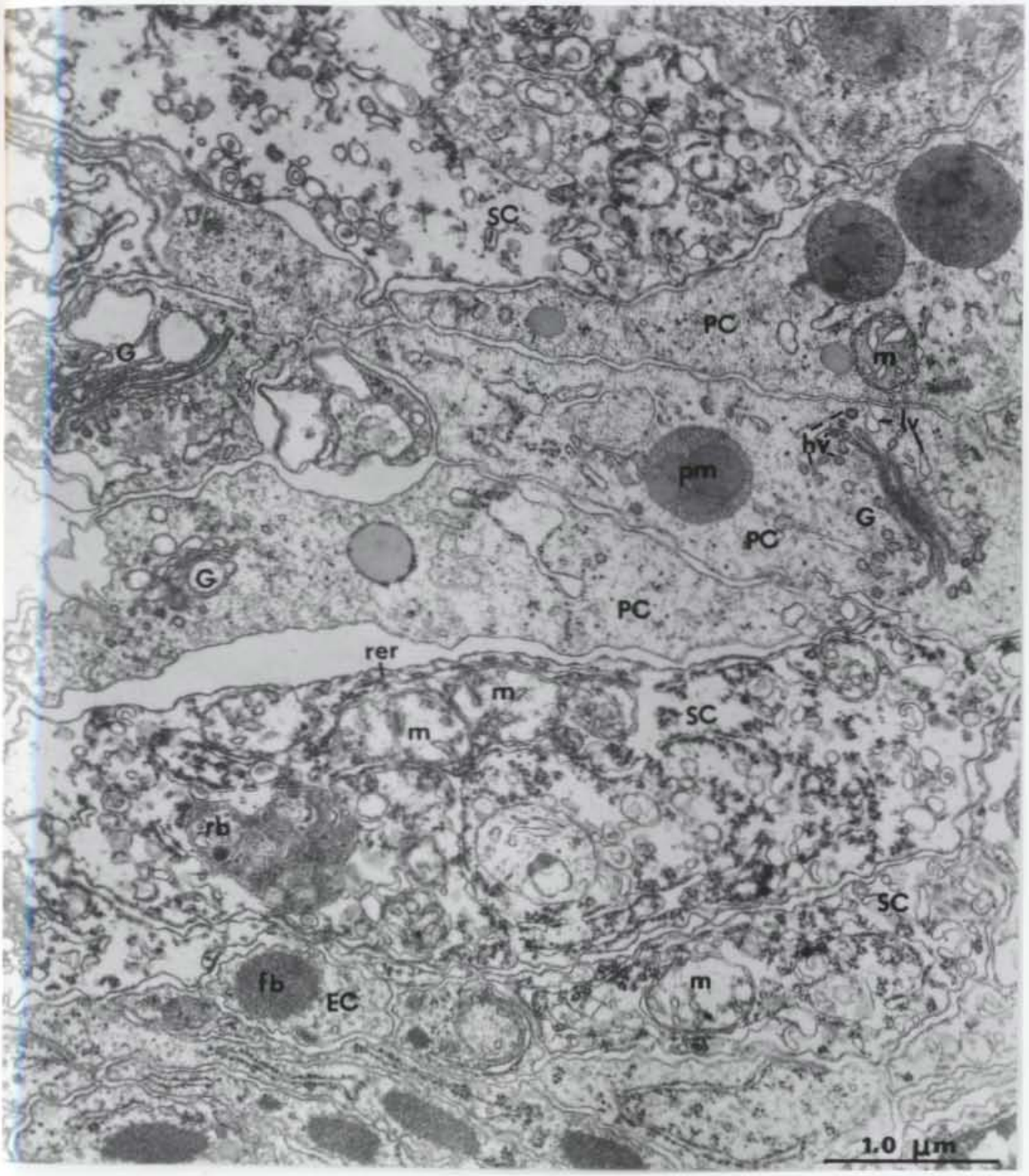

SC
PC
G
m
lv
hv
pm
G
PC
G
PC
rer
m
m
SC
rb
SC
fb
EC
m
1.0 µm

Figure 21. TEM of nuclear region of sensory and pigmented cells. (X25,100)

The differences in nuclear and mitochondrial morphology of the pigmented and sensory cells are illustrated. A few coated vesicles are noted in this region of the sensory cells.

→ - nuclear pore

cv - coated vesicle
m - mitochondrion
N - nucleus
pm - polymorphic body
PC - pigmented cell
rer - rough endoplasmic reticulum
SC - sensory cell

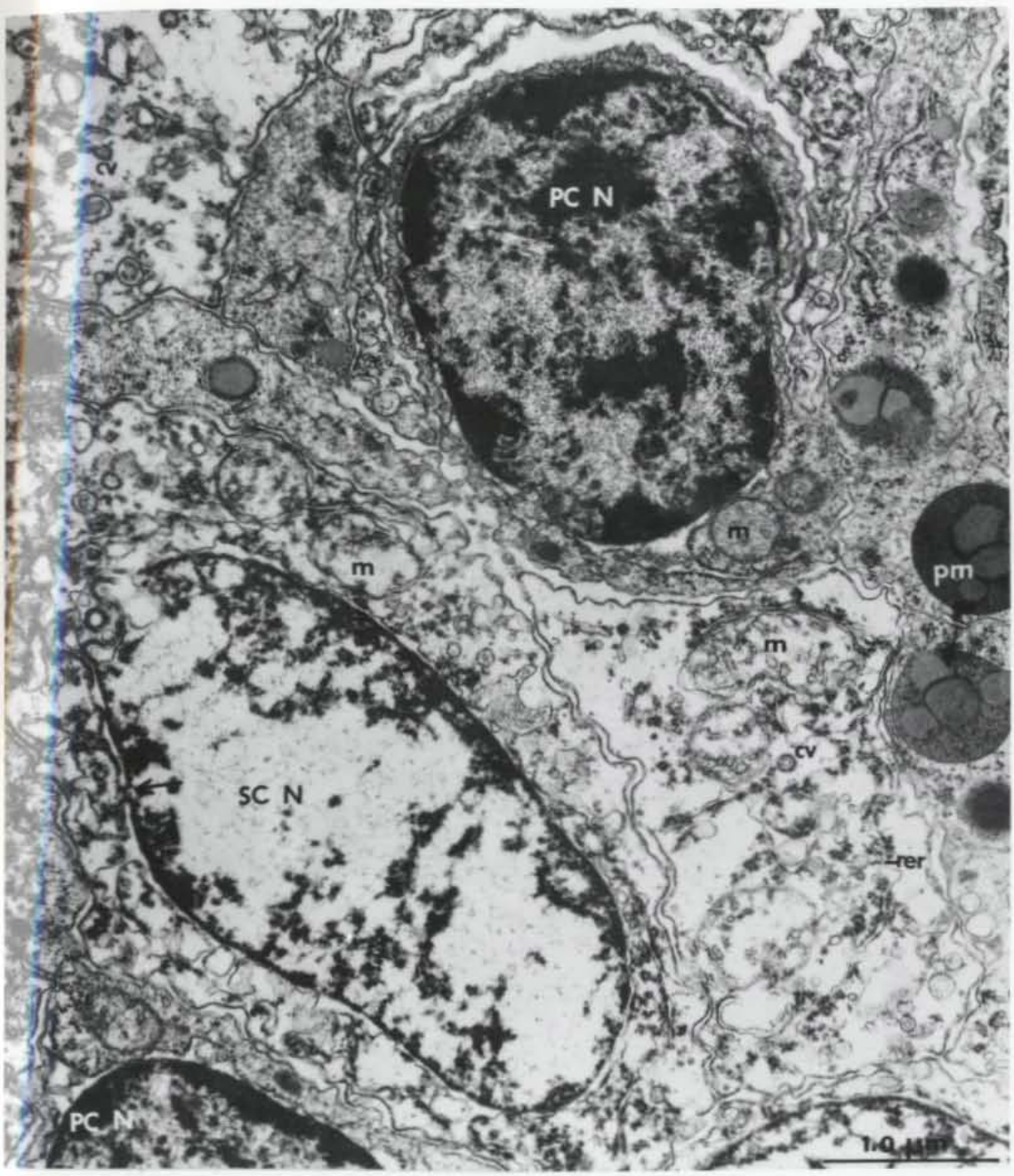

PC N
m
m
pm
m
cv
SC N
rer
PC N

Figure 22. TEM of supranuclear and nuclear regions of sensory cells. (X25,100)

The sensory cell nucleus contains an eccentric nucleolus. High and low density vesicles are associated with the extensive Golgi apparatus.

cv - coated vesicle
G - Golgi apparatus
hv - high density vesicle
lv - low density vesicle
m - mitochondrion
n - nucleolus
N - nucleus
pm - polymorphic body
rer - rough endoplasmic reticulum
SC - sensory cell

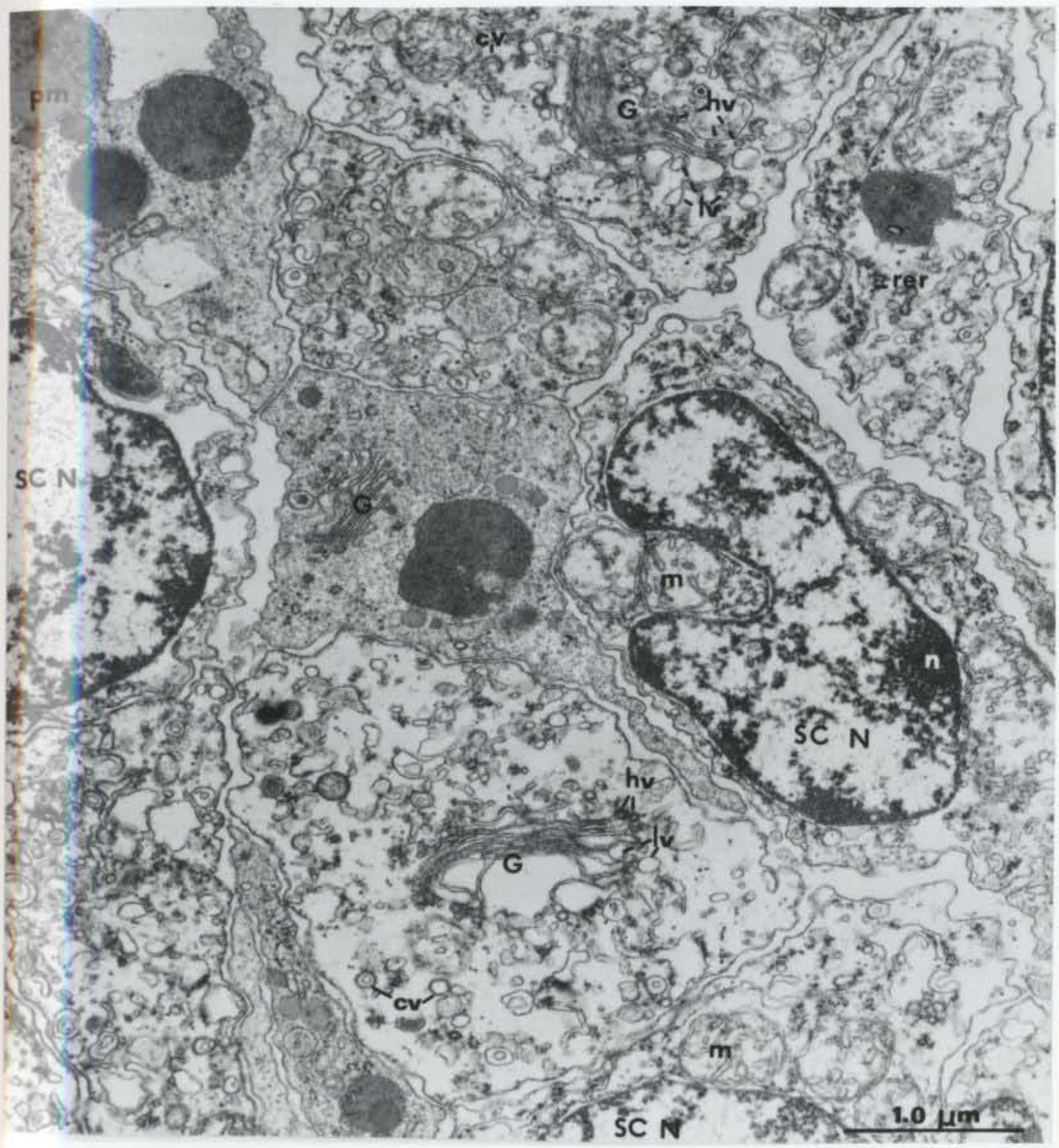
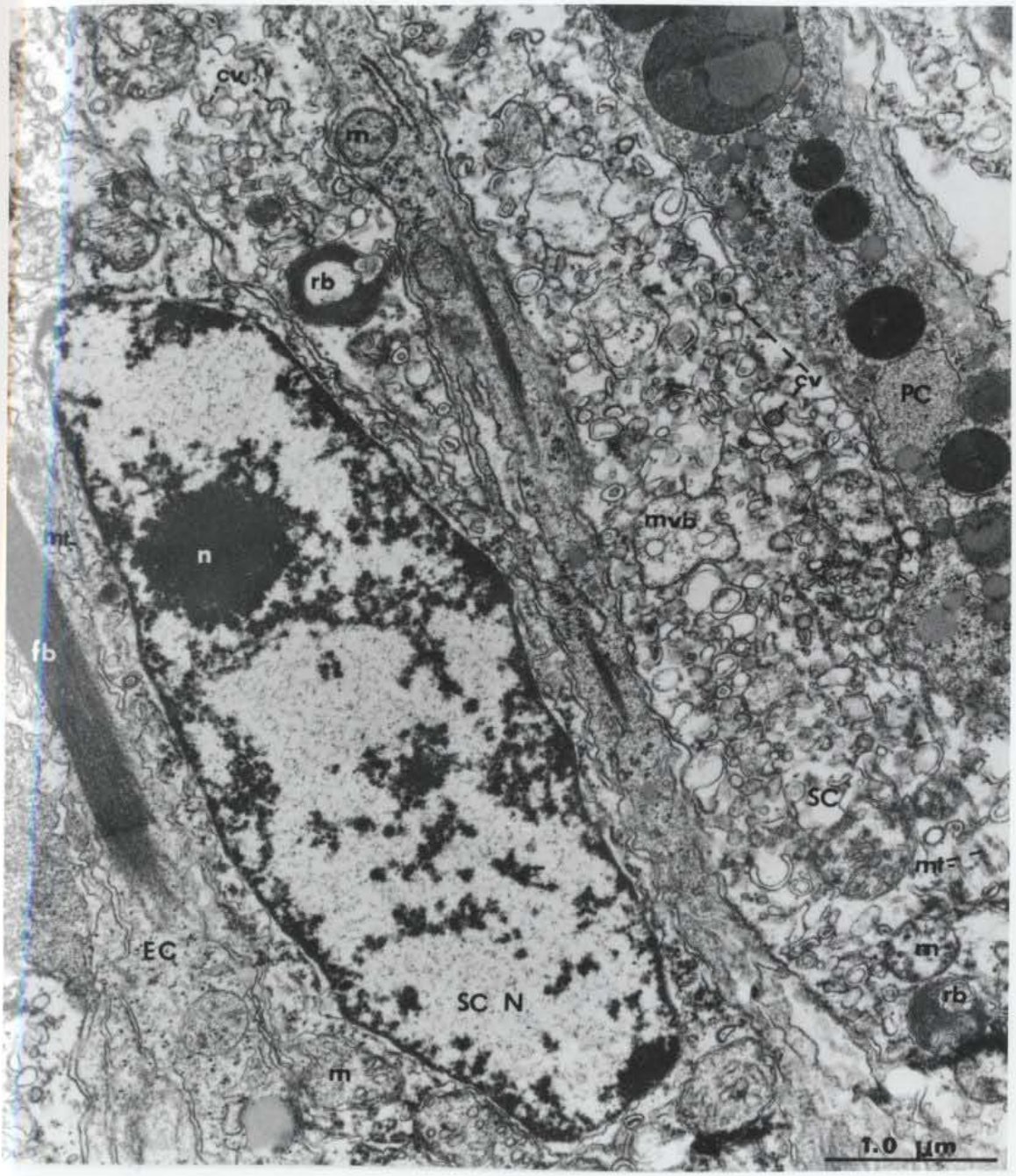

cv
pm
G
hv
lv
rer
SC N
G
m
n
SC N
hv
lv
G
cv
m
SC N
1.0 µm

Figure 23. TEM of L/S through nuclear region of sensory cells. (X25,100)

Vesicular profiles are numerous in this region, and microtubules extend past the nucleus which displays a prominent nucleolus.

cv - coated vesicle
EC - epithelial cell
fb - filamentous bundle
m - mitochondrion
mt - microtubule
mvb - multivesicular body
n - nucleolus
N - nucleus
PC - pigmented cell
SC - sensory cell
rb - residual body


cv
m
rb
cv
PC
mvb
mt
n
fb
SC
mt
m
rb
EC
SC N
m
1.0 µm

Multivesicular bodies, a few residual bodies (Fig. 23), and multilamellar whorls are noted in this area of the cell.

The nuclei of the sensory cells are elongate, usually measuring seven μm in length and two to three μm in width (Fig. 23). In cross-section, the nuclei are rounded or indented (Fig. 22). The ground substance of the nuclei is finely filamentous and generally appears less electron-dense than the nucleoplasm of the pigmented, corneal, and epidermal cells (Figs. 19, 21). Chromatin is condensed to some extent within the nucleoplasm, but especially so at the periphery. A granular nucleolus, one μm in diameter, is visible in the nuclei, and usually occupies an eccentric position near the nuclear membrane (Figs. 22, 23). The outer layer of the nuclear membrane, coated externally with ribosomes, is pulled away from the nucleus, and contacts the inner membrane at the sites of nuclear pores (Fig. 21).

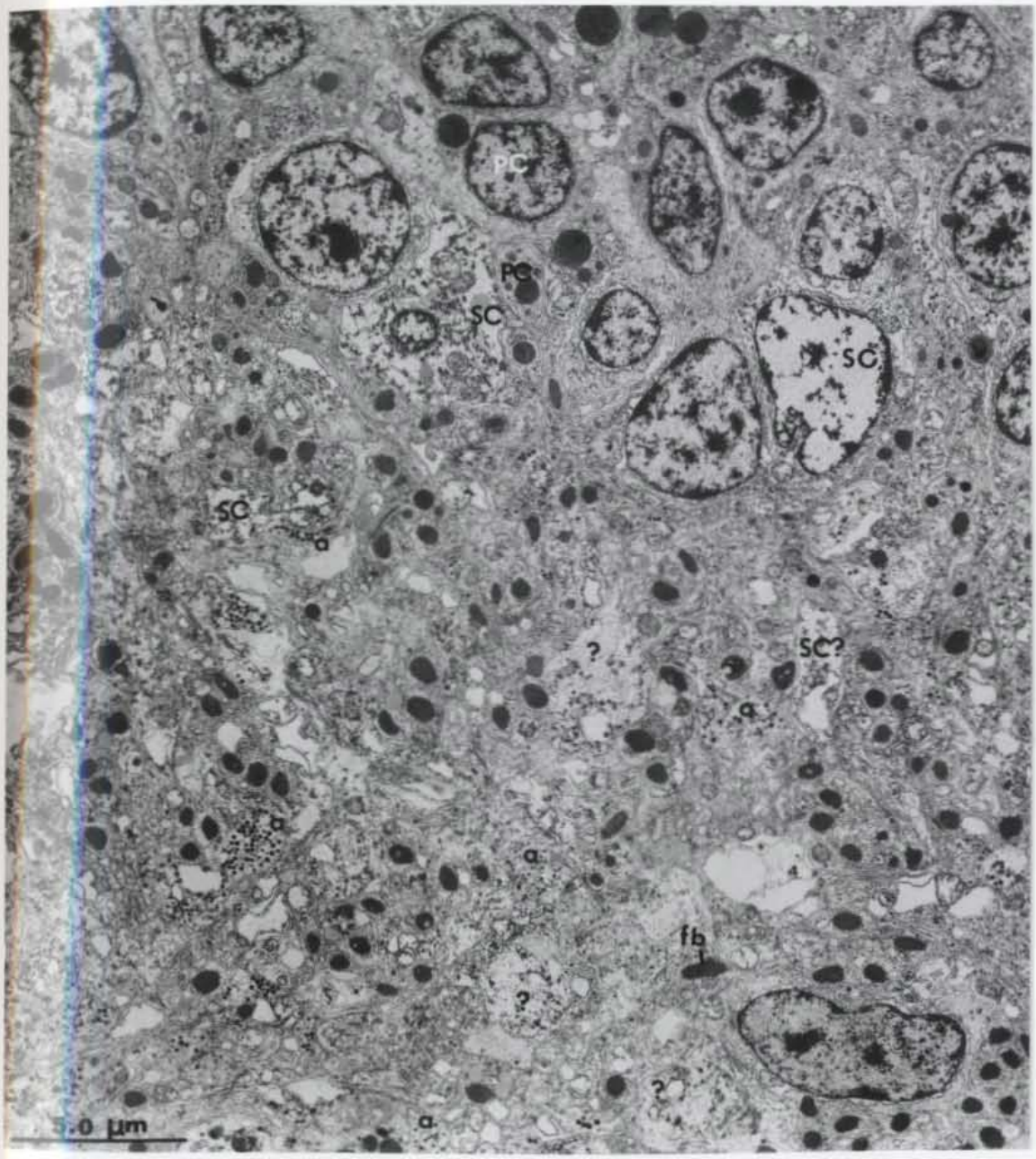
(iv) Basal Portion

Below the level of the nucleus, the sensory cells taper and continue into the nerve plexus. The very large numbers of intertwined axons and proximal portions of epidermal and ocellar cells in this region, make the interpretation of the precise termination of these sensory cell processes difficult. For example, in a plane at which various levels of cells have been sectioned (Fig. 24), some cell types, such as the pigmented and sensory cells, are recognized in the region of their nuclei (at the top of Fig. 24). In more proximal regions, to the bottom, the bases of the epidermal support cells are easily identified by the presence of longitudinally oriented fibre bundles in their

Figure 24. TEM showing ectoneural plexus and basal regions of sensory, pigmented, and epithelial cells. (X5050)

Many processes (?) are difficult to distinguish as axons or sensory cell processes.

a - axon
fb - filamentous bundle
PC - pigmented cell
SC - sensory cell


PC
PC
SC
SC
SC
SC
a
?
SC?
a
a
a
fb
?
?
a
1.0 µm

cytoplasm. Similarly, the occasional pigment granule serves to denote some processes as being derived from the pigmented cells of the ocellus. However, the electron-lucidity of the cytoplasm and the presence of microtubules and vesicles in both sensory cell processes and in axons, make their distinction increasingly difficult.

The processes of sensory cells traced most proximally into the plexus display the following features (Fig. 25). The cytoplasm of these processes is very electron-lucent and in regions of some cells, appears almost empty. In the cytoplasm are found scattered microtubules as well as ribosomes and some rough endoplasmic reticulum. Mitochondria and occasional multilamellar whorls are also present (not illustrated).

In addition, vesicles are a common component in the cytoplasm. Clear vesicles and vacuoles of various sizes are found in this region, though they are not as abundant as in the more distal portions of the sensory cells. Also found, however, in these proximal processes, are vesicles, 60 to 160 nm in diameter, with dense contents sometimes separated from the vesicle's limiting membrane by a clear space.

(d) Pigmented Cells

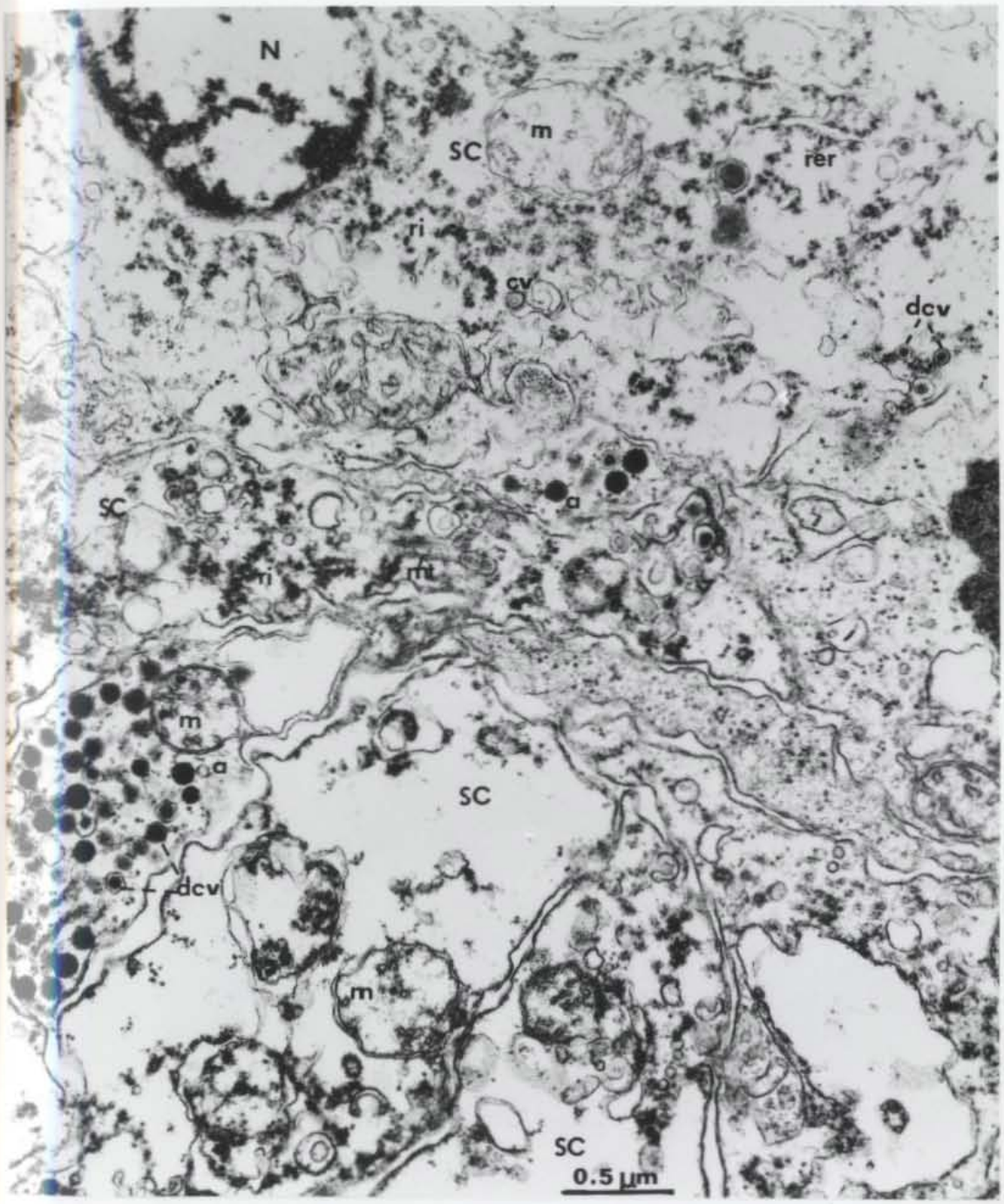
Pigmented cells form the bulk of the ocellar wall bounding the lumen (Fig. 13). These cells are large, often measuring seven μm in width, and display zonulae adhaerentes and septate desmosomal regions with adjacent pigmented, corneal and sensory cells (Figs. 10A, 14).

Microvilli project from the pigmented cells up to three μm into the lumen (Fig. 17A, 18). These microvilli are occasionally seen to be

Figure 25. TEM of basal regions of sensory cells at the level of nerve axons. (X32,000)

Axons contain large, dense-cored vesicles, mitochondria, and scattered ribosomes. Sensory cells contain large mitochondria, rough endoplasmic reticulum, microtubules, and some dense-cored vesicles.

a - axon
cv - coated vesicle
dcv - dense-cored vesicle
m - mitochondrion
mt - microtubule
N - nucleus
rer - rough endoplasmic reticulum
ri - ribosomes
SC - sensory cell


N
SC
m
rer
ri
cv
dcv
SC
a
ri
mt
m
a
SC
dcv
m
SC
0.5 µm

branched and contain cytoplasm of granular nature similar to that of the cell body, but do not contain organelles or microtubular inclusions. Fine short filaments are often seen to coat the surface of these microvilli (Fig. 17A).

The pigmented cells are characterized by the presence in their cytoplasm of numerous inclusions which fall into two main sizes and morphological classes. The first class is very numerous and is spherical, ranging in diameter from 0.1 to 0.5 μm, with most being approximately 0.3 μm in diameter. They appear uniformly and moderately electron-dense (Fig. 14). These pigmentary inclusions are not membrane-bound, but most display a densely staining interface with the cytoplasm. The smaller inclusions of this type have indistinct peripheries and are more electron-lucent than larger ones. Low density vesicles are occasionally seen to be located near these inclusions, and vesicles with granular contents are also occasionally present nearby (Figs. 11, 14, 15C).

Less numerous, but still prominent in the pigmented cells of L. polaris ocelli, is a second group of inclusions which typically measure one μm in diameter. These large inclusions present a variety of appearances suggesting stages of elaboration. One variety, designated as type 'A' for purposes of description, comprises individual small pigment granules, or more extensive granular areas of cytoplasm which may contain pigment granules and other inclusions, partially enclosed by a membrane or by a grouping of low density vesicles (Fig. 14). In some cases, these low density vesicles are apparently fusing

with the bounding membrane.

Completely delimited profiles (B) are spherical in shape (Fig. 14). Low density vesicles may still be closely associated with their enclosing membranes. Occasionally, there appears to be a double membrane delimiting these profiles (Fig. 12, 18). The matrix of these bodies is more electron-opaque than in A profiles, and possesses one to several homogeneously stained spherical patches that are identical in appearance to the small pigment granules in the cytoplasm. Small, dense, 40 nm diameter spherical profiles may be noted in the matrix of some A and B forms or in the surrounding cytoplasm (Fig. 11).

Up to eight lighter patches may be visible in sections of some profiles (C), some patches appearing to be fusing with (or being extruded from) the main body (Figs. 11, 14). The bounding membranes of these large polymorphic bodies are often indistinct. The electron-dense matrix frequently possesses groups of parallel membranes. Denser bodies (D) and large multilamellar bodies (E) are more rarely noted in the cytoplasm of these cells.

There are various other inclusions in the cytoplasm of the pigmented cells. Large vacuoles containing a small amount of flocculent material are frequently noted, as are high and low density vesicles (Figs. 11, 14). Mitochondria are rounded with a moderately electron-dense matrix and tubular cristae (Fig. 11). Low density profiles and vesicles, 60 nm in diameter with dense contents, are associated with the Golgi apparatus which is located in the middle, more tapered portion of the cell (Fig. 20). Free ribosomes, and cisternae of rough endoplasmic

reticulum are present in the cytoplasm, and microtubules are occasionally sectioned (Fig. 15C).

The nuclei are located in the proximal portions of the cells, which narrow and pass radially through the epidermal layer of the optic cushion (Figs. 19, 21, 24). The nuclei have a denser matrix than those of the sensory cells. Slender processes with the occasional pigment granule can be seen among axons at the level of the nerve plexus. The eventual 'fate' of these processes has not been clearly determined, although the presence of a polymorphic body in a cellular process near the basal lamina (Fig. 28A), suggests that the pigmented cells may terminate at the connective tissue layer in the manner of the epithelial supporting cells.

(e) Radial Nerve Cord

The radial nerve cord of each ray is composed of an ectoneural portion which is separated by a connective tissue layer from a thinner hyponeural portion. The ectoneural component of the radial nerve cord lies beneath, and is contributed to by, elements of the external epithelium. It measures up to 75 μm in thickness on the optic cushion, and consists of large numbers of axons directed primarily longitudinally along the ray (Figs. 7C, 26A).

The axons vary in diameter, being commonly observed with diameters of between 0.2 and 1.0 μm. Their contents include longitudinally oriented microtubules, large mitochondria with loosely structured cristae, free ribosomes, some large vacuoles, and a variety of vesicles (Fig. 26A, 27).

Figure 26. TEM's of ectoneural plexus.

A. Section shows numerous axons traversed by the filamentous bundle-containing epithelial cells. Localized accumulations of vesicles occur in some axons ⟶ . (X5050)

B, C. Higher magnification TEM's of such localized accumulations of vesicles. (X6200)

fb - filamentous bundle

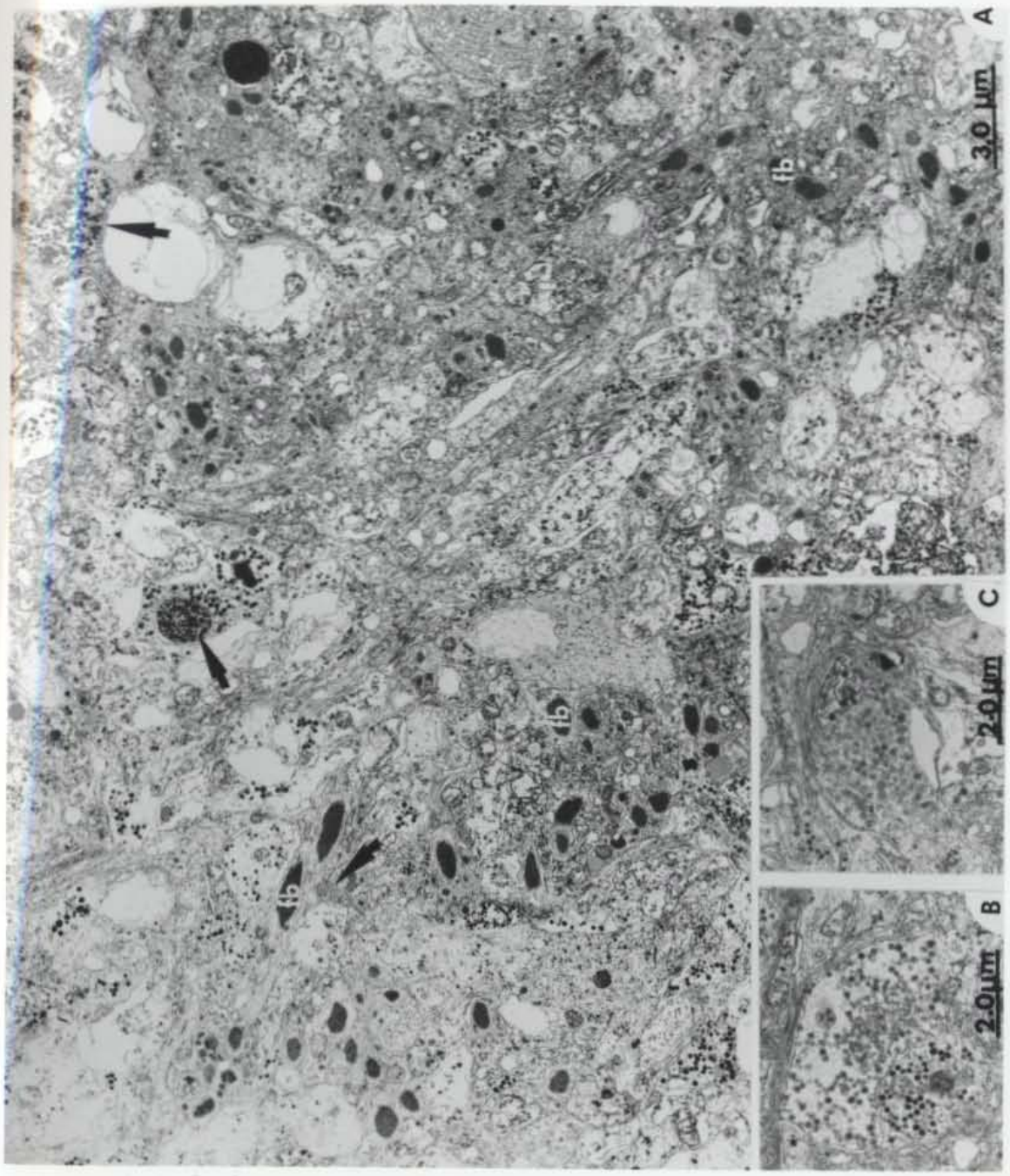
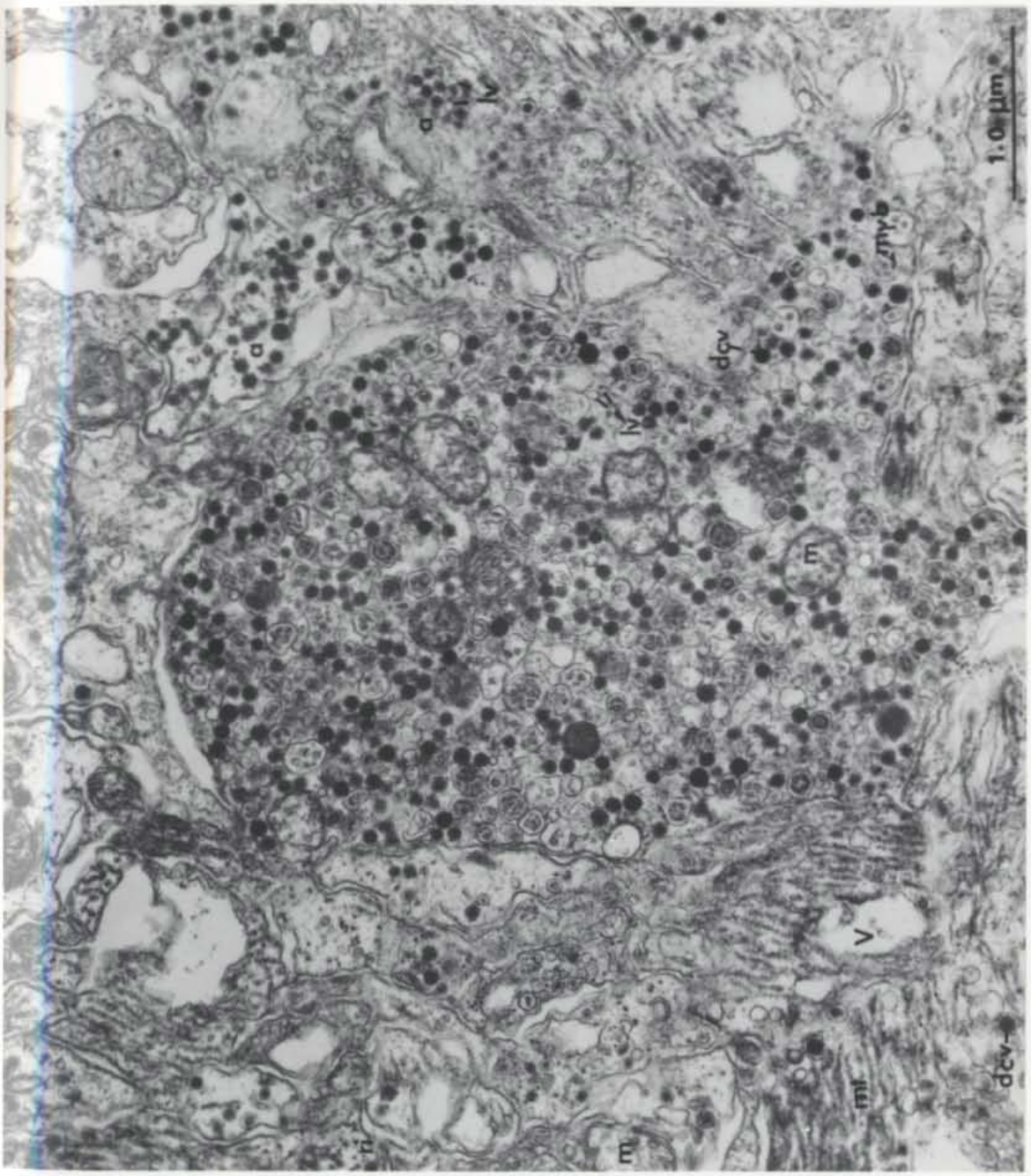

A
3.0 µm
fb
fb
fb
C
2.0µm
B
2.0µm

Figure 27. TEM of presumptive neurosecretory cell in ectoneural plexus. (X25,100)

Neurosecretory cell contains large numbers of dense-cored vesicles, low density vesicles, multivesicular bodies, and mitochondria. Axons contain microtubules, ribosomes, mitochondria, vesicles, and vacuoles.

a - axon
dcv - dense-cored vesicle
lv - low density vesicle
m - mitochondrion
mt - microtubule
mvb - multivesicular body
ri - ribosomes
V - vacuole


1.0 µm
dcv
V
E
dcv
m
m
m

Some vesicles range from 50 to 120 nm in diameter (most averaging 60 to 80 nm) and are clear or have faintly staining contents. Other vesicles, which tend to be larger, up to 160 nm in diameter, have electron-dense contents. These contents fill the vesicles completely or are separated from the limiting membrane by a clear space (Fig. 27).

Large axonal profiles often contain numerous microtubules with scattered mitochondria and a few dense-cored or clear vesicles. Other profiles show larger concentrations of vesicles of the dense-cored type (Figs. 26A, B, C). Clear or lightly stained vesicles, in particular, are frequently confined to localized and often expanded axonal profiles.

Also obvious in the nerve cord are cells that contain numerous dense-cored vesicles, 80 to 160 nm in diameter, other vesicles with less dense contents, and multivesicular bodies (Fig. 27). Many long mitochondria with electron-dense matrices are common in these cells as well, and occasional microtubules may be present. These cells are broad, often exceeding six μm across, and may possess several processes. Other than these presumptive neurosecretory cells, cell bodies are rarely encountered in the ectoneural portion of the radial nerve cord of the optic cushion.

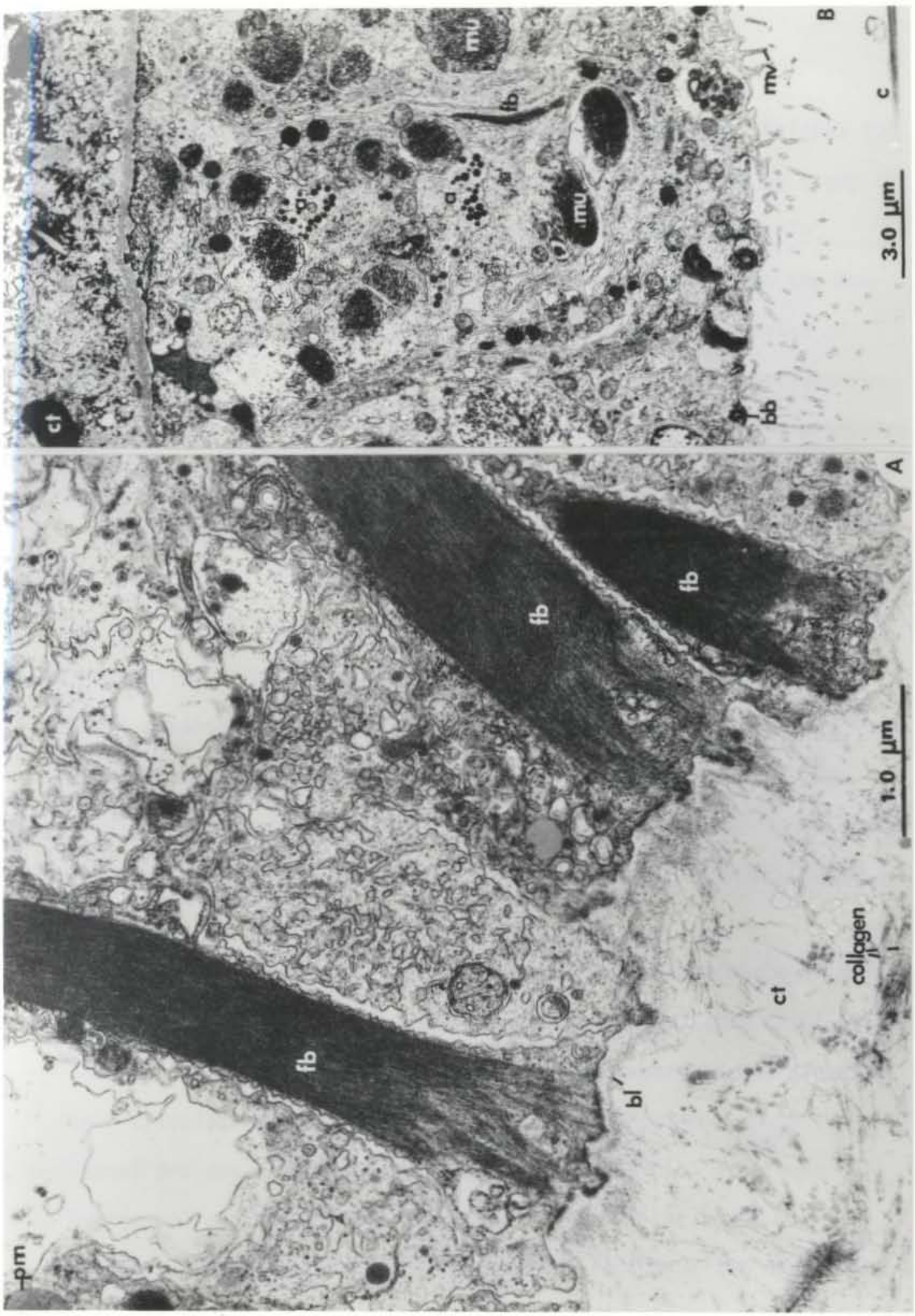
The ectoneural plexus is traversed by the proximal portions of epithelial supporting cells with fibre bundles in their cytoplasm (Fig. 28A). These bundles spread out at their proximal end and contact the cell's plasma membrane adjacent to the basal lamina delimiting the connective tissue layer. There is an accumulation of electron-dense material on the internal face of this portion of the cell membrane at

Figure 28A. TEM of L/S of bases of epithelial cells terminating at the connective tissue layer. (X25,100)

The filamentous bundles terminate at thickened regions of the epithelial cell membrane. Following the contours of the epithelial cell termini is a basal lamina. The connective tissue layer contains collagen, and filamentous material. A polymorphic body is present in a cell at the left of the figure.

28B. TEM of connective tissue layer, hyponeural and muscular tissues, and ciliated coelomic epithelium bordering on the radial water canal of the optic cushion. (X6050) L/S.

a - axon
bb - basal body
bl - basal lamina
c - cilium
ct - connective tissue
fb - filamentous bundle
mu - muscle
mv - microvillus
pm - polymorphic body


pm
fb
fb
fb
bl
ct
collagen
1.0 µm
A
ct
mu
fb
mu
bb
mv
3.0 µm
B
c

the site of contact of the fibre bundles.

The basal lamina, approximately 50 nm in thickness, follows the proximal contours of these cells and is separated from them by a gap of about 50 nm (Fig. 28A). The lamina itself is composed of finely fibrillar material and from this layer, filaments extend into the connective tissue. Much of the connective tissue layer is composed of collagen fibrils, up to 150 nm in diameter, with a banding pattern repeating every 60 nm. Fine filaments and fibroblasts with long processes are also found in the connective tissue layer (not illustrated).

At the level of the optic cushion, the reduced hyponeural component of the radial nerve cord lies between the connective tissue layer and the epithelial layer lining the radial water canal (Fig. 28B). This epithelium is composed of ciliated cells joined by desmosomes. Each cell possesses microvilli, several of which form a palisade around the long cilium of the cell. There is no extracellular revestment surrounding the microvilli such as there is on the external epithelium. Some of the coelomic epithelial cells possess bundles of fibres which extend toward the connective tissue layer.

Muscle cells are located between the coelomic epithelium and the connective tissue layer and are aligned primarily along the longitudinal axis of the ray (Fig. 28B). The large numbers of myofilaments in their cytoplasm have a somewhat irregular orientation. Only a few axonal processes with dense-cored or other vesicles are found in the hyponeural portion of the radial nerve cord in the optic cushion, and are particularly rare in the mid-sagittal region of the nerve cord.

B. DEVELOPMENT OF LARVAE, OPTIC CUSHIONS, AND OCELLI

GENERAL OBSERVATIONS ON SPAWNING, BROODING, AND LARVAL DEVELOPMENT

In 1974, ten egg masses were spawned between February 24 and March 13 in the laboratory, and in the following year, 16 egg masses were produced between February 3 and March 27. These eggs were abandoned by the females within two weeks, with the exception, in 1975, of two females in a different wet-table (less crowded with sea stars), which continued to brood for 5½ and 6½ months, the larvae having fully metamorphosed during that time.

Several hundred eggs, yellow to bright orange in colour and approximately one mm in diameter, were deposited under the females which then assumed a 'pin-wheel' configuration (Fig. 29A). Eggs were laid on a variety of substrates in the laboratory--on the sides of the tank, rough rocks, empty scallop shells, mussel shells, a brick, a glass beaker, and a plastic water hose. The two females that continued to brood for several months had deposited their eggs on the smooth, vertical, dull green, fibreglass wall of a wet-table.

Males spawned during this same period, often several doing so concurrently. The egg patches remained adhering to the substrate if the coiled females were removed, or themselves left the eggs, and no case of a female returning to brood eggs was observed in the laboratory.

The embryological development of L. polaris parallels morphologically that described for L. hexactis by Chia (1964, 1968). The

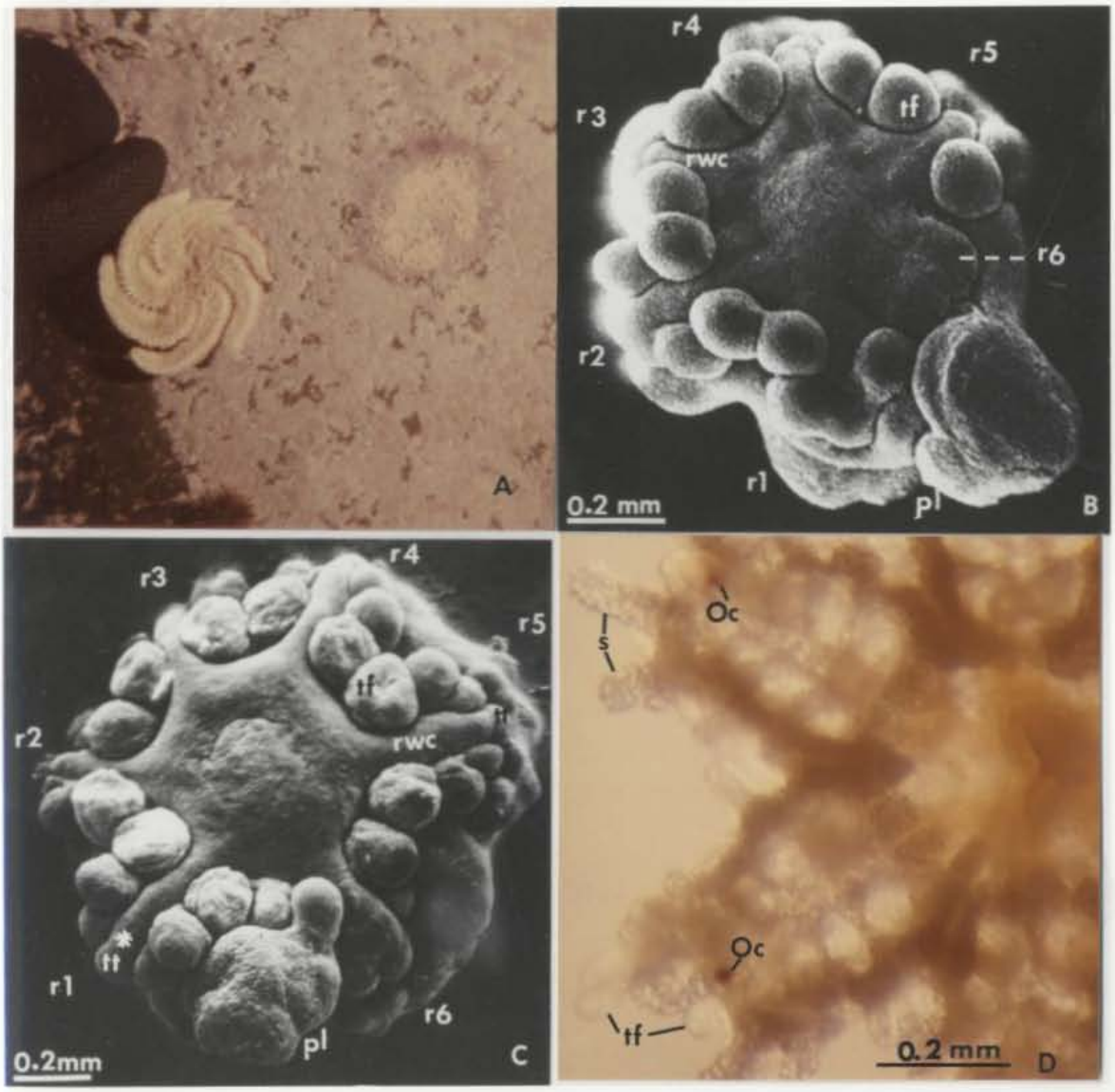
Figure 29A. Underwater photograph of brooding female removed from her eggs. Photograph by Ian Emerson.

Figure 29B. SEM of larva with five rays that possess one to two pairs of tube feet, and a rudimentary sixth ray next to the preoral lobe. (X70)

Figure 29C. SEM of larva with three pairs of tube feet bordering the radial water canals that terminate as short tentacles. * denotes site of optic pigmentation. (X55)
(Fixed in Karnovsky's in Millonig's phosphate buffer)

Figure 29D. Colour photograph of metamorphosed larva with pigmented optic cushion, numerous spines, and suckered, extensible tube feet. (X90)

Oc - optic cushion
pl - preoral lobe
r - ray
rwc - radial water canal
s - spine
tf - tube foot
tt - terminal tentacle


A
r4
r5
r3
tf
rwc
r6
r2
r1
pl
0.2 mm
B
r3
r4
r5
tf
r2
rwc
tt
r1
r6
pl
0.2mm
C
Oc
ls
Oc
tf
0.2 mm
D

pronounced difference is that of rate of development, with L. polaris requiring a considerably longer period of time for embryogenesis. The following chart (Table 1) will summarize the sequence and timing of events of the embryological development of L. polaris, and will serve as a comparison with L. hexactis (data from Chia 1964, 1968) and L. ochotensis (information from Kubo 1951) for later discussion.

In more detail, some of the events accompanying the elaboration of the pigmented eyespots are as follows. After the development of five hydrocoelic pouches on the flattened larval body, five rays, corresponding in position to these pouches, were established. A first, and then distal to these, a second pair of tube feet developed on each of these five rays, initially on rays two, three, and four, and then on rays one and five. At this stage, the preoral lobe began to become reduced in size, and the formation of a sixth ray was noted between rays one and five, as were also the radial water canals ending as terminal tentacles on rays one to five (Fig. 29B).

A third pair of tube feet was established, again as was the case for the two earlier pairs of tube feet, initially on rays two, three, and four, and shortly thereafter on rays one and five. The beginning of the first pair of tube feet on ray six was noted in most cases at this stage.

After three pairs of tube feet were well established on rays one through five (Fig. 29C), bright orange-red flecks of pigment became visible on the oral surface of the terminal tentacles just distal to the third pair of tube feet on rays two, three, and four. Within two

TABLE 1

TIMING OF THE LARVAL DEVELOPMENT FOR *LEPTASTERIAS OCHOTENSIS*, *L. HEXACTIS*, AND *L. POLARIS*

Morphological Event	Time elapsed in days from previously listed event				
	L. ochotensis	*L. hexactis*	*L. polaris*		
			1973	1974	1975
Spawning	0	0		0	0
Smooth morula	3	3		5	4-7
Network of egression tracts		2		3-5	4-7
Gastrula	3	3			7-12
Elongation of embryo, brachiolar grooves	12	7-9			14-40
Brachiolaria hatching		6		0*	9-15
Larval body flattened, 5 hydrocoelic lobes		5-7	0*	19	25-39
1 pair tube feet on rays 1-5		2	5	8	↓
2 pairs tube feet on 1-5; 6th ray forming; radial water canal & terminal tentacle visible; preoral lobe reducing		2	4-6	5	↓
3 pairs tube feet on 1-5; 1 pair on 6		↓	10-15	4	14-16
Pigmentation on terminal tentacles of rays 2, 3, 4		↓	↓	↓	4
3 pairs well developed tube feet on 1-5; *pigmentation* on 1-5; 1-2 pairs tube feet on ray 6		8 (4 pairs tube feet 1-5; 3 on 6)	18 ↓	25	2

TABLE 1 (CONTINUED)

Morphological Event	Time elapsed in days from previously listed event				
	L. ochotensis	L. hexactis	L. polaris		
			1973	1974	1975
3-4 pairs tube feet on 1-5; 3rd pair forming on ray 6; pigmentation on ray 6		5		9	8-14
4 pairs tube feet on 1-5; 3 pairs on 6; eyespots well developed; sign of mouth				11	5-9
4 pairs tube feet on 1-6; mouth open		4	40	15	17

0*--indicates the beginning of timing sequence for a new embryo collection.

more days, pigment was noticeable on the terminal tentacles of rays one and five. Rarely had the fourth pairs of tube feet begun their development before the pigmentation of the eyespot was noted with the dissecting microscope.

The concentration of pigment increased with time in the eyespot, which was now noticeably located on a raised pad on the terminal tentacle. As the fourth pair of tube feet began development on rays one through five, a second and then a third pair of tube feet were established on ray six. Pigmentation was then visible on the terminal tentacle of ray six, one to two weeks after the appearance of pigment on the other five rays.

Specifically, in 1973, the appearance of eyespot pigmentation was first noted in laboratory-raised larvae on July 7. In 1974, eyespots were noticeable on larvae on July 2, 127 days after spawning first occurred in the laboratory, and in 1975, pigmentation was first visible in some larvae on May 26, 103 days following fertilization.

Larvae at this stage in their metamorphosis had completely lost any sign of the preoral lobe, and were becoming whitish, having assimilated much of their orange yolk supply (Fig. 29D). Skeletal plates and spines had formed on the aboral surface, and ray six had approached the other five rays in the extent of its development. The tube feet were very extensible with suckers on the first two older pairs, and the larvae could manoeuver about and right themselves when turned over.

The two female adults that had continued to brood their young in the laboratory in 1975, began to shift their rays frequently, disrupting

the 'pinwheel' configuration. When they left their broods, the larvae possessed four pairs of tube feet on rays one to five, and three or four pairs on ray six. The optic cushions were brightly pigmented, the mouth had broken through, and pyloric caecae were visible in the translucent larvae. Eight hundred and ninety young were collected in one brooding location.

Comparisons of artificially reared larvae with larvae collected later in the season from the field, and with those larvae that continued to be brooded in the laboratory in 1975, indicated that development of larvae 'in vitro' closely kept pace with those raised in the wild. However, cleavage and elaboration of early larval stages were not synchronous within populations of larvae reared 'in vitro', and these larvae tended to display more deformity, e.g. failure or delay in development of one or several rays, than was evident in samples from natural populations.

DETAILED OBSERVATIONS ON THE LARVAL DEVELOPMENT OF THE OPTIC CUSHION AND OCELLI

The dates of spawning and fertilization in the laboratory varied for those larvae raised through to metamorphosis, and the times of these events were unknown for the developing larvae collected from the field for subsequent culture in the laboratory. It was thus impossible to assign such absolute and specific time markers as, "in 22 week old larvae", or, "18 weeks after fertilization", to describe stages of development. However, the following descriptions of the elaboration of the larval ocellus are arranged chronologically and the times between certain stages are determined from the morphological event of the

appearance of the orange-red pigmentation on the optic cushion region of the terminal tentacle. Such time intervals are themselves not rigorous, as the rates of larval development differed in the three years of the study, and to a lesser degree between culture populations in any one year. They serve, rather, as a general indication of the time spans involved.

The descriptions include observations from whole mounts, histological material, thick Epon sections, and SEM and TEM studies of larval tissues.

(a) About two to three weeks before the visible appearance of the eyespot pigmentation, SEM views show the sixth ray being established between ray five and the diminishing preoral lobe (Fig. 29B). Rays one through five possess two pairs of tube feet, and their radial water canals project distally beyond the tube feet as terminal tentacles (Fig. 30A).

The oral surface of the terminal tentacle just distal to the place of attachment of the most recently formed pair of podia, i.e. the future site of the optic cushion, has an epidermis characterized ultrastructurally as follows (Figs. 30B, 31A). The epidermal cells are columnar in form and display zonulae adhaerentes between adjacent cells. Below this region, the cell membranes are closely aligned with each other, and in some sections, septa are seen to bridge the space between the cells for a distance of one to two μm.

The epidermal cells possess microvilli which project through the

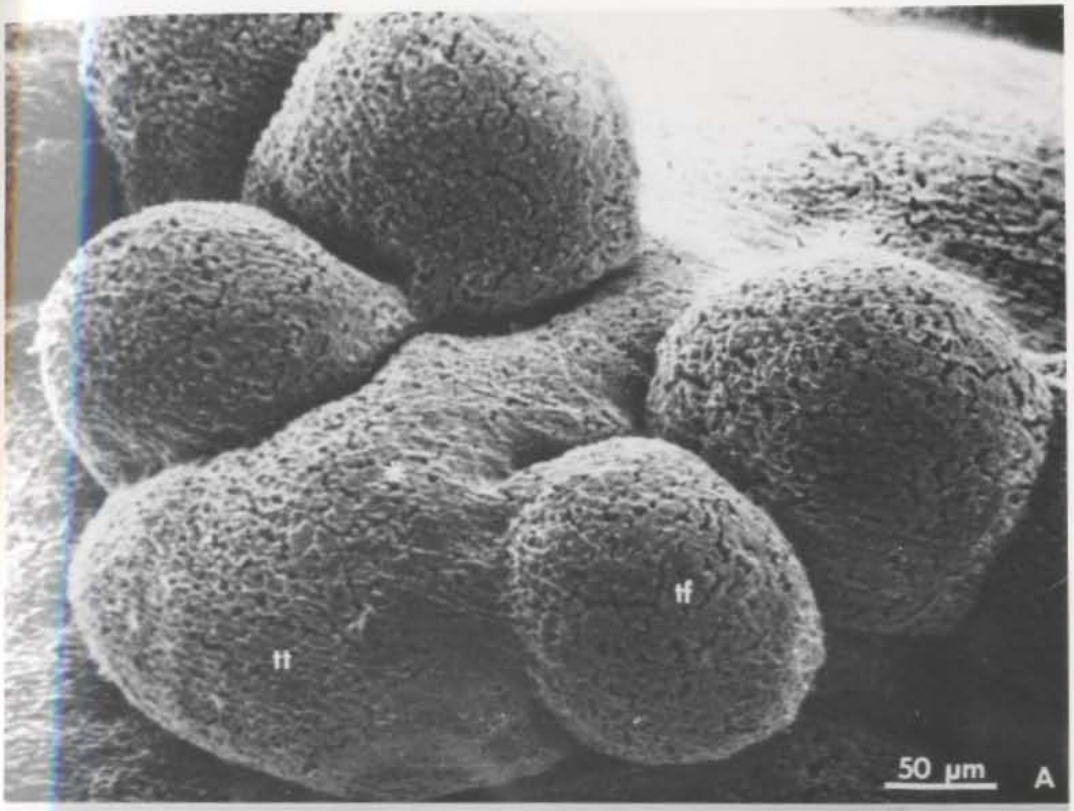
Figure 30. EM's of optic region in larvae, two weeks before the appearance of optic pigmentation.

A. SEM of larval ray showing short terminal tentacle and two pairs of tube feet. (X300)
* site of future optic cushion

B. TEM of epithelium at future optic site on terminal tentacle. (X 15,850)

C. TEM of ciliary basal apparatus of epidermal cell. (X28,000)

ac - accessory centriole
bb - basal body
fb - filamentous bundle
mt - microtubule
mv - microvillus
mvb - multivesicular body
ri - ribosomes
sr - striated root
V - vacuole
za - zonula adhaerens
re - revestment


tf
tt
50 µm
A

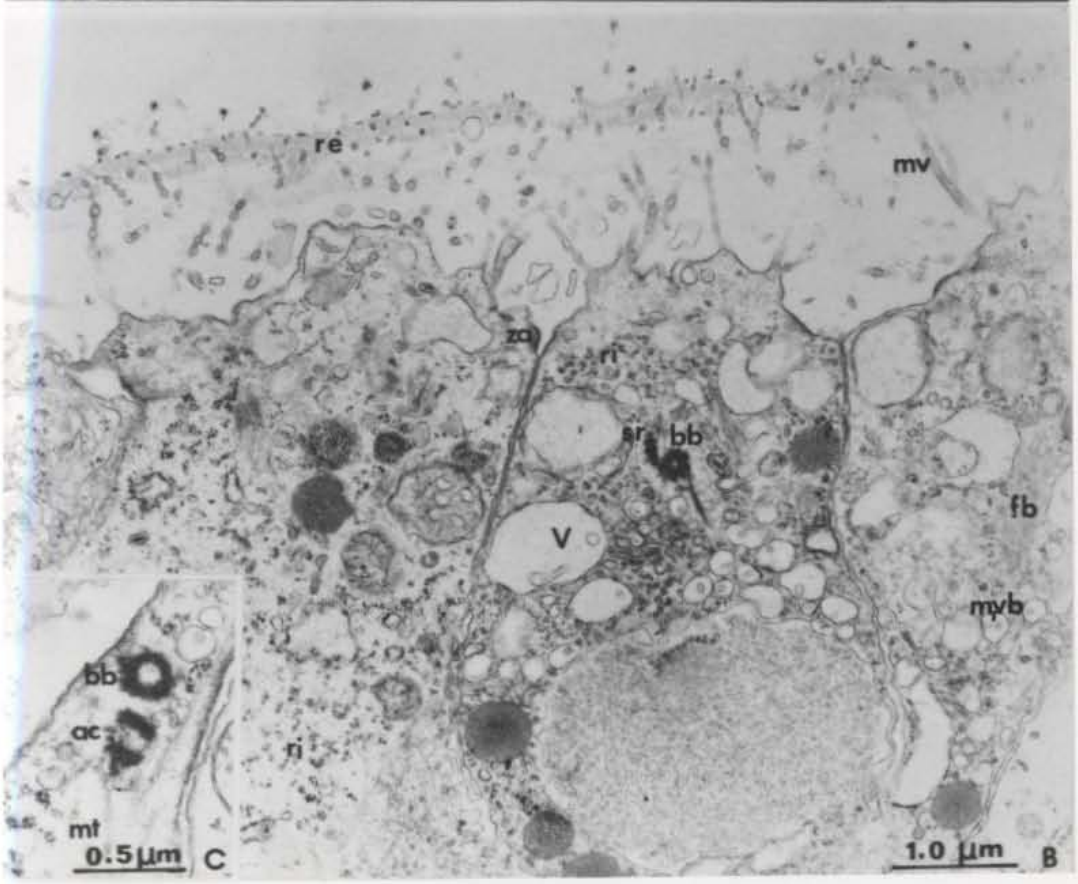
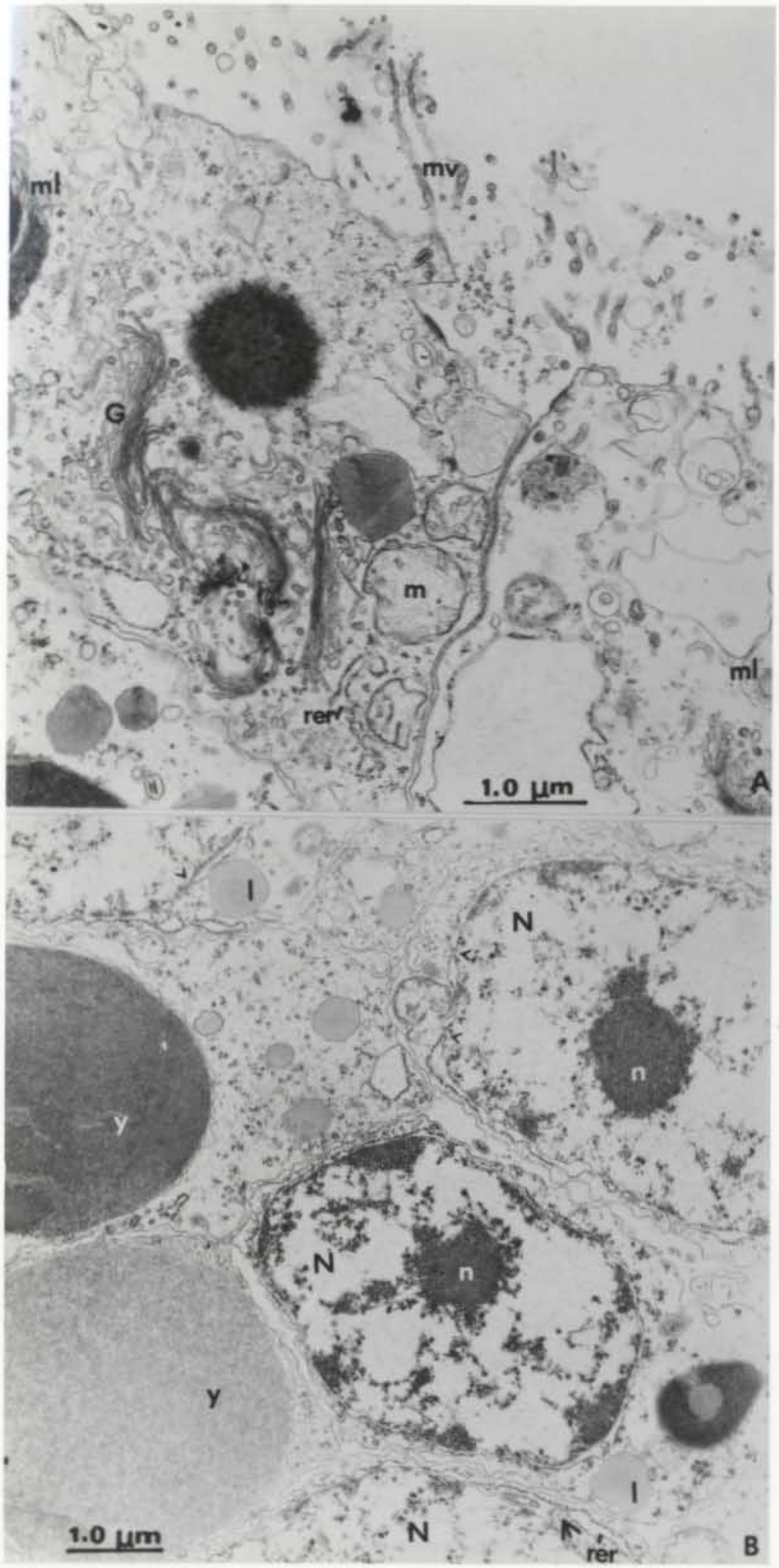

re
mv
zo
ri
sr
bb
V
fb
mvb
bb
ac
mt
ri
0.5µm
C
1.0 µm
B

Figure 31. TEM's of tentacular epithelial cells two weeks before appearance of optic pigmentation.

A. Tentacular epithelial cell contains multiple Golgi apparatuses and numerous ribosomes. (X16,500)

B. Tentacular cells contain nuclei with prominent nucleoli, large yolk granules, ribosomes, lipid droplets, and rough endoplasmic reticulum extending from the nuclear membrane (→). (X12,900) > nuclear pore.

G - Golgi apparatus
l - lipid droplet
m - mitochondrion
ml - multilamellar whorl
mv - microvillus
n - nucleolus
N - nucleus
rer - rough endoplasmic reticulum
y - yolk granule


ml
mv
G
m
rer
ml
1.0 µm
A
l
N
n
y
N
n
y
l
1.0 µm
N
rer
B

thin revestment. Many epidermal cells possess a cilium with a basal body, accessory centriole, striated root, and radiating microtubules (Figs. 30B, C).

Large vacuoles containing finely filamentous material are common in the apical portions of these cells. There are also numerous smaller vesicles, some multivesicular. Small electron-dense vesicles are associated with the Golgi apparatus, which is typically present in the apical portions of the cells (Fig. 31A). In some epidermal cells, this organelle is very extensive, presenting profiles of many groupings of cisternae. Multilamellar bodies are also occasionally found in these cells.

The cytoplasm contains numerous ribosomes, polyribosomes, and cisternae of rough endoplasmic reticulum. Large mitochondria are numerous throughout the cells and few scattered microtubules run longitudinally down the cells. Many cells also contain microfilaments constituting a longitudinally oriented fibre bundle.

The nucleus, four to five μm in diameter, is rounded or slightly indented in form, with a lightly staining matrix (Fig. 31B). A centrally located nucleolus, often exceeding one μm in diameter, is prominent in the nucleus and displays a fibrous interior and granular cortex. Except in the region of nuclear pores, the outer nuclear membrane, coated with ribosomes on its cytoplasmic face, is pulled away from the nucleus and is continuous with some cisternae of rough endoplasmic reticulum.

The tentacular cells of larvae at this stage of development also possess numerous membrane-bound yolk granules up to five μm in

diameter (Fig. 31B). Some granules show an internal structure of parallel striae, while cross-sections of these striae present a granular appearance. Other bodies of homogeneously medium electron density, that are presumably lipid droplets, measure approximately 0.5μm in diameter, and are numerous in these cells.

(b) At the time that pigmentation of the optic cushion regions was first noted with a dissecting microscope in some larvae, the metamorphosing sea stars possessed the remnants of the preoral lobe and six recognizable rays (Fig. 29C). Rays one to five had three pairs of tube feet arranged on either side of the conspicuous radial water vessels, and ray six had two pairs of tube feet. A circumoral water ring joined these radial canals and circled the region destined to become the mouth. The terminal tentacle was short and displayed a slight swelling on its oral surface at the level of the most distal pair of tube feet (Fig. 32A).

A sagittal histological section (Fig. 32B) through a larva at this stage illustrates the aboral skeleton to be well established with the presence of terminal spines over the ray tips. The developing digestive system was present as a mass of endodermal tissue, with the oesophagus and caecae of the pyloric stomach beginning to form. The mouth had not yet broken through.

At higher magnification, the radial water canal is seen to extend into the short terminal tentacle (Fig. 32C). In histological preparations of this stage, other than a swelling of the region in some specimens, no particular features distinguish the area that will bear

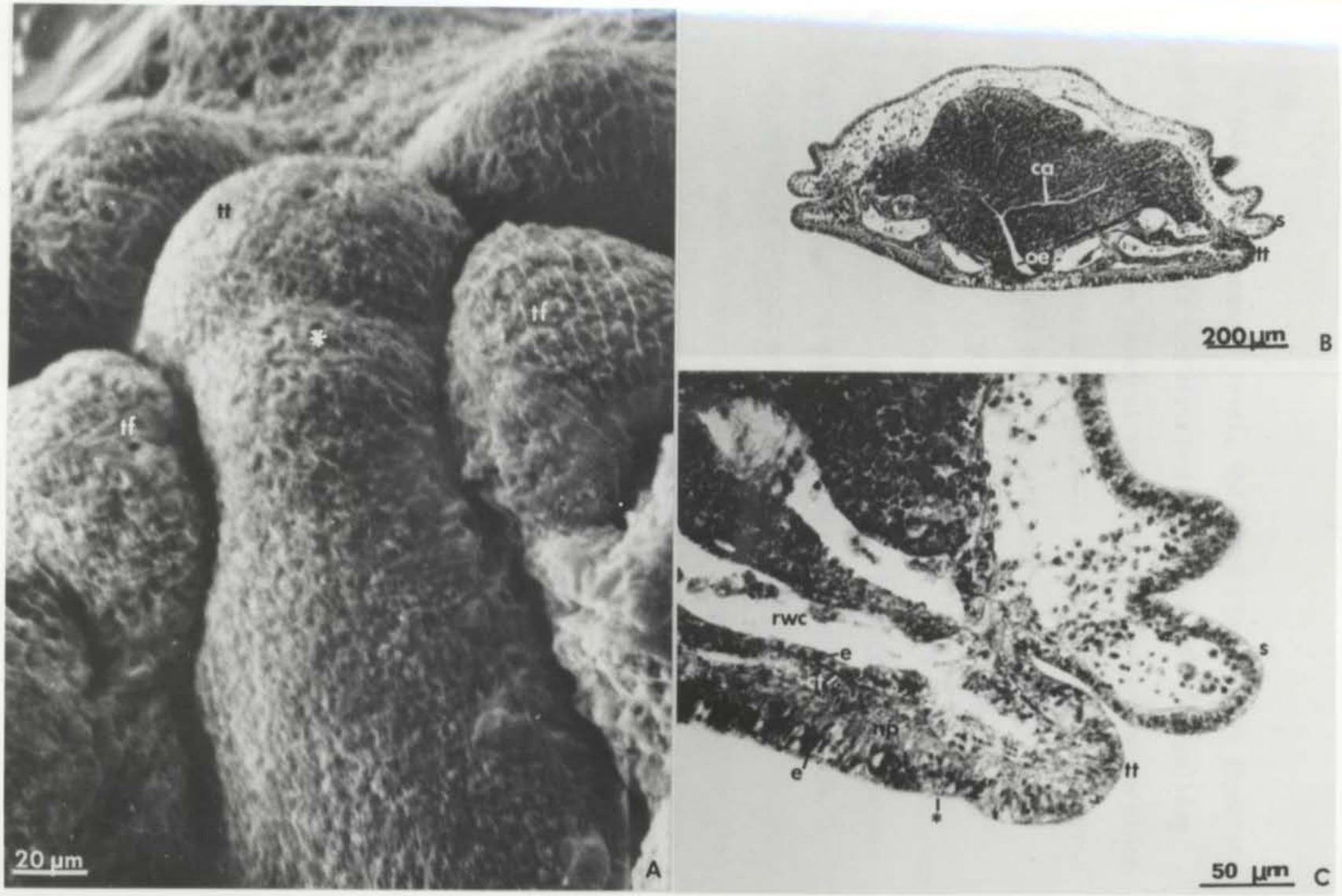
Figure 32. Micrographs of larvae when optic pigmentation was first noted.

A. SEM of tentacular region of larval ray denoting site of optic pigmentation *. (X620)
(Fixed in Karnovsky's in Millonig's buffer)

B. LM of S/S shows mesodermal tissue surrounding the developing oesophagus and caecae of the digestive system. A terminal spine extends over the short terminal tentacle. (X65)
(Mallory-Heidenhain's stain)

C. Higher magnification of B shows the differentiating tissue layers in the region of optic pigmentation (*) at the base of the terminal tentacle. (X300) (Mallory-Heidenhain's stain)

ca - caecum
ct - connective tissue
e - epithelium
np - nerve plexus
oe - oesophagus
rwc - radial water canal
s - spine
tf - tube foot
tt - terminal tentacle


tt
tf
tf
20 µm
A
ca
s
tt
200µm
B
rwc
e
s
np
e
tt
50 µm
C

the ocellus.

At the ultrastructural level, the first signs of the appearance of an ocellus are noted in the distal-aboral region of the swelling cushion at the base of the terminal tentacle. In this region, a plate of cells begins to differentiate into cells recognizable as the developing pigmented and sensory components of the ocellus (Fig. 33). Still other cells project slightly over some of these elements and correspond in position and other characteristics to corneal cells. The central area of this grouping of cells becomes indented, with the revestment continuous over the entire surface of the optic cushion.

The three cell types of the developing ocellus are morphologically similar in some features (Figs. 34, 35). The pigmented and presumptive corneal cells possess large vacuoles with contents consisting of a sparse and finely filamentous material. Similar vacuoles are occasionally noted in proximal regions of the sensory cells. The three ocellar elements also possess ribosomes and polyribosomes in their cytoplasm. Microtubules, routinely present in sensory and corneal cells, are also seen in pigmented cells. Mitochondria in the three cell types are irregular in shape with sparse matrix and few cristae. Vesicular profiles appear similar in the cells and multilamellar bodies are also noted in each of the differentiating cell types.

Certain cells are presumed to be corneal cells by their location, and by their processes which extend to lie over the slight indentation of the developing ocellus (Fig. 35). These cells possess microvilli that extend through the revestment. The presumptive corneal cells bear a

Figure 33. TEM of ocellus developing as a group of differentiating cells when optic pigmentation was first noted. (X8500) (Fixed in Karnovsky's in Millonig's buffer)

c - cilium
CC - corneal cell
fb - filamentous bundle
n - nucleolus
N - nucleus of presumptive sensory cell
PC - pigmented cell
re - revestment
SC - sensory cell
tt - terminal tentacle

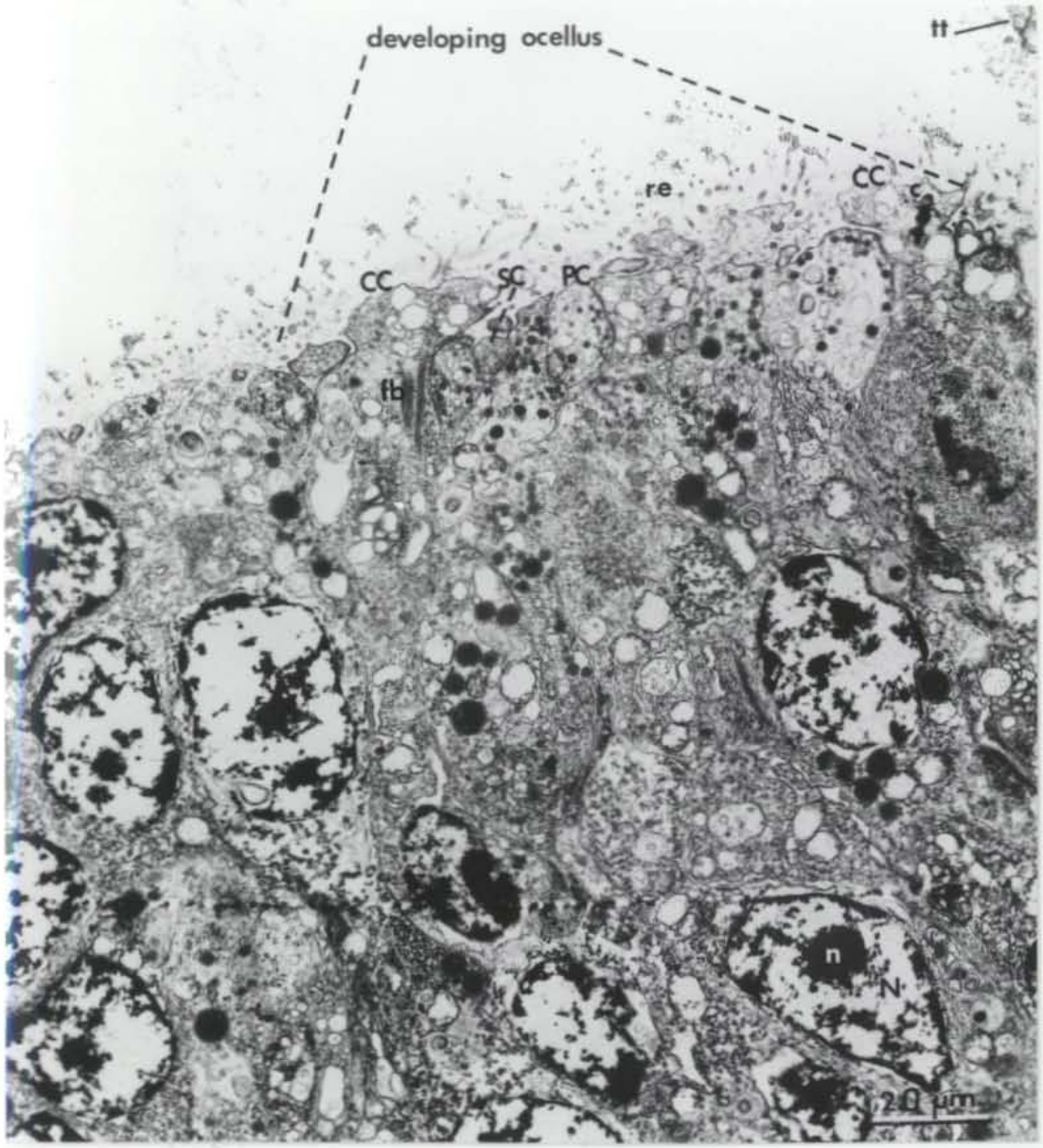

developing ocellus
tt
re
CC
CC
SC
PC
fb
n
N
20 µm

Figure 34. TEM montage of developing ocellus showing the shared and distinctive features of the differentiating cell types. (X20,100)
Processes of ciliated corneal cells extend over a shallow lumen. Small pigment granules distinguish the pigmented cells and in the apical regions of the cells, they are in close association with granular vesicles (*). Sensory cell necks display microtubules and short regions of subsurface cisternae. Proximally, coated vesicles, RER, and multivesicular bodies are noted in the sensory cells. (Fixed in Karnovsky's in Millonig's buffer.)

bb - basal body
ci - subsurface cistern
cv - coated vesicle
Cp - corneal cell process
m - mitochondrion
ml - multilamellar whorl
mt - microtubule
mv - microvillus
mvb - multivesicular body
ol - ocellar lumen
p - pigment granule
PC - pigmented cell
re - revestment
rer - rough endoplasmic reticulum
ri - ribosomes
sd - septate desmosome
SC - sensory cell
v - vesicle
V - vacuole

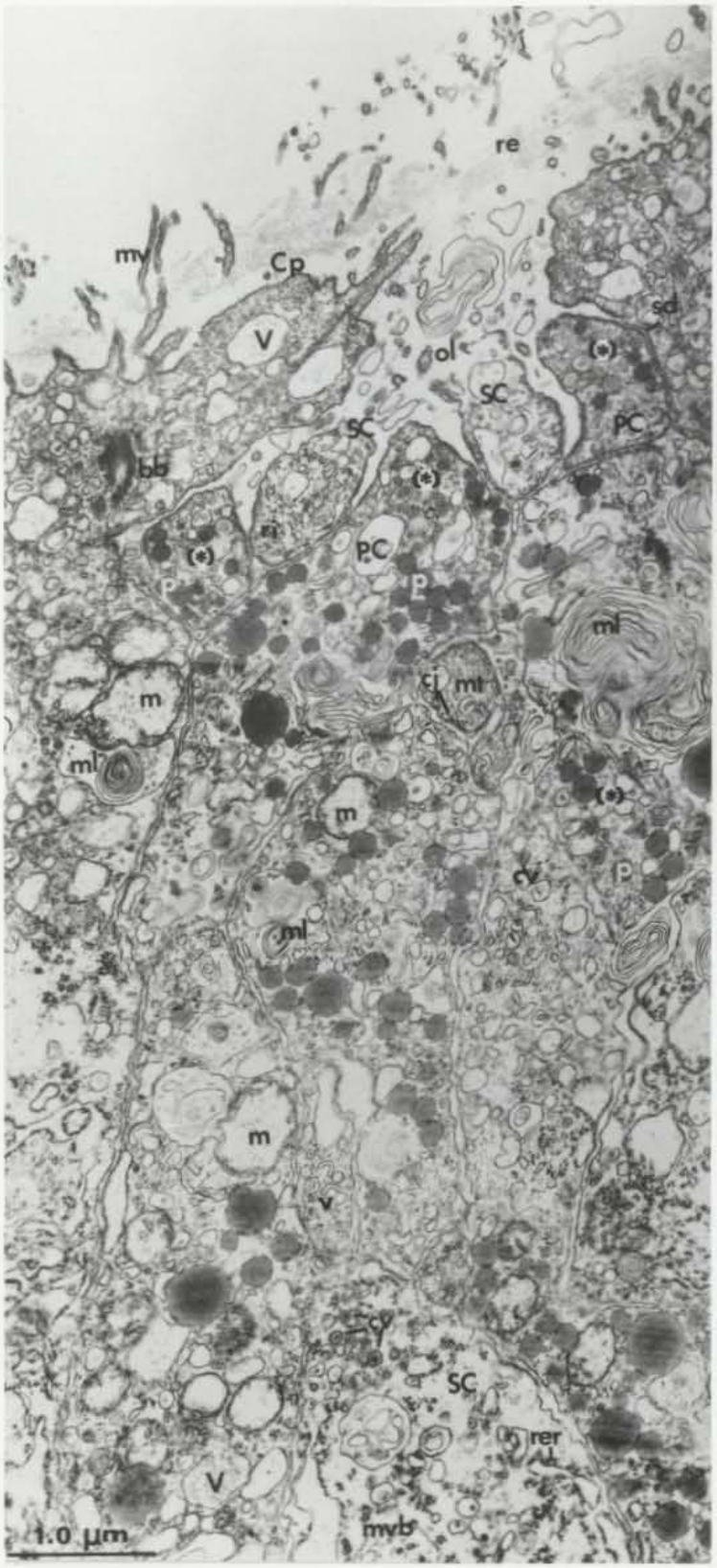
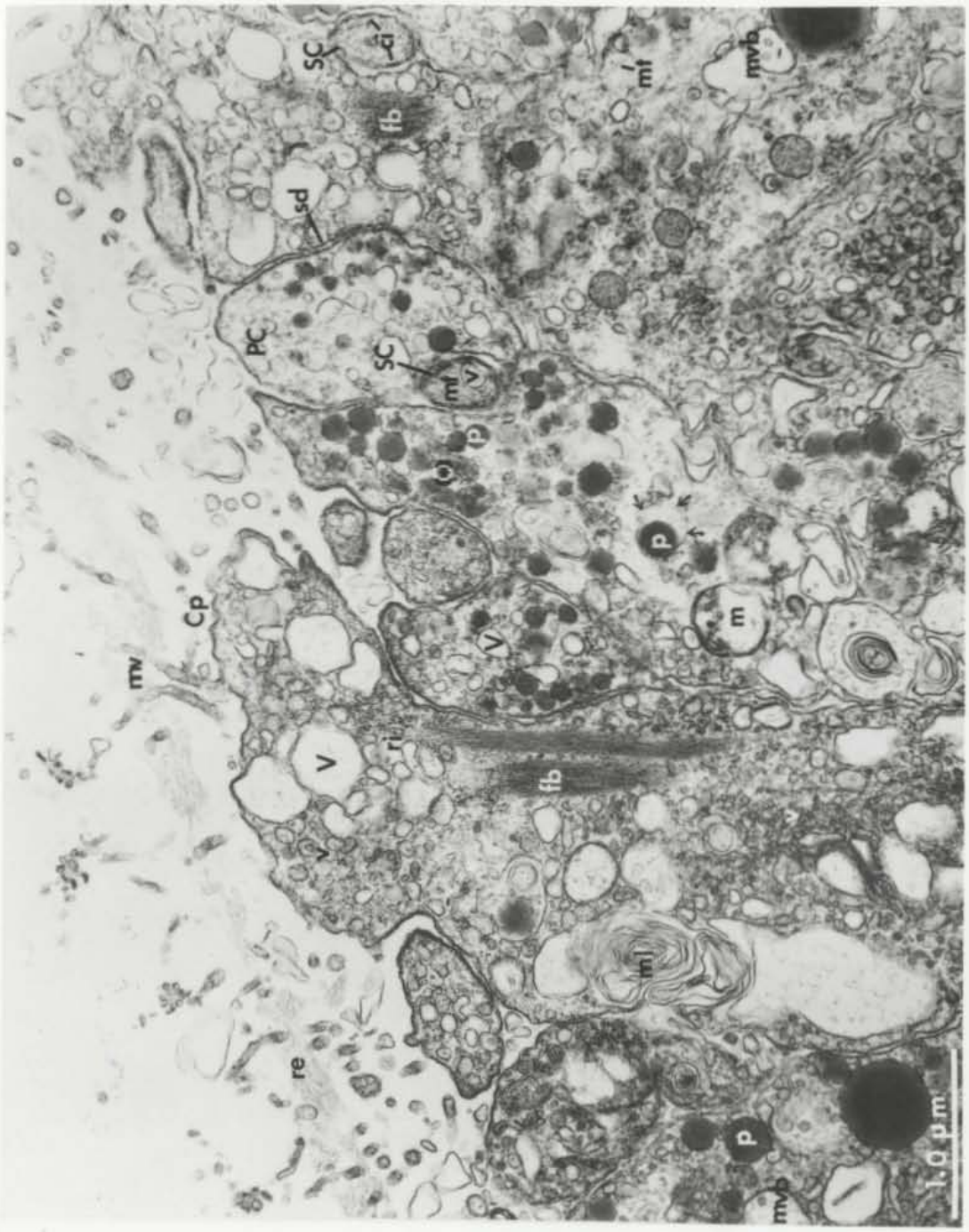

re
my
Cp
V
ol
sd
(*)
SC
PC
bb
ri
P
ml
ci
mt
m
cv
1.0 µm
rer
mvb

Figure 35. TEM of same developing ocellar region as in Fig. 33, 34, showing the three differentiating types of ocellar cells. (X25,100).

The corneal cells possess a filamentous bundle and numerous vesicles and a few large vacuoles. In the pigmented cells, small pigment granules are often seen close to granular vesicles (*), and to clear vesicular profiles (⟶). (Fixed in Karnovsky's in Millonig's buffer.)

ci - subsurface cistern
Cp - corneal cell process
fb - filamentous bundle
m - mitochondrion
ml - multilamellar whorl
mt - microtubule
mv - microvillus
mvb - multivesicular body
p - pigment granule
PC - pigmented cell
re - revestment
ri - ribosomes
SC - sensory cell
v - vesicle
V - vacuole


SC
ri
mi
mvb
fb
sd
PC
SC
mv
p
p
Cp
mv
V
V
ri
V
fb
E
V
mi
p
mvb
re
1.0 µm

single cilium with a secondary centriole, and microfilaments, some of which are associated as a bundle that extends proximally down the cell (Figs. 33, 34). As well as the large vacuoles noted above, numerous smaller vesicles, some clear, are also present.

Developing pigmented cells, associated with each other and with the presumptive corneal and sensory cells by short desmosomal connections (Figs. 34, 35) are characterized by the presence of numerous granules in their cytoplasm identical to the small class of pigment inclusions present in adult ocellar pigmented cells. These granules are of a homogeneous and medium electron density and range in diameter from 0.1 to 0.5 μm, with the smaller ones concentrated apically in the cells. A series of inclusions located near these small granules present a variety of appearances from completely or partially membrane-bound vesicles (approximately 100 nm in diameter) with granular contents, through vesicles with more condensed interiors approaching the appearance of the small pigment granules (Figs. 34, 35). Many of the small pigment granules display a less homogeneous peripheral area suggestive of the incorporation of a granular substance. Other very pale inclusions appear to be condensing toward the small class of pigment granule from material in the cytoplasm independent of any bounding membrane.

Some pigment granules are closely associated with clear vesicles and the occasional one appears to be contained within a vacuole (Fig. 35). Vesicles with clear profiles are numerous in the cytoplasm of these cells and multivesicular bodies are occasionally seen. Multilamellar bodies are numerous and are contained within the cell or apparently

interposed between cells.

Present between the apical ends of the pigmented cells are narrow regions of differentiating sensory cells (Figs. 34, 35). These portions contain ribosomes, numerous longitudinally oriented microtubules, and a variety of vesicles. Some cisternae are flattened against the plasma membrane for part of the cells' circumference, and the space between these cisternae and the cell membrane is filled with an electron-dense material. More proximally, the sensory cells are expanded in diameter and possess cytoplasm with sparse ground substance (Fig. 34). Mitochondria are numerous in these regions. Clumps of ribosomes are scattered in the cytoplasm and cisternae of rough endoplasmic reticulum are common. Multivesicular bodies, clear vesicles, and vesicles up to 80 nm in diameter with coated outer membranes and cored interiors, are also present in the expanded region of the sensory cells.

Nuclei in the ocellar region are located more proximally in the cushion than are those of the rest of the epithelium (Fig. 33). Most are oval in shape with a prominent nucleolus and pale matrix.

The small indentation of the developing ocellus contains a few scattered microvillous profiles and membranous whorls (Fig. 34). Microvilli are rarely seen to arise from the presumptive pigmented or sensory cells at this stage of elaboration, and no sensory cell cilium was noted in the material sectioned.

(c) In other, more advanced larvae, the ocellar lumen is present as a more prominent identation, two to three μm deep and seven μm across

(Fig. 36). Situated over the lumen with small gaps between them are several extensions of developing corneal cells. These processes contain large vacuoles, and by this stage also possess numerous tubules and vesicles (80 to 100 nm in diameter) that characterize the corneal cells of fully differentiated ocelli.

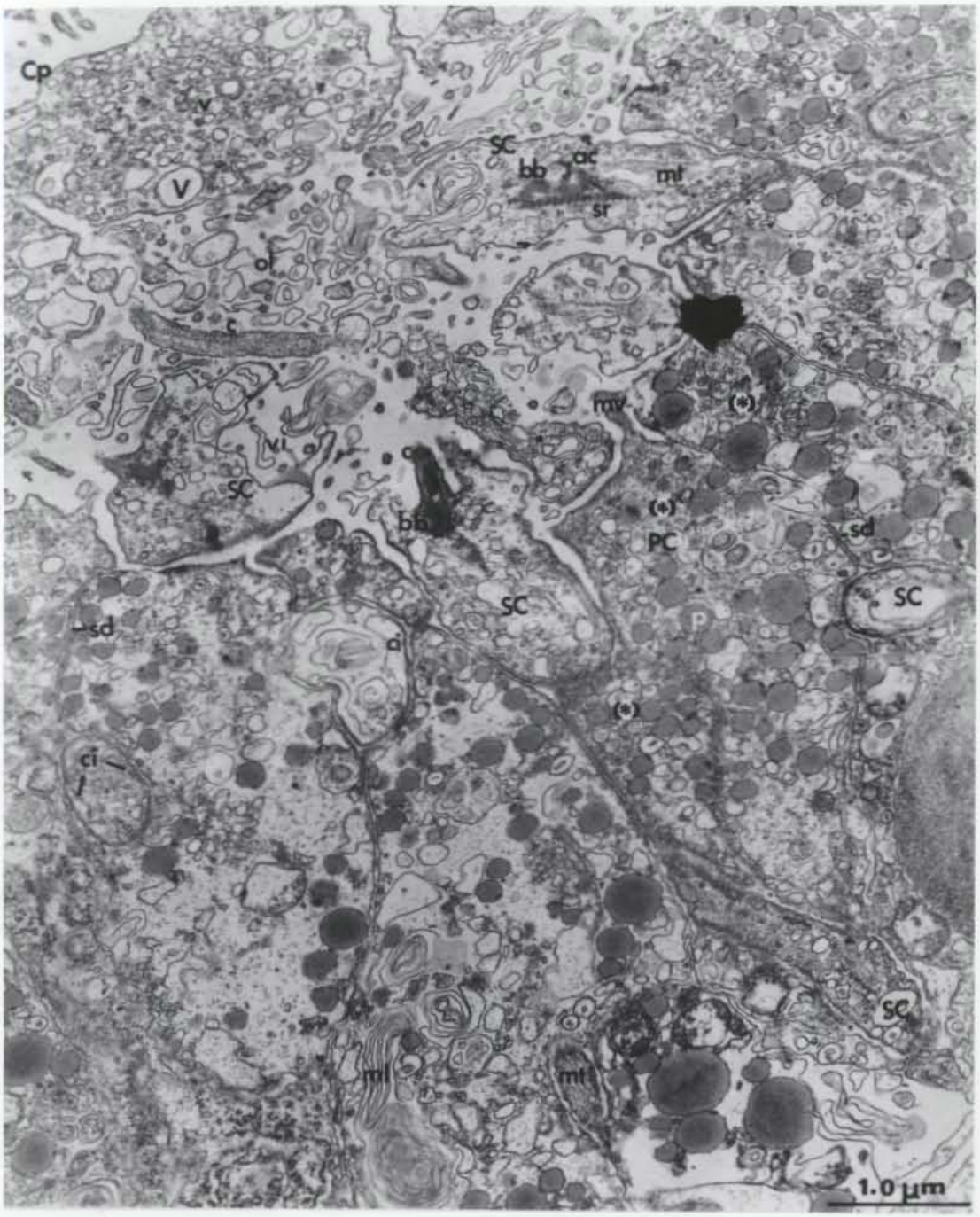
The pigmented cells, over one μm in width at the lumen, are associated with neighbouring cells for distance up to two μm by septate desmosomes, and the cells' apical-lateral borders project a few short microvilli into the lumen. The small pigment granules have become a numerous constituent in the cytoplasm. Again, vesicles filled with a granular material, some with ruptured membranes, are also numerous in the apical portions of the pigmented cells near the very small pigment granules. Granular vesicles, most of them approximately 50 nm in diameter, are also located in the immediate vicinity of the Golgi cisternae (not illustrated).

The sensory cells are expanded apically from their neck region into the developing indentation of the lumen beyond the level of the adjacent pigmented cells (Fig. 36). A single cilium, with a basal body,accessory centriole and striated root, extends from a depression in this apical portion. Microtubules are aligned alongside the one μm long root and extend down the neck of the sensory cell. A 1.5 μm length of ciliary shaft sectioned in the lumen showed no extension from its sides. Elsewhere from the apical sensory cell surface, however, there are irregular villous extensions into the lumen. This expanded region of the sensory cell contains clear vesicles, and the neck region

Figure 36. TEM montage of an invaginating ocellar lumen covered by corneal cell processes and lined by sensory and pigmented cells. (20,100)

The corneal cell processes possess numerous low density vesicles. The sensory cells expand distally and give rise to cilia and villi in the lumen. The pigmented cells possess numerous pigment granules and apical concentrations of granular vesicles (*). (Fixed in Karnovsky's in Millonig's buffer)

ac - accessory centriole
bb - basal body
c - cilium
ci - subsurface cistern
Cp - corneal cell process
ml - multilamellar whorl
mt - microtubule
mv - microvillus
ol - ocellar lumen
p - pigment granule
PC - pigmented cell
sd - septate desmosome
sr - striated root
SC - sensory cell
v - vesicle
vi - villus
V - vacuole


Cp
V
SC
bb
ac
mf
sr
ol
c
mv
(*)
vl
SC
bb
(*)
PC
sd
SC
SC
sd
ci
P
(*)
ci
SC
mf
mf
1.0 µm

is characterized by numerous microtubules, vesicles, ribosomes, and cisternae applied to the plasma membrane, some of which are studded with ribosomes on their cytoplasmic side.

Proximally, the sensory cells expand and are characterized by the presence of numerous vesicular profiles (Fig. 37). Particularly plentiful above the nucleus are multivesicular bodies, approximately 0.5 μm in diameter. Their contents include a sparse filamentous matrix, small dense vesicles averaging 40 nm in diameter, larger vesicular profiles with clear or cored interiors, and occasionally a whorl of membranous material. High and low density vesicles are especially numerous in the regions of the Golgi apparatus.

At this level in the cell there are numerous polyribosomes and cisternae of rough endoplasmic reticulum. Mitochondria are present in the region of the nucleus, and longitudinally oriented microtubules can also be seen at this level. Sensory cell nuclei, located more proximally in the optic cushion tissues than those of the epidermal and developing corneal cells, are rounded in form, up to four μm across.

(d) Five weeks post-pigmentary stage ocelli from 1973 larvae showed markedly less developed features than similarly 'timed' larvae of the following year when development occurred more rapidly. Their features are thus better described here preceding that of 1974 larvae at three weeks post-pigmentation.

Fig. 38 illustrates a longitudinal section through a developing ocellus present on the optic cushion near a fold in the terminal tentacle. The epidermal cells adjacent to the ocellus possess conspicuous

Figure 37. TEM montage of mid region of sensory cells. (X22,500)

Numerous vesicles and multivesicular bodies fill the neck and supranuclear region. Mitochondria, cisternae of RER, and ribosomes are numerous around the broad nucleus. (Fixed in Karnovsky's in Millonig's buffer.)

G - Golgi apparatus
m - mitochondrion
mt - microtubule
mvb - multivesicular body
N - nucleus
rer - rough endoplasmic reticulum
ri - ribosomes
v - vesicles

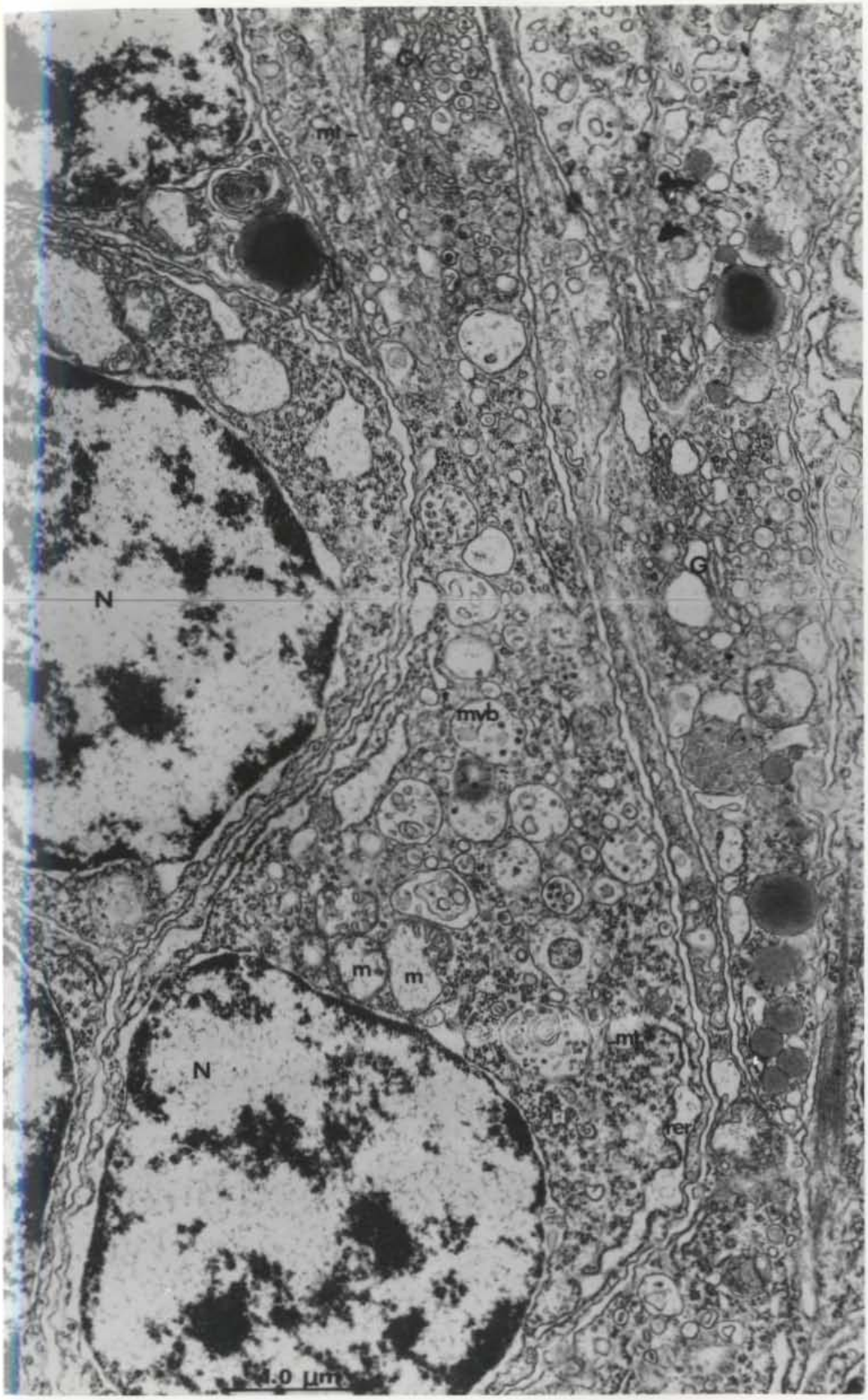
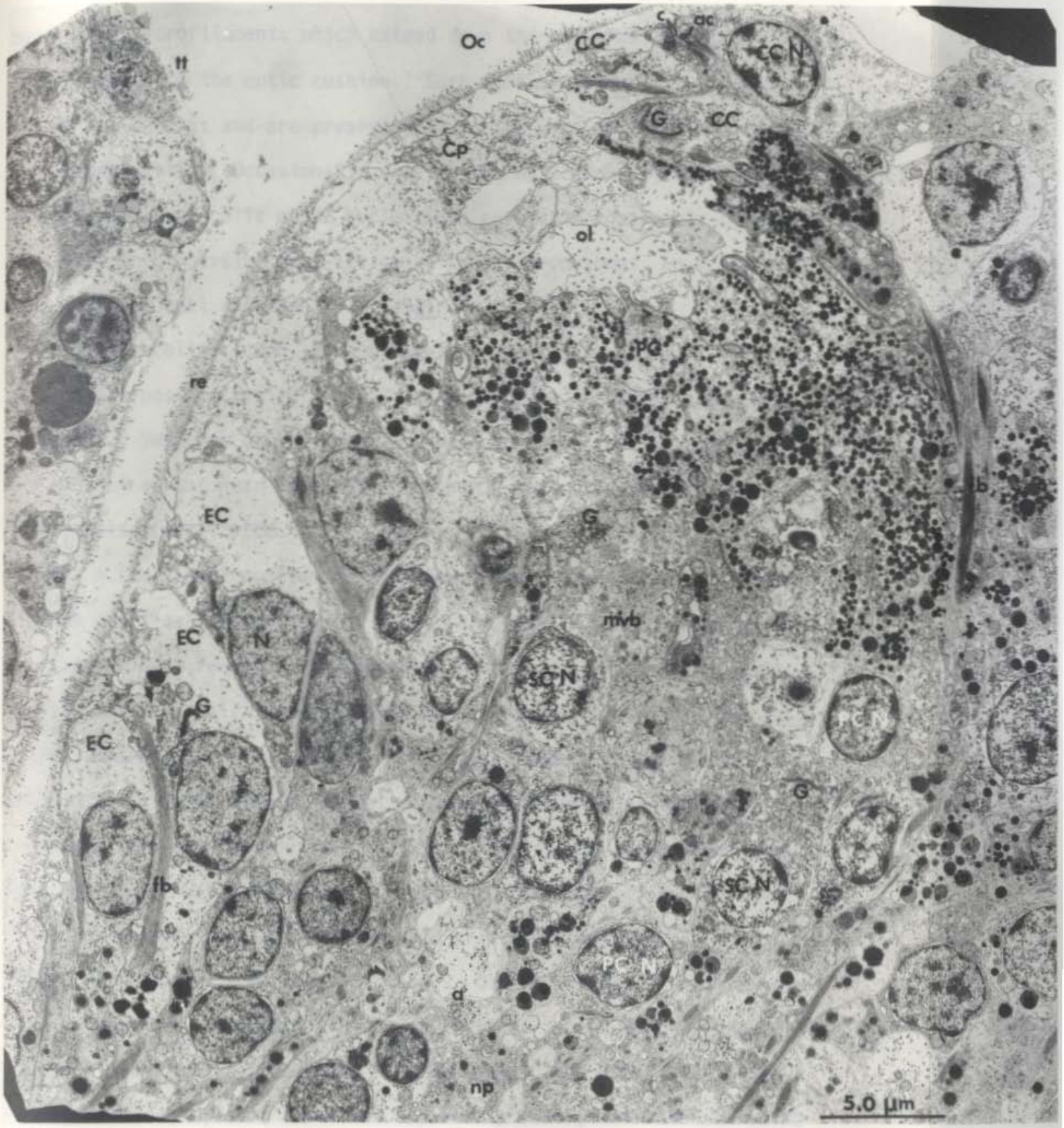

GV
mt
N
G
mvb
m
m
mt
N
rer
1.0 µm

Figure 38. TEM montage of five week post-pigmentary optic cushion. (X4,600)

A prominent ocellus with differentiated corneal, sensory, and pigmented cells can be observed in the leading edge of the cushion. Axons of the radial nerve cord are present deeper in the cushion. The sensory cell nuclei appear less densely stained than those of the other cells. Multivesicular bodies fill the supranuclear region of the sensory cells, and a prominent Golgi region is present as well. The pigmented cells possess large polymorphic bodies in addition to the small pigment granules.

a - axon
ac - accessory centriole
c - cilium
CC - corneal cell
Cp - corneal cell process
EC - epithelial cell
fb - filamentous bundle
G - Golgi apparatus
mvb - multivesicular body
np - nerve plexus
N - nucleus
ol - ocellar lumen
Oc - optic cushion
PC - pigmented cell
re - revestment
SC - sensory cell
tt - terminal tentacle


Oc
CC
ac
CC N
tt
G
CC
CP
ol
re
EC
G
mvb
EC
N
SC N
G
EC
PC N
G
fb
SC N
PC N
a
np
5.0 µm

bundles of microfilaments which extend down the cells as they taper and travel deep into the optic cushion. Such fibrous elements form a layer around the ocellus and are present also in the developing extensions of the corneal cells. Occasionally seen in the epidermal region of the optic cushion are cells whose nuclei have chromatin apparently condensing into or dispersing from its constituent chromosomes, an appearance suggestive of cell division (Fig. 39A).

The ocellar lumen, ten μm deep, is overlain by corneal extensions which possess vesicular and tubular profiles in their cytoplasm (Fig. 39). Vesicles are numerous in the Golgi region also. Centrioles and part of a root near the apical border of one cell, indicate that these cells are ciliated.

A few distal portions of sensory cells, microvilli, and a few ciliary profiles extend into the lumen (Fig. 39B). The lumen is primarily bordered by a few broad pigmented cells that contain small pigment granules, and at this stage of development, larger, membrane-bound polymorphic bodies (Fig. 40). Again, some small pigment granules are in close physical proximity in apical regions to vesicles with a range of condensing granular contents (Fig. 39B). Some of the polymorphic bodies have a granular matrix with small electron-dense inclusions, and some show more heavily stained and condensed areas in their matrices and contain portions of membranes (Fig. 40). Others possess several regions similar in shape, size, and staining properties to the small pigment granules found in the cytoplasm. In some cases, low density vesicles, common in the cytoplasm, are found apparently fusing with the enclosing

Figure 39A. TEM of optic cushion epidermal cell with nuclear morphology suggestive of division. (X13,300)
(→) condensed chromatin

39B. TEM of ocellar wall of five week post-pigmentary larva. (X16,500)

Sensory cells display the features characteristic of the neck regions of mature ocelli. The apical regions of the pigmented cells contain pigment granules and granular vesicles (*). Low density vesicles are also seen (→).

c - cilium
ci - subsurface cistern
m - mitochondrion
mt - microtubule
N - nucleus
ol - ocellar lumen
p - pigment granule
SC - sensory cell
vi - villus

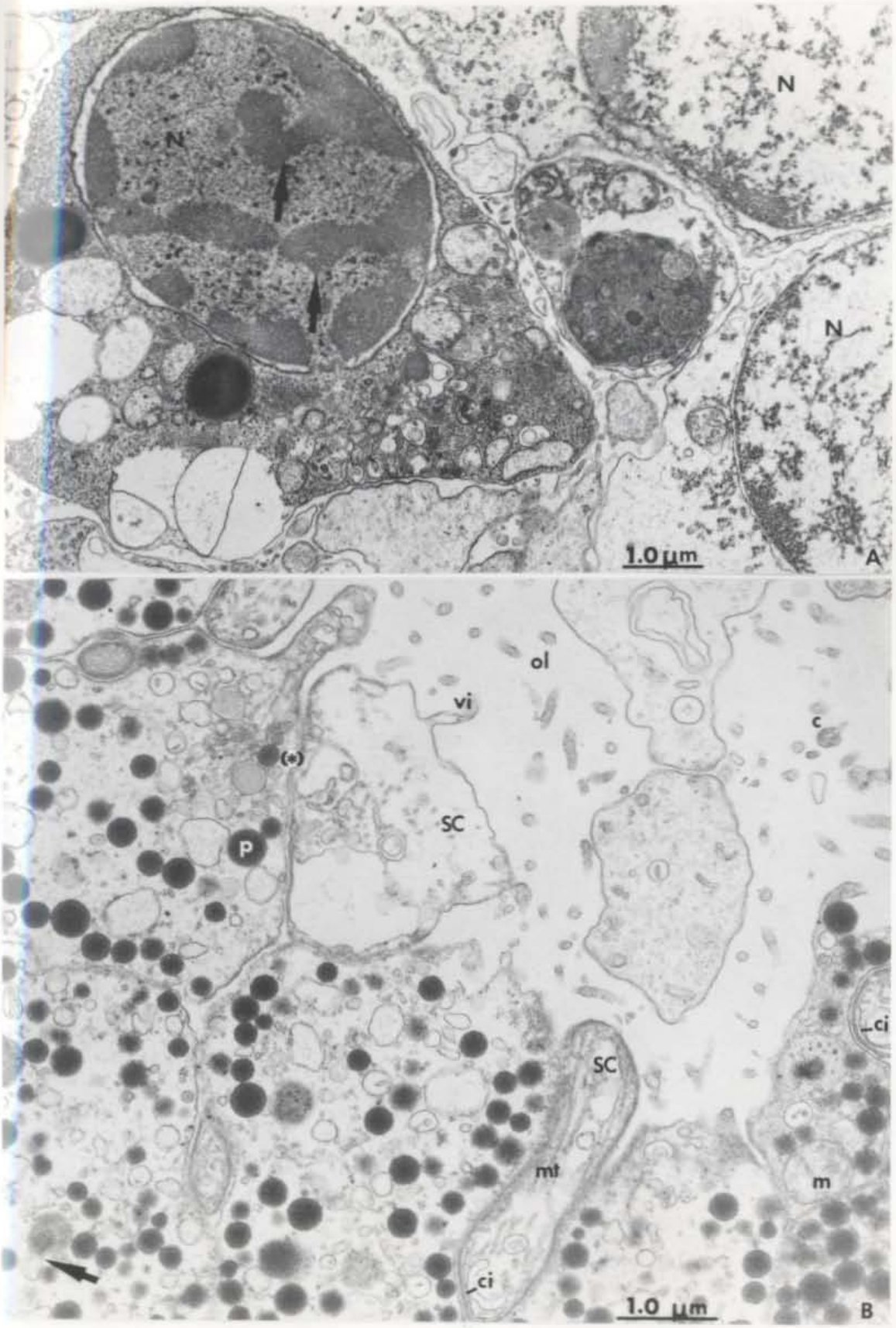
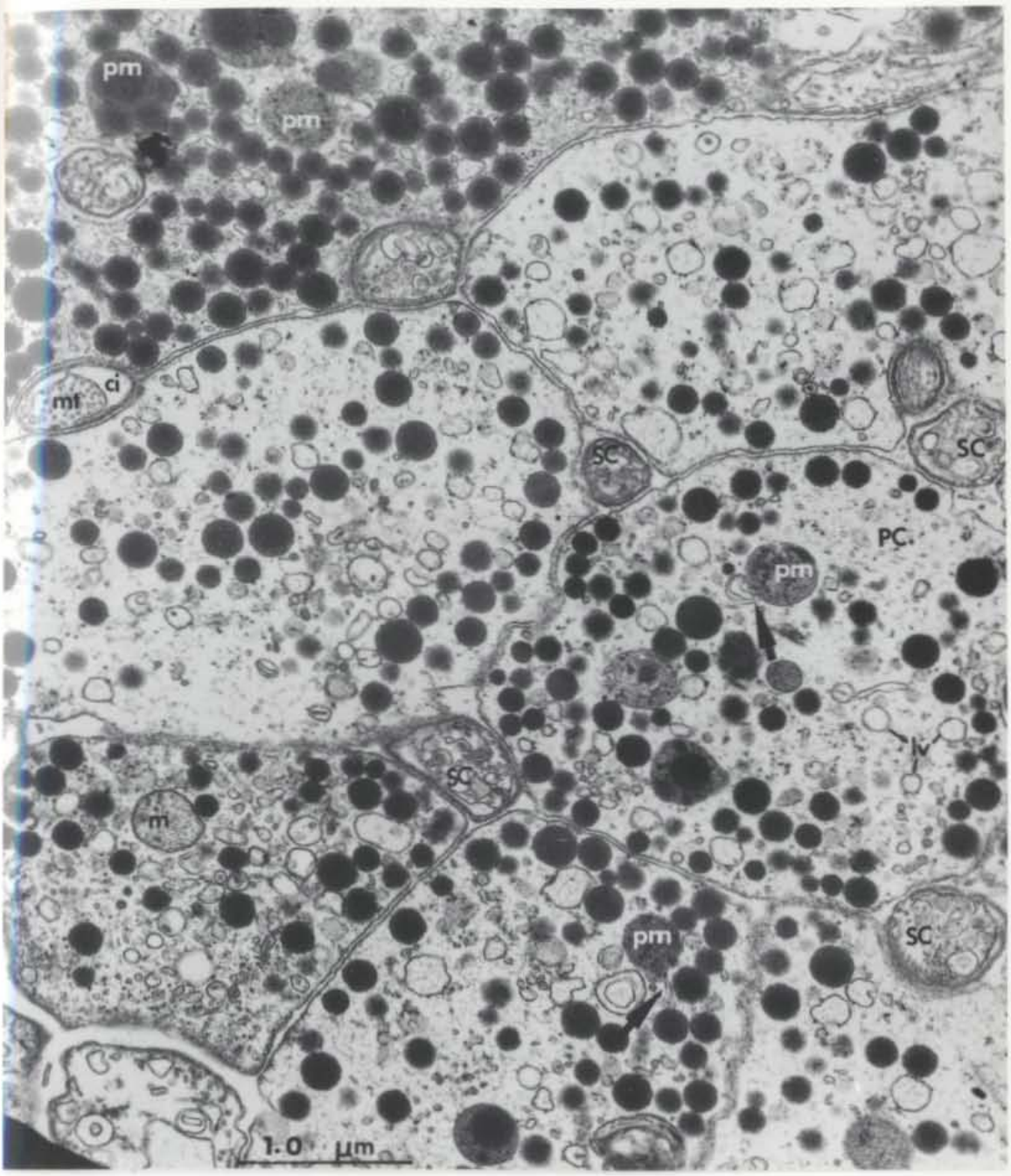

N
N
N
1.0 µm
A
ol
vi
c
(*)
SC
P
SC
ci
mt
m
ci
1.0 µm
B

Figure 40. TEM of T/S of ocellar wall showing pigmented cells surrounded by several sensory cell necks. (X25,100)

Sensory cells display subsurface cisternae, microtubules, and vesicles. Pigmented cells possess small pigment granules and polymorphic bodies, with some associated low density vesicles (→).

ci - subsurface cistern
lv - low density vesicle
m - mitochondrion
mt - microtubule
pm - polymorphic body
PC - pigmented cell
SC - sensory cell


pm
pm
mt
ci
SC
SC
PC
pm
lv
m
SC
pm
SC
1.0 µm

membranes of these bodies.

Mitochondria are becoming more regularly rounded in cross-section with more electron-dense matrices than in earlier stages. Tapered portions of pigmented cells are seen at the level of the nerve axons (Fig. 38).

A transverse section of the components of the ocellar wall, shows each pigmented cell to be surrounded by four or five narrowed sensory cells (Fig. 40). Higher magnification micrographs of this region (Figs. 41A, B), illustrate subsurface cisternae to be flattened around the circumference of the sensory cell neck with a 20 nm gap between the outer cisternal membrane and the plasma membrane. This gap is filled with an amorphous material. The membrane of the cisternae facing the cytoplasmic core of the neck typically has microtubules arranged adjacent to it. These microtubules are approximately 25 nm in diameter and are readily distinguished from the microfilaments in the fibre bundle of adjacent corneal and epithelial supporting cells (Fig. 41B). Also visible in the sensory neck are C-shaped sacs, coated on their convex surface.

The supranuclear region of the sensory cells is very strikingly filled with numerous vacuoles, vesicles, and particularly, multivesicular bodies which frequently measure up to one μm in diameter. The Golgi region is also prominent (Fig. 38).

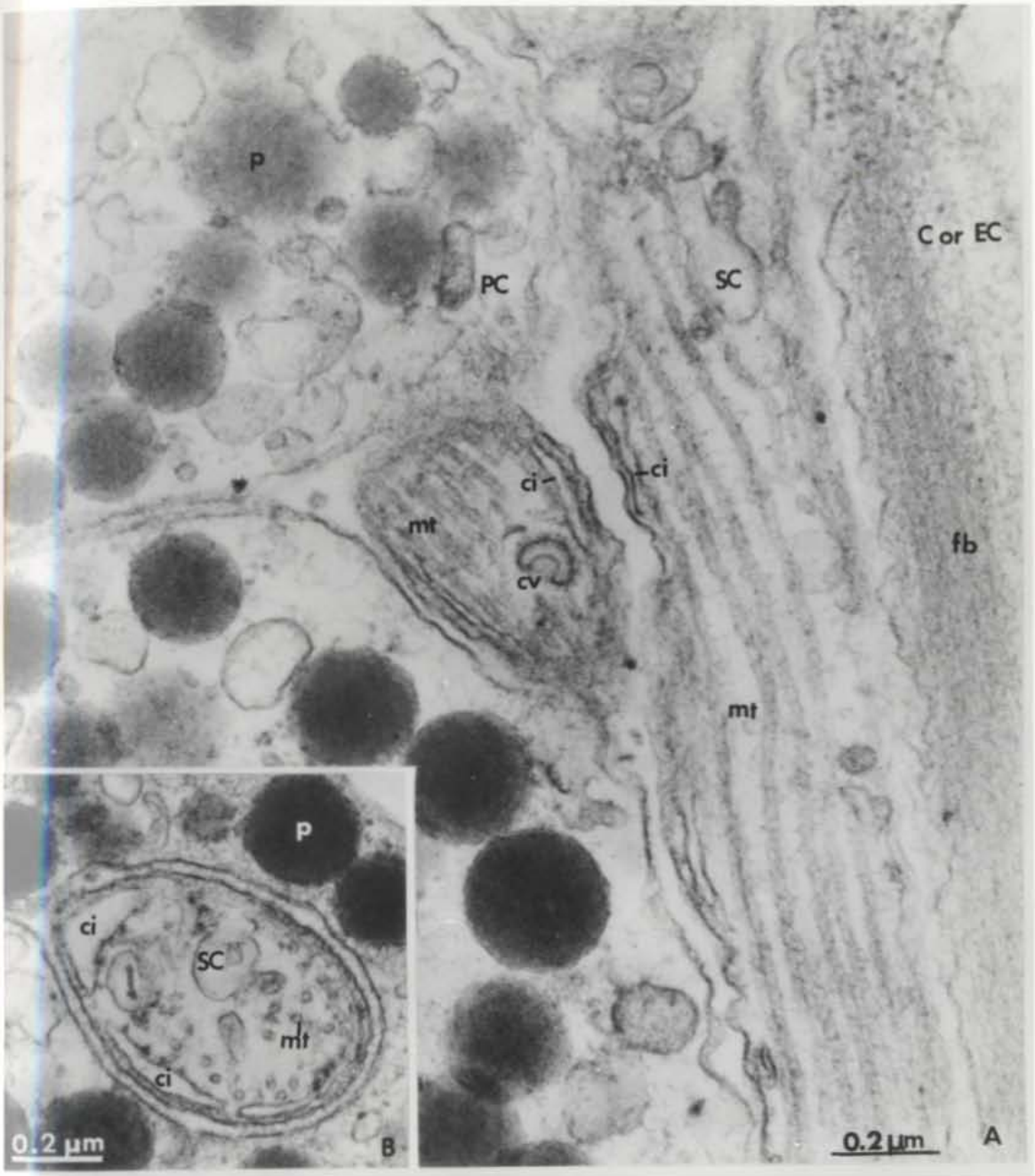
The sensory and pigmented cell nuclei are rounded in form and smaller in size than the epithelial cell nuclei (Fig. 38). Some sensory cell nuclei display a more lightly stained matrix than other nuclei

Figure 41. TEM's of sensory cell neck region.

A. Neck contains a coated vesicle, subsurface cisternae, and microtubules. An adjacent cell possesses microfilaments constituting a bundle. (X75,000)

B. In T/S, subsurface cisternae are seen to line the sensory cell neck, with microtubules running longitudinally. (X64,000)

ci - subsurface cistern
cv - coated vesicle
CC - corneal cell
EC - epithelial cell
fb - filamentous bundle
mt - microtubule
PC - pigmented cell
SC - sensory cell


P
PC
SC
C or EC
ci
ci
mt
cv
fb
mt
P
ci
SC
mt
ci
0.2 µm
B
0.2 µm
A

in the optic cushion. Proximal portions of sensory cells extend to the level of the nerve plexus.

In the terminal tentacle, the ectoneural portion of the radial nerve cord may exceed ten μm in thickness, and is composed of numerous axons (Fig. 42). As in the adult, the axons contain dense-cored vesicles and vesicles with clear or poorly staining contents. Also present are mitochondria, microtubules, and occasional ribosomes and clear vacuoles.

(e) In 1974 larvae, three weeks after the orange-red pigmentation was noted at the base of the terminal tentacle, the ocelli were much more developed with marked changes in many aspects of their morphology, as compared to the larvae illustrated in section (d).

The lumen itself extends for more than 20 μm into the optic cushion and is six μm in breadth (Fig. 43). Six or seven corneal extensions, longer and thicker than in previous stages, arch over the lumen, with small gaps present between them. These extensions are now more densely packed with tubules and vesicles. A 0.5 μm wide bundle of microfilaments is also prominent in these extensions. A few microvilli extend from these cells, and Fig. 43 illustrates a section in which the cilium of one of the corneal cells projects into the lumen of the ocellus.

Constituting most of the wall of the ocellus are pigmented cells, more numerous, and broader (up to four μm), than in earlier developmental stages. These cells project a few microvilli into the lumen and are very obviously characterized by the numerous small pigment granules noted in differentiating pigmented cells in younger larvae. Also

Figure 42. TEM of axons of the ectoneural plexus in the terminal tentacle of five week post-pigmentary specimen. (X24,000)

The axons contain low density and dense-cored vesicles, microtubules, mitochondria, and the occasional vacuole.

dcv - dense-cored vesicle
lv - low density vesicle
m - mitochondrion
mt - microtubule
V - vacuole

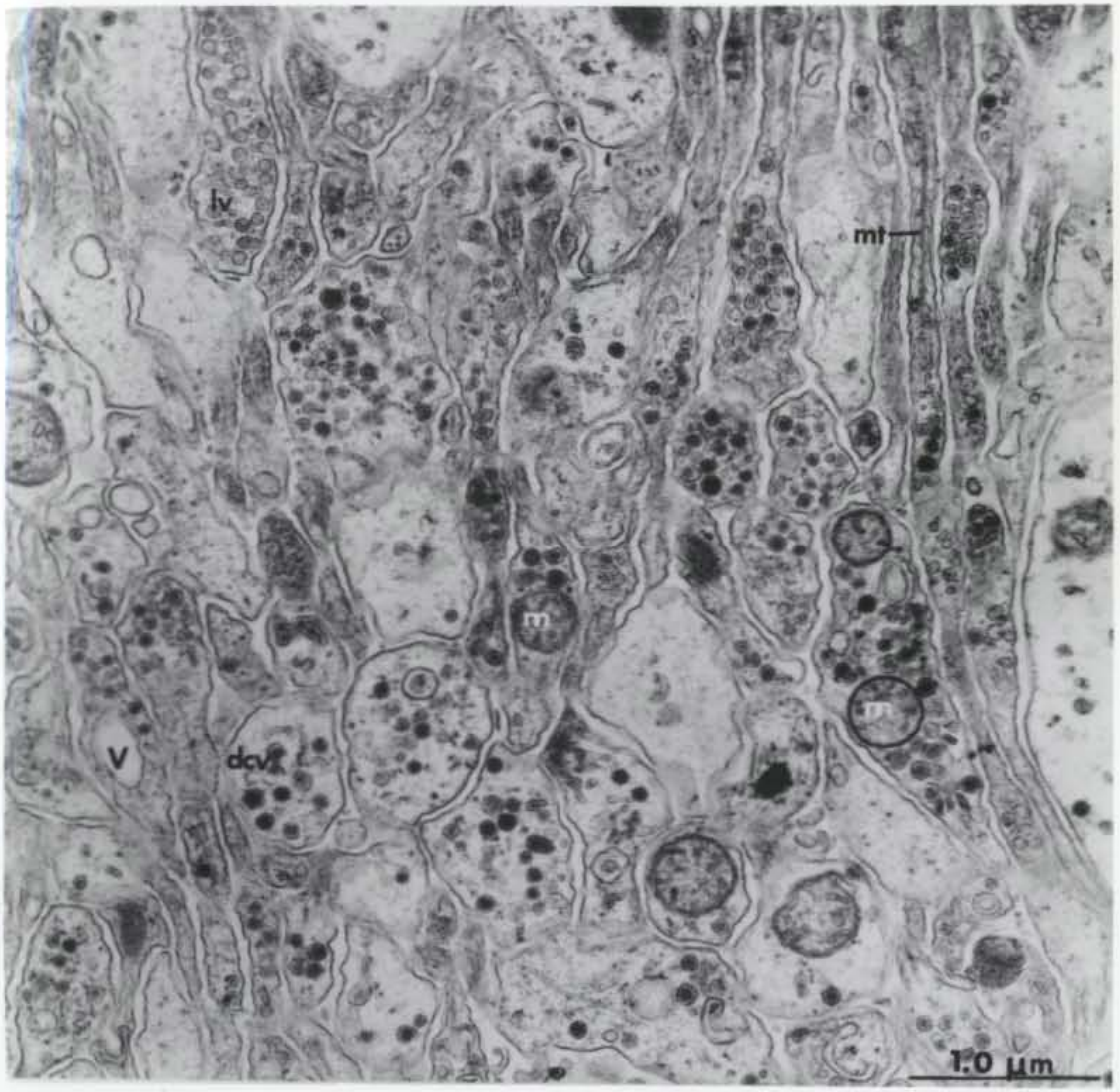
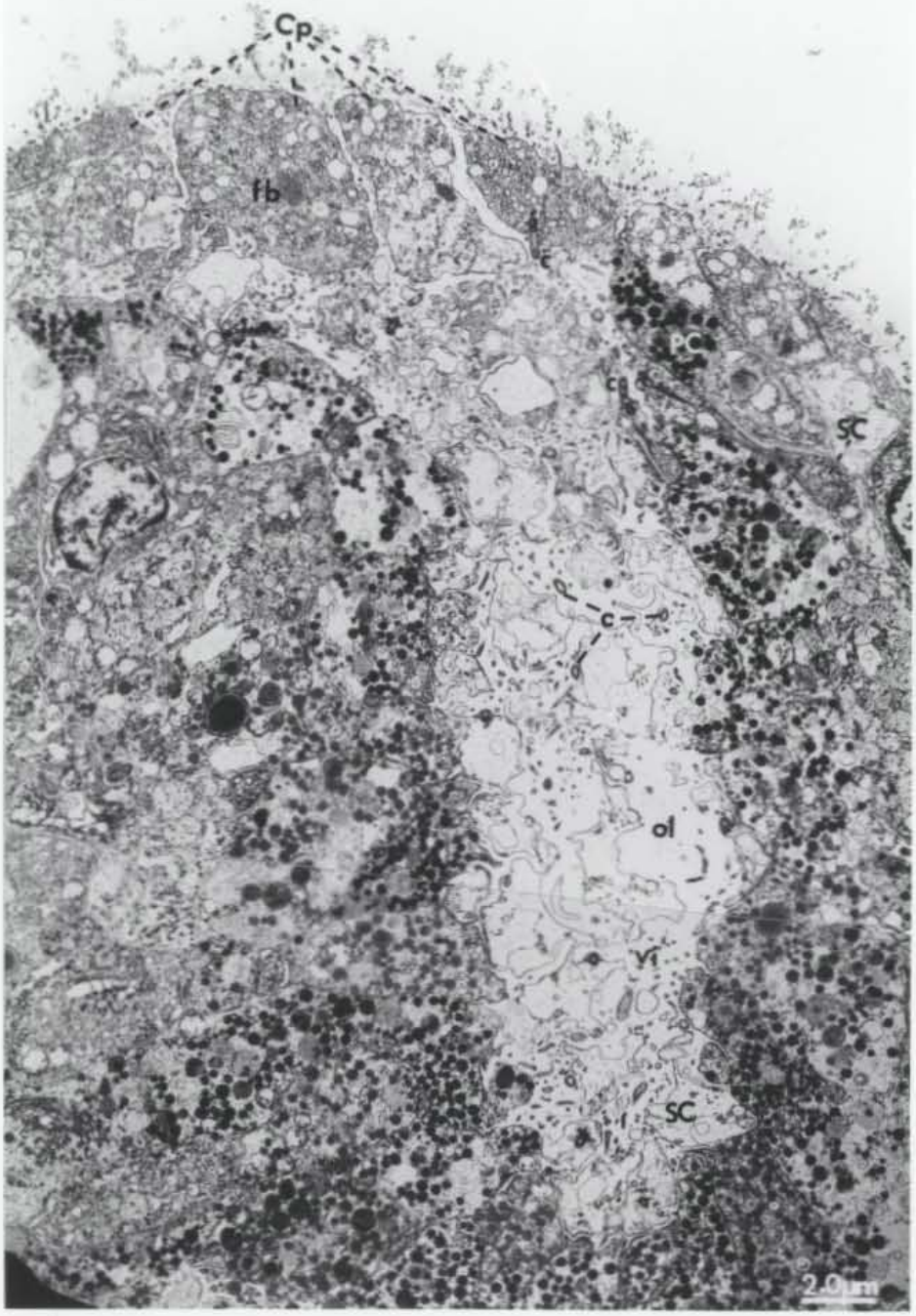

lv
mt
m
m
V
dcvl
1.0 µm

Figure 43. TEM montage of well developed ocellus. (X5050)

The deep lumen contains the distal portions of sensory cells and their cilia and villi, and is covered by several thick corneal cell processes. The lumen is lined principally by numerous pigmented cells with pigment granules and polymorphic bodies. (Fixed in Karnovsky's in Millonig's buffer.)

Cp - corneal cell process
c - cilium
fb - filamentous bundle
ol - ocellar lumen
SC - sensory cell
vi - villus


Cp
fb
PC
SC
c
ol
Yl
SC
2.0 µm

noticeable in these cells, however, though fewer in number than the small pigment granules, are a variety of the larger, membrane-bound, polymorphic bodies which are often seen to contain areas similar to the small pigment granules (Figs. 44, 45).

Occasionally, one or more small pigment granules or region of cytoplasm, is seen to be surrounded by an investing membrane, or is closely associated with flattened low density vesicles (A) (Fig. 44, 45). Larger membrane-bound bodies (B) may contain several of these homogeneously electron-dense patches in a surrounding granular matrix, or may be totally granular. Other profiles (C) are more coarsely granular and contain some membranous components as well as areas similar to the small pigment granules. Still other profiles (D) display a matrix of varying granular condensation and lamellar whorls, and some bodies (E) are completely filled with these membranous lamellae.

The Golgi complex is located in the mid region of the cells and its saccules extend over two µm in length (Fig. 45). The cisternae of the forming face have low density vesicles associated with their ends, while saccules at the mature edge, together with numerous 40 to 120 nm diameter vesicles, are filled with a dense granular material. The mitochondria are more regularly rounded than those in less developed pigmented cells.

The distal portions of sensory cells at this stage are bulbous at their apical end which projects into the lumen (Fig. 43). Villous extensions that are irregular in size and arrangement, arise from these distal regions. Each sensory cell also gives rise to a single cilium

Figure 44. TEM of mid regions of sensory and pigmented cells. (X25,500)

The sensory cells display numerous vesicles and multivesicular bodies. The pigmented cells possess a variety of polymorphic bodies (A - E). (Fixed in Karnovsky's in Millonig's buffer.)

ci - subsurface cistern
cv - coated vesicle
m - mitochondrion
mvb - multivesicular body
p - pigment granule
rer - rough endoplasmic reticulum
SC - sensory cell
v - vesicle

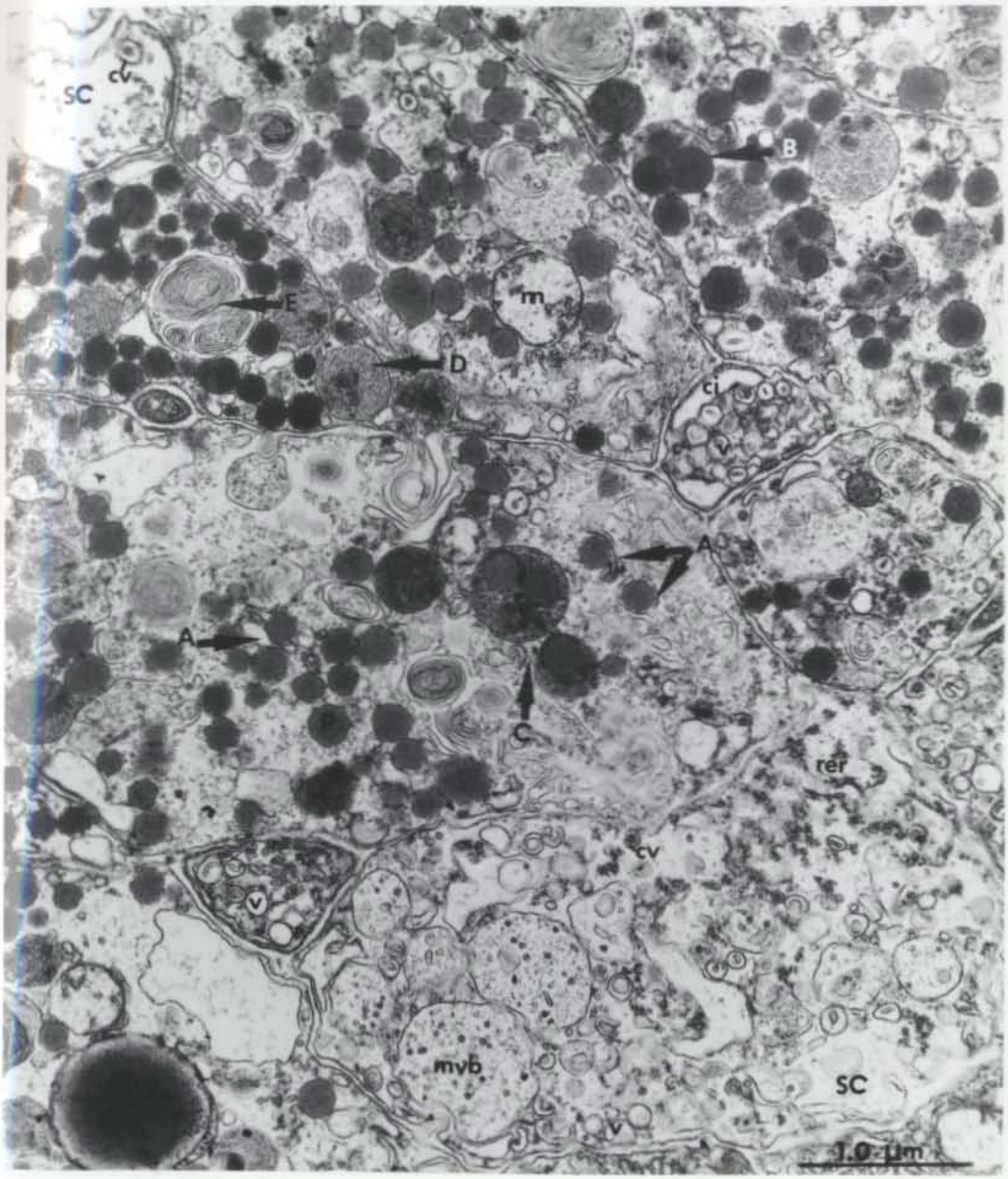
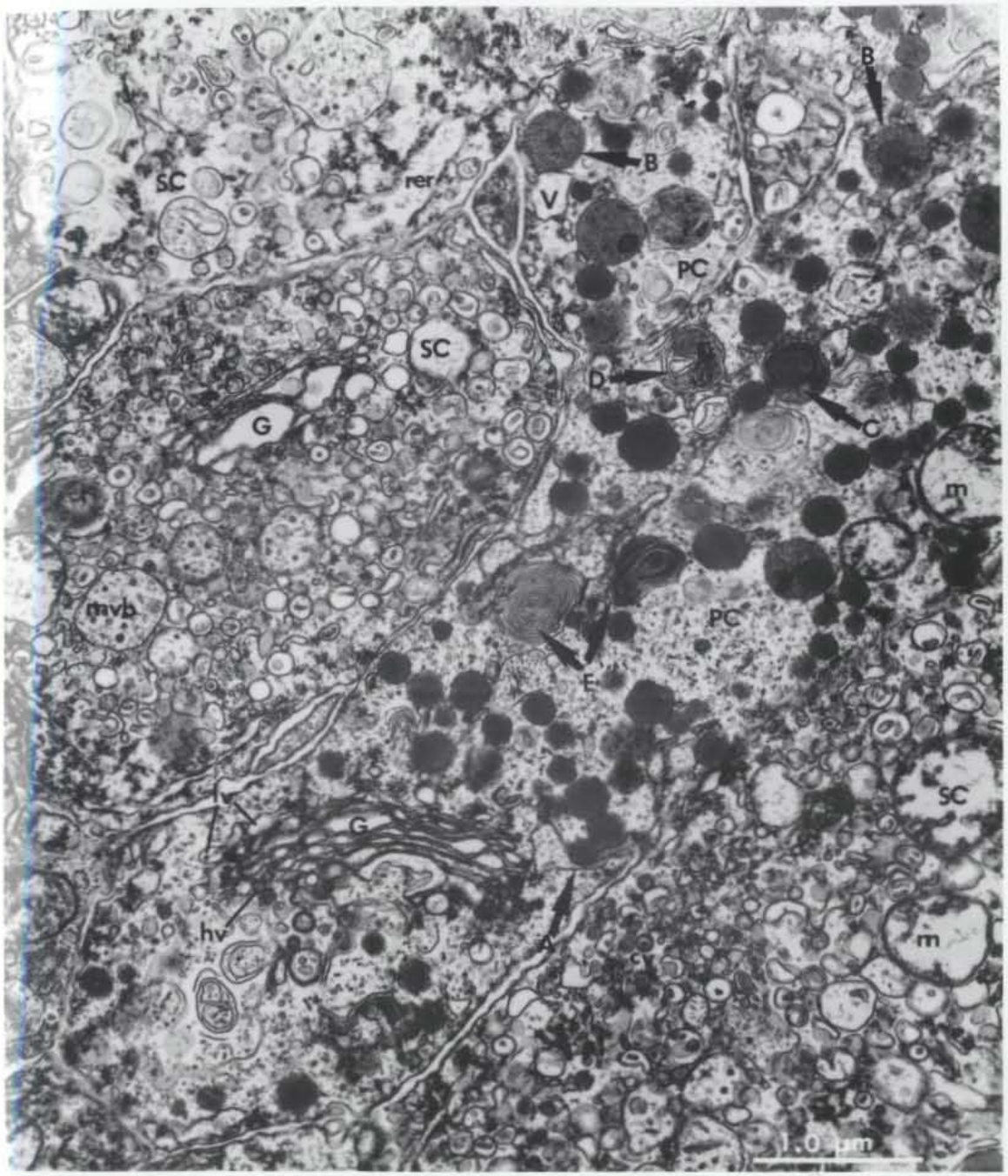

SC
cv
B
E
m
D
ci
v
A
A
C
rer
cv
v
myb
SC
v
1.0 µm

Figure 45. TEM of mid regions of sensory and pigmented cells. (X25,500)

Numerous vesicles, multivesicular bodies, and a Golgi apparatus are present in the sensory cells. The pigmented cells display polymorphic inclusions (A - E) and a Golgi apparatus giving rise to high and low density vesicles. (Fixed in Karnovsky's in Millonig's buffer.)

hv - high density vesicle
lv - low density vesicle
m - mitochondrion
mvb - multivesicular body
PC - pigmented cell
rer - rough endoplasmic reticulum
SC - sensory cell
G - Golgi apparatus


SC
rer
V
B
B
PC
SC
D
C
G
m
mvb
PC
E
SC
G
hv
m
1.0 µm

from a portion of this expansion, or more proximally, at the level at which the sensory cell reaches the lumen from between neighbouring pigmented cells (Figs 46A, B). Each cilium possesses an accessory centriole and a striated root that extends for more than one μm into the neck. The shaft of the cilium generally appears ruffled, and in Fig. 46B, an irregular villous extension of the membrane is seen.

Microtubules noted in the distal expansions of these cells extend down the longitudinal axis of the neck, and vesicles, clear or cored, are very numerous in this area (Fig. 44). Cisternae are flattened around most of the periphery of the neck. Mitochondria with electron-lucent matrices may be present in the neck, as well as in deeper parts of the cells.

Where the sensory cells pass proximally between the pigmented cells and expand in diameter, clumps of ribosomes are numerous, while other ribosomes are associated with short segments of endoplasmic reticulum (Figs. 44, 45). The sensory cells at this level are filled with very numerous multivesicular bodies. Also numerous are vesicles with cored interiors, and some of these are coated. Clear vesicles and smaller denser vesicles are concentrated in the vicinity of the Golgi apparatus (Fig. 45).

(e) Five weeks after pigmentation was initially noted at the base of the terminal tentacle, the larvae have differentiated considerably. The skeleton possesses numerous spines and pedicellariae aborally (Fig. 47A), and the digestive system at this stage consists of an

Figure 46. TEM's of L/S of sensory cell cilia.

A. Basal body, accessory centriole, and striated root can be observed. (X81,000)

B. A villus arises from the shaft of the cilium. (X25,000)

ac - accessory centriole
bb - basal body
c - cilium
PC - pigmented cell
ri - ribosomes
sr - striated root
SC - sensory cell
vi - villus

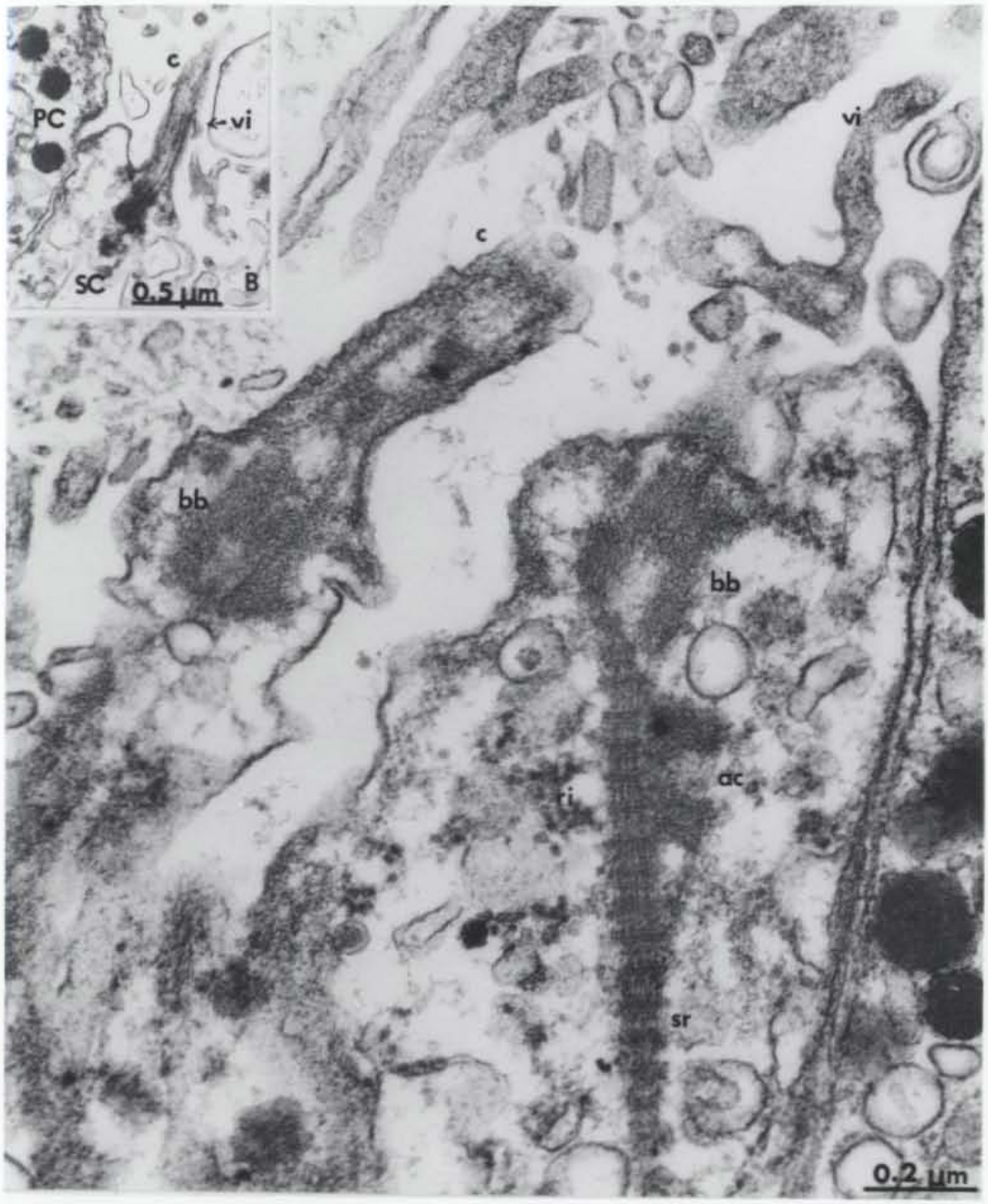

c
PC
←vi
SC
0.5 µm
B
vi
c
bb
bb
ac
ri
sr
0.2 µm

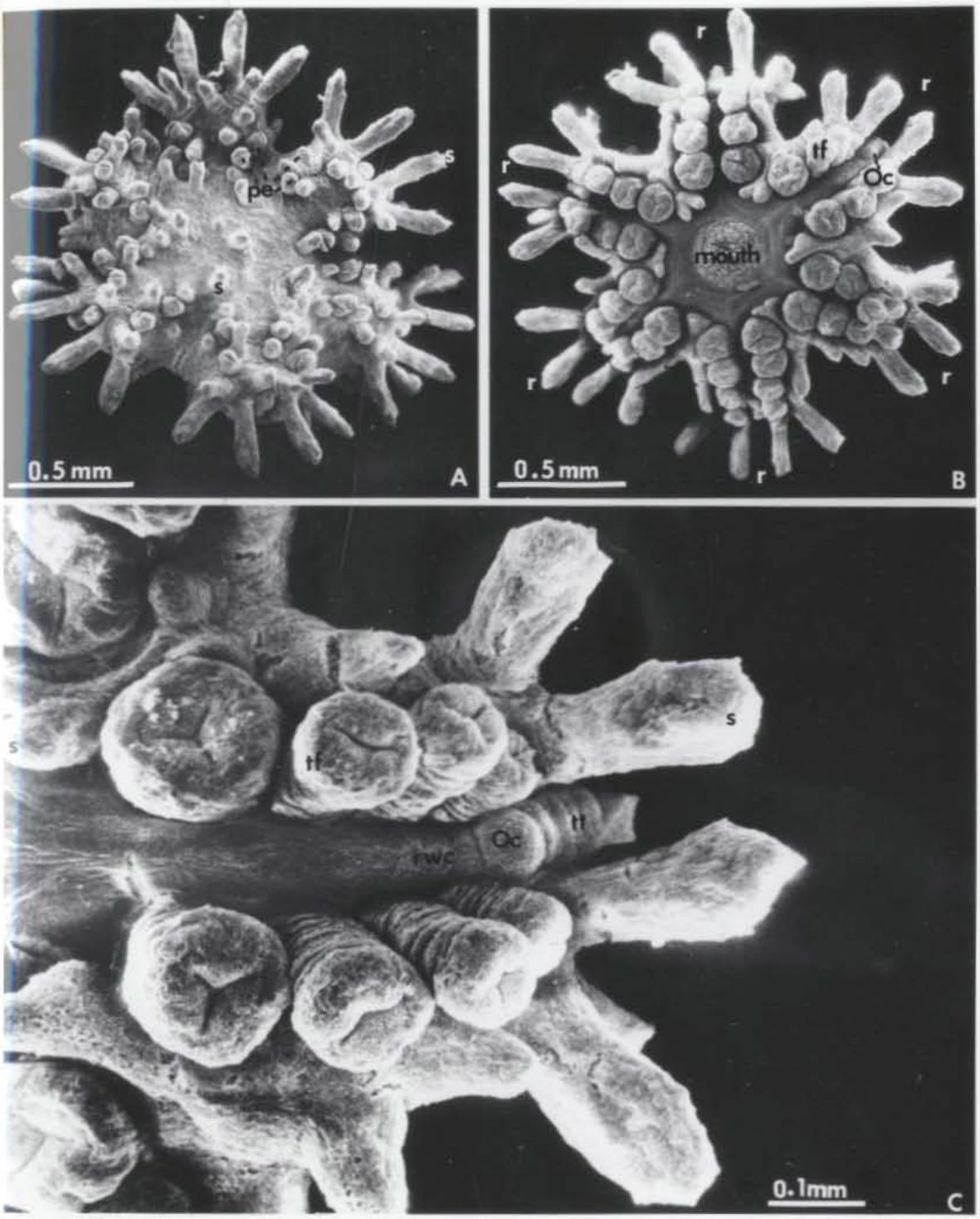
Figure 47. SEM's of five week post-pigmentary larvae.

A. The six rays are well developed, and spines and pedicellariae are noted aborally. (X40)

B. An oral view shows the developing mouth and four pairs of tube feet bordering the radial water canals. (X40)

C. A higher magnification micrograph shows the terminal tentacle, swollen optic cushion, and well developed tube feet. (X150)

Oc - optic cushion
pe - pedicellaria
r - ray
rwc - radial water canal
s - spine
tf - tube foot
tt - terminal tentacle


pe
s
s
0.5 mm
A
r
r
r
tf
Oc
mouth
r
r
r
0.5 mm
B
s
s
tf
tf
Oc
rwc
0.1mm
C

oesophagus, cardiac stomach and developing extensions of caecae from the pyloric stomach (Fig. 48A). The position of the future mouth is evident (Fig. 47B).

Orally, the radial nerve cord is thickened beneath the external epithelium, and four pairs of tube feet are arranged to either side of the water canal (Fig.48A,47B). The terminal tentacle has lengthened and when contracted by its longitudinal muscles becomes folded in appearance. At its base of the oral surface, is a noticeable cushion (Fig. 47C), which in histological sections is seen to contain an ocellus, apparent as a lucent area measuring approximately 25 μm in depth (Fig. 48B). The ocellus is covered externally by the transparent revestment over the epidermis, beneath which lies a thin band of stained tissue, the corneal extensions. Cells with very lightly stained cytoplasm and basally located nuclei are arranged in a radiating fashion around the lumen of the ocellus which contains filamentous structures. Below the nuclei is a fibrous area continuous with the well-developed subepidermal nerve plexus of the rest of the ray.

The cellular components of the ocellus are readily distinguished at the ultrastructural level, exhibiting essentially the same morphological features that characterize them in the adult eyespot (Fig. 49).

Beneath a well-developed revestment, the corneal cells abut closely to the adjacent epidermal, pigmented, and sensory cells, while their processes are less tightly applied to each other. More so than in earlier stages, the corneal processes are densely packed with vesicular and tubular profiles. Their proximal portions together with those

Figure 48. LM's of S/S of five week post-pigmentary larvae.

A. The section show the well developed digestive system and bulbous optic cushion at base of terminal tentacle. (X170)

B. Higher magnification LM of another section, shows the features of the well developed ocellus. (X535) (Mallory-Heidenhain's stain)

ca - caecum
Cp - corneal cell process
e - epithelium
oe - oesophagus
ol - ocellar lumen
Oc - optic cushion
re - revestment
rnc - radial nerve cord
rwc - radial water canal
s - spine
tt - terminal tentacle

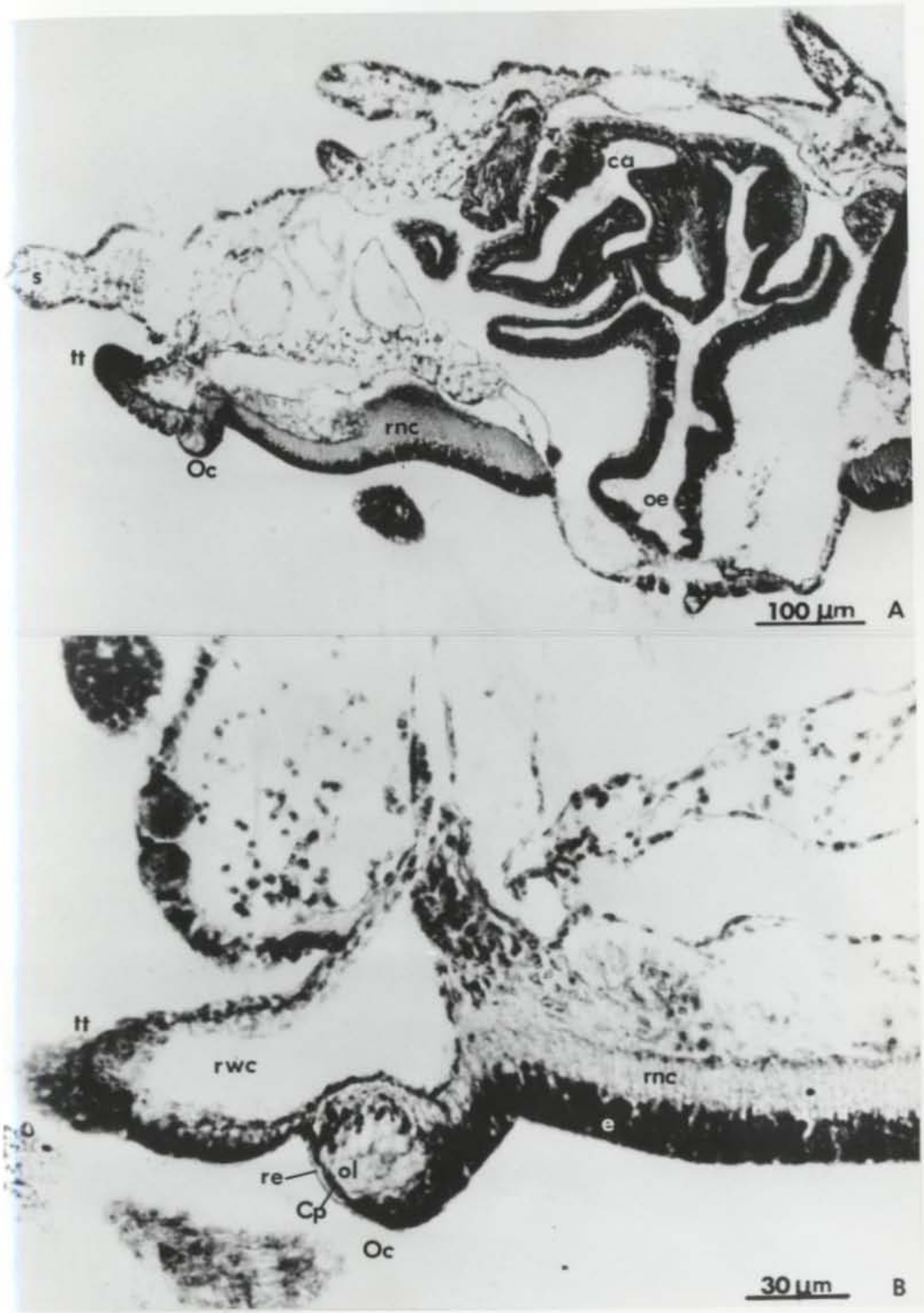
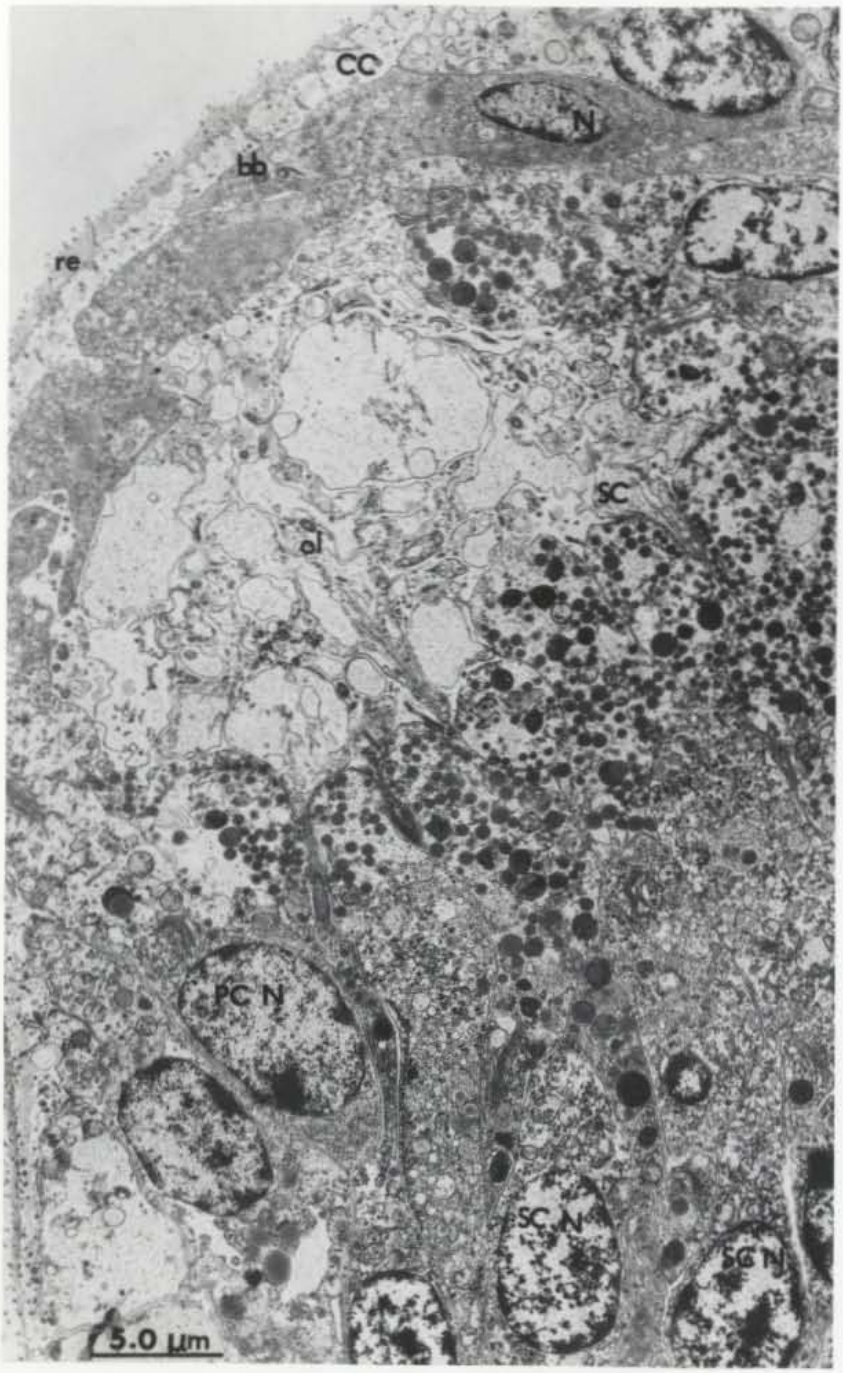

ca
s
tt
rnc
Oc
oe
100 µm
A
tt
rwc
rnc
e
re
ol
Cp
Oc
30 µm
B

Figure 49. TEM of five week post-pigmentary ocellus showing well differentiated components. (X3300)

bb - basal body
CC - corneal cell
N - nucleus
ol - ocellar lumen
PC - pigmented cell
re - revestment
SC - sensory cell


CC
N
bb
re
SC
al
PC N
SC N
SG N
5.0 µm

of neighbouring epidermal supporting cells, extend down around the ocellus and 'encapsulate' it. Their nuclei display matrices of medium electron density and are located in the mid region of the cells at the side of the ocellus.

In the larvae at this time, the small pigment granules are numerous in the portion of the pigmented cells that forms the wall of the ocellus. Also numerous and prevalent in mid and proximal regions are the larger polymorphic bodies (Fig. 50). Multilamellar bodies are not encountered as commonly in these cells as those at the three week stage, and endoplasmic reticulum is not conspicuous.

The lumen of the ocellus is largely filled with distended distal portions of sensory cells and their irregular villous extensions (Fig. 49). A single cilium also arises from this area of each sensory cell. Most sections show the cilia to have a slightly fluted configuration of the shaft membrane. Two cilia transversely sectioned in Fig. 50A illustrate an axonemal complement of nine pairs of peripheral microtubules and a single central microtubule. Other, less distinct ciliary sections suggest the presence of two central microtubules, and still others indicated that the central microtubules and some of the peripheral ones are missing.

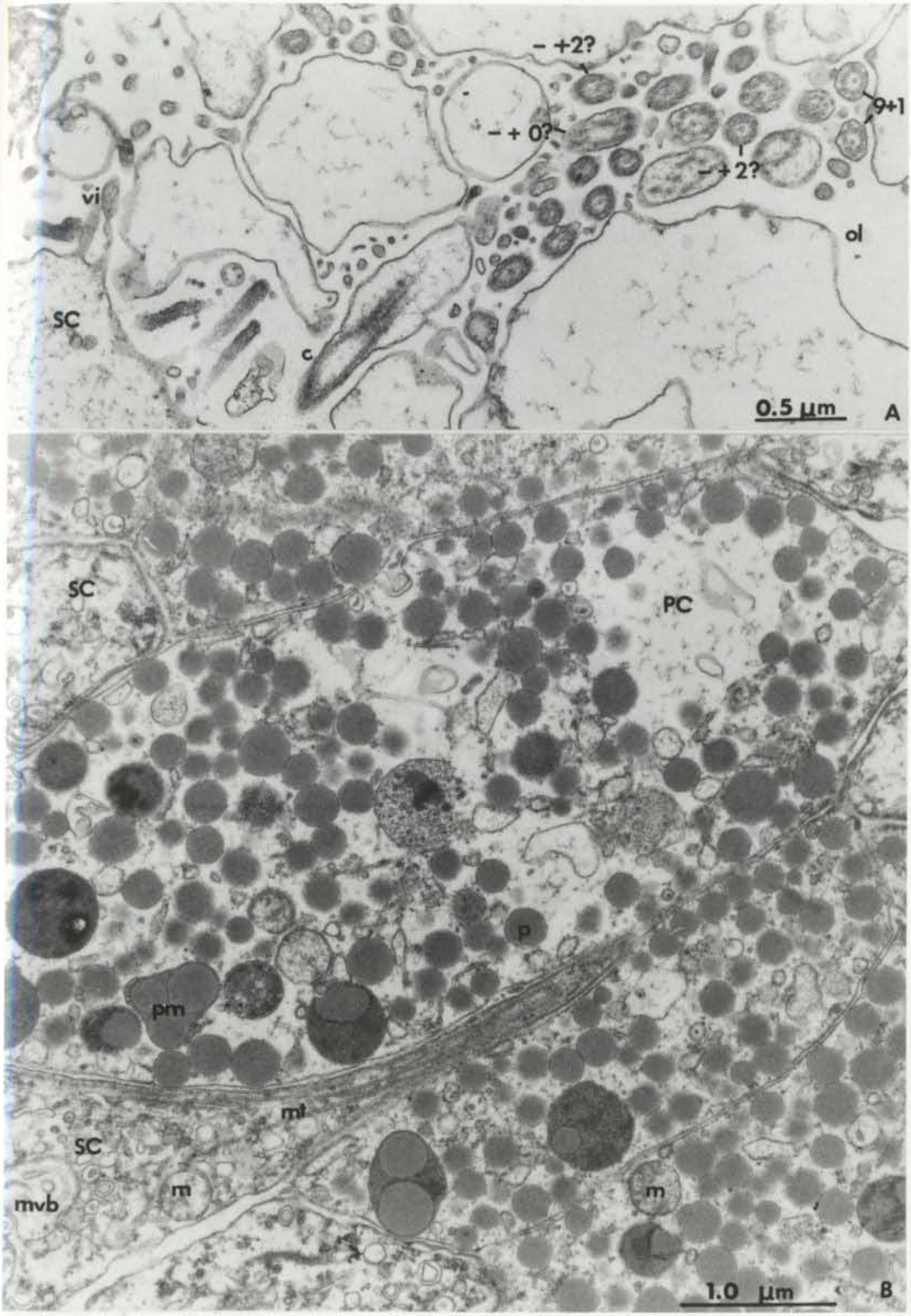
Several micrometres from the lumen, below the broad portions of the pigmented cells, the sensory cells expand and contain the organelles described in earlier stages, with some features to be noted here (Fig. 50B). The sensory cell mitochondria usually have a less densely stained matrix, and a more irregular form than those of the

Figure 50. TEM's of sensory cells of five week post-pigmentary larva.

A. T/S of cilia in the lumen. (X29,000)

B. Mid region of sensory cells between pigmented cells in the ocellar wall. (X25,500)

c - cilium
m - mitochondrion
mt - microtubule
mvb - multivesicular body
ol - ocellar lumen
p - pigment granule
pm - polymorphic body
PC - pigmented cell
SC - sensory cell
vi - villus


- +2?
9+1
- + 0?
- +2?
vi
ol
SC
c
0.5 µm
A
SC
PC
P
pm
mi
SC
mvb
m
m
1.0 µm
B

pigmented cells. Ribosomes and rough endoplasmic reticulum continue to be common in this region of the cell, and multivesicular bodies are still numerous in many sensory cells apical to the nucleus.

Sensory cell nuclei have become more elongated in form than in less developed ocelli and have maintained a matrix lighter in its staining properties than pigmented, corneal, and epithelial cell nuclei (Fig. 49). Proximal portions of the sensory cells extend into the nerve plexus and are noted to lie very close to and be similar in appearance to axons.

(f) The larval ocellus has essentially established its adult morphology by the five week stage, with minor changes in dimensions and some morphological features to be noted in larvae at eight, 12, 18, and 23 weeks post-pigmentation.

By the eight week stage, the elaborated digestive system opens from the oesophagus to the exterior through the mouth (Fig. 51A). The optic cushion is an obvious swelling at the base of the terminal tentacle (Fig. 51B) and in thick Epon sections, the accumulation of pigment granules in this region is readily noted (Fig. 51C).

Twelve weeks after the appearance of pigmentation, the optic cushion has increased in size, particularly along the longitudinal axis of the ray (Fig. 52A). The lumen of the ocellus has also elongated to approximately 35 μm in length (Fig. 52B). Thick Epon sections of 18 week post-pigmentary larvae illustrate this broad and deep ocellus in the optic cushion (Fig. 52D). The extracellular revestment and thick corneal processes are obvious. Numerous pigmented cells line the wall

Figure 51. LM's of S/S through eight week post-pigmentary larvae.

A. The section shows four pairs of tube feet and terminal tentacle with optic cushion. The digestive system consists of an oesophagus, cardiac and pyloric stomachs, and caecae, and opens to the exterior through a mouth. (X120). Delafield's Haematoxylin and Eosin.

B. Higher magnification micrograph illustrates the extensive ocellus in the optic cushion. (X360). Mallory-Heidenhain's stain.

C. LM of epon-embedded section shows pigmented cells lining the deep ocellus. (X700)

ca - caecum
car - cardiac stomach
e - epithelium
np - nerve plexus
o - ocellus
oe - oesophagus
Oc - optic cushion
p - pigment granule
pyl - pyloric stomach
rwc - radial water canal
tf - tube foot
tt - terminal tentacle

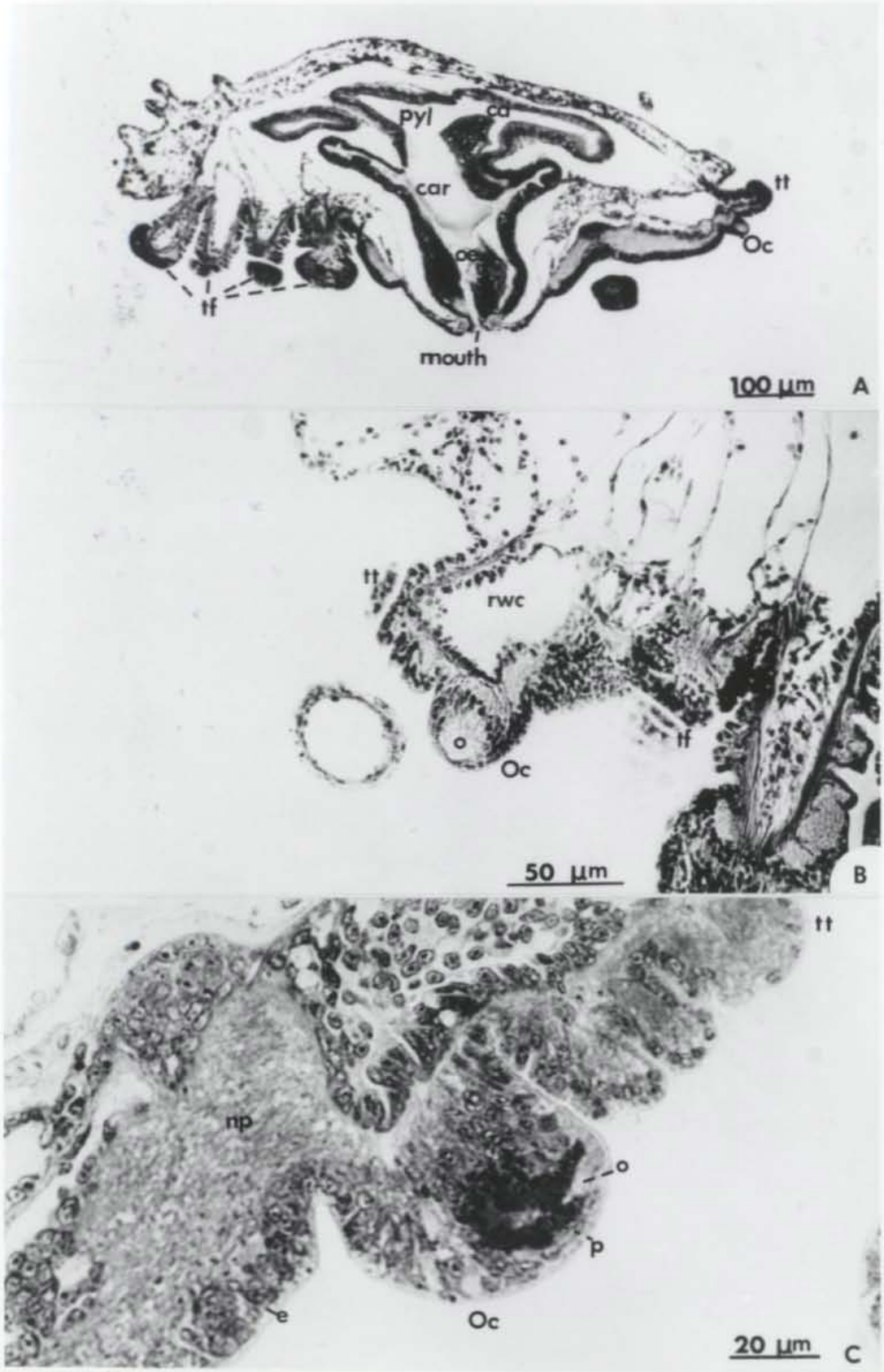

pyl
cd
car
tt
Oc
oe
tf
mouth
100 µm
A
tt
rwc
o
Oc
tf
50 µm
B
tt
np
o
p
e
Oc
20 µm
C

Figure 52. Micrographs of the optic region of well developed larvae.

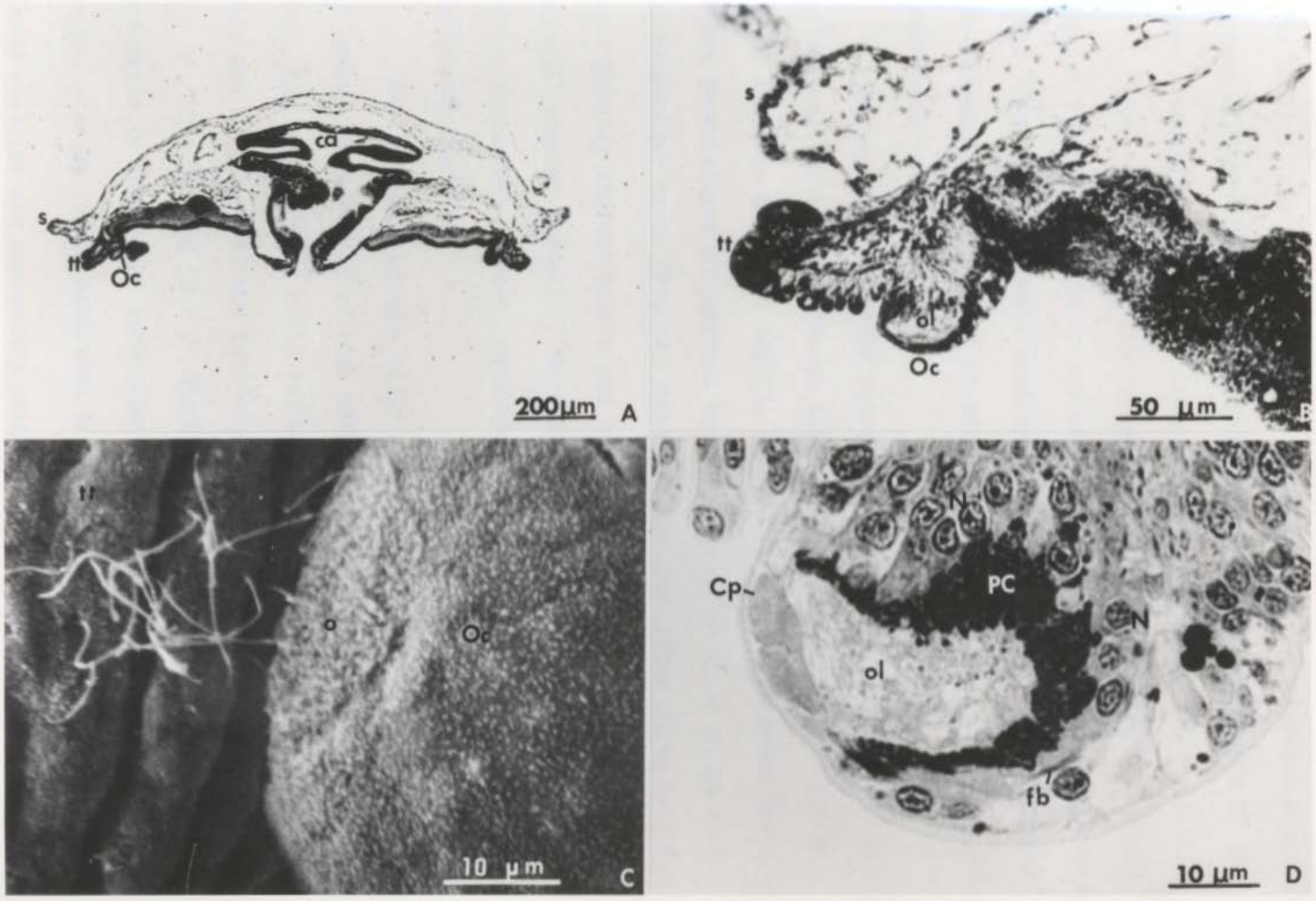
A. LM of S/S of twelve week post-pigmentary larva showing complete digestive system and elongated optic cushion. (X65) Mallory-Heidenhain's stain.

B. Higher magnification LM of same specimen to show elongated ocellus in optic cushion. (X370). Mallory-Heidenhain's stain.

C. SEM of 19 week post-pigmentary larva shows surface irregularity denoting the position of the large single ocellus in the optic cushion. (X1900) (Fixed in Karnovsky's in Millonig's buffer)

D. LM of epon-embedded section of 18 week post-pigmentary larva shows ocellar lumen overlain by thick corneal processes and lined by numerous pigmented cells. (X1450)

ca - caecum
Cp - corneal cell process
fb - filamentous bundle
N - nucleus
o - ocellus
ol - ocellar lumen
Oc - optic cushion
PC - pigmented cell
s - spine
tt - terminal tentacle


ca
s
tt
Oc
200µm
A
s
tt
ol
Oc
50 µm
B
tt
o
Oc
10 µm
C
N
Cp
PC
N
ol
fb
10 µm
D

of the ocellus and the lumen is filled with finely filamentous material. SEM views of the bulbous cushion reveal an irregular appearance of the epidermis denoting the site of the single ocellus (Fig. 52C).

Ultrastructurally, the corneal cells, as in the adult, project processes filled with tubular and vesicular profiles and microfilamentous bundles (Fig. 53). The well-developed revestment possesses the typical inner filamentous stratum and an outer, bilayered, stratum of more densely fibrous material.

The numerous and broad pigmented cells have microvilli extending into the ocellar lumen, although they are not yet as numerous or as long as those in the adult ocelli. The cells possess numerous small pigment granules and polymorphic bodies. A section of an eight week post-pigmentary larva (Fig 54) shows many of the latter to be profiles with a densely stained matrix and one or more patches of material like the small pigment granules. More coarsely granular areas of cytoplasm possess incomplete bounding membranes occasionally associated with low density vesicles.

The numerous sensory cells are greatly expanded apically into the lumen with numerous villous extensions from their cell membranes (Figs. 55A, B). Single cilia arise from these expansions, with transverse and slightly oblique sections showing axonemal complements of '8 + 1', '8 + 2', and '9 + 2' microtubules (Fig. 55A). Also very noticeable in the distal expansions of the sensory cells in these later stages, are the coated membranes of many of the clear and cored vesicles. Coated vesicles are apparently phagocytosing bits of villous material

Figure 53. TEM of corneal cell processes of eight week post-pigmentary larva. (X25,100)

Processes contain numerous vesicles, and overlie the ocellar lumen under a well developed revestment.

Cp - corneal cell process
fb - filamentous bundle
mv - microvillus
ol - ocellar lumen
PC - pigmented cell
re - revestment
SC - sensory cell
v - vesicle

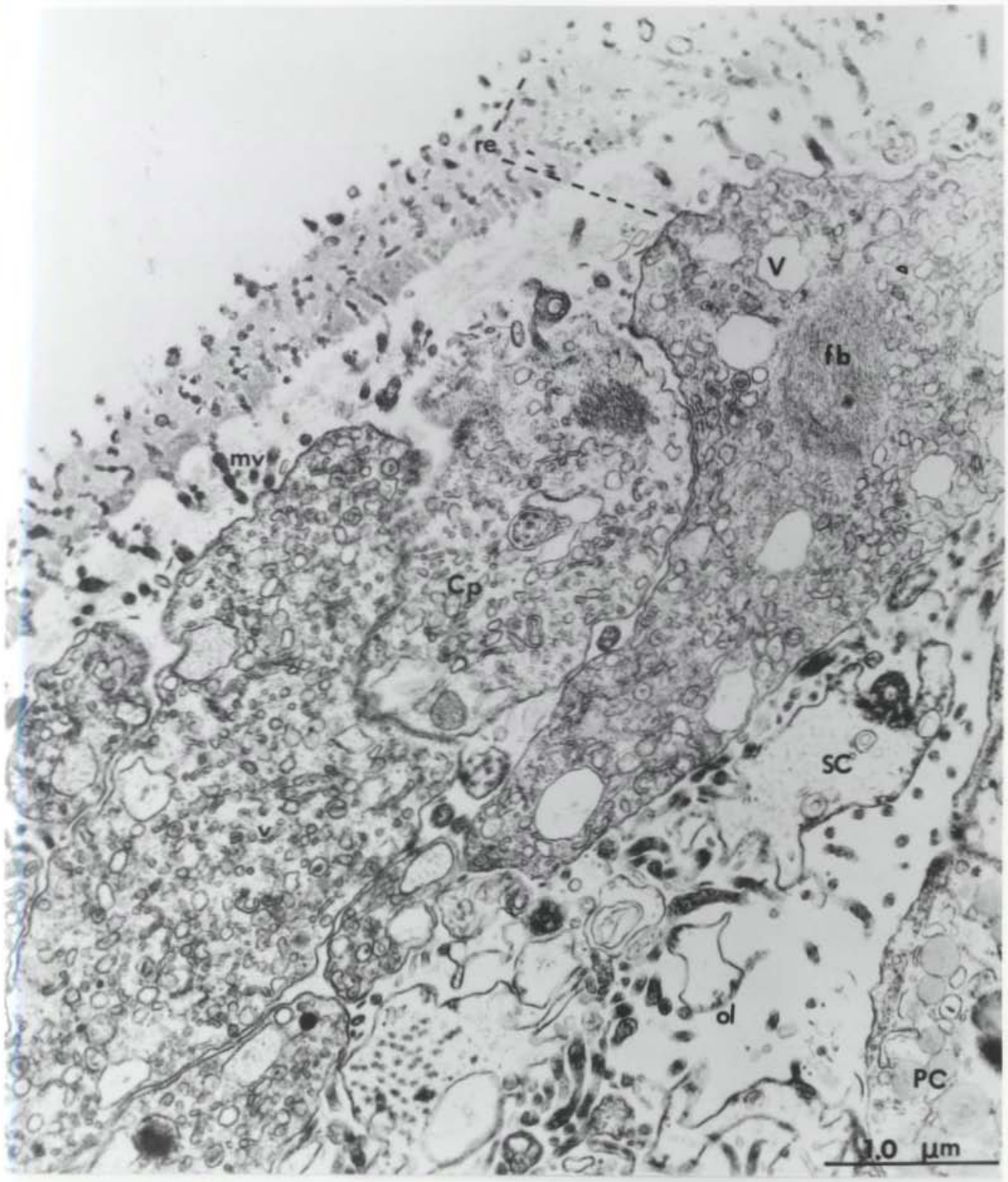

re
V
fb
my
Cp
SC
v
ol
PC
1.0 µm

Figure 54. TEM of mid regions of pigmented and sensory cells in eight week post-pigmentary larva. (X25,100)

The pigmented cells display numerous low density vesicles (⟶) in association with pigment granules and with granular regions of the cytoplasm. The Golgi apparatus is prominent. The sensory cell contains coated vesicles, and a coated invagination of the plasma membrane (⟶⟩) can be noted.

cv - coated vesicle
dcv - dense-cored vesicle
G - Golgi apparatus
hv - high density vesicle
m - mitochondrion
mvb - multivesicular body
N - nucleus
p - pigment granule
pm - polymorphic body
SC - sensory cell

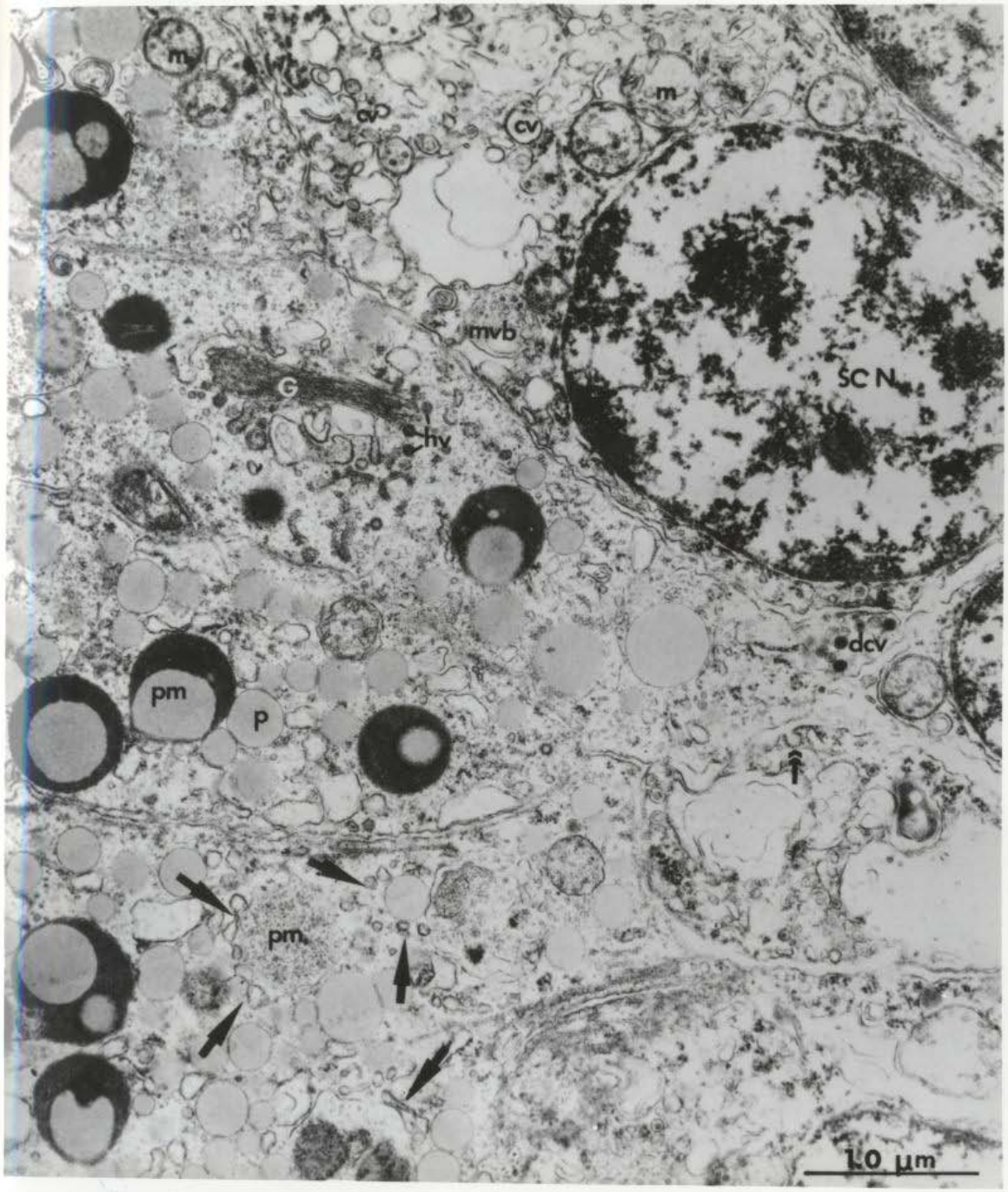
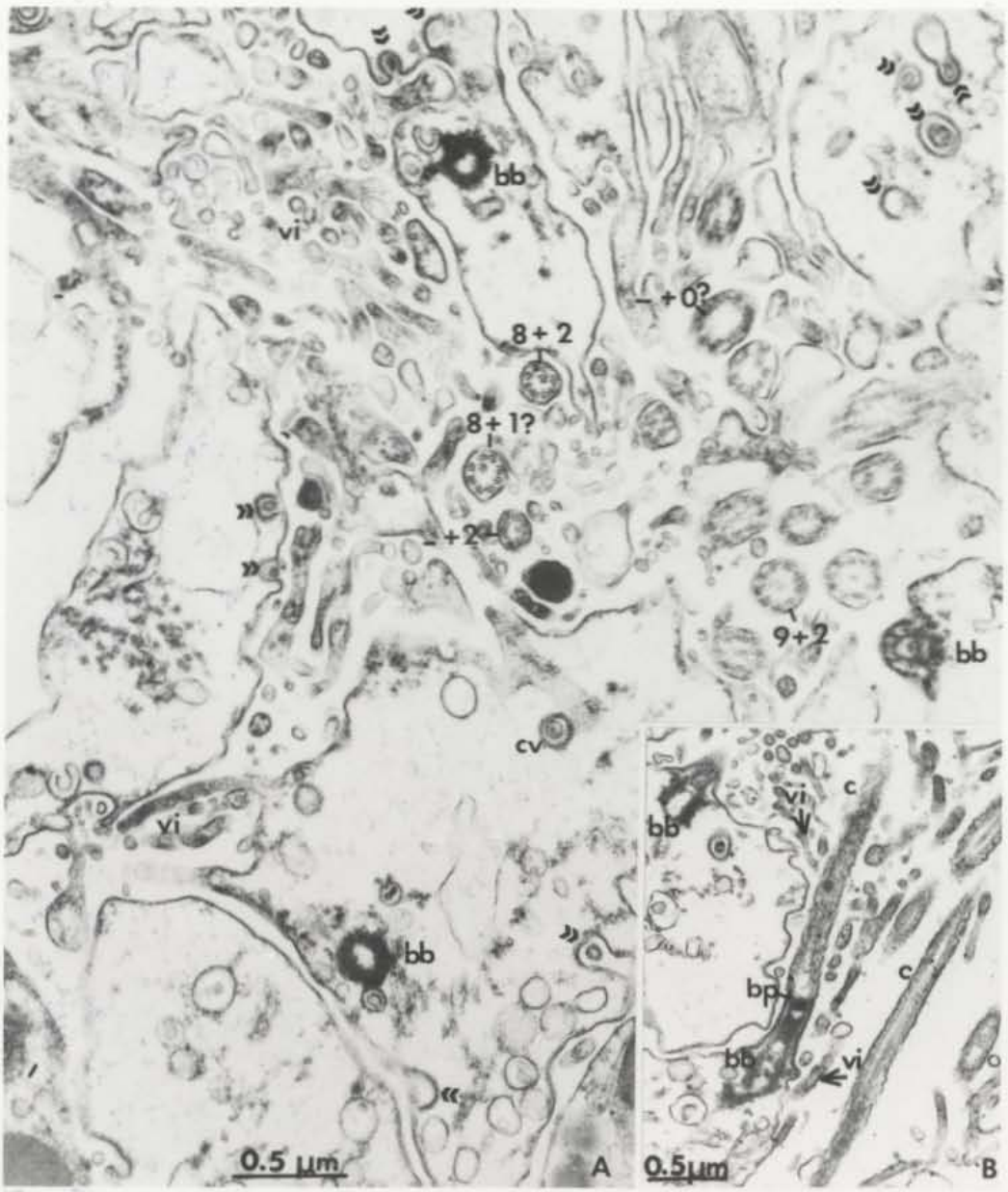

m
cv
m
cv
mvb
G
hy
SC N
dcv
pm
P
pm
1.0 µm

Figure 55. TEM's of distal sensory cell expansions with cilia, villi, and coated invaginations of the plasma membrane.

A. The lumen of an 18 week post-pigmentary ocellus possesses cilia with various microtubular counts. Coated invaginations (≫) of the sensory cell membrane apparently represent the phagocytosis of villous debris into coated vesicles. (X29,000)

B. A 23 week post-pigmentary sensory cell shows a villus arising from the cell membrane, and another villus perhaps having arisen from the ciliary shaft membrane. (X21,600)

bb - basal body
bp - basal plate
c - cilium
cv - coated vesicle
vi - villus


bb
vi
8+2
8+1?
9+2
cv
0.5 µm
A
c
bp
0.5µm
B

noted free in the ocellar lumen, and such material is similar to the inclusions of the cored, coated vesicles.

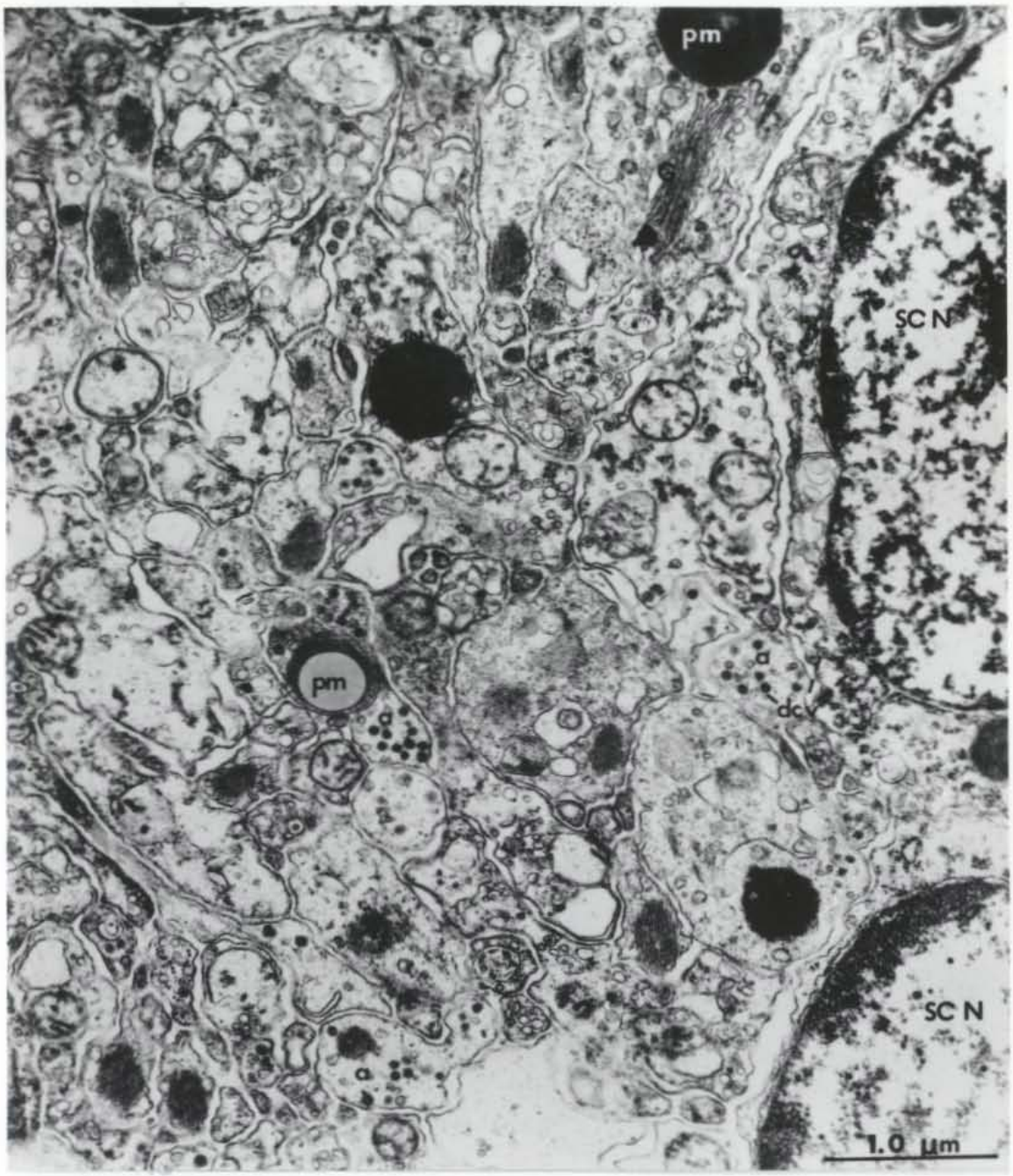
Cored, and clear vesicles continue to be very numerous in the neck and supranuclear regions of the sensory cells. Some of these display coated membranes, and a profile in Fig. 54 illustrates that coated vesicles may occasionally also arise from the plasma membrane in this region of the cell.

Multivesicular bodies are present, though not as numerous in most of these stages as in some earlier ones (Fig. 54). The peripherally located subsurface cisternae of the neck region extend for some distance down the cell and terminate distal to the nucleus. Proximally, a few dense-cored vesicles are occasionally present in the cytoplasm at the level of the ectoneural axons (Fig. 56). Tapered portions of pigmented cells are present in this region also.

Figure 56. TEM of eight week post-pigmentary larva at level of sensory cells and pigmented cells intermingling with axons. (X25,100)

The sensory cell to the right possesses a few dense-cored vesicles in its cytoplasm.

a - axon
dcv - dense-cored vesicle
G - Golgi apparatus
N - nucleus
pm - polymorphic body
SC - sensory cell


pm
SC N
pm
a
dc
SC N
a
1.0 µm

C. REGENERATIVE DEVELOPMENT OF THE ADULT OPTIC CUSHION AND OCELLI

1. RATE OF REGENERATION

Regeneration data were collected for specimens whose terminal tentacles including eyespots were excised. Observations were continued of the regeneration of those regions that were left after severance of the distal several millimetres of the ray tip for material to use for light and electron microscopy. General observations of the times required for the appearance of the pigmentation of the eyespot site, and for the development of a recognizable optic cushion, were noted by periodic examination of the specimens with a dissecting microscope.

A significant factor correlated with regeneration rate was the sea water temperature during the period of redevelopment. Table 2 lists data indicating the state of development of pigmentation in specimens, 30 to 34 days after their terminal tentacles had been removed, and serves to illustrate this temperature relationship. Within approximately the same length of time, specimens regenerating in warmer sea water displayed more rapid appearance of pigmentation and subsequent elaboration of the optic cushion than did those under colder temperature regimes.

Regeneration times were also related to some extent with the amount of ray tissue initially removed. For example, from the data recorded in Table 3, for Group III specimens with terminal tentacles excised, optic cushions regenerated in an average time of less than 25

TABLE 2

DATA TO ILLUSTRATE A RELATIONSHIP BETWEEN REGENERATION RATE AND SEA WATER TEMPERATURE

Group I Excision 13 May 1974 Examined 12 June 1974--30 days post-excision Average Sea Water Temperature: 2.5°C (Range 0.5°C to 3.5°C)		
Specimen	Number of Rays Displaying	
	Pigmentation	Optic Cushion
8	1/4[1]	0/4
10	0/4	0/4
11	0/4	0/4
12	1/4	0/4
13	2/3	0/3
14	2/4	0/4
15	0/4	0/4
16	2/4	0/4
	8/31	0/31
Group II Excision 3 July 1974 Examined 5 August 1974--33 days post-excision Average Sea Water Temperature: 6.5°C (Range 5.5°C to 9.5°C)		
Specimen	Number of Rays Displaying	
	Pigmentation	Optic Cushion
9	4/4	0/4
17	4/4	3/4
18	4/4	2/4
19	4/4	1/4
20	4/4	3/4
21	2/4	0/4
	22/24	9/24

TABLE 2 (CONTINUED)

Group III Excision 14 August 1974 Examined 17 September 1974--34 days post-excision Average Sea Water Temperature: 11.0°C (Range 8.5°C to 12.0°C)		
Specimen	Number of Rays Displaying	
	Pigmentation	Optic Cushion
22	4/4	3/3
23	4/4	0/4
24	4/4	4/4
25	4/4	3/3
26	3/4	3/4
27	2/2	1/2
28	4/4	3/3
	25/26	17/23[2]

[1]Expressions are the number of rays displaying the morphological feature of the total number of rays excised on the specimen.

[2]At some date preceding 17 September 1974, three rays displaying pigmentation were removed for microscopy and were thus not available for examination on 17 September 1974 for the presence of well elaborated optic cushions.

TABLE 3

THE RATE OF DEVELOPMENT OF OPTIC CUSHIONS (O.C.) OF SPECIMENS WITH TERMINAL TENTACLES (T.T.) REMOVED AS COMPARED WITH THOSE WITH RAY TIPS (R.T.) REMOVED

Specimen	Ray	Date of T.T. Excision	No. of Days Until O.C. Noted	Date of R.T. Excision	No. of Days Until O.C. Noted
22	1	14.8.74	13	--	-
	4	"	34	17. 9.74	41
	5	"	--	27. 8.74	42
	6	"	34	8.10.74	55
23	2	"	--	27. 8.74	42
	3	"	55	28.10.74	101
	4	"	--	17. 9.74	41
	5	"	55	8.10.74	121
24	1	"	13	17. 9.74	41
	4	"	13	--	--
	5	"	13	--	--
	6	"	13	27. 8.74	42
25	1	"	34	8.10.74	65
	4	"	--	27. 8.74	42
	5	"	34	17. 9.74	41
	6	"	34	28.10.74	101
26	1	"	--	27. 8.74	42
	2	"	13	2.12.74	66
	4	"	13	8.10.74	55
	6	"	13	17. 9.74	41
27	3	"	34	17. 9.74	41
	4	"	--	27. 8.74	42
28	3	"	13	17. 9.74	41
	4	"	13	--	--
	5	"	--	27. 8.74	42
	6	"	34	8.10.74	65

days for 19 rays. In cases where the tips of these rays were then removed for microscopical study, regeneration occurred in an average time of less than 47 days for 19 rays (excluding three observations exceeding 100 days where regeneration continued over the winter period and temperature might be an additional factor).

Again, as for the larval situation, because development times may vary between individual sea stars, and because the rate of regeneration is likely to be affected by sea water temperature, time sequences will serve to order events chronologically, and to give a general estimate of the time spans involved for eyespot elaboration in some individual sea stars.

2. TISSUE REMOVAL AND WOUND HEALING

Routine excision resulted in complete removal of the optic cushion and of the rest of the terminal tentacular tissue (Fig. 57A). Damage to skeletal tissues of the terminal ossicle overhanging the tentacular region also occurred in some cases. In all specimens of L. polaris whose terminal tentacular tissues were excised, the wound area was observed to be contracted somewhat and completely covered by a non-pigmented epithelium within several days.

3. DIFFERENTIATION AND REGENERATION (HISTOLOGICAL OBSERVATIONS)

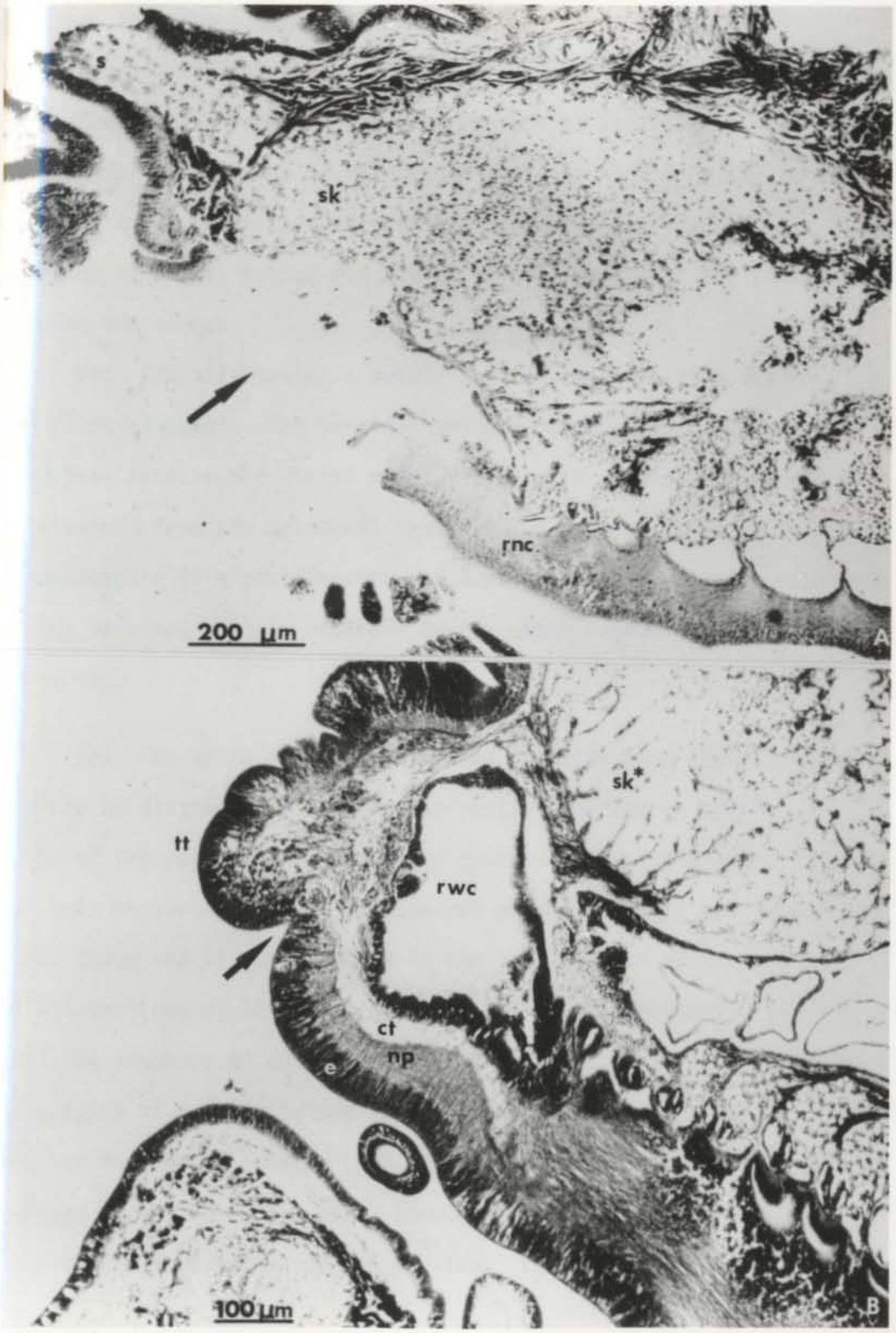
(a) Within approximately a week after excision, the tissues of the distal portion of the oral surface of the ray can be distinguished as epithelial, nervous, connective, muscular, and coelomic epithelial layers. The most distal region of the regenerative tissue presents the

Figure 57. LM's of adult ray tips at various stages of regeneration.

A. S/S of post-operative ray tip shows complete removal of terminal tentacle and optic cushion, and some damage(→) to the skeletal tissue. (X95) Mallory-Heidenhain's stain.

B. S/S of Group IV, 10 day post-operative specimen shows short terminal tentacle. (X125) Mallory-Heindenhain's stain
→ -- groove; sk* -- proliferation of new skeletal tissue.

ct - connective tissue
e - epithelium
np - nerve plexus
rnc - radial nerve cord
rwc - radial water canal
sk - skeletal tissue
tt - terminal tentacle


s
sk
rnc
200 µm
A
sk*
tt
rwc
ct
np
e
100 µm
B

beginnings of a short terminal tentacle.

Coincident with the outgrowth of the terminal tentacle is the establishment of a groove on the oral surface of its base. This groove separates the distal edge of the future optic cushion from the terminal tentacle proper. A further sign of the development of the eyespot is the appearance of small, orange-red flecks on the surface of the cushion bordering the groove.

Fig. 57B illustrates a nearly sagittal section of a ray in this stage of development. The terminal tentacle extends 150 μm beyond the groove that denotes the distal edge of the optic cushion. Supporting fibres extend from the epidermal layer to the connective tissue layer. Also noticeable is a proliferation of dermal tissue as strands extending into the terminal ossicle adjacent to the other regenerative tissue of the ray tip.

(b) The optic cushion, delimited distally by a groove, now commences to be distinguishable from the rest of the ray by another infolding of the epithelium. The optic cushion in Fig. 58A has a longitudinal axis measuring 300 μm. Orange-red pigmentation is very noticeable at this stage and is concentrated in the leading edge of the cushion. Sagittal sections of 10 and 13 day post-operative specimens (Fig. 58B, C) reveal the presence of an ocellus in this region. Beneath the revestment, a thin strip of darkly stained tissue denotes the corneal processes overlying the inpocketed ocellus (Fig. 58B). Fibrous components extend from the edges of the epidermal cells through the nerve plexus and also extend around the perimeter of the ocellus. The lumen of the ocellus is

Figure 58. LM's of adult ray tips at various stages of regeneration.

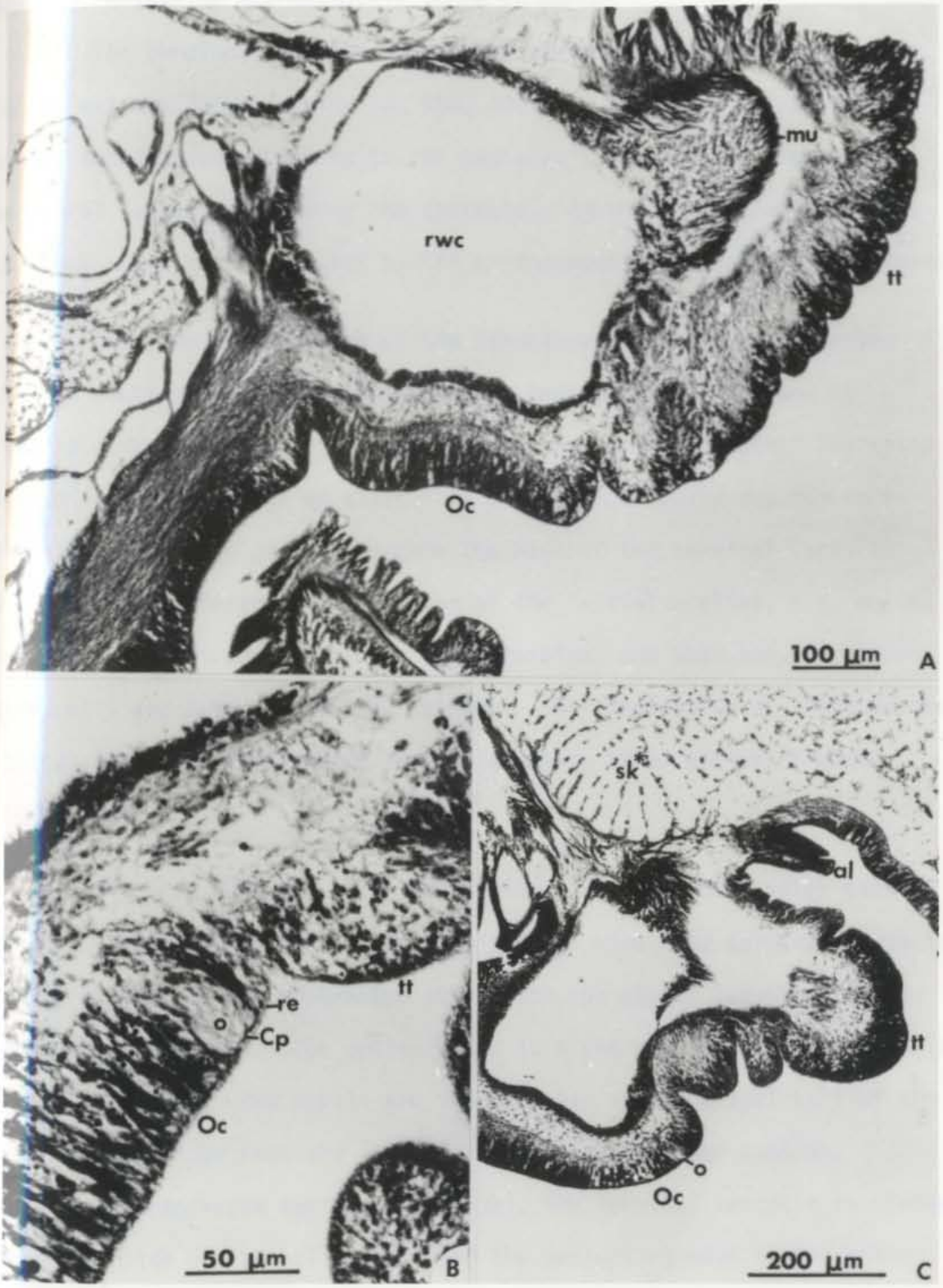
A. Group IV, 16 day post-operative ray tip shows demarcation of optic cushion at base of short terminal tentacle. (X135)

B. Group IV, 10 day post-operative ray tip shows presence of an ocellus in the distal edge of the optic cushion. (X345)

C. Group III, 13 day post-operative ray tips shows terminal tentacle with aboral lappet, and optic cushion with ocellus in its leading edge. (X105).
sk* -- proliferation of skeletal tissue

All stained with Mallory-Heidenhain's stain.

al - aboral lappet
Cp - corneal cell process
mu - muscle
o - ocellus
Oc - optic cushion
re - revestment
rwc - radial water canal
sk - skeletal tissue
tt - terminal tentacle


mu
rwc
tt
Oc
100 µm
A
tt
re
o
Cp
Oc
50 µm
B
sk
al
tt
o
Oc
200 µm
C

shallow, measuring approximately 10 μm in depth.

The terminal tentacle proper has continued to lengthen. In Fig. 58C it extends 300 μm and in Fig. 58A, 500 μm. The longitudinal muscle sheath imparts contractility to the tentacle (Fig. 58A). There is also an aboral lappet overhanging the tentacle. Again, proliferation of the terminal ossicle is suggested by the arrangement of dermal tissue strands.

(c) The regeneration of the structures of the distal portion of the ray continues rapidly. The terminal tentacle lengthens and is clearly contractile, and the lappets also increase in length. The optic region is noticeable as an elliptical pad that gradually expands in a distal direction to project beneath the base of the terminal tentacle. New ocelli proliferate in the region of the initial ocellus, i.e. on the leading or distal portion of the optic cushion, and then they form more proximally and laterally on the cushion. The progression of these morphological events is illustrated in the following three groups of photomicrographs.

(i) Fig. 59A and B are of specimens fixed 34 days after the standard excision procedure. In longitudinal view, the terminal tentacle is 400 μm long in its contracted state with the aboral lappet extending about 70 μm above it. The optic region is a pad with a longitudinal axis measuring 450 μm. Two ocelli are identifiable in the distal half of the cushion. New tube feet are forming just proximal to the cushion.

In transverse section (Fig. 59B), the terminal tentacle is cloaked on either side by lateral lappets and the projecting edge of the optic

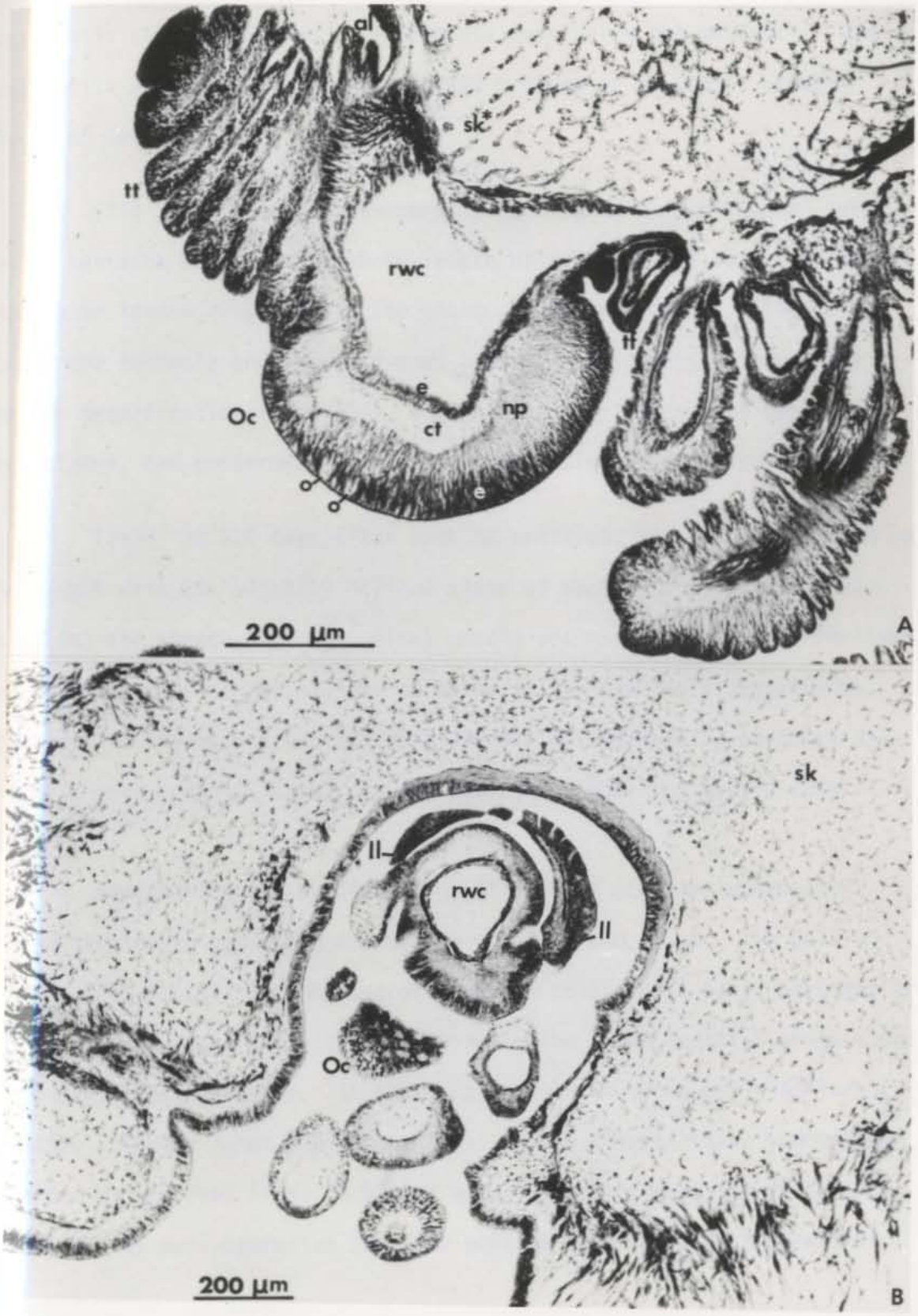
Figure 59. LM's of adult ray tips at various stages of regeneration.

A. Group II, 34 day post-operative ray shows contracted terminal tentacle and expanded optic cushion with several ocelli. (X120)

B. T/S of Group II, 34 day post-operative ray tip shows lateral lappets, and protruded optic cushion with numerous ocelli. (X80)

Mallory-Heidenhain's stain.

al - aboral lappet
ct - connective tissue
e - epithelium
ll - lateral lappet
np - nerve plexus
o - ocellus
Oc - optic cushion
rwc - radial water canal
sk - skeletal tissue
tf - tube foot
tt - terminal tentacle


al
sk
tt
rwc
tt
e
Oc
ct
np
e
200 µm
A
sk
ll
rwc
ll
Oc
200 µm
B

cushion is seen in section as a separate mass below the tentacle. The cushion is 200 μm in width and displays a dozen or so ocelli in this level of section.

(ii) In a 55 day post-operative specimen, the contracted terminal tentacle measures 500 μm in length with the aboral lappet exceeding 100 μm in length (Fig. 60A). The optic cushion projects for 200 μm out under the tentacle and shows several ocelli in this projection. At a higher magnification (Fig. 60B), the cushion displays a well developed revestment, and epidermal, plexal, and connective tissue layers.

(iii) At 120 days after routine excision, the tentacle is 650 μm in length with the slightly oblique plane of section in Fig. 60C illustrating the sheath of longitudinal muscle present in its wall. Portions of approximately ten ocelli can be noted in the cushion's distal projection. A transverse section of a 168 day post-operative specimen displays a very broad (330 μm) cushion containing numerous ocelli (Fig. 60D).

Another 120 day specimen (Fig. 61A) shows ocellar lumens with respective depth and width dimensions of 50 μm and 12 μm. The wall of the ocellus is clearly composed of numerous cells whose basal portions are directed toward the subepithelial plexus. The lumen contains projections from many of these cells. In the section, one of these cells noticeably expands into the lumen beyond the level of the intercellular connectives stained as the dark line. A higher magnification micrograph (Fig. 61B) of a 133 day post-operative sea star ocellus shows tuft-like expansions

Figure 60. LM's of adult ray tips at various stages of regeneration.

A. S/S of Group III, 55 day post-operative ray tip shows lengthened terminal tentacle with aboral lappet, and protruding optic cushion with ocelli. (X85)

B. LM of same specimen as A shows tissues of the optic cushion. (X245)

C. S/S of Group III, 120 day post-operative ray tip shows the lengthened terminal tentacle and numerous deep ocelli. (X100)

D. T/S of Group I, 168 day post-operative ray tip shows greatly expanded optic cushion with numerous ocelli. (X100)

Mallory-Heidenhain's stain.

al - aboral lappet
ct - connective tissue
e - epithelium
mu - muscle
np - nerve plexus
o - ocellus
Oc - optic cushion
re - revestment
rwc - radial water canal
tf - tube foot
tt - terminal tentacle

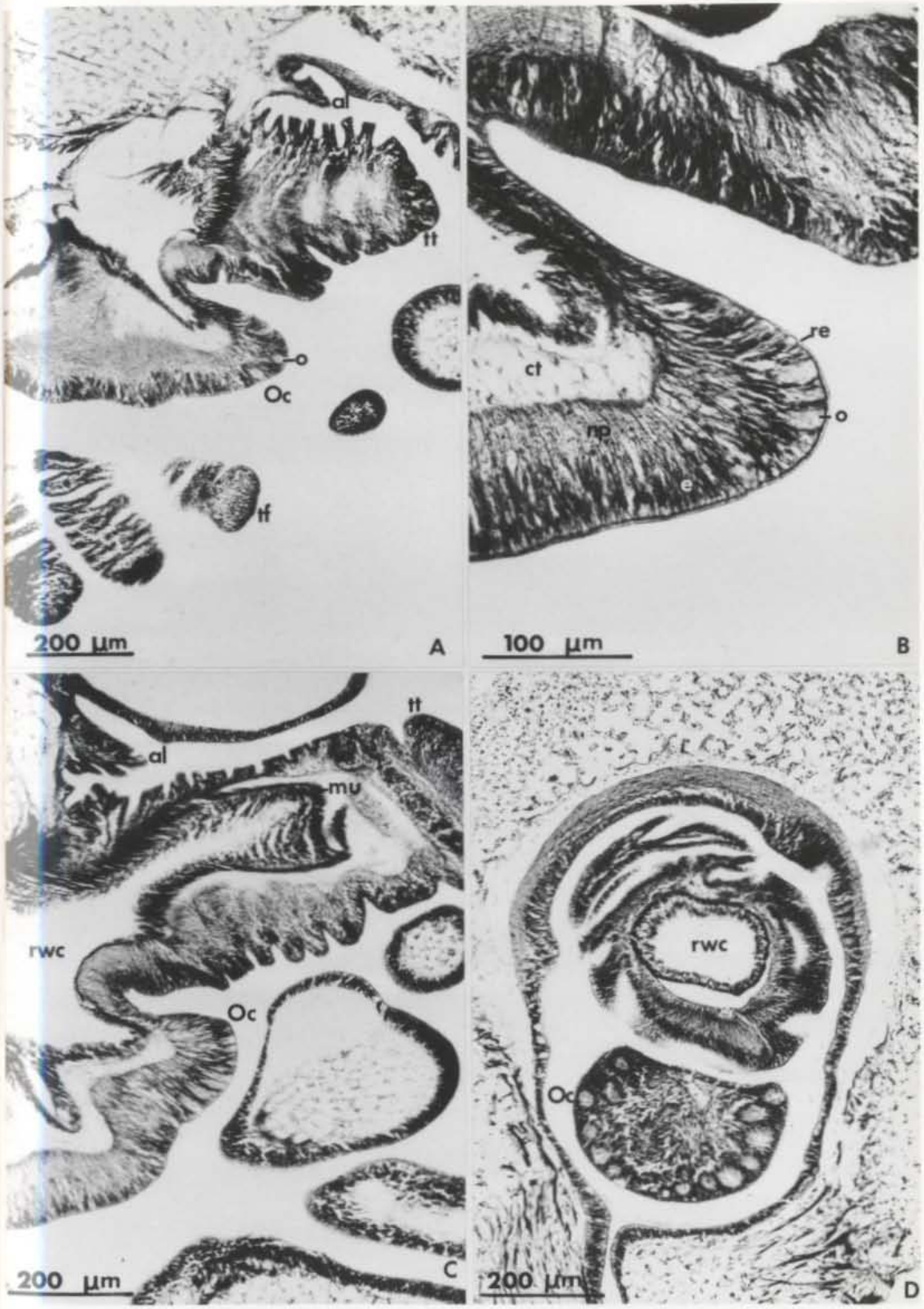

al
tt
o
Oc
tf
200 µm
A
re
ct
o
np
e
100 µm
B
tt
al
mu
rwc
Oc
200 µm
C
rwc
Oc
200 µm
D

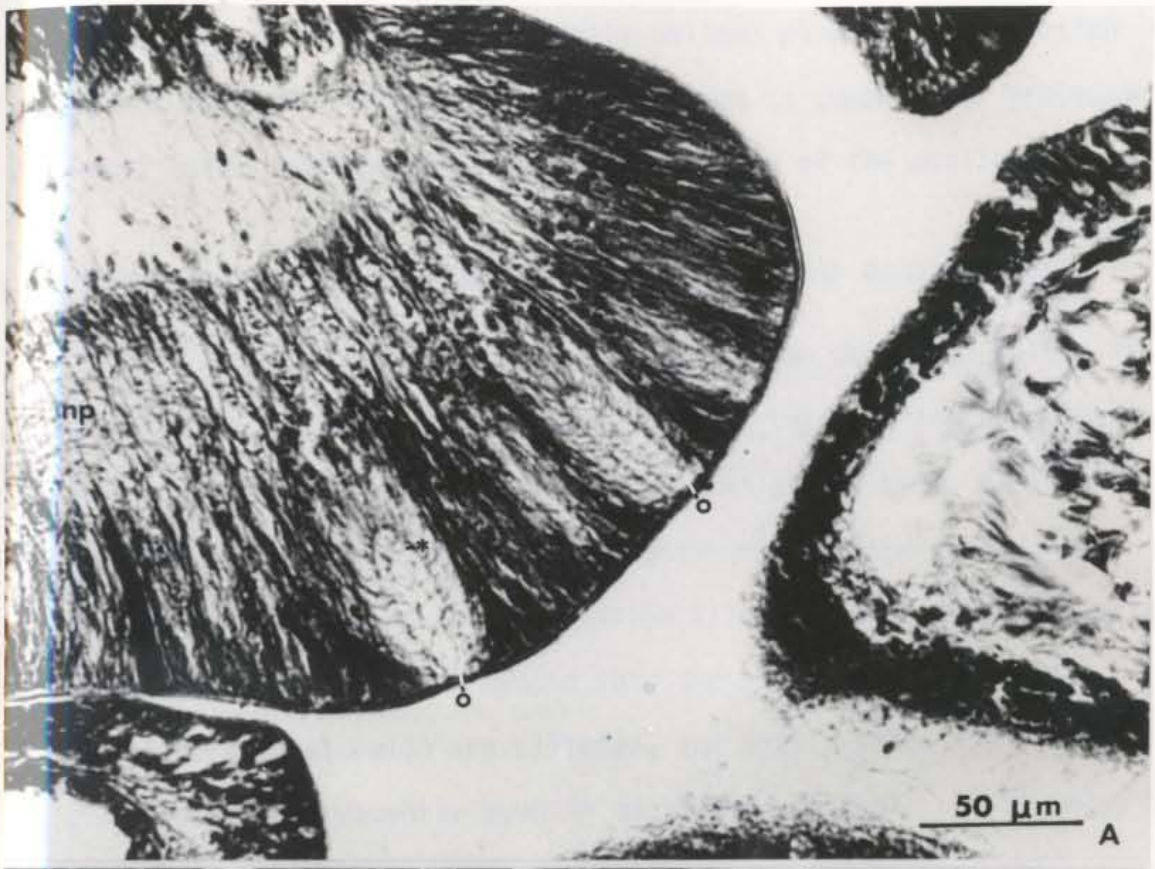
Figure 61. LM's of adult ray tips at various stages of regeneration.

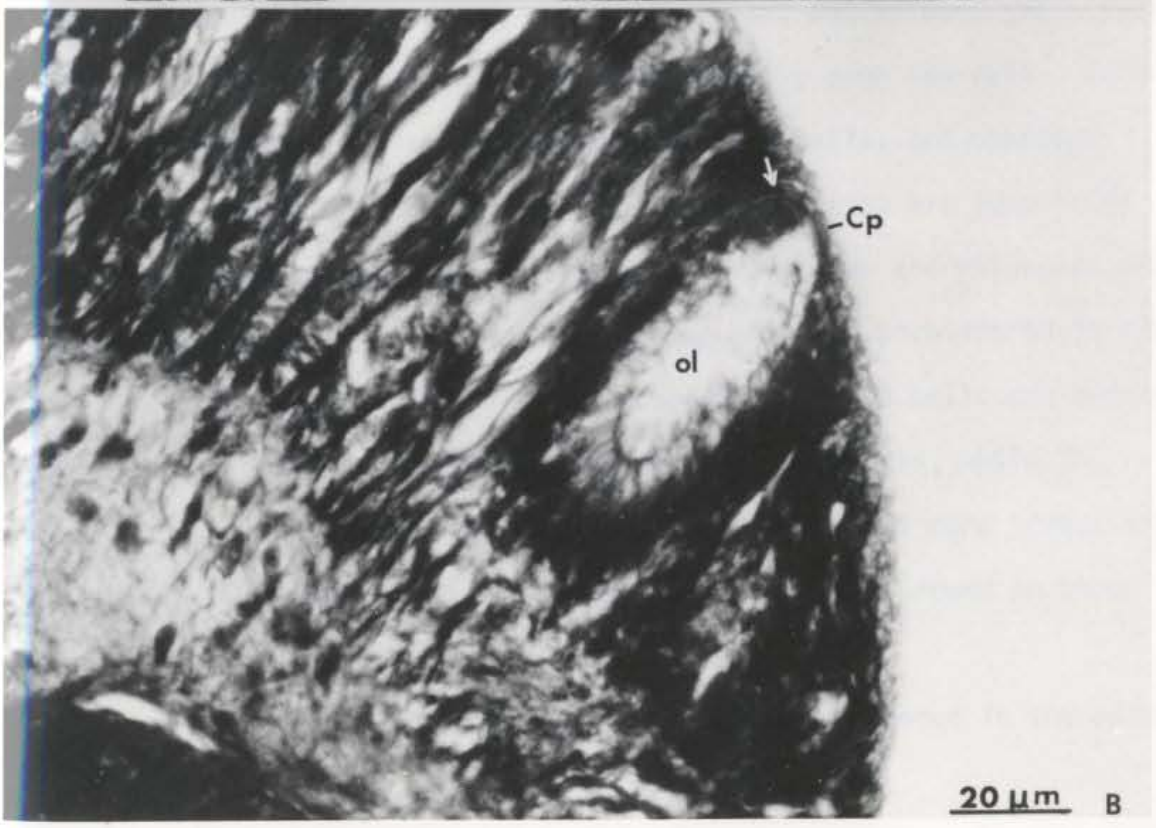
A. S/S of optic cushion of Group II, 120 day post-operative specimen shows well developed ocelli and tissues of the optic cushion. (X410).
* -- sensory cell expands into the ocellar lumen.

B. S/S of optic cushion of Group II, 133 day post-operative specimen shows processes of corneal cells curving (→) to overhang the ocellar lumen. (X750)

Mallory-Heidenhain's stain.

Cp - corneal cell process
np - nerve plexus
o - ocellus
ol - ocellar lumen


np
50 µm
A


Cp
ol
20 µm
B

from cells into the lumen. The slightly oblique plane of this section illustrates that the tissue overlying the lumen is composed of processes of corneal cells, which then bend around the side of the ocellus.

4. DIFFERENTIATION AND REGENERATION (ULTRASTRUCTURAL OBSERVATIONS)

(a) A 13 day post-operative specimen to be described below, displayed under the dissecting microscope, a short terminal tentacle with a minute concentration of orange-red pigmentation at its base, a stage corresponding to the histological preparation represented in Fig. 57B.

The epidermis of the optic cushion is composed of columnar cells with numerous microvilli that extend into the revestment (Fig. 62). Most of the epidermal cells are ciliated, and basal bodies and short striated roots are frequently seen in sectioned material. Small groupings of microfilaments are scattered in the cytoplasm, and in some cells they are organized into a bundle running longitudinally down the cell. Golgi centres are prominent in the apices of epidermal cells, and numerous small dense vesicles, and vesicles with clear profiles, are associated with the Golgi cisternae. Rough endoplasmic reticulum and ribosomes are also present in the cytoplasm, and large vacuoles are encountered in the apical portions of the cells. The mitochondria of some cells are regular in outline and possess a moderately electron-dense matrix, while in others, they display a less electron-opaque interior and more irregular arrangement of cristae. Multivesicular bodies are also found in these cells.

In Fig. 63A, a recognizable pigmented cell is noted in the epidermis.

Figure 62. TEM of optic cushion epidermal cells in Group III, 13 day post-operative specimen. (X25,100)

bb - basal body
fb - filamentous bundle
G - Golgi apparatus
hv - high density vesicle
lv - low density vesicle
m - mitochondrion
mv - microvillus
mvb - multivesicular body
re - revestment
rer - rough endoplasmic reticulum
sd - septate desmosome
sr - striated root
V - vacuole
za - zonula adhaerens

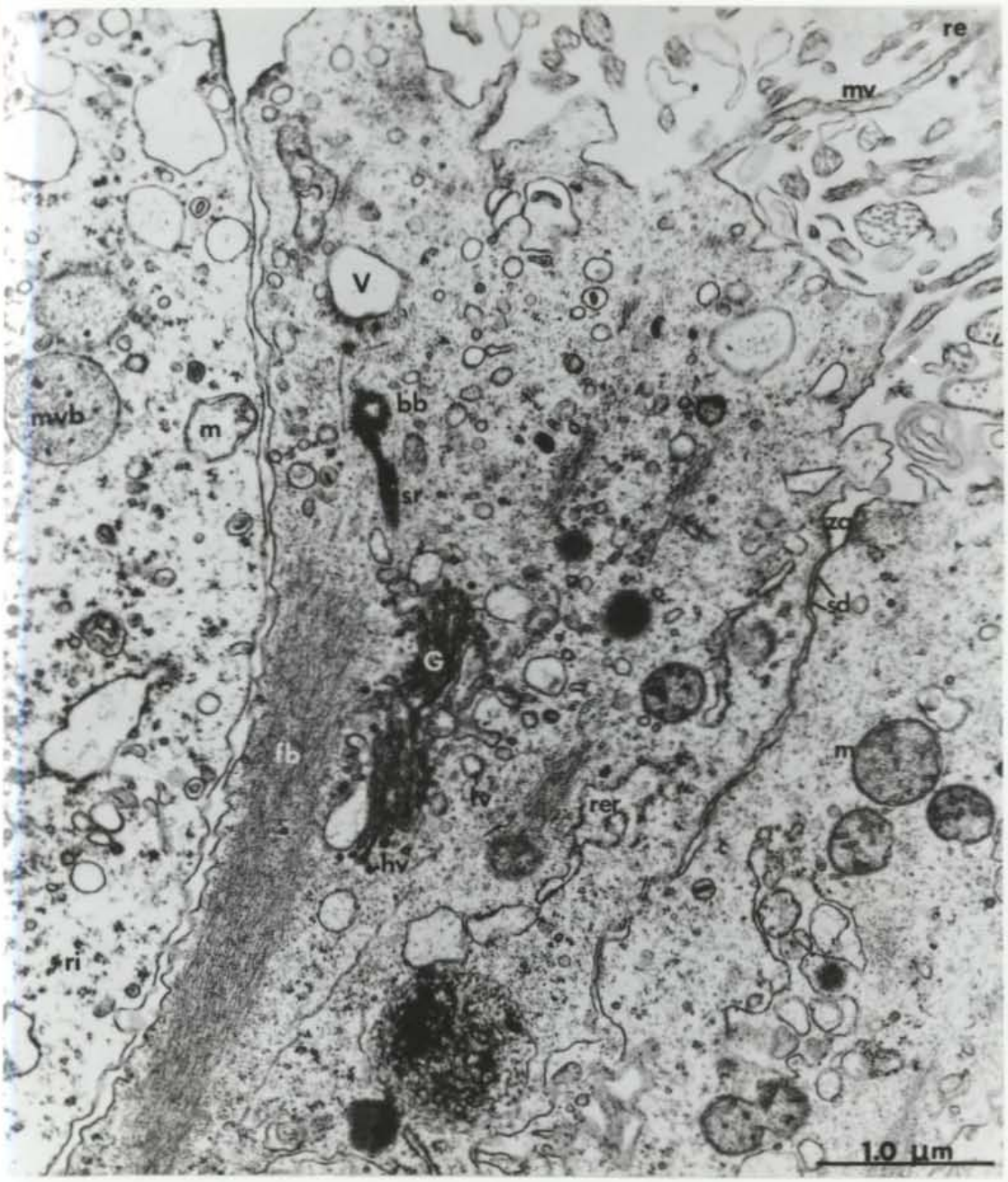
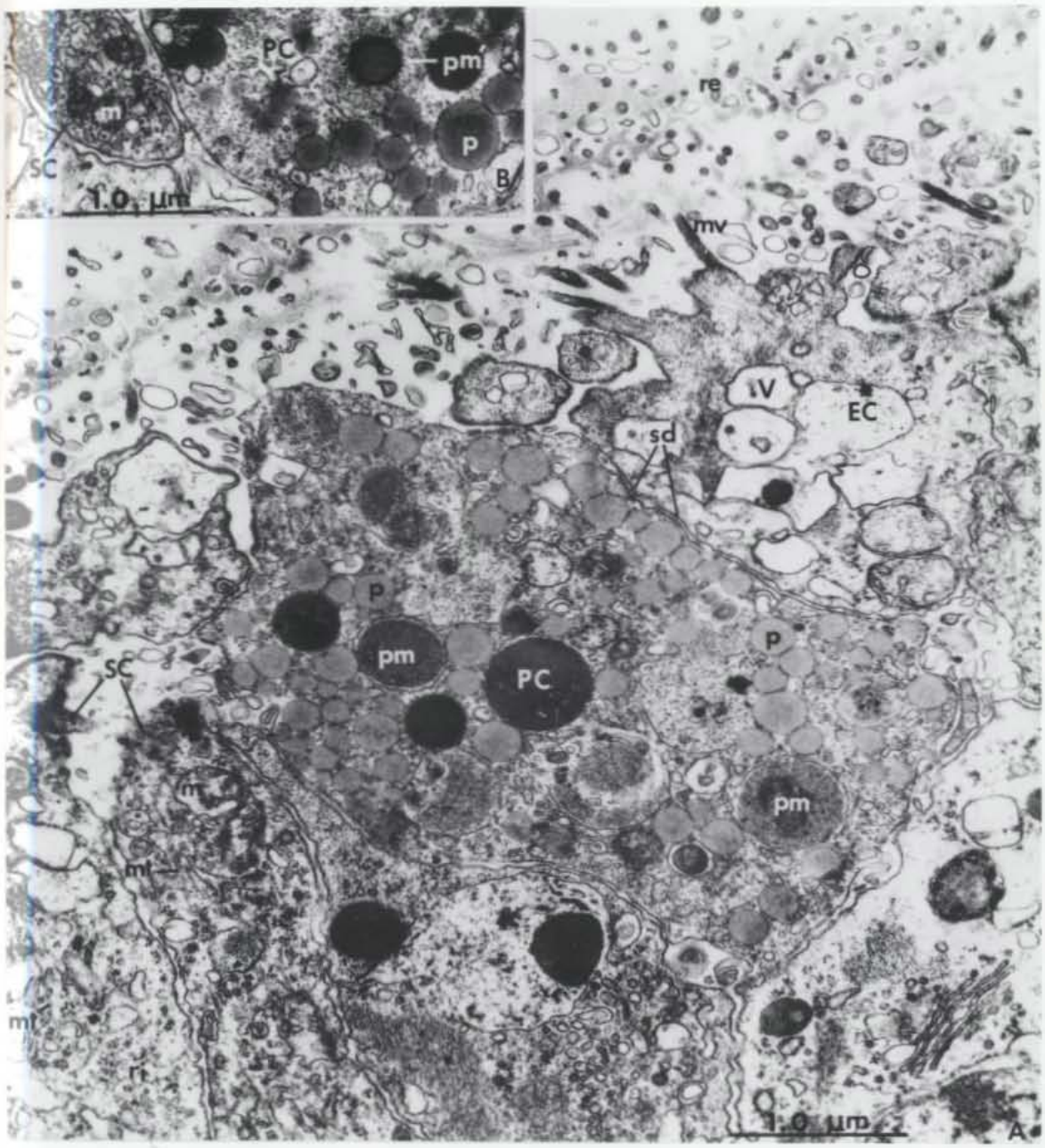

re
mv
V
mvb
m
bb
sr
zo
sd
G
fb
hv
rer
hv
ri
1.0 µm

Figure 63. TEM's of pigmented cells and presumptive sensory cells in 13 day post-operative specimen.

A. Pigmented cell contains small pigment granules and polymorphic bodies, at the optic cushion surface. Sensory cells possess microtubules, mitochondria, some vesicles, and ribosomes. (X25,100)

B. Adjacent to presumptive sensory cell, a pigmented cell contains a polymorphic body (pm') containing region similar to small pigment granules. (X21,500)

EC - epithelial cell
m - mitochondrion
mt - microtubule
mv - microvillus
p - pigment granule
pm - polymorphic body
PC - pigmented cell
re - revestment
ri - ribosomes
sd - septate desmosome
SC - sensory cell
v - vesicle
V - vacuole


PC
pm
m
P
SC
1.0 µm
B
re
mv
iV
EC
sd
P
pm
PC
SC
m
mf
mf
rt
P
pm
1.0 µm
A

It is associated with the apically vacuolated cell on it right by short regions of septae. Characteristic of this cell type are the numerous 0.2 to 0.3 μm diameter homogeneously stained small pigment granules present in the cytoplasm. Several larger (0.6 μm diameter) polymorphic bodies are also noted. Some are membrane-bound with electron-dense interiors, while others have incomplete membranes and varied granular and membranous contents. Other polymorphic bodies (Fig. 63B) have very heavily stained areas present within large, primarily granular regions, and within these dense areas may sometimes be seen a less heavily osmicated patch that resembles the small pigment granules in staining characteristics. The cytoplasm of the pigmented cells is granular.

Some cells in close proximity to the pigmented cells possess features that suggest their elaboration as sensory components of the future ocellus (Fig. 63A, B). Narrow processes contain numerous longitudinally aligned microtubules. Mitochondria and numerous ribosomes are also present, as well as vesicular profiles.

(b) At 34 days, the developing ocellar lumen is covered by several corneal extensions (Fig. 64). These processes possess microtubules and a single cilium, and in addition to large vacuoles, also contain numerous granular and some clear, vesicles and short tubular segments.

The wall of the ocellus is composed of numerous pigmented and sensory cells, associated with each other by septate desmosomes for distances up to 2.5 μm (Fig. 65A). Most of the pigmented cells are a few micrometres broad at their distal edge and possess several microvilli that project into the lumen. The small pigment granules are very

Figure 64. TEM of 34 day post-operative ocellus. (X25,100)

Ocellus is overlain with corneal cell processes, and is lined with sensory cells and pigmented cells with apical concentrations (*) of granular vesicles in the region of pigment granules.

bb - basal body
c - cilium
Cp - corneal cell process
mt - microtubule
ol - ocellar lumen
p - pigment granule
pm - polymorphic body
PC - pigmented cell
sr - striated root
SC - sensory cell
v - vesicle
V - vacuole

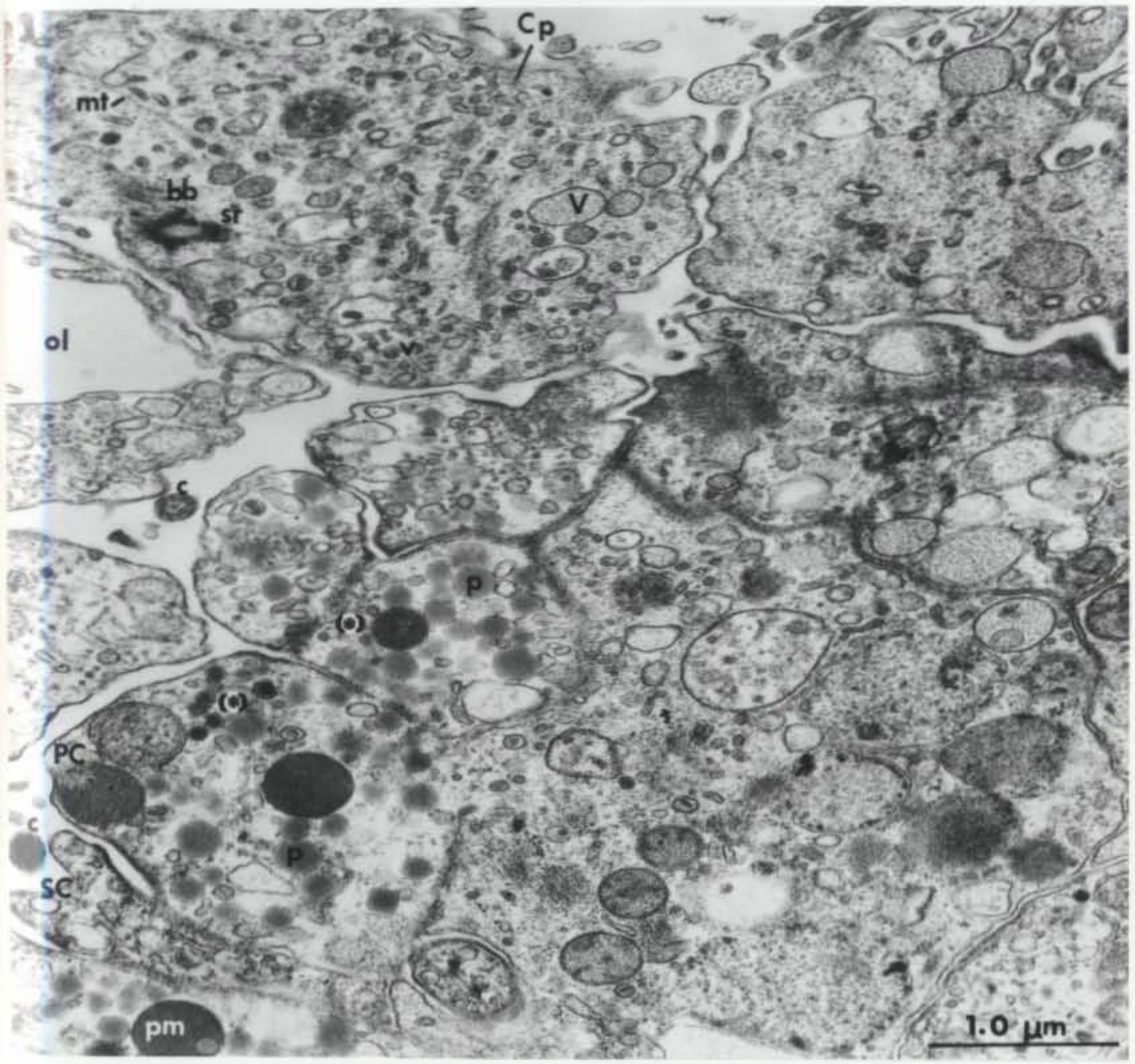
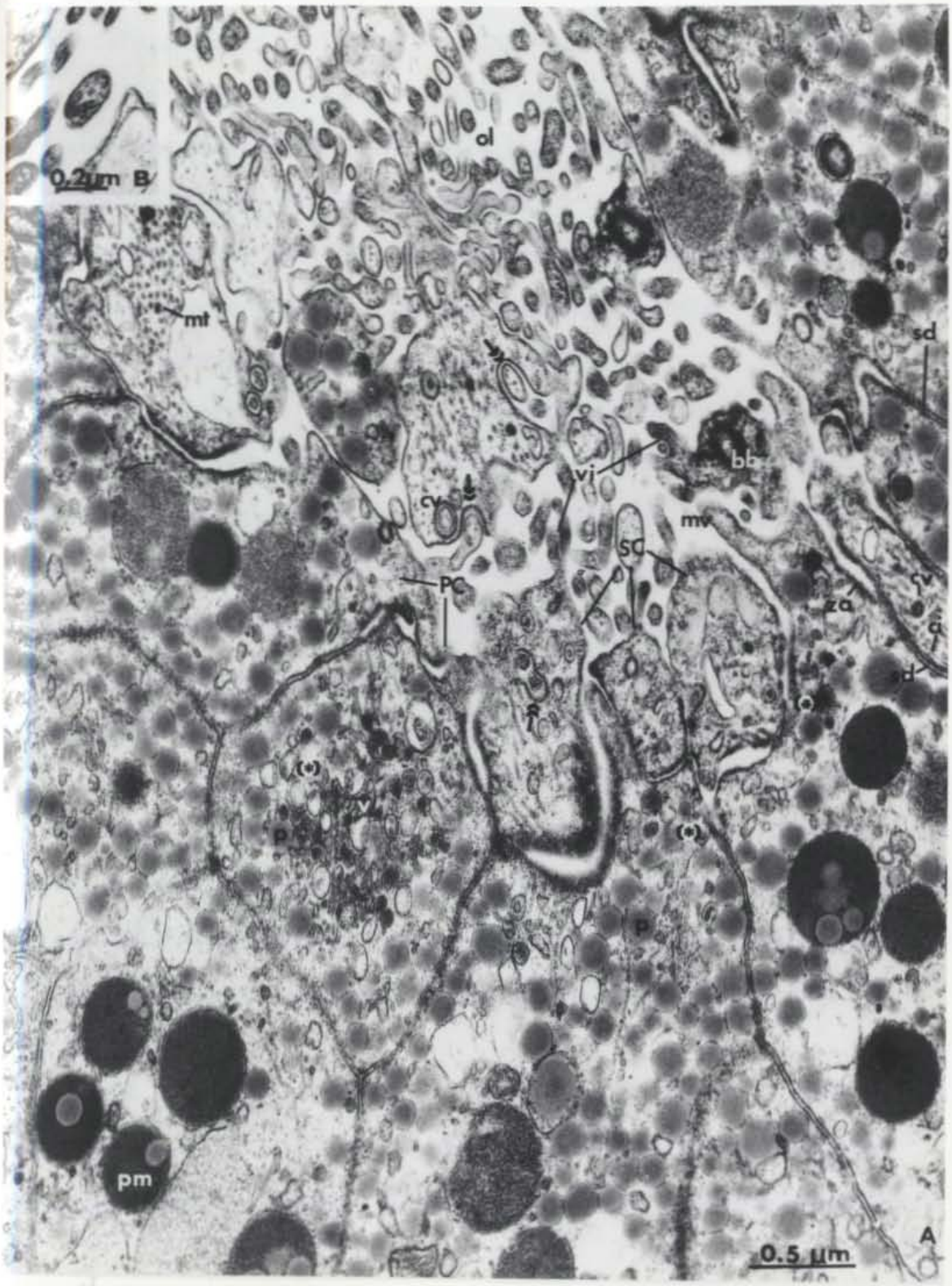

Cp
mt
bb
st
V
ol
v
c
p
(•)
(•)
PC
c
SC
p
pm
1.0 µm

Figure 65. TEM's of 34 day post-operative ocellus.

A. Section shows villi and cilia in the lumen, and apical concentrations (*) of granular vesicles near small pigment granules in the pigmented cells. Coated invaginations (≫) of the sensory cell plasma membrane can be noted. (X32,800)

B. T/S of cilium shows paired central microtubules, and eight or nine peripheral pairs. (X41,500)

bb - basal body
ci - subsurface cistern
cv - coated vesicle
mt - microtubule
mv - microvillus
ol - ocellar lumen
p - pigment granule
pm - polymorphic body
sd - septate desmosome
SC - sensory cell
vi - villus
za - zonula adhaerens


0.2µm B
ol
mt
sd
vj
bb
cv
mv
sc
pc
za
sv
sd
(•)
p
pm
0.5 µm
A

numerous in these cells particularly in their apical portions. These granules are often found in close physical association with smaller, more densely granular vesicles in apical regions of the cells (Fig. 64, 65A), and with numerous densely stained vesicles found with the Golgi apparatus more proximally in the cells (Fig. 66A). Further, similar homogeneously stained patches are located in the midst of finely granular regions which may be partially or completely membrane-bound, and are also noted within the very densely stained polymorphic bodies also characteristic of pigmented cells (Figs. 65A, 66A).

The sensory cells, interspersed among the pigmented cells to form the ocellar wall, project distally into the lumen (Fig. 65A). These expansions contain the usual microtubules, vesicles, and vacuoles. Some vesicular profiles are coated externally and contain an inclusion, and may be seen in endocytotic association with the plasma membrane, engulfing pieces of villous debris. Villi, 60 to 80 nm in diameter extend irregularly from the apical regions of the sensory cells. Also projecting from the sensory cell expansions are single cilia with a central pair and nine peripheral pairs of microtubules (Fig. 65B). The narrowed neck region displays some flattened cisternae against its lateral margins with the gap filled with an electron-dense material (Fig. 65A). Clear and cored vesicles are numerous in the neck as are ribosomes and elongated mitochondria.

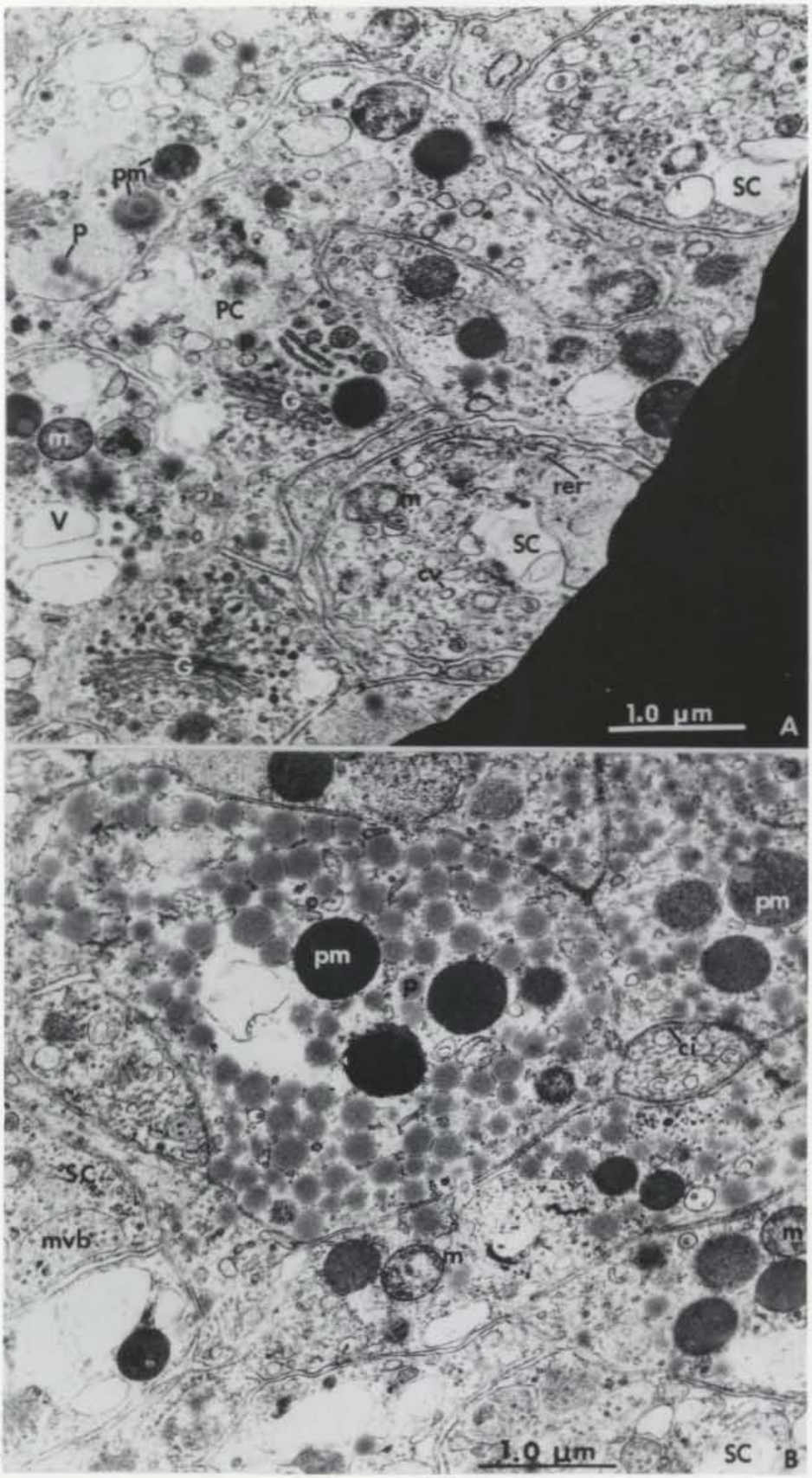
Below this area, the sensory cells expand and in addition to the organelles located in the neck, also possess numerous multivesicular bodies and segments of rough endoplasmic reticulum (Figs. 66 A,B), part-

Figure 66. TEM's of mid regions of sensory and pigmented cells. (X (X20,750)

A. Sensory cells contain vesicles, and pigmented cells contain Golgi apparatus with associated vesicles.

B. Sections shows sensory cell neck region, and multivesicular bodies in more proximal regions.

ci - subsurface cistern
cv - coated vesicle
G - Golgi apparatus
m - mitochondrion
mvb - multivesicular body
p - pigment granule
pm - polymorphic body
PC - pigmented cell
rer - rough endoplasmic reticulum
SC - sensory cell
v - vesicle


pm
P
PC
SC
m
V
rer
cv
1.0 µm
A
pm
pm
P
ci
SC
mvb
m
m
1.0 µm
SC
B

icularly near the nucleus. The Golgi apparatus and associated vesicles are located apical to the nucleus, whose matrix appears less densely stained than that of the pigmented cell nuclei (Fig. 67A).

(c) Specimens at the 55 day post-operative stage of development, possess well advanced ocelli with lumens extending deep into the tissues of the optic cushion. Several long and broad corneal processes overhang the lumen and are morphologically very similar to such cells in the fully differentiated condition (Fig. 67B). Microfilaments associated as a bundle, are present in the extensions and then continue down the more proximal portion of the cells as they curve around the wall of the ocellus. The extensions are somewhat loosely associated with each other over the lumen, but at the level at which they contact the wall of the ocellus, the corneal cells display a septate desmosomal connection with pigmented cells and with adjacent corneal and epithelial cells.

The corneal cell nucleus is located in the mid portion of the cell to the side of the ocellus. Cisternae of rough endoplasmic reticulum and clumps of ribosomes are present around the nucleus. Mitochondria are found in this region as well. The Golgi apparatus is oriented along the longitudinal axis of the cell apical to the nucleus, and associated with its saccules are numerous granular and clear vesicles.

The pigmented cells are often four to five μm wide at their apical border with the lumen, with long and sometimes bifurcated microvilli (Fig. 68). Septate desmosomal associations with other cells extend up to five μm in length. Small pigment granules and the larger polymorphic

Figure 67A. TEM of mid regions of pigmented and sensory cells of 34 day post-operative ocellus, showing staining properties of their nuclei. (X5,100)

B. TEM of 55 day post-operative specimen showing features of the corneal cell. (X23,000)

CC - corneal cell
Cp - corneal cell process
fb - filamentous bundle
G - Golgi apparatus
m - mitchondrion
N - nucleus
pm - polymorphic body
PC - pigmented cell
rer - rough endoplasmic reticulum
sd - septate desmosome
SC - sensory cell

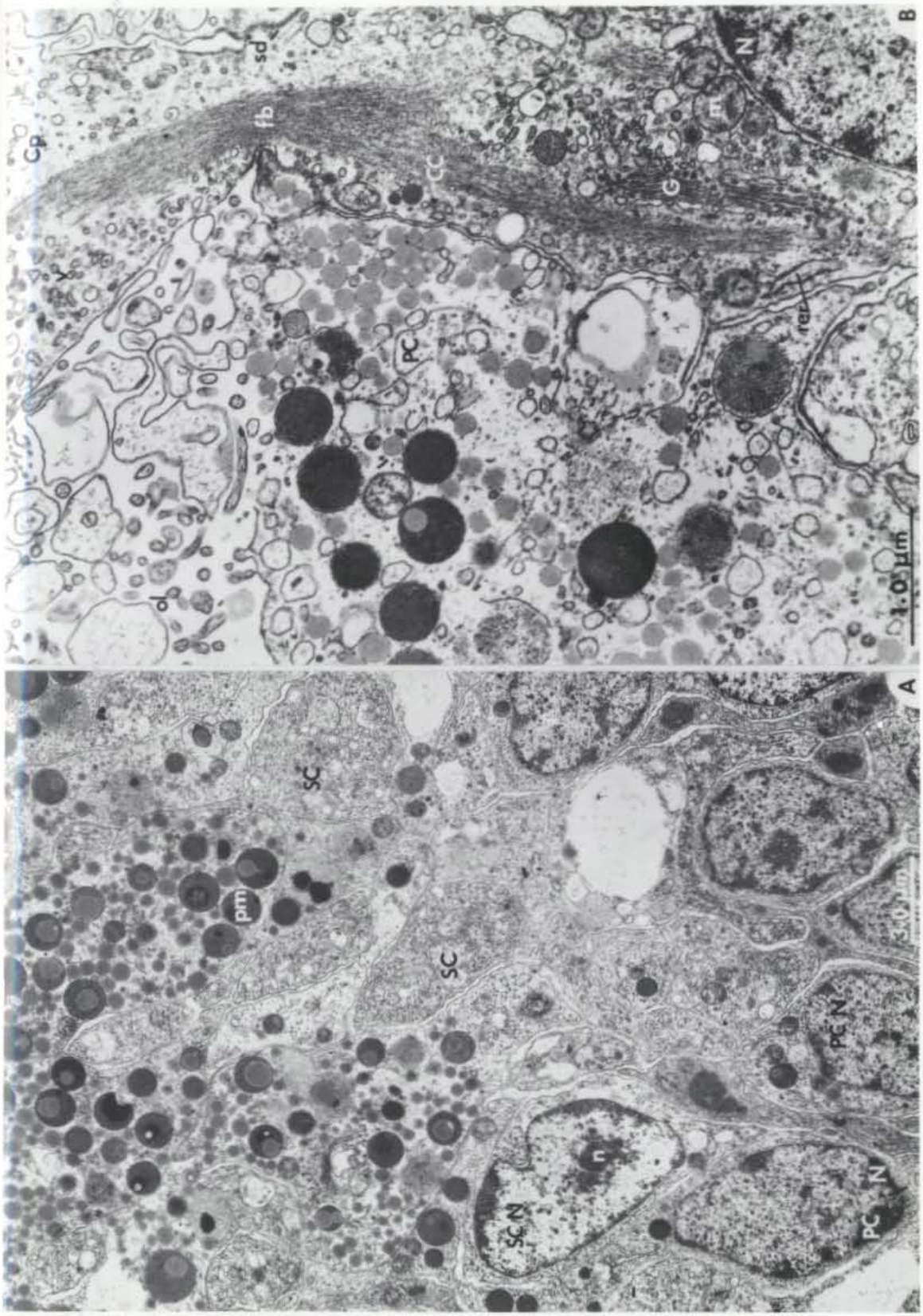
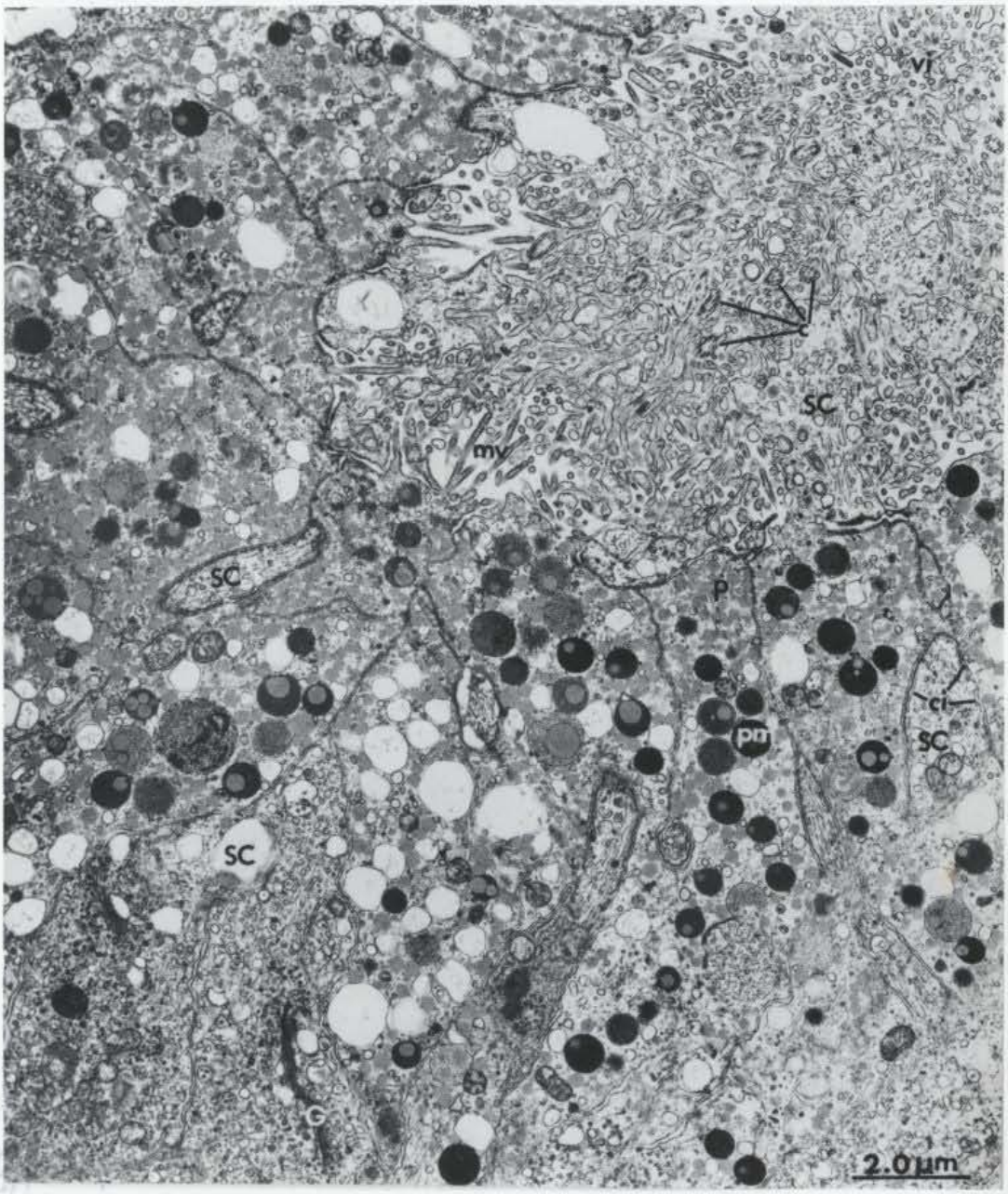

A
B
SC
SC
pm
PC N
PC N
SC N
G
PC
Cp
sd
fb
cc
G
N
rer
1.0 µm

Figure 68. TEM of 55 day post-operative ocellus showing extensive lumen filled with villi and cilia from distal expansions of sensory cells. (X8000)

Below the pigmented cells, which make up the bulk of the ocellar wall, the sensory cells expand.

c - cilium
ci - subsurface cistern
mv - microvillus
p - pigment granule
pm - polymorphic body
SC - sensory cell
vi - villus


vi
SC
my
SC
P
ci
pm
SC
SC
G
2.0µm

bodies abound in these cells.

Some sections suggest the possible origin of this cell type. In Fig. 69A, adjacent to a clearly recognizable pigmented cell near the surface of the optic cushion, is a cell with some features characteristic of an epidermal cell, such as vacuoles and a microfilamentous bundle. Also present, however, is a profile, 0.5 µm in diameter, with two homogeneously stained areas within it, like the larger polymorphic bodies found in pigmented cells.

In another section taken at the periphery of an ocellus (Fig. 69B), is a pigmented cell with its typical complement of small pigment granules and polymorphic bodies. Also prominent, however, is a 1.5 µm long ciliary root with the characteristic striated pattern repeating every 60 nm. Examination of several serial sections did not confirm the presence of a cilium.

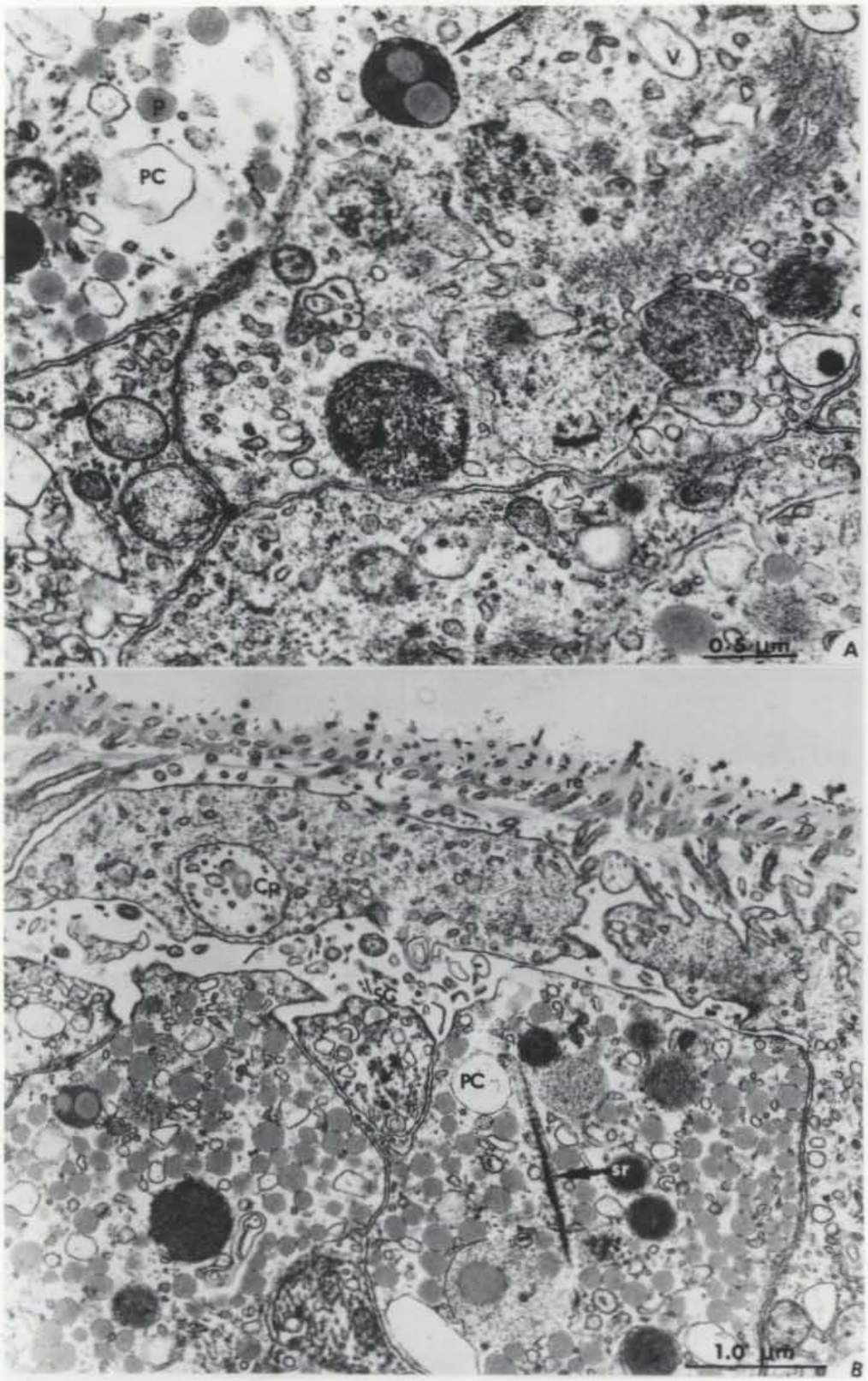
The sensory cells give rise to most of the structures that fill the lumen (Fig. 68). Such structures include distal expansions of these cells, villous extensions, and numerous cilia. The neck regions possess subsurface cisternae which at this stage extend for several micrometres, and the usual microtubules, vesicles, and mitochondria. Multivesicular bodies also markedly fill the cytoplasm of the sensory cells in the supranuclear region, and a prominent Golgi centre is present in this region also.

Figure 69. TEM's of 55 day post-operative ocelli showing differentiating pigmented cells.

A. Section at ocellar edge shows a cell with characteristics of an epidermal cell, and a polymorphic inclusion (⟶) characteristic of pigmented cells. (X33,500)

B. Section of ocellar border shows a pigmented cell with a striated root in its cytoplasm. (X25,100)

Cp - corneal cell process
fb - filamentous bundle
p - pigment granule
PC - pigmented cell
sr - striated root
V - vacuole


P
PC
V
0.5 µm
A
CR
PC
SF
1.0 µm
B

DISCUSSION

The light microscopical and ultrastructural observations of developing and fully differentiated ocelli of the sea star, Leptasterias polaris, provide the basis for discussion of numerous and varied topics. Certain morphological features of the adult tissues, with emphasis on the three ocellar types and their constituents, will be discussed and compared with previous studies of echinoderm tissues and other photoreceptors. Particular attention will be paid to the cellular structures present, or modified, as an expression of the sensory cells' function of photoreception. Discussion of the pigmented cells will primarily concern the small pigment granules and the identity of the large class of polymorphic cytoplasmic inclusions.

The sections on embryology and regeneration will stress the origins of the ocellar cells, and will compare these two pathways of ocellar development, with discussion of ocellar differentiation in other animals. These sections will also provide further discussion of the significance of the cilia of the sensory cells, and of the polymorphic inclusions of the pigmented cells. More general observations of larval development of L. polaris will also be compared with that of other species of this genus.

A. ADULT OCELLAR STRUCTURE

1. EPIDERMAL CELLS

The epidermis of the optic cushion surrounding the ocelli was

characterized by the presence of columnar epithelial cells whose ultrastructure has been described in numerous other studies, e.g. Kawaguti and Kamishima (1964), Menton and Eisen (1970), Nørrevang and Wingstrand (1970), Huet (1972a).

Brief comment will be made on the extracellular revestment present over the apical portions of these cells. The presence of distinct inner and outer zones in the L. polaris revestment is similar to that described in other instances (e.g. Menton and Eisen 1970; Engster and Brown 1972), and the distinction of the outer layer itself into two components was noted by Souza Santos and Silva Sasso (1970). In this latter study on Asterina stellifera tube feet, however, the space between the distal surface of the epidermal cells and the revestment contained various inclusions. In L. polaris, with the exception of sections through microvilli, no organelles or other structures were seen to lie in this area.

Further, Souza Santos and Silva Sasso proposed that the revestment was not contributed to by most of the epithelial cells, but extended from certain T-shaped cells that secreted it and possessed most of the microvilli present in it. No evidence for such a system of T-cells was found in this study. Rather, it was apparent that all of the epithelial cells protruded numerous microvilli into the revestment.

2. OCELLUS

The ocelli of L. polaris consisted of three types of cells--sensory and pigmented cells lining the deeply invaginated lumen, and corneal cells covering the external opening of the lumen and extending

down around the side of the ocellus. The cells of the ocelli are in close physical association with a thickened subepithelial nerve plexus. Consideration of morphological and other aspects of the ocellar elements will be given separately.

(a) Corneal Cells

As Smith (1937) had concluded from his light microscopical study, and Eakin and Westfall (1964) also later affirmed by transmission electron microscopy, the structures overlying the external opening of the ocelli of L. polaris are extensions of some of the epithelial cells that closely surround the ocelli. Smith (1937) concluded that this "lenticular body" was composed of extensions of epithelial cells, from his observation of supporting fibres extending into it. Neither von Harnack (1963), nor Eakin and Westfall (1964) made mention of such cellular inclusions. Transmission electron and light micrographs made in the present study of L. polaris ocelli, showed that the bundle of microfilaments, with closely associated microtubules, present in the mid and proximal portions of the supporting cells surrounding the ocellus, also extended for some distance into their apical corneal processes.

Like other epithelial cells, and as von Harnack had described for the corneal cells in Asterias rubens, these extensions in L. polaris each possess a single cilium. Eakin and Westfall (1964) suggested that they may have missed sectioning this organelle in their material. Microvilli also extend from these cells through the revestment that lies over all the epidermis including the ocelli.

Eakin and Westfall (1964) described desmosomal connections

between the corneal extensions and proposed that they would provide a tight seal against the entry of sea water into the lumen. The corneal cells in this study were seen to be joined to neighbouring pigmented and epidermal cells by septate desmosomes, but many of the extensions directly over the lumen were more loosely associated. Von Harnack (1963) in figure 17 of her paper, also showed corneal processes with gaps between them, but to the side of the ocellus, the main portions of corneal cells were closely apposed to other cells.

The large clear vacuoles observed by Eakin and Westfall in the corneal cells in the sea stars they examined, were also noted in the corneal extensions and apical portions of the epithelial cells of L. polaris. Further, as both von Harnack's and Eakin and Westfall's studies had described, numerous clear vesicles (and in this examination, tubular profiles), 80 to 150 nm in diameter, fill the portion of the corneal cells overhanging the lumen. Clear vesicles of a similar size were also present near the extensive Golgi complex.

The cornea in sea stars is less complex than that described for some other invertebrate ocelli. For example, in the annelid, Nereis vexillosa and the gastropod Helix aspersa, the cornea is composed of one or two cellular layers plus strata of extracellular material such as fibres, granules, or collagen (Eakin and Westfall 1964). Simpler corneas have been described in a variety of animals. In the mollusc, Pecten maximus, for example, the cornea lies near the lens and is one cell thick but comprises several complete cells including their nucleated portions, associated by desmosomes (Barber et al 1967). Singla (1974) in the hydromedusan, Bougainvillia principis, which possesses ocelli morphologically very like those of sea stars, also illustrated

a single layer of several epithelial cells covering the opening of the ocellus. Bouillon and Nielsen (1974) in another hydromedusan, Cladonema radiatum, described a corneal structure similar to the asteroid situation in that it is composed of vacuolated extensions of epidermal cells. In that case, however, the elongations overlapped and interdigitated to make up a multilayered, yet still thin cornea. These latter two authors suggested that in addition to allowing light to enter but excluding sea water from the lumen, the cornea might also serve to mechanically protect the photoreceptoral apparatus and lens of the eyecup.

The corneal cells, exclusive of their apical extensions, probably also serve a supportive role because their mid and basal portions encapsulate the ocellus. The corneal cells possess a broad bundle of microfilaments and some include peripherally located microtubules, continuous down the length of the cell. Engster and Brown (1972) made observations similar to those in the present study and noted the bundles to be primarily filamentous with a few microtubules extending longitudinally as well. Most workers have previously described such bundles as being fibrous or filamentous, (Bargmann et al 1962; von Harnack 1963; Kawaguti and Kamishima 1964), while Huet (1972a) described them as being composed of microtubules.

(b) Pigmented Cells

(i) Small Pigment Granules

The presence of large numbers of pigment granules is a characteristic immediately noted in microscopical observations of the sea star

eyespot. As several early workers determined, they are a component of a group of pigmented cells distinct from others that serve as the photoreceptors (Hoffman 1873; Hamann 1883; Smith 1937). While Cuénot (1887) described the granules as being most numerous in the enlarged, superior portions of these cells and Smith (1937) stated that they were restricted to the outer third of the cells, well above the nucleus, Millott and Vevers (1955) and Yoshida (1966) using fresh material, noted abundant pigment throughout the cytoplasm. Pigment granules in L. polaris eyespots were abundant particularly in the apical and mid portions of the pigmented cells and were also scattered in the basal regions of the cells.

As noted in the Introduction, Millott and Vevers (1955) isolated and identified two pigments and suggested that both were present in at least some pigmented cells. The first TEM study of sea star ocelli, that by Philpott and Chaet (1960) on Asterias vulgaris, described two different sized classes of pigment granules, some 0.2 x 0.1 μm and others 1.3 x 0.8 μm. Von Harnack's (1963) light and TEM examination of A. rubens also indicated the presence of two classes of pigment granules: small (0.1 to 0.16 μm in diameter) densely stained spheres and larger, membrane-bound polymorphic bodies. Eakin, Brandenburger, and Westfall's studies on Henricia leviuscula, Leptasterias pusilla, and Patiria miniata, summarized by Eakin (1963, 1968, 1972), however, revealed only one class of pigment granule corresponding to the small type of von Harnack. They are membrane-less, typically less than 0.3 μm in diameter, and present a homogeneous appearance.

This present TEM study has illustrated that small pigment

granules identical in size and appearance to those described by von Harnack and Eakin, abound in the pigmented cells of L. polaris. They presumably also correspond to the small granules noted by Philpott and Chaet.

While in the eyes of most animal species, melanin is the pigment present in such cells, reddish-orange pigments have been noted in the pigmented cells of a few other invertebrates. In some of these species, the pigment is localized in membrane-bound vesicles, as in the naupliar eye of the copepod Macrocyclops albidus, (Fahrenbach 1964).

In several other copepod species, however, the red pigment is localized in cellular inclusions similar to the small pigment granules described in sea stars. In the naupliar eye of several species of the copepod genus Sapphirina, for example, Elofsson (1969) noted membraneless, moderately electron-dense spheres, 0.1 to 0.5 μm in diameter. Other copepods of the genus Copilia, also have pigmented cells in their eyes, which contain spheres less than 0.3 μm in diameter, with similar staining characteristics to the sea star small pigment granules (Wolken and Florida 1964). Another copepod species, Doropygus seclusus, displayed this single class of pigmentary inclusion that ranged in diameter from 0.3 to 1 μm (Dudley 1969). Dudley inferred an association of lipid with the pigment granules from their general appearance in micrographs, the intense reactivity with osmium at their edge, and their appearance in certain staining procedures.

No attempts were made to further clarify the nature of the constituent(s) of these granules in L. polaris ocelli. As in D. seclusus, they do display the central homogeneity of staining, and the

peripheral more intense osmiophilia that Dudley noted as being characteristic of lipids. The possible pathway(s) by which they arise in these cells will be discussed in detail in the larval section.

(ii) Polymorphic Bodies

As in the ocelli of Asterias rubens examined by von Harnack (1963), the pigmented cells of L. polaris also contain numerous bodies of a larger (approximately 1 μm in diameter) and polymorphic form. Her micrographs show some irregularly granular profiles with often incomplete limiting membranes, as well as bodies whose matrix varied in appearance and contained multiple spherical inclusions. These inclusions in L. polaris material consistently displayed the same staining characteristics and size range as the small pigment granules, and in some sections appeared as though fusing with or being extruded from the larger bodies.

Although the information yielded by the conventional TEM methods used in this study is admittedly 'static', it is still possible and useful to order the various forms these polymorphic bodies are seen to display into a likely developmental sequence, and to speculate on their identity, function, origin, and fate in the pigmented cells.

First, previous investigators have made suggestions as to the identity of these structures. Von Harnack (1963) and Philpott and Chaet (1960) referred to them as a second class of pigment granules, the latter workers stating that they may contain one of the two pigments isolated and identified in Marthasterias glacialis ocelli by Millott and Vevers (1955). In view of the larger profiles never having

been described in the ocellar pigmented cells of L. pusilla, Henricia leviuscula, and Patiria miniata examined by Eakin and his co-workers, Eakin (1972) suggested that they are not pigment granules, but are perhaps "cytoplasmic bodies", which they noted in another sea star, and which also varied in size and appearance. He did not elaborate further on the significance of these forms.

The staining characteristics of the inclusions in many of the large profiles might suggest that they represent a stage in small pigment granule formation whereby small pigment granules condense from concentrations of precursors isolated within these membrane-bound bodies, and are then eventually released back into the cytoplasm. This relationship with the small pigment inclusions would certainly be rejected, however, on the basis of their not being seen in the ocelli of the three species of sea stars examined by Eakin and his colleagues. Further, small pigment granules, but no large polymorphic bodies, were present in very early differentiating pigmented cells of L. polaris larvae.

Such bodies could still bear a relationship to the small pigment granules, however, if they represent stages of autophagocytosis of cellular components including the small pigment granules and/or their precursors. Such a proposal is being made with evidence based on the morphology of these polymorphic bodies and their physical associations with other structures in the pigmented cells. Although this proposal and its significance will be discussed at greater length in the larval section, certain ultrastructural observations noted in the adult, fully formed ocelli, are important to note at this point, in support of an

autophagic involvement of the large polymorphic bodies.

Granular areas of cytoplasm, occasionally with other inclusions, are seen in various stages of enclosure by a membrane. Such profiles are frequently surrounded with clear (light density) vesicles, some of which are apparently fusing with the membrane. Some areas within these profiles appear similar to the small pigment granules, and the small dense vesicles, contained within or near these bodies, are probably primary lysosomes. Other profiles contain more electron-dense contents and membrane stackings characteristic of telolysosomes, and very densely stained residual bodies are also present in these cells.

Lysosomes have been implicated in a wide variety of routine physiological as well as pathological processes in cells, with cellular autophagy occurring under normal circumstances and being enhanced during active metabolic states, e.g. differentiation, as well as in times of stress (de Duve and Wattiaux 1966). A constant turnover of pigment granules and/or removal of excess precursors from the cytoplasm in these cells might thus involve a high degree of autophagy, with the processes of growth and differentiation of pigmented cells in larval and regenerating sea stars further emphasizing such a process.

(iii) Functions of Pigmented Cells

Eakin (1972) in his review of invertebrate photoreceptor structures discussed several functions for pigmented cells. Based on morphological information, these could readily apply to those of L. polaris. An important function, inferred by the presence of the large numbers of pigment granules in their cytoplasm and the arrangement of

pigmented cells about the ocelli, is to permit light to strike the photoreceptoral surfaces from primarily one direction, and to absorb light that passes by those surfaces in the lumen. This function was also proposed by Singla (1974) for similar ocelli of two species of coelenterates. 'In vivo', sea stars raise the tips of their rays, and the corneal covered openings of the ocelli on the optic cushions would thus be exposed to light from the environment. In addition, Smith (1937) suggested that the pigmented nature of those cells could reflect light onto the photoreceptoral surfaces of the sensory cells, perhaps at a wavelength approximating the maximal absorption of the photosensitive pigment. Millott and Vevers (1955) also suggested that the carotenoids of the pigmented cells might act as filters in permitting light of certain wavelengths to reach the lumen, a role documented for astaxanthin in some other eyes (Wolken 1971). The prominence of these cells in comprising the bulk of the ocellar wall, and their attachment to each other and to the interspersed sensory cells by septate desmosomes, also confers on the pigmented cells a supportive function, a role widespread in the eyes of numerous species.

Further, the pigmented cells of some invertebrates have been implicated in the manufacture and secretion of the precursors of humor and lens material noted to fill some ocellar lumens (Eakin 1972). Smith (1937) proposed that the pigmented cells of Marthasterias glacialis probably possessed the function of secreting the lightly staining droplets of fluid which he noted to fill the optic cup cavity. No ultrastructural detail of the humor filling the lumen of the ocelli was revealed in this study, however, and there is no lens in asteroid eyecups.

(iv) Origin of Pigmented Cells

Some aspects of pigmented cell morphology reveal similarities with epithelial cells. The distal surface of the pigmented cells bears microvilli identical in their dimensions and internal structure to those borne by the cells of the epidermis. Von Harnack (1963) found ciliary roots in some pigmented cells of Asterias rubens, though none were noted in sections of the fully elaborated ocelli of adult L. polaris. Some microtubules were present in pigmented cells and a few large vacuoles with sparse, irregular contents were located distally in both these and epithelial cells. The mitochondria and nuclei of pigmented cells also have similar morphology and staining characteristics to those in most epidermal cells. Smith (1937) had noted that the pigmented cell nuclei of Marthasterias glacialis were smaller (2.5 μm in length) than the 4 μm long epithelial cell nuclei. However, those in A. rubens (von Harnack 1963) and in L. polaris were elongate and of a similar dimension (7 to 10 μm) to those of epidermal cells. The implications of these observations of similarity between these cells and those of the general epidermis will be discussed further in the section of larval development.

(c) Sensory Cells

(i) Distal Photoreceptive Apparatus

Thurm (1969) described the general organization of sensory receptors and divided such cells functionally and morphologically into a) a receptive region involved with the sensory transducer function, that is, reception of a stimulus and its subsequent transduction into a receptor current, b) a perikaryon engaged primarily in protein synthesis

and cell maintenance, c) an axon (in primary sense cells), d) and a pre-synaptic region. In his description of the receptive region, Thurm considered that there are certain features characteristic of most receptors regardless of the modality to which they respond, and that there are also structural modifications that are typically modality-specific. As an example of this latter point, he stated that the correlation between expansive membranous surfaces and a photoreceptive function has been documented in so many cases, that when such features are noted in cells that also have neuronal characteristics, they are generally proposed to be photoreceptors.

An electrophysiological response to light by ocelli of an unidentified species of Asterias (Hartline et al 1952) and behavioural and biochemical experiments on the asteroid Asterias amurensis, (Yoshida and Ohtsuki 1966, 1968) have demonstrated that ocelli do function as photoreceptors. No similar evidence is specifically available for Leptasterias polaris. Rather, the assumption that the ocelli are photoreceptive is made from morphological considerations as will be discussed below.

The presumed photoreceptoral surfaces of the asteroid ocellus are the extensions of the plasma membrane of the distal portions of the sensory cells, and their cilia (Eakin 1963). The cilia of these cells in L. polaris possess a typical basal morphology with basal body, accessory centriole, striated root and associated cytoplasmic microtubules. The shaft for part of its length displays a '9+2' arrangement of microtubules. Those sections with variant microtubular patterns may represent the situation as in olfactory cilia (Reese 1965), where a considerable distal

portion of those sensory cilia displayed atypical microtubular arrays. Often some axonemal microtubules terminate near the distal extremities of typical motile cilia as well (Satir 1965).

Most ciliary photoreceptors differ from motile kinocilia in having a different microtubular count, usually '9+0' (Eakin 1972b). The ATPase containing arms extending from one of the peripheral paired tubules of motile cilia are also often lacking in photoreceptoral cilia. In L. pusilla, Eakin (1963) noted an axonemal complement of '8+1', and in Henricia leviuscula, '9+0'. However, in a later publication (Eakin 1968), he illustrated this latter species as possessing nine peripheral pairs plus two central microtubules in its sensory cilia. Von Harnack (1963), in Asterias rubens, also talked of the cilia showing a '9+2' arrangement.

A few other invertebrate ciliary photoreceptors possess '9+2' cilia -- a coelenterate (Eakin and Westfall 1962), a polyplacophoran mollusc (Boyle 1969), a bryozoan (Woolacott and Zimmer 1972), an entoproct (Woolacott and Eakin 1973), and an enteropneust (Brandenburger et al 1973). In addition, Hughes (1970) suggests that '9+2' cilia of some retinal cells of the arthropod Aplysia punctata may be photosensitive.

Polyorchis penicillatus (Eakin and Westfall 1962b), and two other coelenterate species described by Singla (1974) and Bouillon and Nielsen (1974) are also similar to asteroids in their possession of villous extensions of the ciliary membrane. But whereas the coelenterate photoreceptoral cilia display these extensions along the length of the ciliary shaft, Eakin and Westfall (Eakin 1963) found that the

asteroid villi originate from the raised surface of the sensory cell itself and from the base of the cilium, the rest of the ciliary shaft being fluted but otherwise unmodified.

Brandenburger et al (1973) in their description of photoreceptoral cells in tornarian larvae of two species of enteropneusts, found them to possess both a cilium and irregular microvilli, which projected laterally from the cell body below the cilium. Both features were proposed to be photoreceptive. The cilium was only slightly modified, being short and occasionally bulbous distally. Like the asteroid sensory cell cilia, they displayed a fluted shaft membrane above the basal plate.

The villous extensions form an interwoven mass in the lumen of L. polaris ocelli. Von Harnack (1963) described the tangled villi in the ocelli of A. rubens as originating from the sensory cell apex. In this study of L. polaris, the majority of the extensions are derived from the plasma membrane of the distal expansions of the sensory cell proper, while a few were noted to irregularly protrude from the base of the ciliary shaft.

Eakin has postulated that very little structural modification may be required to impart photosensitivity to ciliary structures (Brandenburger et al 1973). This suggestion may be particularly relevant to the echinoderm situation where dermal sensitivity has been demonstrated in all classes of the phylum (Yoshida 1966; Millott 1968). This sensitivity is a property of much of the body surface whose epithelium possesses large numbers of ciliated cells, with particular sensitivity being associated with echinoid nerves (Yoshida and Millott

1959), and with ciliated cells lying in pits on the upper surface of the sea urchin Diadema antillarum (Millott and Coleman 1969). These latter cilia appeared normal with no modifications of the membrane noted.

(ii) Vesicles

Photoreception may also be associated with the membranes of some of the 100 to 200 nm diameter clear vesicles found in the distal expansions of the sensory cells. In his scheme for the evolution of various specialized photoreceptive surfaces, Eakin (1965) proposed as an early step, the production of small vesicles by the infolding of the ciliary membrane, and noted as examples of these structures, the vesicles in sea star and coelenterate ocelli, 140 nm diameter clear vesicles in a ctenophore apical organ (Horridge 1964), and 120 nm diameter vesicles in the ependymal cells of amphioxus cerebral vesicle (Eakin and Westfall 1962a).

The origin of these smooth-surfaced vesicles in asteroid ocelli is not clear. Various possibilities would include, among others, their origin as speculated above, by the infolding of the cell or ciliary membrane. As will be elaborated upon in greater detail, many vesicles do form by endocytosis and these are initially typically externally coated. Alternatively, some vesicles may derive from intracellular organelles, for example, the Golgi complex. Thurm (1969) noted the abundance of clear vesicles in receptive regions to be a common feature of receptor cells in general.

In addition to smooth surfaced vesicles and larger clear vacuoles, the apices of the sensory cells in fully formed and adult

regenerating L. polaris ocelli, and particularly in developing larval ocelli, also contained vesicular profiles with a 'bristled' coating of their external membranes and these often possessed an inclusion, apparently a bit of villous material. Many of these vesicles were in a size range of 100 to 200 nm and arose by invaginations (also coated) of the distal sensory cell plasma membrane, some engulfing material from the lumen. Though not referred to, von Harnack's Fig. 7 (1963) illustrates C-shaped profiles approaching 200 nm across with externally coated membranes and Eakin's Fig. 11 (Eakin 1968) of Henricia leviuscula, similarly shows some coated profiles.

These vesicles correspond in morphology and apparent origin to the pinocytotic vesicles initially described by Roth and Porter (1964) and shown to be involved in the uptake of yolk protein in mosquito oocytes. Similarly, Friend and Farquhar (1965) demonstrated that a population of endocytotic vesicles incorporated protein in rat vas deferens cells, then travelled proximally in the cells, losing their coats and fusing to become multivesicular bodies and eventually dense bodies.

Endocytotic vesicles have been noted in echinoderm epithelia, e.g. in the ciliary bands of young brachiolaria larvae of Asterias rubens (Nørrevang and Wingstrand 1970), and vacuolated cells of the tube foot epithelium in a species of Astropecten (Engster and Brown 1972). This latter paper referred to other echinoderm studies, which described the pinocytotic uptake of amino acids and glucose, with the suggestion that it was an important mechanism for obtaining nutrients from the environment.

Pinocytotic vesicles have been reported in a number of rhabdomeric eyes (see Eakin 1973, for summary). White (1968) observed, for example, that in larval mosquitoes exposed to light, coated vesicles were formed by endocytosis mainly at the bases of the sensory microvilli. These profiles formed multivesicular bodies more proximally in the cells. One function suggested for vesicles is the intake of extracellular fluid and macromolecules such as protein (White 1967). Another, perhaps more probable function, because of the increase in numbers of pinocytotic vesicles with exposure to light, is the removal of microvillar membrane containing the metabolites of the initial photoreactions (Eguchi and Waterman 1967).

A recent publication by these latter authors cites freeze-etch and histochemical evidence for endocytosis functioning as part of a recycling sequence of crayfish photoreceptoral membranes (Eguchi and Waterman 1976).

In the case of L. polaris ocelli, portions of tubular elements, probably villi, were endocytosed by these vesicles. While the sensory cells in larval and regenerating L. polaris ocelli often contain large numbers of multivesicular bodies, those of the adult, although they possess large numbers of coated vesicles, do not have particularly numerous multivesicular bodies.

Other coated vesicles less than 100 nm in diameter and often displaying denser contents than the endocytotic forms, correspond to Friend and Farquhar's (Friend and Farquhar 1965) second population of coated vesicles in morphology and apparent origin from the Golgi apparatus. Schjeide (1970) and Morré et al (1971) in reviews of

organelle assembly, also included Golgi membranes as a source of small coated vesicles. A major function attributed to these vesicles is the carrying of lytic enzymes to the multivesicular bodies to form heterolysosomes (Friend and Farquhar 1965).

Behrens and Krebs (1976), in addition to the initially coated endocytotic vesicles, also described small coated vesicles in the rhabdomeric eye of Limulus polyphemus. They proposed too that these were primary lysosomes originating from the Golgi apparatus, and they were numerous especially when endocytosis and formation of multivesicular bodies were prevalent.

It is also possible that some vesicular profiles may be involved in supplying membranous material to the villous extensions of the sensory cells, perhaps also supplying the photopigment or its precursors. One clearly documented example of such a pathway is the rhabdomeric cells of the snail, Helix aspersa, summarized by Eakin and Brandenburger (1975).

Clearly there have been many questions raised concerning the origin and function(s) of the various vesicular profiles noted in the sensory cells of the sea star ocelli, and the information provided solely by routine transmission electron micrographs does not answer these questions as satisfactorily as one would like. Several additional types of study would be necessary to unravel some aspects of these problems. For example, an ultrastructural comparison of the ocelli of light and dark adapted animals might reveal differences in the number and types of vesicles present in the sensory cells. Such studies in rhabdomeric structures (reviewed by Eakin 1972) have first of all,

provided support for the microvilli being the photoreceptoral surfaces of these eyes, and also described the synthetic and autophagic organelles and processes in the sensory cells.

Studies involving the introduction of radioactively labelled photopigment precursors have also localized the sites of production and transport of photopigment, e.g. in the snail, Helix aspersa (Eakin and Brandenburger 1968). Although the precise identity of the photopigment of asteroid ocelli is still not clear, those proposed as such, are carotenoid substances (Yoshida and Ohtsuki 1966) derived from vitamin A, so that similar experiments using tritiated vitamin A might be useful.

Similarly, Eakin and Brandenburger (1970) utilized the fact that osmium tetroxide reacts with unsaturated compounds such as aldehydes, and by long periods of osmium impregnation at raised temperatures a localized staining was produced in vesicles and the microvilli. Again, a carotenoid molecule is composed of two vitamin A derivatives and is unsaturated, and would presumably react in a similar manner. Finally, the introduction of ferritin particles into the ocelli to identify pinocytotic activity, and histochemical tests for acid phosphatase would further elucidate the roles of some of the coated vesicle population.

(iii) Microtubules

Microtubules are numerous in L. polaris ocellar sensory cells, as in many other photoreceptors (Eakin 1972). They extend from the vicinity of the basal apparatus of the cilium in the distal expansion

of the sensory cell, longitudinally down the cell to continue into the tapered proximal process. Axons also possess longitudinally oriented microtubules.

De Robertis et al (1975) list among several functions attributed to microtubules, determination of cellular polarity with respect to the arrangement of structures within the cell and movement of the cell itself, and circulation and transport of molecules. These would seem to be prime candidates for the possible role(s) of the microtubules prominent in the sensory cells, axons, and indeed for those fewer microtubules found in pigmented and epidermal cells.

Allison (1973) in a review of some aspects of cell polarity and locomotion, further summarized that microtubules were necessary for the directional movement of endocytotic vesicles. Such vesicles are directed to areas of the cell where they will most likely be contacted by primary lysosomes. The numerous endocytotic vesicles which arise in the distal portions of L. polaris sensory cells, may thus be guided proximally down the neck region by microtubules which are noticeably concentrated in longitudinal array.

Another review by Dustin (1972) lists numerous instances of various structures being transported in cells in association with microtubules, including vesicles, lysosomes, lipid droplets, mitochondria, and ribosomes. In this regard, Eakin (1972) suggested that transport of low molecular weight macromolecules to and from the photosensory apparatus in sensory cells was an attractive function for microtubules, rather than their serving primarily as a cytoskeleton.

In the case of neurons, Dustin (1972) mentions movement of the

cytoplasm itself, of catecholamines, and of synaptic vesicles. Most of the proteins of axons are synthesized in the cell body proper and transported as macromolecules by so-called "axon flow" (De Robertis et al 1975). Numerous studies that compared movement of labelled molecules in normal axons, and in axons whose neurotubules were selectively disrupted, have confirmed that intact neurotubules are necessary for axonal transport. Specifically in radial nerve cord axons of the asteroids Asterias vulgaris and A. forbesei, Gamache and Gamache (1975) identified both fast and slow phase transport of labelled proteins, and demonstrated that disruption of neurotubules disturbed fast transport.

Finally, Thurm (1969) suggested that the microtubules of the ciliary connecting piece in vertebrate retinae act as an energy transmitting 'bridge' between mitochondria and the outer segment of the rod, but did not elaborate on this function.

(iv) Subsurface Cisternae

Von Harnack (1963) described but did not speculate on the significance of oblate sacs lying directly beneath the plasma membrane of the neck region of the sensory cells of Asterias rubens ocelli. Similarly, transmission electron micrographs of sensory cells of the sea star Henricia leviuscula (Eakin 1968) illustrated short sacs closely applied to the cell membrane in the septate desmosomal zone.

Such structures are found also in the neck region of the sensory cells of Leptasterias polaris and correspond in morphology to organelles first designated by Rosenbluth (1962) as subsurface cisternae (SSC's). SSC's have been identified in numerous vertebrate and

invertebrate neurons, and are characterized by being flattened, membrane-limited sacs closely applied to the internal surface of the plasma membrane. The gap between the SSC and the cell membrane is of a constant width, though the dimensions of the gap vary among the cell types thus far examined, e.g. Rosenbluth described a range of 4 to 10 nm in his material, Siegesmund (1968) mentioned a space of 13 nm, and in this study, the gap was 15 to 20 nm in width.

The gap is also characterized by the presence of an intermediate substance which may vary in appearance depending to some extent on fixation, e.g. septal-like bridges (LeBeux 1972), a 'horizontal line' (Rosenbluth 1962), granular or opaque material (Siegesmund 1968; Takahashi and Wood 1970). The material in L. polaris sensory cells generally appeared electron-opaque.

Athough they may occasionally be subsynaptic in location, SSC's are almost always located in opposition to glial or support cells, which have never been seen to possess SSC's (LeBeux 1972; Siegesmund 1968; Rosenbluth 1962). They have also been found in some sensory cells, e.g. the hair cells of vertebrate inner ear cochlea (Smith and Sjöstrand 1961), goldfish photoreceptors (Stell 1967), planarian photoreceptors (Carpenter et al 1974a), and in developing cones of human fetal retina (Fisher and Linberg, in Fisher and Goldman 1975). Rosenbluth (1962) noted the similarity of such cells to neurons in their functioning as generators or conductors of changes in electrical potential.

In the asteroid, Asterina stellifera, Dolder (1972) noted sacs beneath the cell membrane of one or both members of adjacent smooth muscle cells in the tube feet. These were identified as subsarcolemmic cisternae with an electron dense accumulation between them and the cell

border, and were noted to become somewhat inflated upon glutaraldehyde fixation. She speculated that they were important in the transmission of nervous impulses.

SSC's have also been identified in the gastrodermis of coelenterates, and in the salt-absorbing epithelia of crustacea (Copeland 1966), and are found in close association with septate desmosomes, specialized cell junctions that have been described as regions of low electrical resistance (Lowenstein and Kanno 1964).

SSC's in most of the material examined have been shown to be continuous with cisternae of the rough endoplasmic reticulum, and where the cisternae are expanded, they may be studded with ribosomes (Rosenbluth 1962). In the adult L. polaris sensory cells, ribosomes were occasionally seen to be associated with the cisternae, and no clear examples of a physical connection with RER were discerned. The SSC's usually terminated in the supra-nuclear region where the RER was primarily localized.

In L. polaris photoreceptoral cells, some organelles were found in close association with the SSC's, principally numerous microtubules. Mitochondria were also occasionally noted adjacent to them, as also observed in other studies (Rosenbluth 1962; Takahashi and Wood 1970).

Rosenbluth (1962) proposed several ways in which SSC's, by virtue of their morphology, location, and association with other organelles, might influence the activities of the cells which possess them, cells that are all involved with changes in electrical potential. The first suggestion is that a stacking of membranes and creation of cytoplasmic compartmentalization affects the mobility and concentration of ions,

and will create ionic gradients and thus electrical potentials across these membranes. LeBeux (1972) hypothesized that SSC's may thus transmit impulses from surface membranes into deeper regions of the cell. In L. polaris sensory cells, this proposed conductance of electrical information presumably generated by photoreaction with the pigment-bearing membranes of the villous and possibly ciliary extensions, might be further aided in being channelled by the presence of septate desmosomes in this region.

Secondly, Rosenbluth pointed out that the cytoplasmic volume of neurons is large, as one could also suggest for the ocellar sensory cells with their cilia and numerous extensions. Specializations beneath the plasma membrane might thus serve to facilitate the influx of material into the endoplasmic reticular complex of the cell. Conversely, metabolites, enzymes, or specific ions might be channelled from the cell interior to contact the cell membrane where they could play a role in its activity or reconstitution. As a final suggestion, Rosenbluth pointed out that the SSC might play a more direct part in plasma membrane restoration after stimulation, by serving as a store of membrane material or sodium ions which would be extruded.

All of these processes involve a high energy expenditure, a fact which could explain the close physical association of mitochondria with SSC's that is observed in some cases. There might also be a facilitation of metabolite transport implicated by microtubules being found adjacent to the innermost membrane of these organelles in L. polaris sensory cells.

(v) Mitochondria

Sensory receptors, in general, are characterized by large numbers of mitochondria, reflecting the high energy requirements necessitated by the operation of the ion pumps in the transduction process. Photoreceptors in particular, must further greatly amplify the stimulus current to produce an adequate receptor current (Thurm 1969). Consequently mitochondria are characteristically numerous in distal portions of photosensitive cells, supplying the energy needed for transduction of the photoimpulse, as well as for the maintenance of the cell (Eakin 1963).

The specific distributional pattern of mitochondria in invertebrate photoreceptors is varied, but, as a rule, mitochondria are aggregated between the membranous expansions of the receptive area and the origin of the axon (Thurm 1969). Thus, while the mitochondria of L. polaris sensory cells are relatively scarce in the neck region, in contrast to the situation in many other photoreceptors with similar morphologies (Eakin 1973), their large numbers in the supra- and perinuclear regions of the cells conform with Thurm's generalization.

The mitochondria of L. polaris sensory cells are swollen in appearance with irregular outlines, and have relatively electronlucent matrices, and long tubular cristae, in contrast to the fewer, more compact, and electron-dense mitochondria of the adjoining pigmented cells. Woolacott and Eakin (1973) noted two mitochondrial configurations for sensory and pigmented cells of an entoproct, with those of the sensory cells displaying a more electron-opaque matrix, as well as better developed cristae. These differences in the entoproct study may perhaps

be a reflection of the derivation of photoreceptoral cells from ectodermal cells, and the pigmented elements from mesodermal tissue, as well as the possibility of the expression of different functional states.

Since the ocellar elements in L. polaris are derived from the same tissue type, the variation in mitochondrial morphology is more likely a result of their respective energy roles, a suggestion further supported in larval development where the mitochondria of the three ocellar types are very similar in their early stages of differentiation, and those of the pigmented and corneal cells become somewhat more compact at a later stage.

There is further support for this proposal in a study by Carpenter et al (1974a), who noted in planarian photoreceptors an interesting specialization in the presence of two distinct populations of mitochondria within sensory cells. Those mitochondria located distally tended to be larger than those found more proximally in the cells, and differed in their internal morphology as well. They described the change in the apical population of mitochondria in light-adapted planarians from large forms with matrices of low electron density, and poorly defined, widely spaced cristae, to small, electron-dense structures with more clearly delineated cristae (Carpenter et al 1974b). Such changes were reversible. They suggested a correlation between the apparently 'swollen' mitochondria in the conical portions of light adapted eyes to the intense level of oxidative phosphorylation required to amplify the photoreceptive stimulus. They cited other references to the 'swelling' phenomenon of very active mitochondria even in isotonic media. They further speculated that the more compact mitochondria

located more basally in the visual cells provided the energy for more generalized cellular functions.

A comparison of the mitochondrial morphology in the sensory cells of light adapted and light deprived sea stars would be useful in determining whether this similar correlation between structure and function holds in the asteroid case.

(vi) Axonal Processes and Radial Nerve Cord

Eakin (1972) generalized that most photoreceptive cells were characterized by a basal axonal process that commonly contained microtubules, mitochondria, and vesicles, and that merged with a nerve tract.

While Cuénot (1887) maintained that the asteroid ocellar elements terminated on the connective tissue layer beneath the nerve plexus, Hamann (1883) proposed that the receptor cells merged with the nerve plexus, an observation that was later confirmed by Smith (1937). The previous ultrastructural studies of sea star ocelli did not examine the proximal portions of the photoreceptor cells. Cobb (1968) in his ultrastructural examination of a variety of echinoderm tissues, concluded that all the epithelial sensory cells presumably gave rise to axonal processes that entered the nerve tracts.

The sensory cells of L. polaris ocelli send a proximal process into the subepithelial nerve plexus, but whether these then synapse with interneurons within a relatively short distance, or travel further as axons is not known. These basal processes contain a complement of organelles characteristic of the axons of the subepithelial plexus of this and other echinoderms (see Pentreath and Cobb 1972), namely,

neurotubules, mitochondria, the occasional multilamellar whorl, scattered ribosomes, and clear and dense-cored vesicles. Cobb (1968) suggested that the microtubules associated with the ciliary base of the sensory epithelial cells were continuous with the neurotubules of the axonal processes. Microtubules were observed to extend proximally past the nucleus in L. polaris ocellar sensory cells.

A study by Cobb (1970), and a review by Pentreath and Cobb (1972), summarized available information on the radial nerve cords of echinoderms. The ectoneural plexus consists of numerous axons (0.2 μm to 10 μm in diameter) and there is some evidence that small axons may represent sensory neurons and the larger ones, motor neurons (Cobb and Laverack 1966). The axons in L. polaris radial nerve cord contain neurotubules, large mitochondria, some vacuoles and ribosomes, and both clear and dense-cored vesicles.

As Pentreath and Cobb (1972) have pointed out, echinoderm tissues have provided only extremely rare examples of synapses with the specializations found in "advanced" groups of animals. Cobb (1970) in areas of neuropile in the echinoid radial nerve cord, proposed some associations as being synaptic and these were characterized by accumulations of large numbers of clear vesicles against the axonal membrane, which may appear thickened. Either clear or dense-cored vesicles were seen to be densely packed in certain axonal profiles in L. polaris nerve plexus beneath the optic cushion and may represent synaptic areas.

Certain elements scattered in the ectoneural nerve of L. polaris are proposed to be neurosecretory cells on the basis of morphological similarity to those described in previous echinoderm studies, e.g. von

Hehn (1970), Pentreath and Cottrell (1971), and Millott and Coleman (1969), this latter study being of the nerve plexus beneath proposed photosensory regions in the echinoid Diadema antillarum. Using histochemical techniques, Atwood and Simon (1973) localized neurosecretory substances in sensory nervous tissues of asteroids as well as in their motor nervous component.

Cobb (1970) has proposed a scheme for the structure and functioning of the echinoderm nervous system, with evidence drawn from morphology, behaviour, and some electrophysiology. Numerous sensory epithelial cells send axons directly to an area of neuropile from which motor neurons then run to local effector organs. In addition, interneurons connect areas of neuropile and extend also to the connective tissue layer separating the ecto- and hyponeural portions of the cord. It is proposed that these interneurons release chemical synaptic transmitters across the connective tissue to stimulate hyponeural nerves, which then synapse with mesodermal muscles.

Yoshida and Ohtsuki (1968) showed that the behavioural response of sea stars to sensory information perceived by the ocelli requires integrity of the radial nerve cord. Further, the physical presence of the proximal portions of ocellar and other epithelial cells in the ectoneural plexus of the radial nerve cord has long been established in light microscopical work (e.g. Smith 1937), and similar observations from ultrastructural work (e.g. Cobb 1968), have been supported in this study. Whether these photoreceptive cells are primary or secondary sensory cells, however, has still not been determined and probably only serial sectioning will illustrate any involvement of interneurons in

the system. By Cobb's proposed scheme, some sensory cells may directly contact motor neurons to affect nearby organs, while others may have their ultimate effects through one or more interneurons.

The importance of the circumoral and radial nerve cords to the development of the optic cushion will be discussed in the regeneration section.

B. LARVAL DEVELOPMENT

1. SPAWNING AND BROODING BEHAVIOUR

(a) The spawning periods for several species of Leptasterias, based on published information with data on the brooding duration, location, and water temperature, where available, are summarized in Table 4. In general, the genus spawns in the winter months, although the water temperatures at the time of breeding vary with the location.

As noted in the Results section, female L. polaris in the laboratory, spawned and brooded young on a variety of substrates. The two specimens that completed a brooding cycle in 1975, did so positioned on a vertical, dull coloured, smooth surface. L. polaris females in the field are usually seen brooding on top of rocks (Emerson 1973). Chia (1966) showed that brooding females of the west coast species L. hexactis could distinguish surface texture and colour tone of available substrates and selected dark rough rocks. That species was noted to spawn and brood under rocks, behaviour suggested to be related to its method of holding the young and its habitation of the intertidal zone with exposure at low tides (Chia 1966). L. ochotensis similarly breeds under stones (Kubo 1951).

TABLE 4

SUMMARY OF INFORMATION ON SPAWNING AND BROODING IN THE GENUS *LEPTASTERIAS*

Species	Location	Spawning Period	Spawning Temp.	Brooding Period	Reference
L. pusilla	Monterey Bay, Calif.	Jan. to March			Smith 1971
L. pusilla	California	Jan.		till Feb.	Fisher 1930
L. hexactis	Puget Sound, Wash.	Feb. to March		till April	Osterud 1918
L. hexactis	Puget Sound, Wash.	Nov. to April	9-13°C	3 months	Chia 1964
L. mulleri	British Isles	March to April			Mortensen 1927
L. ochotensis	Japan	April to May	5-7.5°	till mid June	Kubo 1951
L. littoralis	New Hampshire	Oct. to Nov.	<9°C	through winter	O'Brien 1972
L. aequalis	Calif. to Wash.	Feb.		till April, May	Fisher 1930
L. arctica	Aleutians		3°C	till at least June	Fisher 1930
L. groenlandica	Bering Sea	May	2-3°C	till August	Fisher 1930
L. polaris (field)	Newfoundland	Jan. to Feb.	-1°C	till July	Emerson 1973
L. polaris (lab.)	Newfoundland	Feb. to March	-1°C	till July	this study

The eggs laid by L. polaris typically number several hundred, with one instance in this study of approximately 900 young collected from one female. L. hexactis were observed to produce 52 to 1600 eggs per female depending on the size of the female (Chia 1968). Osterud (1918) found the largest number of young taken from an individual of L. hexactis to be 1160, and Kubo (1951) observed up to 1560 eggs per female L. ochotensis. This relatively small number of large yolky eggs is characteristic of brooding species. Chia (1968) discussed the evolutionary, nutritional, and morphological implications of the phenomena of brooding and direct development, and Menge (1974, 1975) further elaborated on the importance of the physical and biological factors which affect the reproductive strategies of brooding and broadcasting species of asteroids.

(b) Brooding

The brooding habits of species of the genus Leptasterias are varied, with L. polaris differing from the rest in this regard. L. hexactis encloses its young in a chamber formed by the arching of the rays (Chia 1966). When the tube feet of the young are functional, the female flattens out and remains on the larvae for an additional 20 days or so. The larvae of L. arctica are carried in a similar pouch and are attached to central masses by a thread (Fisher 1930). L. aequalis, L. pusilla, L. mulleri and L. littoralis also carry their young clustered over the mouth of the female (Mortensen 1927; Fisher 1930; O'Brien 1972). L. groenlandica broods its young under the central disc for a time and they are then taken into the female's cardiac stomach to complete their

development (Fisher, 1930).

L. polaris females, after the eggs are laid, remain flat against the substrate and coil their rays around in one direction to form a 'pinwheel'. Emerson (1973) noted that during the brooding period of L. polaris, the female can reverse the orientation of the rays. Unlike L. hexactis, which can carry its embryo mass to another location during the brooding period (Chia 1966), L. polaris females do not naturally leave their embryos when they are attached to the substrate, and if the females are disturbed and abandon their young, they do not return to brood them. They also do not feed, at least in their normal manner. O'Brien (1972) has, however, suggested that brooding L. littoralis may feed on particulate matter through aboral skin digestion, or on larvae near the mouth that have failed to develop.

The length of brooding period also varies for different species of Leptasterias. Osterud (1918) indicated that brooding extends for six to eight weeks for L. hexactis, while for the same species, Chia (1968) found that females remained brooding for three months. L. ochotensis broods for approximately four to six weeks according to Kubo (1951), and L. aequalis found also in north Pacific waters maintains its young for two to three months (Fisher 1930). In two Arctic species, L. arctica and L. groenlandica, Fisher has observed that brooding continues into late summer, though the precise spawning times are not indicated. Emerson (1973) observed one individual of L. polaris brooding in the field from mid January to mid July, and in other years, females were observed still brooding at the end of July. The two females that successfully brooded their embryos in the laboratory, did so for 5 1/2

and 6 1/2 months until the 19th of June and the 17th of July, respectively.

Thus, for L. polaris, the brooding period of some six or seven months, is considerably longer than that clearly documented for any of the other species of Leptasterias. Here, water temperature might be considered a major factor in the slow development of the young sea stars, and by direct or other means result in the long brooding period of the females.

(c) 'In Vitro' Maintenance of Larvae

For the purposes of studying morphological phenomena expressed during metamorphosis, L. polaris larvae were best procured from the field in their brachiolar stage and subsequently maintained in the laboratory. Disintegration due to the accumulation of debris on the sticky surfaces of early embryonic stages followed by protozoan and bacterial infection as noted also by Chia (1966) in his culture techniques, are thus avoided. Populations of advanced larvae obtained from the natural environment were in a healthy condition, and when held in screened containers that provided circulation of ambient sea water, the larvae were not as subject to rotting and infection, or to predation by invertebrates as in other 'in vitro' systems tried.

2. GENERAL OBSERVATIONS OF LARVAL DEVELOPMENT

The general embryological development of L. polaris larvae from fertilization to metamorphosis, morphologically paralleled the detailed description of events for L. hexactis (Chia 1964, 1968), and the briefer accounts for L. hexactis (Osterud 1918), for L. ochotensis (Kubo 1951),

and for L. aequalis (Gordon 1929).

In the six-rayed species, L. hexactis and L. polaris, the basic pentameric plan of echinoderms is expressed by the initial development of five hydrocoelic lobes almost simultaneously on the larval body. The sixth lobe does not appear in L. hexactis until four days later and in L. polaris until at least two weeks later. Also as Chia noted in L. hexactis, development of the lobes themselves, and of tube feet of rays two, three and four, shortly precedes that of rays one and five. This was also the case for the appearance of the optic pigmentation, that for rays two, three, and four being noticeable two days before that on rays one and five. Chia (1968) speculated that the slight lag in development of rays one and five might be associated with their proximity to the preoral lobe.

Both L. hexactis (Chia 1968) and L. polaris develop a third pair of tube feet on rays one through five as the first pair is appearing on ray six. The next stages of elaboration differ at this point, however, for the two species. In L. hexactis, a fourth pair of tube feet appears on rays one to five and a second followed by a third pair on ray six, and at this stage eyespot pigmentation was noted on rays one through five. In L. polaris, however, optic pigmentation was typically observed on rays one to five when they possessed only three pairs of tube feet. Similarly, one to two weeks later, pigmentation is visible on ray six when it has developed its third pair of tube feet.

The general observations of the embryology of L. polaris would thus appear to support Kubo's surmise, "that the developmental course

of Leptasterias would be, as a rule, similar in each species." However, the rate of development is strikingly different in L. polaris and L. hexactis. As indicated in Table 1, L. polaris larvae take considerably longer to elaborate morphologically. For instance, Chia (1964, 1968) observed eyespots on metamorphosing L. hexactis 40 days after fertilization. For L. polaris, in 1973, 1974, and 1975, optic pigmentation was noted 174, 127, and 103 days after spawning had first been observed in the field or laboratory. Field observations of larvae confirmed that the rate of development in the laboratory situation approximated that in nature.

The factor which would seem to bear considerable importance in delaying the development of L. polaris as compared with L. hexactis is sea water temperature. In the case of L. hexactis, Chia (1964) observed cleavage of eggs in 9°C water with subsequent development of embryos indicated to have occurred at temperatures of 10 to 15°C. Kubo (1951) listed in a table, water temperatures of 5.5 to 6.0°C during development of embryos of L. ochotensis to early brachiolaria, and noted that the temperature of the sea water in the field during the breeding season was approximately 7.5°C. For L. polaris, sea water temperatures remained below 0°C from the time of spawning until April, and then water temperatures slowly rose to, for example, a mean of 4.5°C for June of 1975 (see Appendix I).

3. OPTIC CUSHION AND OCELLAR DEVELOPMENT

(a) General Observations

The tentacular epidermal cells in the larvae, preceding any sign

of ocellar development are in many ways similar to those of the adult. For example, many are ciliated, an observation to be expected, since Chia (1968) noted that the cells of L. hexactis embryos possess cilia in their gastrula stage. Septate desmosomal connections are present between the cells and become more extensive in older larvae.

Certain other features are worthy of mention, however. The presence of extensive rough endoplasmic reticulum,ribosomes, polysomes, and often multiple Golgi centres, indicates that these cells are engaged in active synthesis of constituents. As is typical of cells in early stages of differentiation, nuclei are round and possess prominent nucleoli with a thick granular cortex indicating production of ribosomal sub-units (Boilly 1968).

The radial nerve cords of larvae were also well developed before the appearance of the ocellus. Chia (1964, 1968) made a few observations of the developing nervous system of L. hexactis. The circumoral and radial nerve cords were first noted in metamorphosing L. hexactis larvae two days before the appearance of the eyespots on rays one to five. Chia (1964) commented that the nervous tissue apparently "derived in situ by thickening of the epidermis."

The ocellus of larval L. polaris differentiates on the oral surface of the base of the terminal tentacle just distal to the origin of the terminal pair of tube feet. When pigmentation is first noted in this region with a dissecting microscope, the site of the eyespot is recognized ultrastructurally as a group of epidermal cells which are beginning to differentiate into the three types of ocellar cells: sensory, pigmented and corneal.

Briefly, this plate of cells invaginates while the bordering corneal cells extend to cover the opening of the lumen. The region of the tentacle surrounding the ocellus expands to form a cushion with the ocellus located in its distal portion. The sensory and pigmented elements further differentiate toward the adult condition and increase in number to border the lumen, which expands in breadth and also especially so in depth. Such observations describe the general sequence of events for ocellar development (e.g. Brandenburger et al 1973; Bocquet and Dhainaut-Courtois 1973; Barnes 1974). The pigmented cells reach their differentiated state more rapidly than the sensory cells, an observation made in these studies as well. The lumen becomes filled primarily with the distal portions and extensions of the sensory cells, and the proximal portions of the sensory cells intermingle with the axons of the thickening radial nerve cord.

Invagination and differentiation of larval epidermal cells to form an ocellus with sensory and supportive elements is the pathway by which almost all eyes arise. A few exceptions have been described, such as the segmental eyes of Armandia brevis (Hermans 1969), and the presumed ocelli of an entoproct (Woolacott and Eakin 1973), where it is thought the pigmented cells may develop from mesodermal tissue and the ocelli of the hydromedusan Tiaropsis multicirrata where the pigmented cells are said to be endodermal in origin (Singla 1974).

The cells first recognized as differentiating pigmented, sensory, and corneal cells, are initially noted as a localized portion of the epidermis, and display at this stage numerous ultrastructural features in common with each other and with the epidermal cells. These

observations support the idea that the ocellar components do in fact develop as specializations of ectodermal tissue.

Smith (1937) had proposed from his studies of asteroid optic cushion that "each of the cups is formed by an invagination of the superficial ectoderm." Cobb (1968) in discussion the significance of echinoid pedicellaria sensory cells, those found in a holothurian statocyst, and the asteroid ocellar cells described by Eakin and Westfall (1964) stated that they were all "clearly derived from the general epithelial cells", and further generalized that these regions have specialized in functions already being carried out by the cells of the general epithelium. That asteroids have been demonstrated to possess a "dermal light sense" (Yoshida 1966) would tend to support Cobb's contention.

Other studies have carefully documented the ectodermal origin of eye tissues. Green and Lawrence (1975) in a study of the hemipteran Oncopeltus fasciatus illustrated that the larval eye develops by recruitment of epidermal cells, and grafting experiments demonstrated that even adult head epidermal cells could also become incorporated as ommatidial elements. This latter observation indicates that proximity to an inductive eye margin is an important factor in development, as well as genetical determination and present location of cells. Experiments by Mouze (1975), also examining the growth and regeneration of another insect larval eye, illustrated the progressive recruitment of adjacent epidermal cells and de-emphasized the idea of a permanent population of stem cells.

A proposed mechanism for invagination of ocelli (see e.g.

Spooner 1975) involves the contraction of the apical circumcellular microfilaments constituting the zonulae adhaerentes of a localized population of cells. The pigmented cells, to some extent, and especially the sensory cells of the indenting ocellus of L. polaris are noticeably narrow. Eakin and Brandenburger (1967) illustrated ectodermal cells with more narrowed apices than the cells in the rest of the epidermis lining the lumen of the developing Helix aspersa eye, which has formed by invagination. Eakin (1973) referring to the work of Baker that hypothesized this mechanism for invagination in gastrulae, suggested that fibrillar contraction results in the constriction of inner segments of reptilian third eyes.

(b) Corneal Cells

In several respects, the corneal cells remain much like other cells of the epidermis. They are ciliated and possess microvilli that extend through the external revestment. The corneal cells lengthen and extend around the deepening ocellus and the 'encapsulating' nature of these cells is emphasized by the lengthening and increase in numbers of microfilaments to form a broad fibrous bundle in the cells.

The corneal cell type shows further differentiation by projecting an extension from its apex over the developing lumen. These extensions increase in length and breadth as the ocellus enlarges, and presumably more cells are recruited during the stage of rapid lumen growth, for these overhanging projections become more numerous. The microfilamentous bundles extend for some distance into these projections and presumably serve to support them.

The most striking change in these cells, however, is the rapid accumulation in the projections, of very numerous, spherical vesicles and more flattened tubular profiles. The presence in the apical region of the main cell body, of a Golgi centre apparently giving rise to numerous vesicles that surround it and extend into the projections, suggests a possible Golgi origin for these corneal vesicles.

Mitochondria are also numerous in the Golgi area indicating a substantial necessity for an energy supply for such a proliferation of vesicles. The changes noted in mitochondrial morphology in these and the pigmented cells as mentioned in the Adult section, may reflect the decrease in energy requirements of these cells after the initial burst of synthetic activity.

(c) Pigmented Cells

In their initial state of differentiation at the epidermal surface and as the lumen commences to form, the pigmented cells are narrow apically, probably as a result of the postulated process of invagination discussed earlier. They broaden considerably in older larvae. Microvilli, absent from their apical borders initially, slowly develop, often at the lateral borders of the cells, and extend into the lumen for short distances. Even in the oldest larvae examined, however, the microvilli were not seen to be as numerous or as long as in the pigmented cells of adult fully formed and regenerating ocelli.

In their earliest discernible state, pigmented cells display some features characteristic of the surrounding epidermal cells, including large vacuoles, and scattered microtubules, and microfilaments.

These structures are still occasionally seen in mature pigmented cells. No cilia or their associated features were evident, suggesting either that such structures resorb, or the course of differentiation of these cells is determined at a stage prior to ciliary development.

The mitochondria of the pigmented cells, initially swollen and irregular in appearance as in the other cells in the young larvae at the early stages of differentiation, are then soon observed in these cells as in the corneal cells, to become more compact and electron-dense with more clearly delineated cristae. This change perhaps reflects a decrease in energy requirements of these cells following the initial remodelling of the cell.

The small pigment granules were prominent constituents permitting initial identification of developing pigmented cells, and possible pathways for their formation are suggested by certain morphological features. Micrographs of these cells in their very early stages of differentiation illustrate stages of apparent condensation of a granular material within vesicles up to 100 nm in diameter to approximate the appearance of the pigment inclusions. Such profiles are typically located in the apical portions of the pigmented cells.

A range of sizes of vesicles with granular contents were also noted elsewhere in the cytoplasm, with numerous ones ranging from 40 to 80 nm in diameter surrounding the Golgi apparatus and perhaps deriving from its inner saccules. Because of the non-membrane-bound nature of 'mature' granules, one would have to presume that the vesicular membrane in some way disintegrates. A few ruptured vesicles were noted. The pigment inclusions may then continue to enlarge by the peripheral

incorporation of material.

The only developmental study of eyes whose pigmented cells contained inclusions identical in morphology to those of asteroids, did not discuss their production other than to comment on their smaller size and greaterosmiophilia than in the mature cells (Dudley 1969). Fahrenbach's study (1964) of the nauplius eye of another species of copepod, described small membrane-bound pigment granules, probably containing astaxanthin, and other lipid-type bodies. In addition, he noted the presence of a few small, spherical, membrane-bound masses of granules. Perhaps these correspond to the similar vesicles discussed above. In a study of developing ocelli in the polychaete Syllis amica, Bocquet and Dhainaut-Courtois (1973) proposed that Golgi-derived vesicles fused to constitute the larger membrane-bound granules of a red pigment assumed to be a carotenoid.

Though formation of small pigment granules within Golgi-derived vesicles may in fact be possible in L. polaris ocelli, many small pigment inclusions appear to gradually condense from granular material in the cytoplasm. Vesicles may still play a role in transporting and releasing pigment precursors into the cytoplasm. The relatively extensive areas of granular cytoplasm that give rise to pigment granules would suggest as an additional possibility, however, the manufacture of pigment precursors in the cytoplasm. Such a site of origin has been suggested for melanin precursors (Eakin and Brandenburger 1967), and for yolk constituents (Kessel 1966), the latter study proposing yolk precursors being of cytoplasmic origin in addition to some being Golgi-derived and vesicle-bound.

The second class of noticeable inclusions characteristic of adult pigmented cells of L. polaris and A. rubens (von Harnack 1963) ocelli, the polymorphic bodies, appear in L. polaris larvae within three weeks of the appearance of orange-red pigmentation, and rapidly increase in number to their proportion in mature cells. Again, as in the adult situation, they presented a variety of morphological profiles which suggest they are stages in autophagy. The areas within them identical to the small pigment granules may in some cases represent engulfed granules, for a few such structures did appear to be fusing with the larger profiles. Similarly, Kessel (1966) observed that small, fully condensed yolk granules were incorporated into larger yolk bodies in ascidian oocytes.

Alternatively or primarily, certain areas of the granular cytoplasm isolated by membranes probably contain pigment precursors, which subsequently condense to form areas identical in their staining properties to the small pigment granules.

Morphological parallels to the observations of L. polaris material are provided by Kessel's (1966) study of oocytes in which he described the formation of yolk by a variety of steps. One pathway is by the enlargement and fusion of vesicles derived from the Golgi apparatus. A second mechanism involves precursors elaborated in the cytoplasm under ribosomal control. Low density (clear) vesicles originating from the outer saccules of the Golgi apparatus, accumulate around the precursor-containing regions and fuse with each other to gradually isolate a volume of cytoplasm. Some profiles thus display incomplete membranes. High density vesicles (primary lysosomes) and other material

may also be sequestered in this process and may also fuse with the large membrane-bound developing yolk granules at later stages.

Eakin and Brandenburger (1967) in a study of the differentiation of the eye of the snail, Helix aspersa, described the formation of melanin granules in similarly varied ways, sometimes at sites remote from the Golgi apparatus. They too suggested that the bounding membranes at least, of the propigment granules were Golgi-derived by the fusion of small Golgi vesicles, and that these membranes subsequently enclosed cytoplasmic particles.

In the eye of Drosophila melanogaster, Shoup (1966) described pathways of pigment granule development and noted in colourless mutants, large (up to 1 μm) bodies, which she proposed were related in an abnormal way to pigment formation for they were restricted to pigmented cells. Shoup also noted their appearance initially in pupae and suggested that an initial accumulation of pigment precursors which failed to continue development because of genetic defects, are converted in more mature stages to autophagic vacuoles into which other cytoplasmic constituents may also be incorporated. The contents of such vacuoles are ultimately hydrolysed. Shoup also noted that some of these forms displayed membranes that were indistinct.

A similar mechanism of sequestering areas of cytoplasm, including granular regions containing pigment precursors producing polymorphic autophagic bodies, would appear to be a continuing process in L. polaris pigmented cells, and may function in the removal of cytoplasmic pigment precursors, and perhaps some small pigment inclusions. Regardless of whether, as proposed, these large polymorphic profiles are a morphological

feature of an autophagic phenomenon and that their contents are ultimately lysed and expelled, they may in fact 'function' as large pigment bodies during those stages when precursors have condensed within them.

In an elegant study involving two staining techniques, Locke and Sykes (1975) confirmed the previously postulated origins of the membranes involved in autophagocytosis, in insect fat body undergoing dramatic morphological remodelling. They determined that the outer saccules of the Golgi complex give rise to clear vesicles that fuse to form isolation membranes, which then sequestered various organelles as isolation bodies. The inner saccules of the Golgi apparatus give rise to small dense vesicles, primary lysosomes, which fuse with the isolation bodies to become autophagic vacuoles in which lysis then occurs.

Fig. 70 summarizes the proposed pathways of formation, and inter-relationships of both the small pigment granules and the larger, polymorphic profiles.

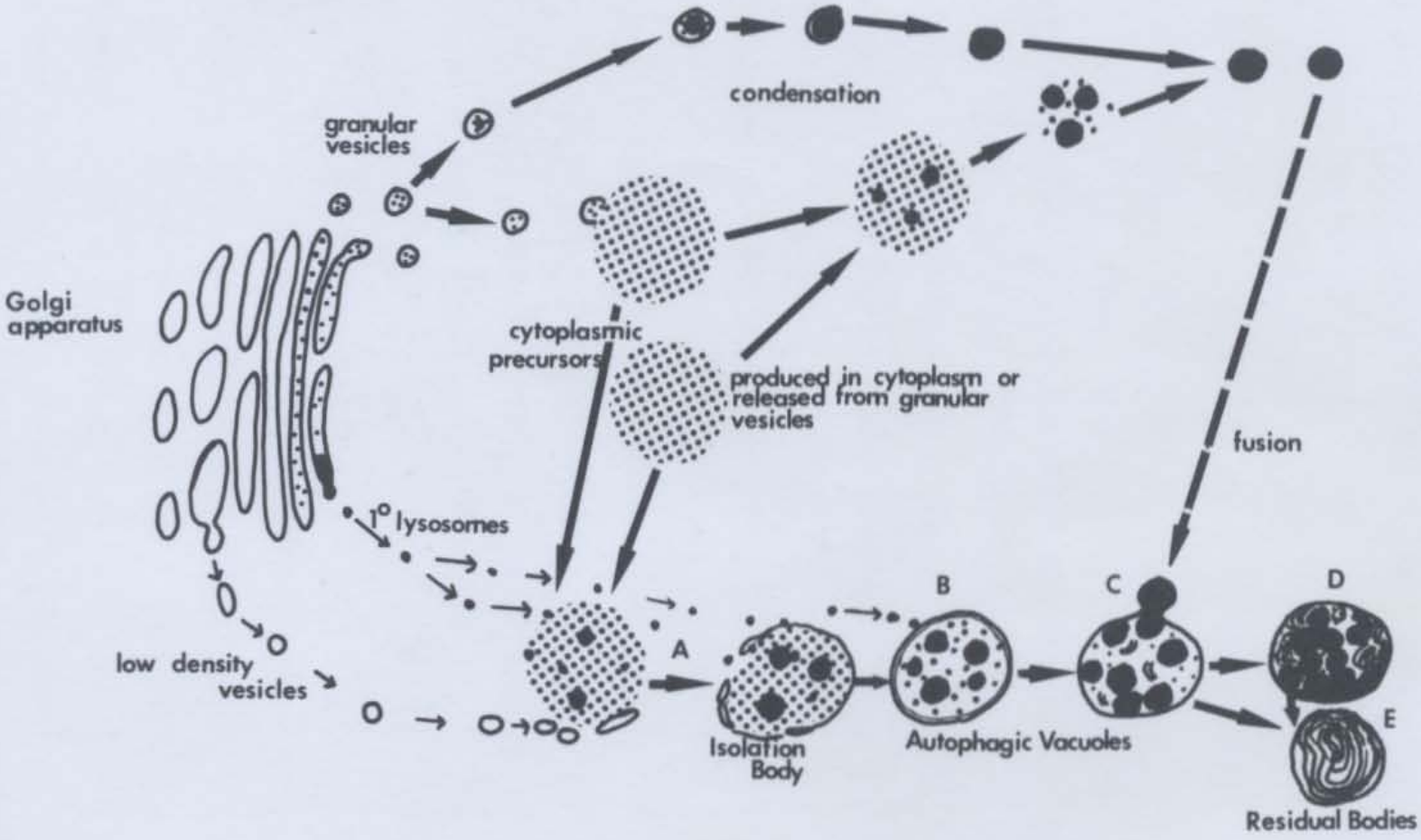
By applying the rationale and procedures utilized by Locke and Sykes (1975) to optic cushion tissue, one could hopefully stain by Friend's hot osmium procedure, the highly osmiophilic components of the Golgi complex (outer saccules) and their derivatives (low density vesicles), and subsequently modified forms (isolation membranes). Similarly, acid phosphatase localization would confirm the site of origin of acid phosphatase (inner Golgi saccules), its packaging as primary lysosomes (high density vesicles), and the product of fusion of the lysosomes with isolation bodies (autophagic vacuoles).

Figure 70. Diagrammatic illustration of proposed pathways of pigment granule and polymorphic body formation and interrelationships.

Pigment granules may form in the cytoplasm by 'condensation' of precursors, either manufactured in the cytoplasm or released there by Golgi-derived vesicles. 'Condensation' may also occur within these vesicles whose limiting membranes subsequently disappear.

It is proposed that the polymorphic bodies represent stages of autophagocystosis, by which regions of cytoplasm are isolated by the fusion of low density vesicles derived from the outer saccules of the Golgi apparatus. Small, high density vesicles arising from the inner saccules are primary lysosomes that are incorporated into the isolation bodies. Sequestered pigment precursors may 'condense' within the polymorphic bodies, and already formed pigment granules may also fuse with them.

PIGMENT GRANULE FORMATION



POLYMORPHIC BODY FORMATION

(d) Sensory Cells

The narrowed neck region of the developing sensory cells is associated with neighbouring pigmented cells by a region of septate desmosomes, which lengthens to extend proximally several micrometres. In addition to the early presence of such junctions imparting structural support, the functioning of these regions as areas of lowered electrical resistance might facilitate 'communication' and the organization of these cells, or indicate a rapidly established photoreceptive ability of the sensory cells. Subsurface cisternae too were present in the earliest stages of ocellar differentiation and rapidly developed to encircle the neck almost completely and extend basally into the supra-nuclear region of the cell.

Microtubules were also present in the neck of the earliest recognized sensory cells and increased in number with time, a phenomenon characteristic of such receptors (e.g. Barnes 1974). Such structures would be of considerable importance in developing ocelli in their capacity for directing materials apically for the assembly of the expanding distal apparatus of the sensory cells. The initially large concentration of ribosomes in this region would further support the idea of enhanced synthesis of materials.

The elaboration of the cilium and its photosensory surfaces in embryological development has been described in many vertebrate photo-receptors and in at least one invertebrate ciliary photoreceptor, the ascidian tadpole (Barnes 1974). The earliest stages in such ciliary development were not identified in the L. polaris larval material examined, but a common pattern has held for those other receptors

investigated. Eakin (1973), for example, for reptilian third eyes, described the migration of the centrioles from the nuclear portion of the cell to the apex, at which time a striated root begins to form. Microtubules commence to extend from the distal centriole toward the plasma membrane, which has elevated slightly above the centriole. As the ciliary membrane evaginates, the nine microtubular doublets continue to lengthen into the developing shaft. The ciliary membrane then differentiates to form the photoreceptoral surfaces characteristic of the particular species.

Sensory cell cilia were not noted in the sections of L. polaris larval material examined, when the cells were first differentiating at the epidermal surface, though a short cilium and paired apical centrioles were observed in presumptive corneal cells. However, by the time a shallow lumen covered by a few corneal extensions has formed, short cilia extend from a pocket in the apical surface of the sensory cells. Eakin (1973) described an invagination of the plasma membrane above the distal centriole to create a depression from which the cilium arises in the vertebrate pineal eye. Echinoderm cilia are frequently described as being situated in 'pits' (Nørrevang and Wingstrand 1970) and this condition is usually seen to persist in adult photoreceptoral cells of L. polaris. An accessory centriole is positioned perpendicularly to the distal centriole, and a short striated root with adjacent microtubules has become established at a very early stage, presumably as described by Eakin (1973) and Barnes (1974), immediately preceding the growth of the ciliary shaft. The root elongates to extend further into the neck region in more mature cells.

The initial phase of ciliary growth appears to be quite rapid, as noted for cilia in general, after which, the growth rate declines. Burns (1973) has suggested that this decline is a reflection of the asymptotic pattern of transport of precursors to the distal growing tip. The ciliary shaft membrane is ruffled slightly but does not appear to give rise to any extensions in its very early development. As the distal portion of the sensory cell protrudes further into the expanding lumen and projects irregular villi from its membrane, a few small villous extensions are noted also to extend from the base of the ciliary shaft.

The ciliary axoneme of nine paired peripheral tubules is seen in some sections and shafts with fewer tubules in others. Similarly the centre of the shaft has been noted to contain none, one, or two, microtubules. Presumably the cilium is of the '9 + 2' configuration and these atypical profiles are of more distal regions of the developing cilia. Those sensory cilia of photoreceptors with other than '9 + 2' axonemes develop with these atypical arrangements (e.g. Eakin 1968; Barnes 1974), rather than a '9 + 2' cilium forming and then subsequently having its microtubular pattern modified.

The sensory cells initially terminate apically at the level of their neighbouring pigmented cells, but they rapidly expand into the lumen as the ocellar elements invaginate. The ciliary basal bodies are usually seen in these apical expansions. Some microtubules and scattered ribosomes are present in these occasionally very voluminous expansions, and smooth-surfaced vesicular profiles such as are seen in the neck, accumulate in them as well. The plasma membranes of the tips of the

expanding sensory cells rapidly give rise to irregular villous extensions. As noted for the lamellae of ascidian tadpole photoreceptors (Barnes 1974) these villi increase in length and in number, and in L. polaris they result in an intermingled complex in the lumen.

The observations of the embryological development of the sensory cell apical regions, as for those of the adult ocelli, do not reveal the presumptive photoreceptoral surfaces, the villi, to be primarily derivations of the ciliary shaft. However, it is clear that the raised distal surfaces of the sensory cells that bear the cilia are extensively modified and that occasional villous profiles arise from the fluted ciliary shaft distal to the basal body. The sensory cell cilia extend over several micrometres in length and are a prominent feature in the lumen of asteroid larval ocelli persisting in the adult sea star.

From similar observations on other asteroid ocelli, Eakin has classified these as being 'ciliary' receptors. Eakin (1968) and others, including a recent study on an arachnid photoreceptor by Muñoz-Cuevas (1975), have also suggested the possibility that cilia, or at least centrioles aligned at the cell surface, may induce the formation of villi perhaps over some distance, and that in rhabdomeric eyes, the cilia may resorb or play no role in photoreception. There are eyes, however, in which neither cilia nor centrioles have been observed in adult or embryological material (Eakin 1968).

Several weeks after ocellar development has commenced, endocytotic invaginations engulfing pieces of villi, and coated vesicles, appear in the distal sensory cell expansions, and in later stages become very numerous. The endocytotic vesicles then pass down the neck

and are seen in deeper regions of the cell. The possible roles of such vesicles discussed in the Adult section--nutrition, phagocytosis of debris, recycling of membranous material--are equally applicable to the larval situation.

When cells are first identified as presumptive sensory cells, the neck contains a few vesicles, while the supranuclear region has a larger number of a variety of profiles--clear vesicles, large vacuoles with flocculent interiors, some multilamellar whorls, granular vesicles (some coated) and a few large multivesicular bodies. These forms all become more numerous, the smaller forms accumulating in the neck as well. Very noticeable, however, as the lumen commenced invagination and continued expansion, was the increase in numbers of multivesicular bodies. There contained some irregular material, and small dense 40 nm diameter vesicles, also noted to be scattered in the cytoplasm. The irregular contents probably represent the phagocytized portions of villi carried by the endocytotic vesicles, which fuse to form the multivesicular bodies. The small, high density vesicles are presumably primary lysosomes. Locke and Sykes (1975) have demonstrated that these pass intact through the limiting membranes of multivesicular bodies.

A range of profiles, including these small dense vesicles, and clear spherical and C-shaped vesicles were clustered about the Golgi cisternae suggesting their derivation from that source. Barnes (1974) also illustrated multivesicular bodies and a few multilamellar whorls in the supranuclear region of elaborating sensory cells in ascidian tadpoles, and multivesicular bodies were noted as well in the differentiating sensory cells in polychaete larvae (Eakin and Westfall 1964;

Holborow and Laverack 1972; Bocquet and Dhainaut-Courtois 1973). Multivesicular bodies are reduced in number in more advanced larval ocelli.

As was indicated earlier, before the invagination of the ocellar plate, the sensory cell nuclei are located basally below the level of those of the general epidermal cells, an observation noted in the eye-cup formation of other eyes, e.g. Eakin and Brandenburger (1967), Bocquet and Dhainaut-Courtois (1973), Barnes (1974).

The nuclei, and other cellular constituents, also undergo certain morphological changes corresponding to changing cellular processes. A very thorough account of the ultrastructural development of cellular organelles is available in the study of Boilly (1968) on the regeneration of annelid segments. The nuclei in their initial stages of differentiation are round to oval with a lightly staining matrix, moderate clumping of chromatin, and a large nucleolus with granular cortex. Ribosomal sub-units produced by the nucleolus, assemble outside the nucleus, and become associated with each other as polysomes, which are very numerous in early stages, and with the endoplasmic reticulum, which is prominent about the nucleus, deriving from its envelope, and extending into other regions of the cell. These observations reflect the high rate of protein synthesis established during the activation and subsequent differentiation of cells.

The ribosomes and polysomes decrease in numbers at later stages, and rough endoplasmic reticulum is typically more restricted to a circum-nuclear distribution. The nuclei become more elongated with time, aligning longitudinally in the cell. The nucleolus generally persists

in L. polaris sensory cell nuclei. Chromatin becomes more noticeably clumped within the nucleus and particularly at its periphery. The nucleoplasm of the sensory cell nuclei, however, remains very lightly stained, while that of the other ocellar cell types and most epithelial cells becomes more electron-dense.

Sensory cell mitochondria are numerous around the nucleus but remain less electron-dense, and generally larger with more extensive cristae than those of the other cells, observations discussed in greater detail previously.

The basal portions of the differentiated sensory cells, which taper to extend into the nerve plexus, display the sparse cytoplasmic contents of a few clear and cored vesicles, scattered ribosomes, and occasional microtubules and mitochondria, like the numerous axons with which they intermingle. Again, as for the adult ocelli, the difficulties in tracing the basal extensions of these cells without examination of serial sections have prevented a description of their precise terminations. In a similar study on ascidian tadpole larvae, Barnes (1974) also described processes extending basally from the photoreceptors after constriction of the neck region and formation of the ciliary extensions, and he identified them as neuronal on the basis of exhibition of similar morphology to adjacent axons.

Clearly the elements of the radial nerve cord are present during ocellar differentiation and are in intimate physical association with the region. The importance of the influence of the nervous system upon development of asteroid ocelli through regenerative processes will be discussed later.

Behavioural studies such as those carried out by Yoshida and Ohtsuki (1966), if modifiable for the larval situation, might prove useful in establishing the time in ocellar development at which the ocellus is functional. Correlative ultrastructural examination would provide further information on the extent of cellular differentiation necessary for photoreception. In a study combining fine structural observations and electrophysiological responses to light in the eyes of developing tadpoles, Nilsson (1969) noted the production of a receptor potential as early as when only a dozen rod discs had developed and some mitochondria were present. No synaptic structures were noted at that point. He found that the elaboration of synaptic vesicles was the last step in development and that only then could the current generated by the receptor be passed further in the retinal complex.

C. REGENERATION STUDIES

1. REGENERATION RATE

Echinoderms as a phylum, and particularly the asteroids, possess a high regenerative capacity, and Hyman (1955) and Swan (1966) summarized much of the information on this topic. Partial removal of sea star rays may occur in nature and serious injury often results in the rejection of the whole arm close to its level of attachment to the central disc (King 1898). Damaged and regenerating arms are in fact frequently observed in sea stars in the field. Marsh (1968), for example, noted that 22% of intertidal Asterias rubens individuals had arm damage, and King (1898, 1900) observed that 11% of the nearly 2000 A. vulgaris specimens she collected in New England waters displayed regenerating arms.

Various authors studying regeneration in sea stars have mentioned the re-appearance of the eyespot. King (1898) induced small specimens of A. vulgaris to throw off arms, and after ten days,observed a short cone of tissue extending from the stump with an eyespot visible at its apex. In cases where arm tips were amputated by transverse or oblique cuts, she noted the presence of an eyespot "at the cut end of the nerve path" after a week. Goldfarb (1909) also cut rays off small specimens of A. vulgaris and observed the appearance of the eyespot after 15 days.

Huet (1966, 1967, 1972a) in his studies of the relationship of the nervous system to the process of regeneration, described the initial appearance of optic pigmentation 30 days after cutting rays of Asterina gibbosa. A line drawing in his 1966 publication illustrates the presence of an optic cushion with several ocelli at the base of the terminal tentacle after 60 days.

The rate of reappearance of the eyespot of experimental L. polaris specimens was comparable to that noted in the studies cited above. Two observations are worthy of comment. First, the regeneration of optic pigmentation in adult specimens of L. polaris was observed to occur more rapidly in those specimens whose tentacular areas only, were removed, as compared with the more extensive excision of the ray tip. Such an observation probably reflects the differences in the area of wound to be sealed off, the amount of the tissue to be phagocytized by coelomocytes, and the region to be recovered by rapidly mitosing and subsequently differentiating epithelial cells. Secondly, the rate of regeneration was seen to be correlated with temperature, with development

over the winter period being slower.

2. OPTIC CUSHION AND OCELLAR REGENERATION

(a) General Observations

The early studies on asteroid regeneration, for example King (1898, 1900), noted the similarity of regeneration to that of the embryological situation in the reconstitution first of the terminal plate, terminal tentacle, optic cushion, and then of the rest of the structures between the ray tip and more proximal parts of the ray. As in the larval development, the initial ocellus became established sagitally by invagination on what becomes the distal edge of the optic cushion. In regenerating tissues, other regions rapidly invaginated peripherally and posteriorly to re-establish several dozen ocelli within a few months. Adult specimens possessed up to approximately 200 such ocelli on normal rays. Von Harnack (1963) suggested that shallow indentations in the cushions of adult Asterias rubens corresponded to stages of ocellar development, commenting that the number of photoreceptoral organs was known to increase with age of the sea star.

Not unexpectedly, ultrastructural as well as general details of ocellar development of regenerating tissues paralleled that of embryological development. Eakin and Ferlatte (1973) similarly illustrated that the eye regeneration in the mollusc Helix aspersa proceeded in the same manner as in embryological development (Eakin and Brandenburger 1967).

The re-establishment of epithelial cells and the differentiation of ocellar cells after excision of the terminal tentacle were similar

to the events described by Huet (1972a) concerning the development of neuroepithelial cells in regenerating Asterina gibbosa rays. That study confirmed his previous observations (Huet 1966) that, in asteroid regeneration, epidermal tissue differentiated from existing epidermal cells rather than from a pool of cells capable of developing into a variety of cell types.

Among the features of differentiating cells in common between Huet's description, and both regenerative and embryological development in L. polaris, are the changing morphology of the nucleus, the production of ribosomes and polyribosomes, the development of rough endoplasmic reticulum, the establishment of the Golgi apparatus, and the increase in length and number of the microtubules which appear in the apical portion of the cells. The cells align with each other and become associated by short desmosomal regions, and microvilli and cilia extend from the apical borders of the cells. It is interesting to note in considering these latter features, that Amemiya (1971) found that the formation of cilia, including their regeneration, in dissociated sea urchin embryos, required establishment of tight intercellular contact among the cells.

The recruitment and differentiation of ocellar elements and invagination of ocelli, proceeded more rapidly in regenerative development as compared with larval elaboration. This difference in developmental rate may reflect the differences in potential supplies of material, energy, and nervous coordination between sea stars with well established and functioning body systems, required to regenerate a proportionally small area of its body, and small larvae which are undergoing major physiological and morphological changes.

(b) Corneal Cells

As described in the larval study, the corneal cells are derived from epidermal cells and specialize by extending processes over the lumen. Microfilaments, associated as a broad bundle, and microtubules, pass from the main cell body into the extensions. The supranuclear Golgi apparatus is prominent, giving rise to numerous vesicles, which may be the source of the numerous profiles, which fill the overhanging processes.

(c) Pigmented Cells

Invagination of the lumen, and its becoming lined with broad pigmented cells with well developed septate desmosomal connectives and microvillous extensions, advanced within a short time span. Large numbers of the small class of pigment granules, and some of the larger polymorphic bodies, were present in pigmented cells differentiating at the epithelial surface. Observations similar to those of larval pigmented cells, of apically located vesicles whose contents varied from granular appearances to that identical to the pigment granules, suggest their transport of a pigment precursor. Densely stained Golgi cisternae and the abscission of numerous granular vesicles, and condensing pigment granules also located in their vicinity, further suggest the possibility of Golgi derivation of such a precursor. Again, however, many other pigment granules apparently transform directly from granular regions in the cytoplasm. Such areas may have resulted from release of the contents of Golgi-derived vesicles, or there is the possibility discussed earlier, that some pigment constituents may be manufactured in the

cytoplasm.

The polymorphic bodies were present at very early stages of differentiation, and appeared to arise by the incorporation of some vesicular elements and granular areas of cytoplasm by the fusion of low density vesicles. These profiles again, correspond morphologically to autophagic vacuoles transforming through their sequestering, hydrolysing, and residual states.

Again, as suggested by examination of the larval states of development, the pigmented cells, by their initial differentiation at the epidermal surface from cells sharing many characteristics with adjacent elaborating epithelial cells, are of epidermal origin, and increase their numbers in the enlarging ocellus by progressive recruitment of ectodermal cells. Further indication of their derivation from cells with potentiality for differentiation as general epithelial cells was the observation in a pigmented cell sectioned at the surface of the cushion, of a striated root. Von Harnack (1963) too, reported occasionally observing striated roots, but no cilia, in A. rubens pigmented cells.

(d) Sensory cells

Development of sensory cells proceeded very rapidly and in a similar manner in regenerating ocelli as compared to the larval situation. Several observations are worthy of re-emphasis.

Cilia are prominent in the distal expansions of the sensory cells and possess paired central microtubules and nine peripheral pairs. Numerous long and branching villi proliferated from the distal expansions, and oblique sections of cilia occasionally suggested irregularities

of the shaft membrane.

In those ocelli with lumens densely filled with villous extensions, phagocytosis of tubular pieces into endocytotic vesicles was particularly evident. Multivesicular bodies were numerous in the expanded mid sections of these cells and have likely resulted from incorporation of these endocytotic vesicles by fusion as described by White (1967) in mosquito larvae rhabdomeres. Further, small dense vesicles appear to arise from the inner saccular region of the Golgi apparatus, and similar vesicles are seen to be contained within the multivesicular bodies. These probably represent the derivation of primary lysosomes containing acid phosphatase from the Golgi apparatus and their incorporation into multivesicular bodies by traversing the delimiting membrane intact. Many of the vesicular profiles that fill the neck region of the sensory cells are endocytotic vesicles which have lost their bristled external coat and are moving proximally to ultimately fuse as multivesicular bodies. Others may possibly be forms derived in the mid regions of the cell and may be involved in transporting photopigment precursors or other constituents and/or membranous material, to the distal surfaces of the sensory cells. The numerous microtubules in the neck may serve to guide these structures or macromolecular material to or from the tips of the cells.

(e) Role of the Nervous System

In L. polaris, regeneration of the eyespot commenced, as in its larval development, following the establishment of a short terminal tentacle into which nerve fibres from the thickening subepithelial

plexus extended as one of its constituent layers.

The presence of nervous tissue at the site of ocellar elaboration is apparently a prerequisite for differentiation of the elements of the optic cushion. Huet (1966, 1967, 1972b, 1975) in a series of experiments demonstrated this to be the case in Asterina gibbosa. He observed that small ocelli developed in the sub-terminal region of regenerating tips where epithelial cells contacted fibres of the radial nerve cord, and found that this cord, with intact connection with the circumoral nerve ring, was necessary for the regeneration of the distal tips of excised arms. By destroying the radial nerve at various times during regeneration, he determined that there is no specific point during development after which the elaboration of the optic cushion will proceed in the absence of the nerve cord. Further, the differentiating tissues must be in a functional state to receive nervous "stimulation" --that is, the presence of a healthy radial nerve could not induce regeneration of tissues damaged by X-radiation. Eakin and Ferlatte (1973) also illustrated that the presence of nervous fibres was necessary for eye reformation in the snail Helix aspersa.

The precise role of the nervous system in regeneration is not clear. Huet (1975), as others have, considered the circumoral nerve ring as a type of central nervous system, and speculated on the importance of neurosecretory activity in communication through the radial nerve cords. In this regard, cells similar to those identified as neurosecretory cells in other echinoderm studies, were found in the nerve plexus in the adult optic region. Huet (1975) suggested that the radial nerve cord induces an intensive round of mitotic division in regenerating

tissues.

Another recent study by Gamache and Gamache (1975) also points to the importance of the radial nerve cord in regenerating tissues by implication of its own enhanced activity. They noted a six- to eleven-fold increase in the amount of labelled protein passing distally along the radial nerve cord of regenerating arms as compared with normal rays of the same specimen. Further, the rate of fast axonal transport had raised from 240-480 mm/day to 400-600 mm/day.

It would thus appear that while the ectoneural nervous system apparently derives embryologically from epithelial elements (Chia 1966), subsequent specialization is such that the elaboration of the optic cushion in regenerating adult tissues, and probably also in larval sea stars, requires the influence of a functioning nervous system. Ocelli do not merely invaginate and their elements 'hook up' with the radial nerve cord.

SUMMARY

1. In Leptasterias polaris, the prominence of cilia in developing and mature ocelli, the atypical microtubular pattern for portions of the ciliary length, and the extension of villi from the base of the shaft, suggest that the cilia supplement the villous extensions of the distal sensory cell surface in a sensory capacity, and support Eakin's proposal that asteroid photoreceptors are 'ciliary'. Other structural features of these cells indicate their sensory nature.

2. Endocytosis, often of villous material, and incorporation of coated endocytotic vesicles and primary lysosomes into multivesicular bodies is observed in L. polaris sensory cells. Such phenomena have been proposed to constitute part of a recycling process for photoreceptoral membrane in a number of rhabdomeric eyes.

3. Subsurface cisternae were identified in the neck region of the sensory cells and together with the adjacent septate desmosomal regions, are proposed to play a role involved with the sensory nature of these cells.

4. The initial 'swollen' appearance of mitochondria in differentiating cells of the ocelli, and the persistence of this configuration in sensory cell mitochondria, while those of the pigmented and corneal cells become more compact with reduced cristae, suggest that the energy requirements of the sensory cells remain at a high level.

5. The corneal components are ciliated epidermal cells which are specialized by (a) the extension of processes over the external opening of the ocellar lumen, (b) the presence of numerous vesicular profiles (perhaps Golgi-derived) in these processes, and (c) the presence of a broad microfilamentous bundle that may confer a supportive function to these cells.

6. Small pigment granules in the pigmented cells correspond in morphology to those described in other asteroids, and they appear to arise from a granular substance transported from the Golgi apparatus by vesicles, and perhaps also manufactured in the cytoplasm.

7. The polymorphic bodies in the pigmented cells are proposed to represent stages in autophagy of cytoplasmic regions including small pigment granules and their precursors. Such an autophagic process is established early in larval and regenerative ocellar development, and is a continuing feature of adult pigmented cells, indicative of a high degree of material turnover in these cells.

8. The larvae of L. polaris develop in a similar manner to other species of the genus with the rate of elaboration being slowed, apparently by the low sea water temperatures.

9. Ocelli develop at the base of the terminal tentacle by the invagination and subsequent recruitment of epidermal cells differentiating as corneal, sensory, and pigmented cells. Cells undergoing differentiation display ultrastructural features that indicate a high level of protein

synthesis and energy expenditure. The region containing the ocellus (i) expands to become the optic cushion.

10. The pattern of ocellar development is the same in embryological and regenerative material, and is similar to the development of other ocellar eyes.

11. Differentiation of ocellar components proceeds more rapidly by regeneration than embryogenesis, and numerous ocelli are established in regenerating adult cushions, while larvae possess only a single ocellus for at least six months.

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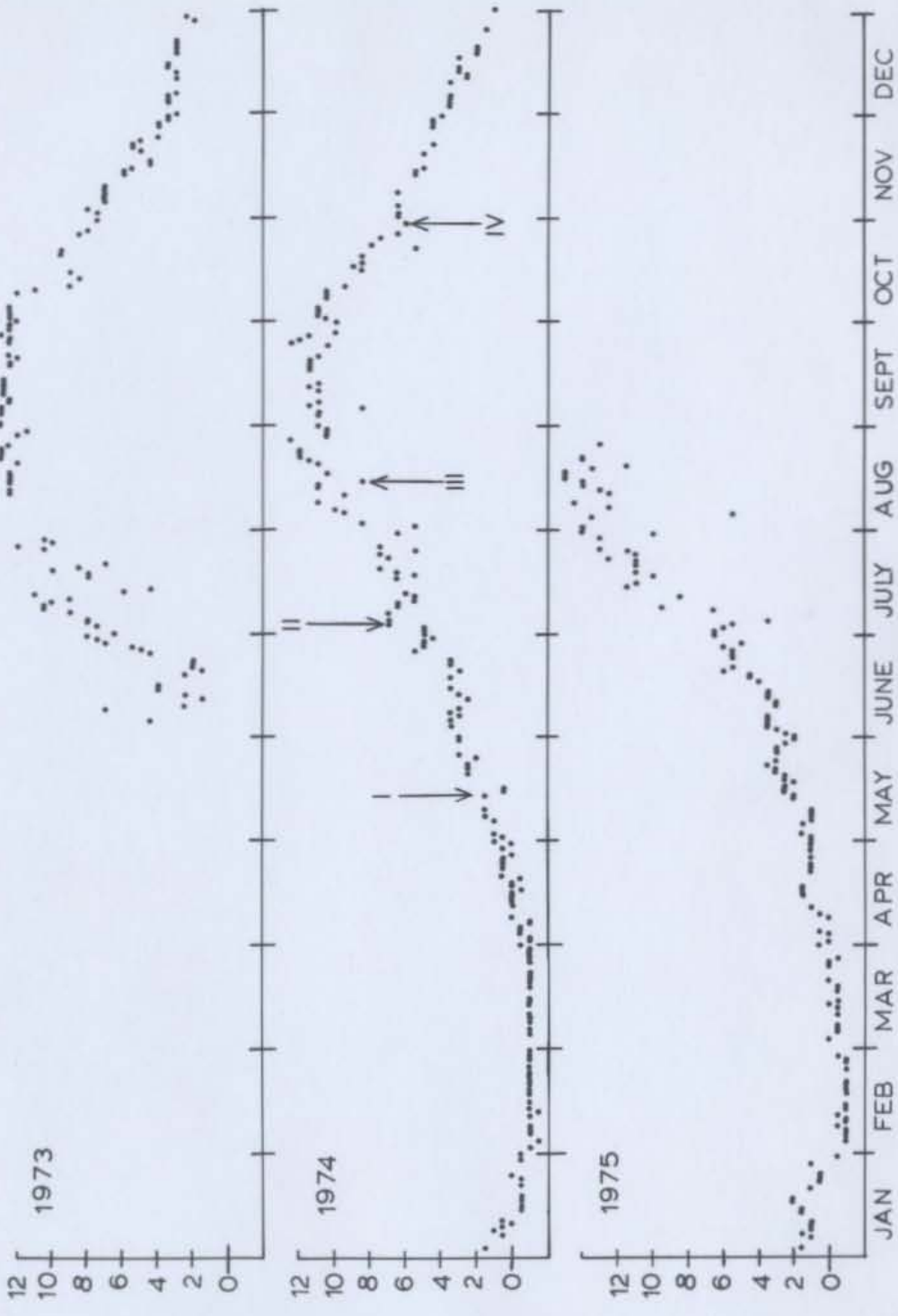
Appendix I. Laboratory sea water temperatures for research period.
(Values in ^{o}C)


1973
1974
1975
12
10
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JAN
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APPENDIX II

MEASUREMENTS OF ADULT *LEPTASTERIAS POLARIS* INDIVIDUALS

Specimen	R (mm)	r (mm)	R:r	Regeneration Group
1	78.0	18.0	4.3	
2	73.0	18.0	4.1	
3	112.0	20.5	5.5	
4	68.0	20.0	3.4	
5	82.0	20.0	4.1	
6	98.5	23.0	4.3	
7	91.0	15.5	5.9	
8	74.5	12.5	6.0	
10	73.0	14.5	5.0	
11	72.0	15.0	4.8	
12	70.0	15.0	4.7	
13	79.0	18.0	4.4	Group I
14	78.5	15.5	5.1	
15	87.5	16.0	5.5	
16	72.0	17.0	4.2	
9	72.5	15.5	4.7	
17	92.5	16.5	5.6	
18	86.5	17.5	4.9	
19	80.5	17.0	4.7	Group II
20	75.5	15.0	5.0	
21	81.0	17.0	4.8	
22	49.5	11.5	4.3	
23	83.0	18.0	4.6	
24	79.5	19.5	4.1	
25	66.5	19.5	3.4	Group III
26	62.5	15.0	4.2	
27	80.0	16.5	4.9	
28	69.5	16.0	4.3	

APPENDIX II (CONTINUED)

Specimen	R (mm)	r (mm)	R:r	Regeneration Group
29	92.0	24.5	3.8	
30	86.0	24.0	3.6	
31	90.5	22.5	4.0	Group IV
32	76.5	21.5	3.6	

$\overline{X} = 4.6$