Final Report

1.Cover Page.

ELECTRON MICROSCOPE STUDY

OF

TISSUES WHICH PRODUCE PEARL SHELL ORGANIC MATRIX

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NON TECHINAL SUMMARY

Pearl oysters of the species *Pinctada maxima* (silver lip or gold lip pearl oyster), *P. margaritifera* (black lip pearl oyster), and *Pteria penguin* (bat wing pearl oyster), are all used in the Australian cultured pearl industry, but *Pinctada maxima* is by far the most important economically. *Pinctada fucata* is of very little commercial interest in Australia but was included in this study for comparative reasons.

While this project was specifically the study of the "electronmicroscopy of tissues which produce pearl shell organic matrix" it is part of a much larger study of these tissues, their secretions and the shell parts formed by their secretions. Also it is an extension of several previous years' work on the light microscopy of decalcified pearl shells and the tissues of the External Mantle and Mantle Margins which produce them. To make this report intelligible some of the results of previous work are included. Similarly, since this work has continued and is continuing, results obtained subsequent to the period of the grant are added where a more complete picture is achieved by their inclusion.

Pearl shells consist of two types of crystalline calcium carbonate. In both cases the individual crystals are enclosed in Organic matrices. These consist largely of sclerified glycoproteins. The pearl shell has three layers - an outer prismatic layer and inside this two nacreous layers. The outer prismatic layer consists of microscopic polygonal prisms the long axes of which are normal to the surface of the shell. The calcium carbonate here consists of calcite crystals in the form of thickish tablets whose largest parallel planar surfaces are normal to the plane of the long axis of the prism in which they are enclosed. The calcite tablets are separated from each other medio-laterally by lace-like sheets of organic matrix.

The outer nacreous layer lies against the inner surface of the prismatic layer and the inner nacreous layer lies between the outer nacreous layer and the external Mantle of the oyster.

The calcium carbonate in both nacreous layers consists of orthorhombic prisms of aragonite twinned to form secondary shapes. These are invariably in the form of flat nacre tiles which lie in nacre sheets. Viewed medially, they may be of various geometric shapes, e.g. regularly hexagonal, diamond shaped, etc. Each nacre tile is enclosed by organic matrix and may also have an internal organic matrix.

The type of calcium carbonate crystal, (aragonite or calcite), the type of aragonite crystal in the nacre tile, and even the direction of the crystallographic axes of the aragonite crystals are all determined by the organic matrices. The organic matrices are formed extracellularly by the combination of precursors secreted by the pearl oyster's external Mantle and Mantle Margin.

The isthmusistic epithelium supplies most, (if not all) of the precursors of the protein rubber of the Hinge.

The various tissues of the External Mantle supply the precursors for the organic matrices of the inner nacreous layer.

The precursors of the organic matrices of the outer nacreous layer are supplied by the tissues and glands between the Distal Folded Region and the Pleated Secretion of Groove F1F2..

The precursors of the organic matrices of the outer shell layer – the prismatic layer – are supplied by the secretory structures on the Mantle Margin between the Pleated secretion of Groove F1F2. and terminal F3.

BACKGROUND.

This project - the electron microscopy of the Shell producing tissues of the pearl oyster Mantle - was part of a larger long term investigation into the biology of the Australian pearl oysters of economic interest - *Pinctada Maxima*, *P.margaritifera*, and *Pteria Penguin*. For comparative reasons the non-economic species *Pinctada fucata* was included in the investigation.

At the outset of the larger investigation it became obvious that then current theories of shell formation in pearl oysters were untenable.

A major problem with investigation of pearl oyster shell production is that the shell producing tissue, the Mantle and Mantle Margin is unattached distal to the Adductor Muscle. Theoretically, therefore, any or all of the tissues and glands from the Adductor Muscle to the Mantle Margin could take place in the production of any of the three shell layers.

An investigation by light microscopy of a large number of bivalves which had shell layers similar to, and dissimilar to pearl oysters, established that the organic matrices of the different layers were usually produced in compartments. For example, in many bivalves the External Mantle and Shell are joined at the Pallial Line. The junction seals off the volume between the External Pallial Mantle and the Shell and the resulting space is called the Pallial Space. Usually a distinctive internal shell layer is laid down in the confines of the Pallial Space. In *Trichoniya hersuta*, e.g., this is an Internal Nacreous Layer. Another compartment may exist between the lateral surface of the Outer Marginal Mantle Fold and the inner surface of an outer shell layer, delimited proximally by the Pallial Line junction and distally by the point at the shell margin where the Outer Shell Layer recurves around the distal extremity of the outer Marginal Mantle Fold.

In *Trishomya hirsuta* again, the External Mantle thus confined produces the Outer Nacreous Layer.

The Outer Shell Layer of *Trichomya hirsuta* is produced in the compartment formed by the Groove between the outer two Marginal Mantle Folds.

Light microscopy of pearl oysters had suggested that the tissues medial to the Outer Marginal Mantle Groove were involved in Prismatic Layer production but had failed to either identify forming nacreous organic matrix in the vicinity of the Outer Marginal Mantle Fold or definite precursors of Prismatic Layer Organic Matrix – nor yet any mechanism whereby the secretion of the precursors of the two could be separated.

The project to investigate pearl oyster mantle under Transmission Electron Nicroscopy was embarked on to solve these and other problems with understanding pearl shell organic matrix secretion.

IV. OBJECTIVES OF THE RESEARCH PROJECT.

To define more accurately than could be achieved by light microscopy the roles of the tissues and glands of the External Mantle and Mantle Margin of pearl oysters in the production of their shells, and secondly to investigate the roles of the Circumpallial Nerve and Circumpallial sinus in pearl oyster shell production.

V. INTRDUCTORY TECHNICAL INFORMATION CONCERNING THE RESEARCH NEED.

Two dimensional growth of a pearl shell which precedes three dimensional growth at least for the greater part of the life of a pearl oyster necessarily means growth of the prismatic layer as the entire lateral surface and the distal part of the medial surface is composed of prismatic layer.

At the commencement of this research the theories then current of prismatic layer production were found to be wrong. Further there was insufficient information as to why the nacre differed between species of pearl oysters and between individuals of the same species. Amidst a general lack of information of the biology of pearl oysters, in particular, as far as this project is concerned, the histology, physiology, biochemistry and extra-cellular chemistry of the secretion of the precursors of the shell organic matrices and their formation was virtually unknown.

Any understanding of pearl oyster growth requires at least a rudimentary understanding of the above.

Which cells, glands and tissues are involved in pearl shell organic matrices precursor secretion is obviously the place to start such an investigation.

This project is a continuation at the ultrastructure level of light microscopy work done on this problem.

VI. RESEARCH METHODOLOGY.
TRANSMISSION ELECTRON MICROSCOPY.

Selection of Tissues, Fixation, Embedding, Sectioning, Staining.

VI.1.Selection of tissues.

. The aim of T.E.M. was to more accurately characterise the glands and secretions, and hence hopefully the functions of the pearl oyster tissues identified with L.M. as likely to be involved in Shell Layer formation. To this end tissue blocks were prepared from the Isthmi, Shoulder Regions, Pallial Gland Regions, Proximal, Middle and Distal Pallial Regions, the Distal Folded Regions and the tissues of the Mantle Margins of *Pinctada maxima, P. margaritifera, P. fucata, Pinctada sp. 1, Pinctada sp. 2, Pinctada sp. 3, Pinctada sp. 4, Pinctada sp. 5, P.albina sugillata, Pteria penguin and Isognomon ephippium.*

VI. 2. Fixation Embedding, Sectioning and Staining.

VI. 2.1. Fixation and embedding.

The animals were removed from their shells into 2.5% glutaraldehyde in 0.1M cacodylate buffer in filtered sea water at 24C. The required tissues were dissected in the fixative as soon as possible into strips less than 1mm wide, and left in the fixative for 1h.

The strips of tissue were then washed twice in 0.1M cacodylate buffer, pH 7.2, in filtered sea water for ten minutes.

They were then post-fixed in 1% osmium tetroxide in 0.1M cacodylate buffer, pH 7.2, in filtered sea water for 1h at 24 C.

The tissues were again washed in cacodylate buffere for ten minutes.

Tissues were then dehydrated through an anascending ethanol series - 50%, 60%, 70%, 80%, 85%, 90%, 95%, 100% - for 2-5 minutes each, then two more

changes in absolute ethanol for ten to twenty minutes each.
The tissues were then placed in equal volumes of ethanol and Spurr's resin and agitated on a rotator for three hours (Spurr, A.R. 1969).

A volume of Spurr's resin equal to that of the solution of Spurr's resin in alcohol was then added and returned to the rotator for a further three hours.

The 75% solution of Spurr's resin in ethanol was then removed and the tissues left in 100% Spurr"s resin on the rotator overnight.

The used Spurr's was then removed and the tissues left in new Spurr's resin for another three hours, and then embedded in moulds in pure Spurr's resin.

The resin blocks were then cured for 16 h at 70 C.

VI. 2. 2. Sectioning and staining.

Sections of 60-100nm were cut with glass or diamond knives using an LKB ultratome. The sections were then mounted on copper grids and stained for 8 minutes in saturated uranyl acetate in 50% ethanol followed by 1 minute in Reynold's lead citrate.

The transmission electron microscope used was a Jeol JEM- 2000FX.

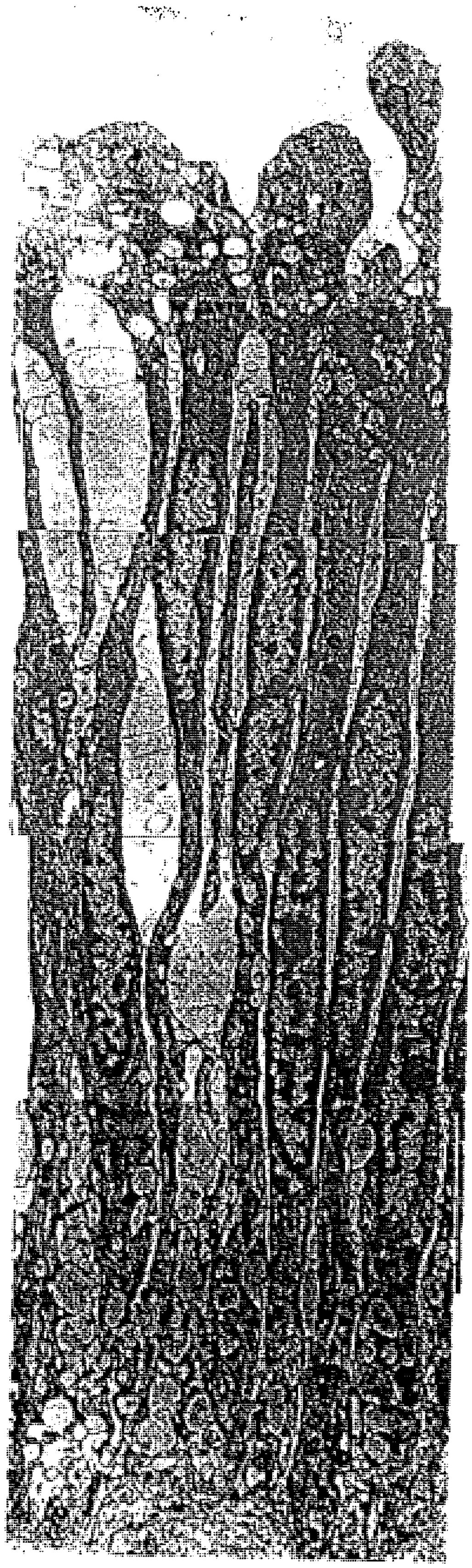
VII. DETAILED RESULTS

Results are presented as photomicrographs with explanatory notes. Only results from the four species *Pinctada maxima*, *P. margaritifera*, *P. fucata* and *Pteria penguin* are included in this report.

P. MAXIMA

T.E.M ISTHMUSISTIC EPITHELIUM (a).

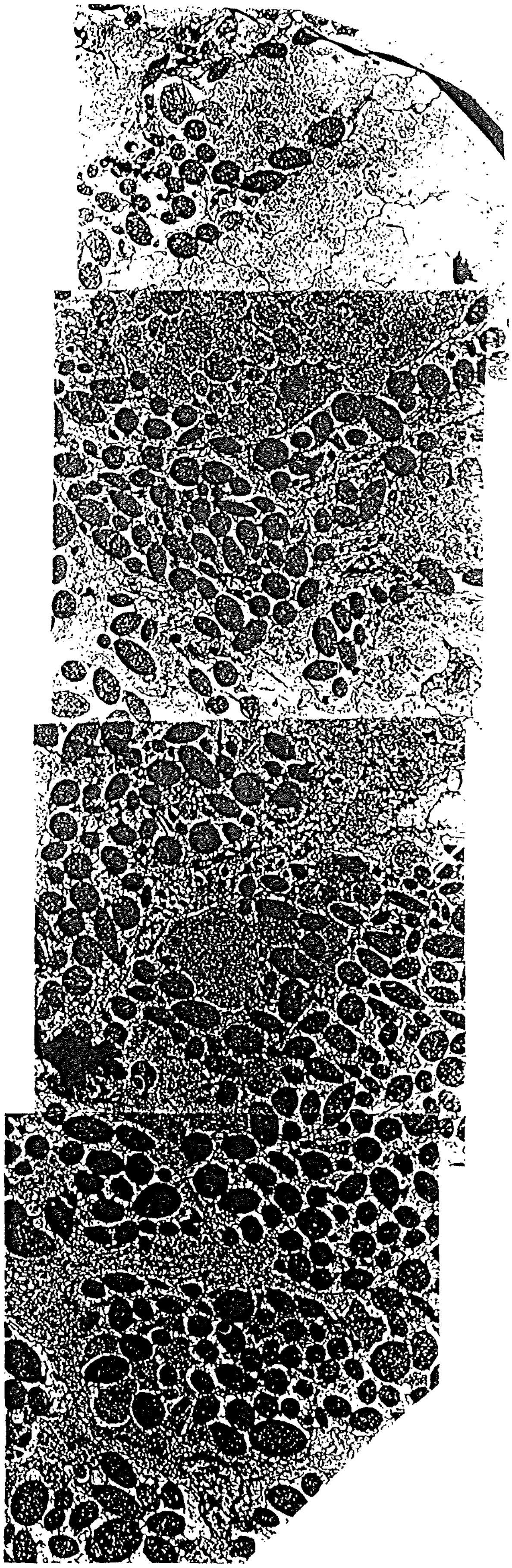
x 5K T.E.M. of Isthmusistic Epithelium from Apical Surface (top) to Basal Lamina (bottom). The cells are greatly elongate columnar epithelium. The nuclei are similarly very elongate and usually situated in the basal half of the middle part of the cell. They commonly display one or two prominent nucleoli.



P. MAXIMA

T.E.M. SHOULDER REGION TISSUES AND GLANDS (b).

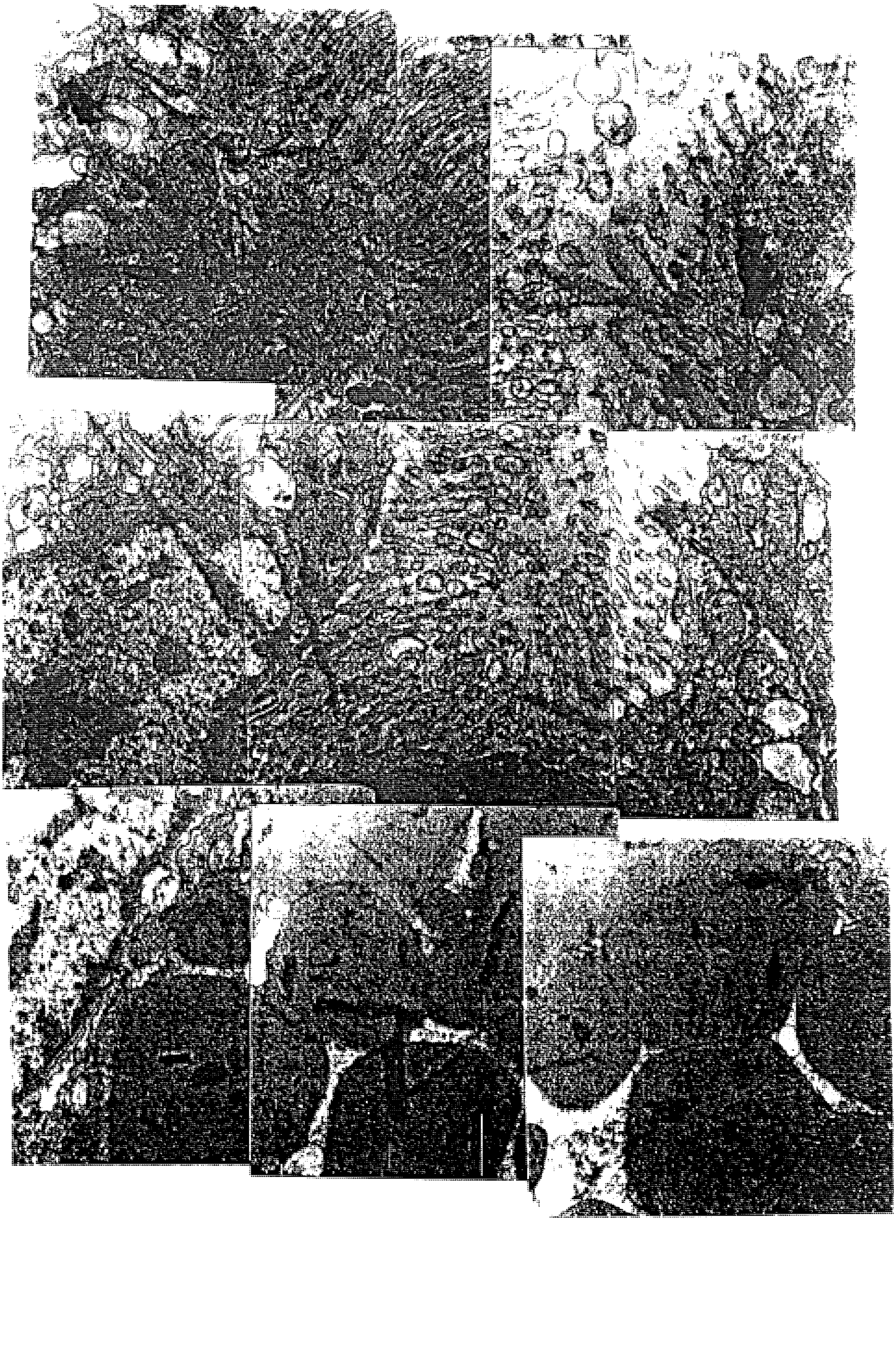
x 1.75K T.E.M. Medial part of Turquoise Gland Layer overlies deeper layer where Granular Cytoplasm Secretory Glands predominate.



P. MAXIMA

T.E.M. VENTRAL PALLIAL GLAND REGION
TISSUES AND GLANDS (d).

x 17.5k T.E.M. At left, part of nucleus and cell body of epitheleal cell bearing microvilli on its apical surface. The swollen end of the microvilli form secreted vesicles in the groove in the epithelium (top right). At the bottom are large granules of a granular cytoplasm secretory gland. Distal to this, nerve fibres reach to near the Epithelial surface.



P. MAXIMA

T.E.M. VENTRAL PALLIAL REGION
TISSUES AND GLANDS (a).

x 6K T.E.M. of Pallial Epithelium and Sub-Epithelial Glands, muscles, connective tissue and nerves showing Laminar Cytoplasm

Turquoise

Glands and a large Granular Cytoplasm Secretory Gland. The surface Epithelium bears short microvilli on the apical membrane.

P. MAXIMA

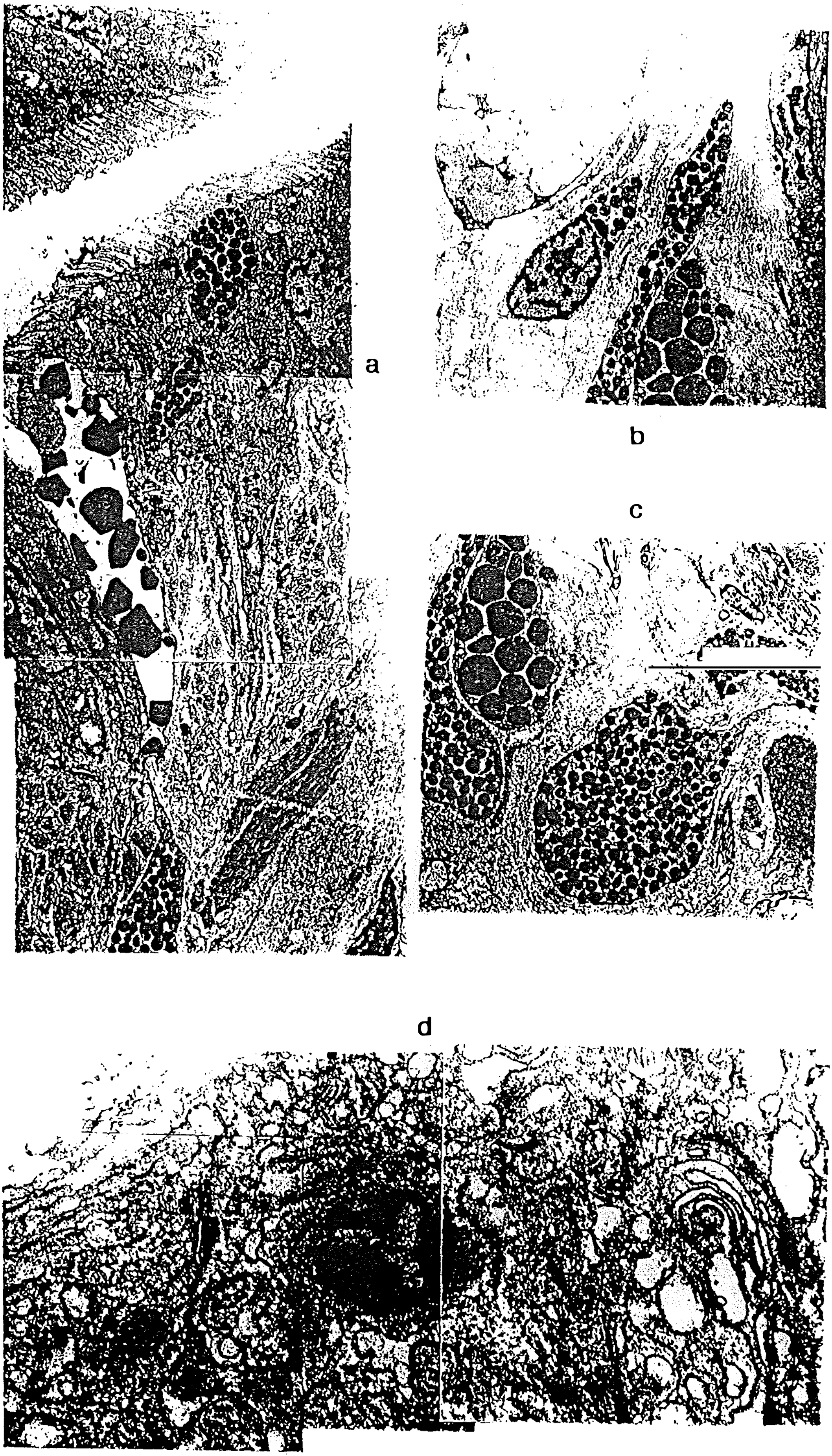
T.E.M. DISTAL FOLDED REGION TISSUES AND GLANDS (a).

a. x 1.75k T.E.M. Deep groove lined with microvilli. Various kinds of Granular Cytoplasm Secretory Glands in Epithelium and Subepithelium.

b. x 1.75K T.E.M. Turquoise Gland and two types of Granular Cytoplasm Secretory Glands in Distal Folded Region Subepithelium.

c. x 1.75K T.E.M. Granular Cytoplasm Secretory Glands in Distal Folded Region subepithelium. (Top of illustration joins bottom of "b" above.).

d. x 7K T.E.M. Of Epithelium Lining Groove in Distal Folded Region with numerous vesicles, mitochondria, and membranous structures. Elongate microvilli cover the apical membrane.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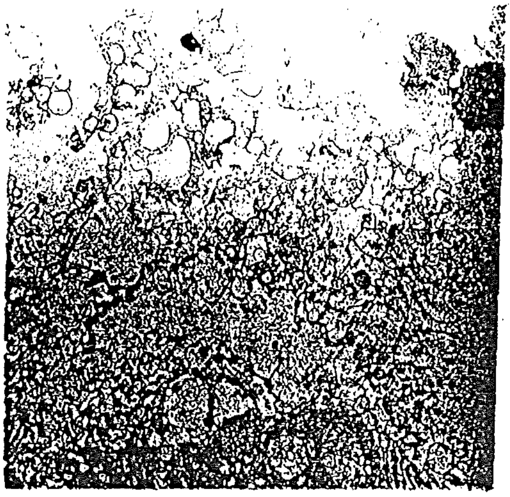
P. MAXIMA

T.E.M. DISTAL FOLDED REGION AND MANTLE EDGE GLAND - TISSUES AND GLANDS.

a. x 1.75K T.E.M. Secretion external to Junction of Distal Folded Region and Mantle Edge Gland.

b. x 1.75K T.E.M. Turquoise Gland and Granular Cytoplasm Secretory Glands in subepithelium beneath "a" above.

c. x 1.75K T.E.M. Of Mantle Edge Gland Epithelium - elongate columnar cells with elongate basal nuclei and a thick, dense Basal Lamina.



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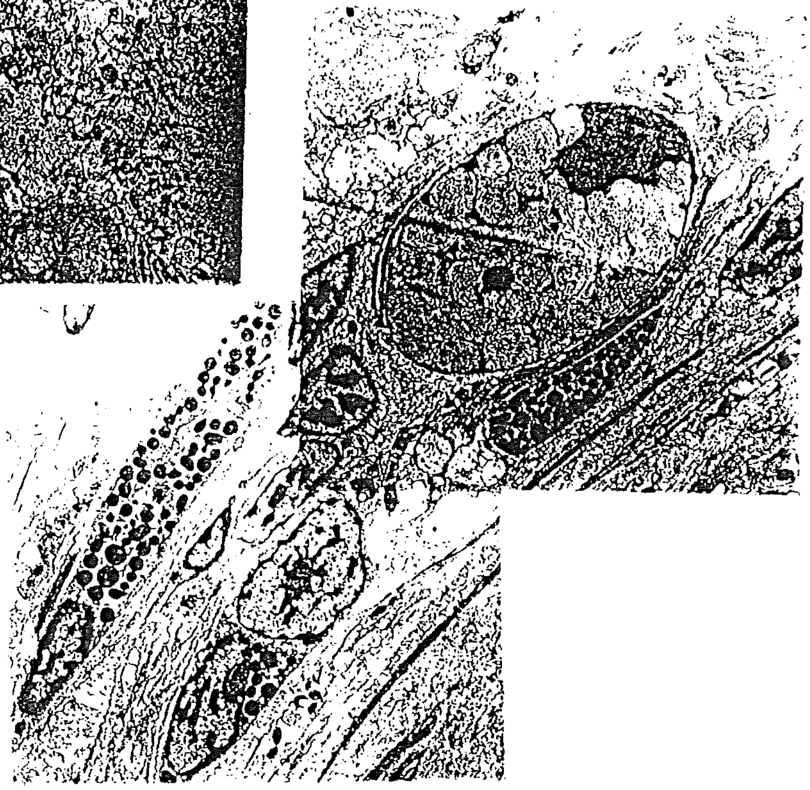

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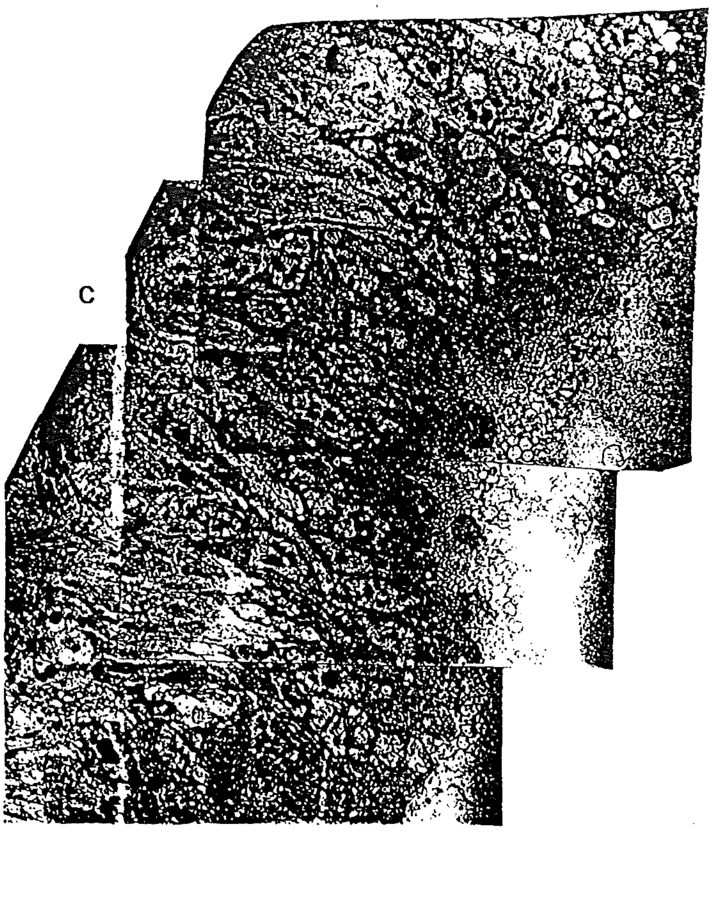
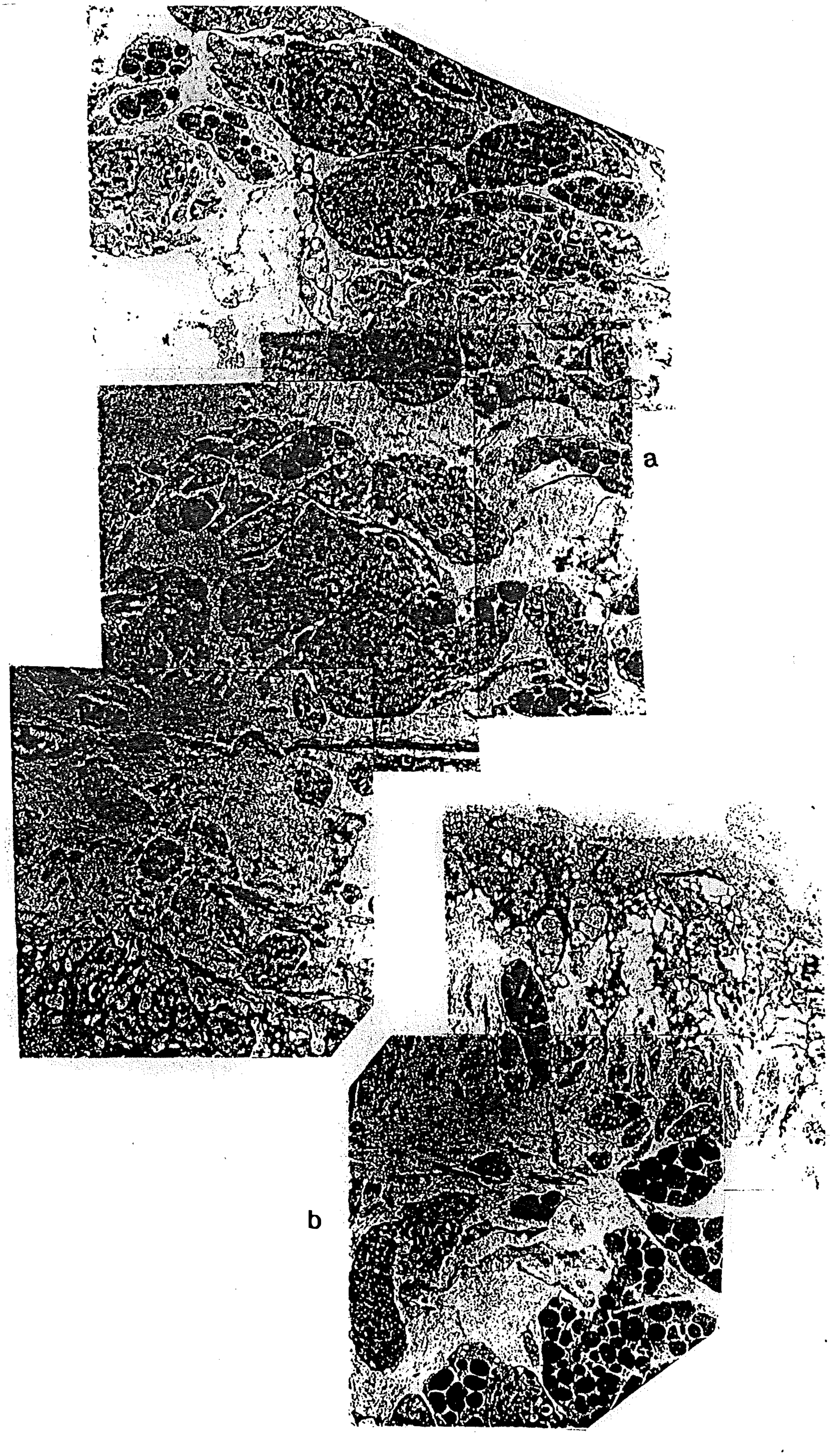


P. MAXIMA

T.E.M. VENTRAL TERMINAL EPITHELIUM AND DISTAL MED FI.

a. x 1.75K T.E.M. Of Ovoid Blue Glands and Terminal Epithelium of Lat F.I. The nuclei of Terminal Epithelium cells are in the bottom left of illustration - the oddly stippled cells with relatively very small rounded nuclei are the Ovoid Blue Glands which are ubiquitous in this location throughout the Pinctada.

b. x 1.75K T.E.M. Distal Med FI. - Epithelium and Subepithelial Glands shows an Ovoid Blue Gland and two types of Granular Cytoplasm Secretory Glands beneath an epithelium with dense microvilli on the apical membrane.


a
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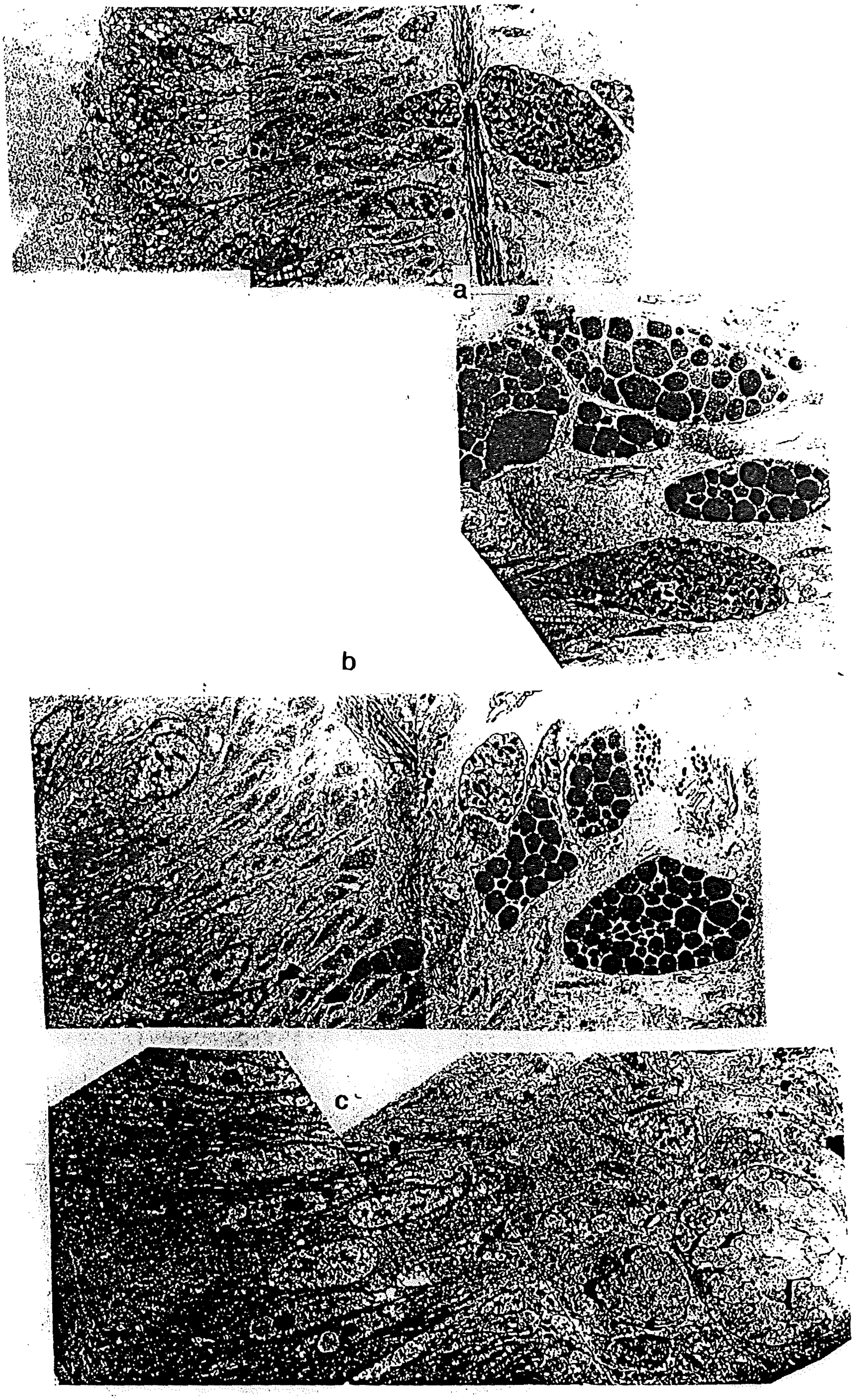
P. MAXIMA

T.E.M. VENTRAL MIDDLE TO PROXIMAL MED FI.

a. x 1.75K T.E.M. of Distal Med FI. Epithelium and Subepithelium showing two Ovoid Blue Glands (which are only found infrequently as proximal as this), and a variety of Granular Cytoplasm Secretory Cells all of whose cell bodies are largely deep to the radial musculature.

b. x 1.75K T.E.M. Middle Med FI. Epithelium and Subepithelium proximal to "a" above. The low columnar epithelium overlies an extensive fibrous connective tissue and muscular tissue beneath which are the cell bodies of a variety of secretory glands.

c. x 1.75K T.E.M. Proximal Med FI. just distal to the Omega Gland showing elongate columnar epithelial cells with elongate ovoid nuclei and associated Turquoise Glands.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P. MAXIMA

T.E.M. VENTRAL OMEGA GLAND AND DACTYLOCTYES.

x 1.75K This section has cut through a folded part of the apical region of Groove F1F2 so that two Apical Channels are seen on either side of an intervening fold of Omega Gland cells. Proceeding from the right margin and following the basal lamina of the various epithelia in a clockwise direction the tissues illustrated are, Black Granule Secretory Cells (with rounded basal nuclei), Dactylocytes which line the right hand side of the opened Apical Channel with prominent elongate nuclei and apical microvilli, Omega Gland cells which comprise all the tissue from the bottom left hand corner to the top centre to the right of the second Apical Channel, and then the second display of Dactylocytes at the top left of the illustration to the left of the second Apical Channel.

P. MAXIMA

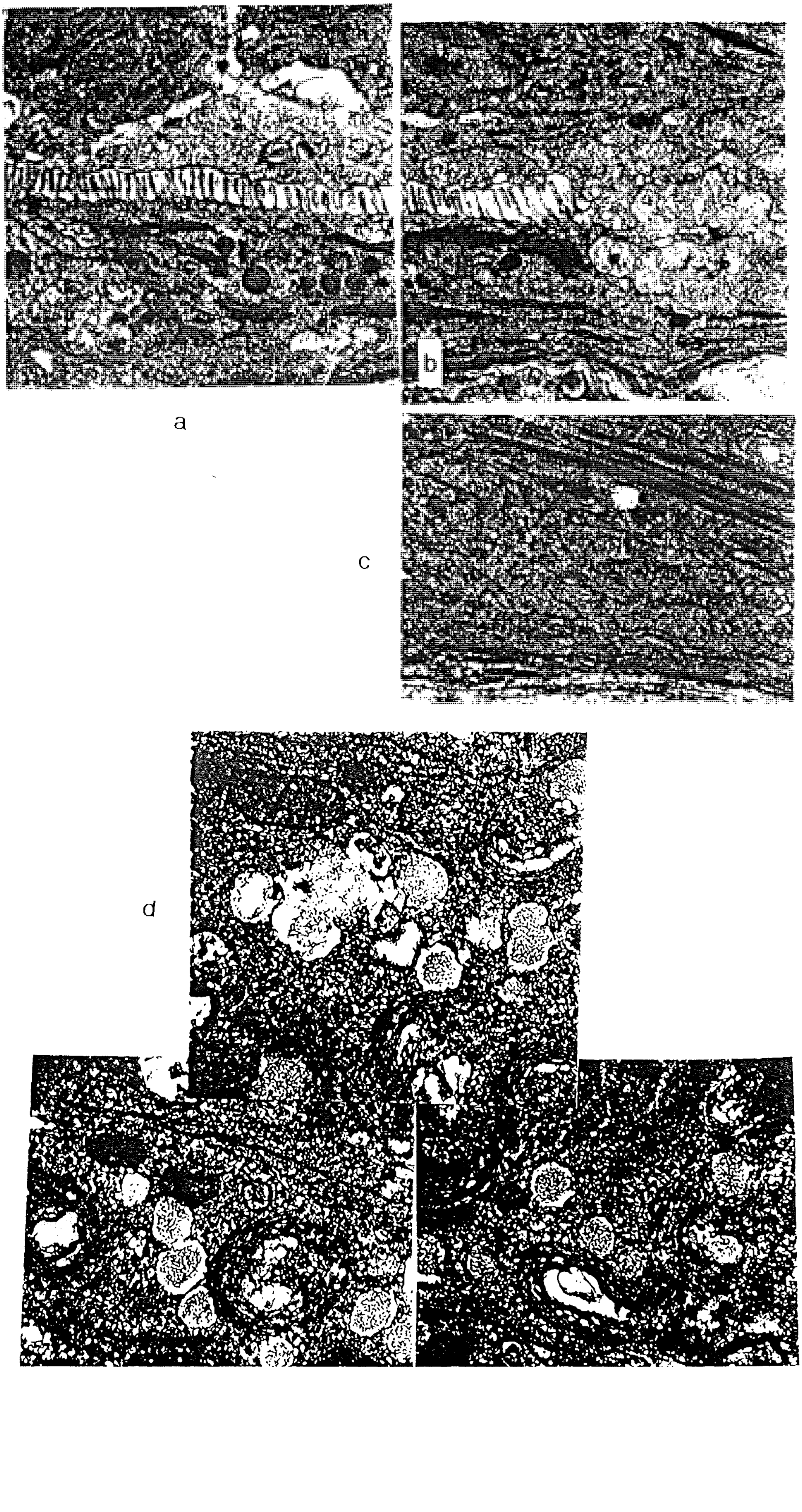
T.E.M. VENTRAL OMEGA GLAND, DACTYLOCYTES AND BLACK GRANULE SECRETORY CELLS.

a. x 7K T.E.M. Forming Pleated Secretion in channel between Dactylocytes (below) and Omega Gland cells (above).

b. x 7K T.E.M. Of Proximal end of forming Pleated Secretion left side of illustration abuts right side of figure "a" above.

c. x 7K T.E.M. Of "membrane bound" substance at proximal end of forming Pleated Secretion. Left side of illustration abuts right side of figure "b" above.

d. x 15K T.E.M Of formation of "black granule" secretion by Black Granule Secretory Cells. The right of the illustration is proximal, left distal.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P. MAXIMA

T.E.M. TISSUES AND GLANDS OF VENTRAL APEX OF GROOVE F1F2. AND THEIR SECRETIONS INTO PROXIMAL GROOVE F1F2.

a. x 1.75K T.E.M. Showing Omega Gland with vesiculate secretions on bottom right, Proximal Med F1. epithelium with somewhat amosphous secretions issuing from it on bottom left; and the Pleated Secretion separating these secretions from the secretions of Proximal Lat F2. at the top of the illustration.

b. x 7K T.E.M. Showing enlargement of the secretions of Proximal Med. F1. and the Omega Gland below the Pleated Secretion at middle left of "a" above. The Omega Gland secretions are here present as vesicles with electron dense material adhering to the inner wall of the surrounding memebrane. Also shown is the amorphous secretion of Proximal Med F1.


b
a

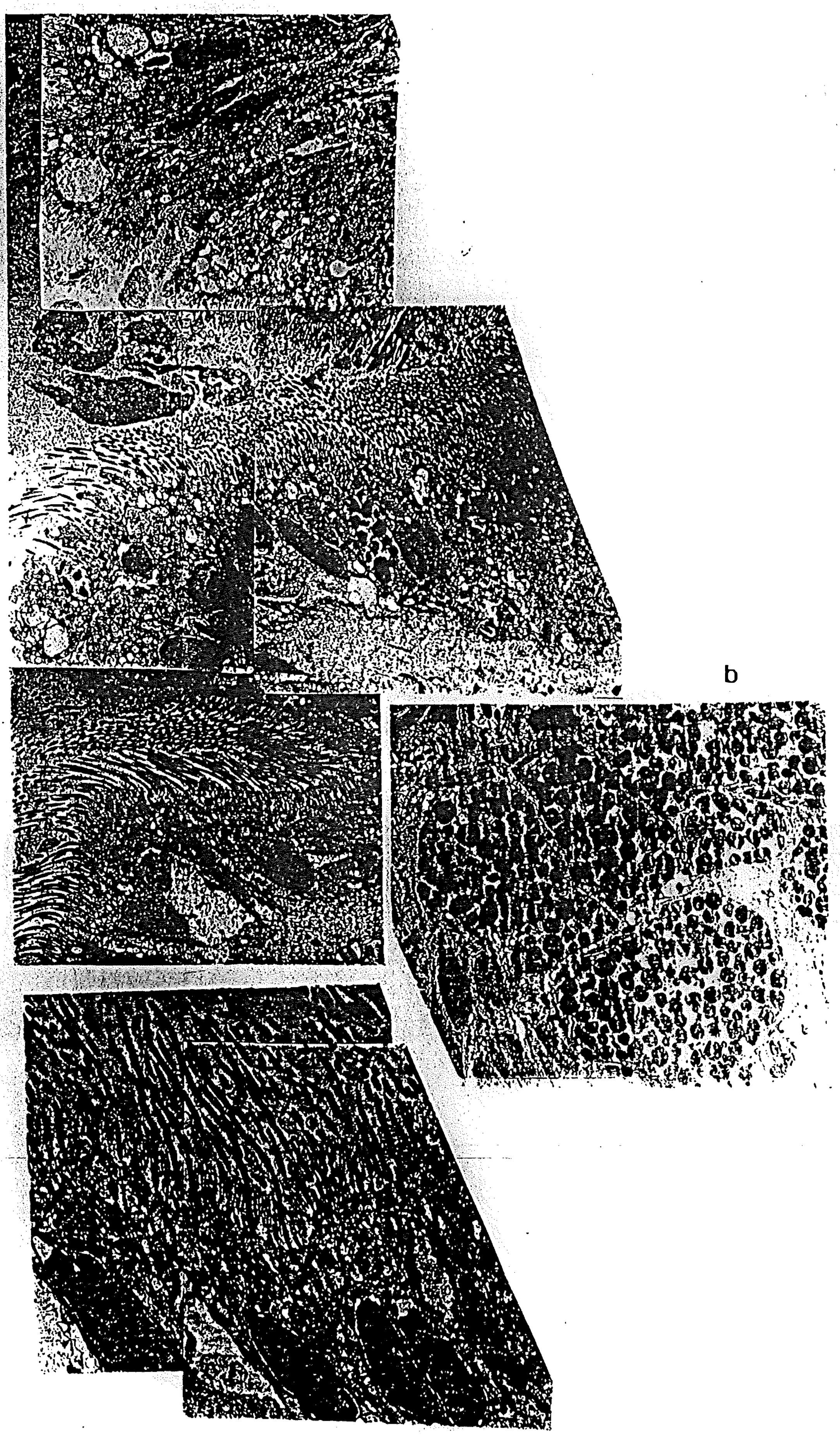
P. MAXIMA

T.E.M. JUNCTION OF VENTRAL PROXIMAL
AND MIDDLE LAT F2 (b).

a. x 1.75K T.E.M. Showing transition from proximal (top) to middle (bottom) Lat F2. Apical mebranes of epithelial cells bear microvilli (Proximal) and microvilli plus elongate cilia (Middle).

b. x 1.75K T.E.M. Of Granular Cytoplasm Secretory Glands of the subepithelim beneath "a" above. These are probably "Light Blue Glands".

c. x 3.5K T.E.M. Elongate cilia and microvilli of the Middle Lat F2. epithelium. Apical cytoplasm displays numerous vesicles and large elongate elipsoid mitochondria like bodies.

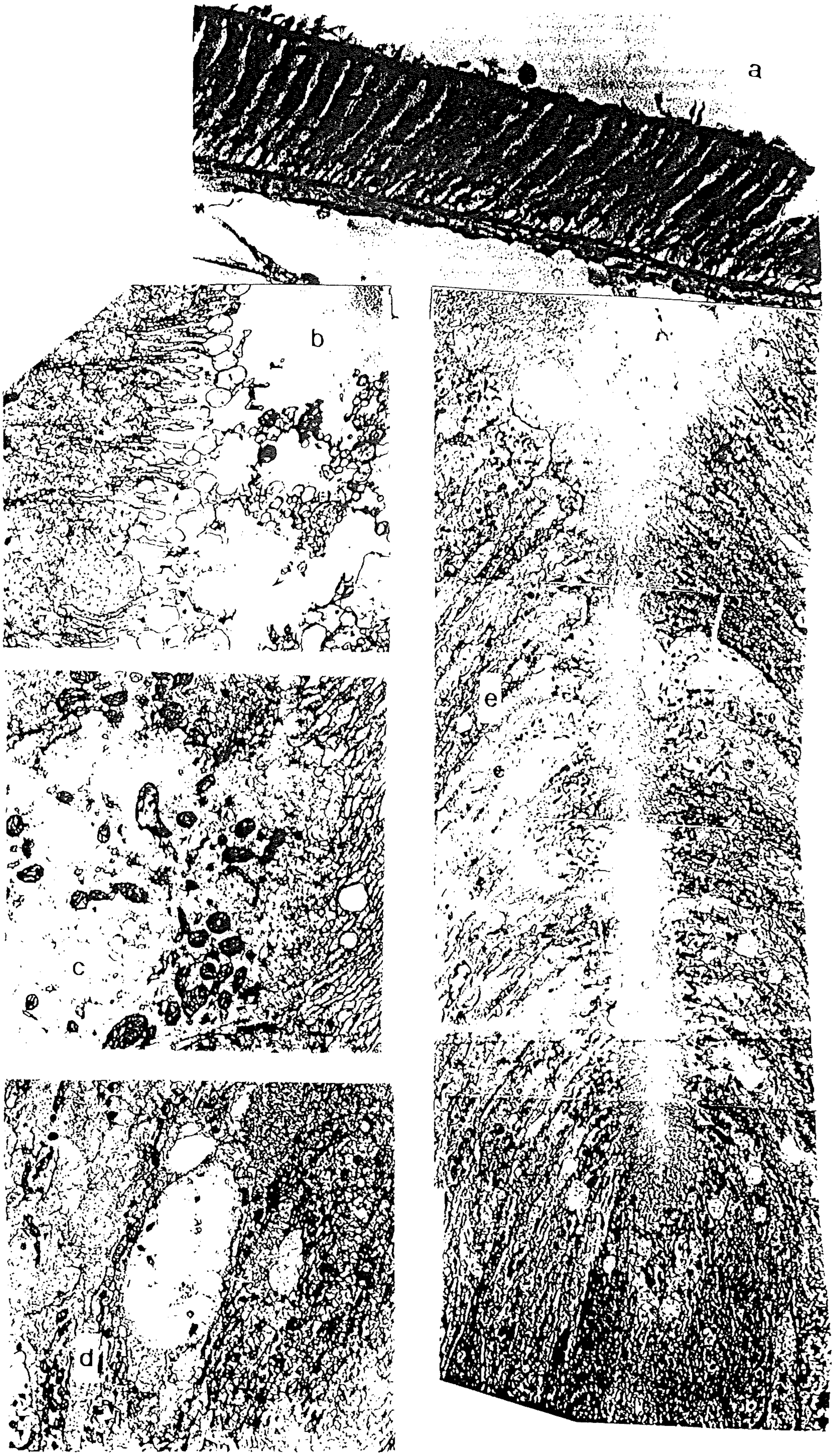


b

P. MARGARITIFERA

L.M. - T.E.M. ISTHMUSISTIC EPITHELIUM

a. x 750	L.M. Mallorys. Ellongate Columnar Isthmusistic Epithelium.
b. x 7K	T.E.M. Secretory vesicles formed by terminal enlargement of apical microvilli of the Isthmusistic Epithelium.
c. x 0.1K	T.E.M. Mitochondria like organelles concentrated in apical part of the isthumsistic epithelium.
d. x 3.5K	T.E.M. Turquoise Gland and concentration of mitochondria-like organelles in the adjacent isthmusistic epithelia.
e. x 1.8K	T.E.M. Central sulcus of Isthmusistic Epithelium showing concentration of mitochondria-like organelles beneath the apical membranes of the elongate colunnar cells.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e

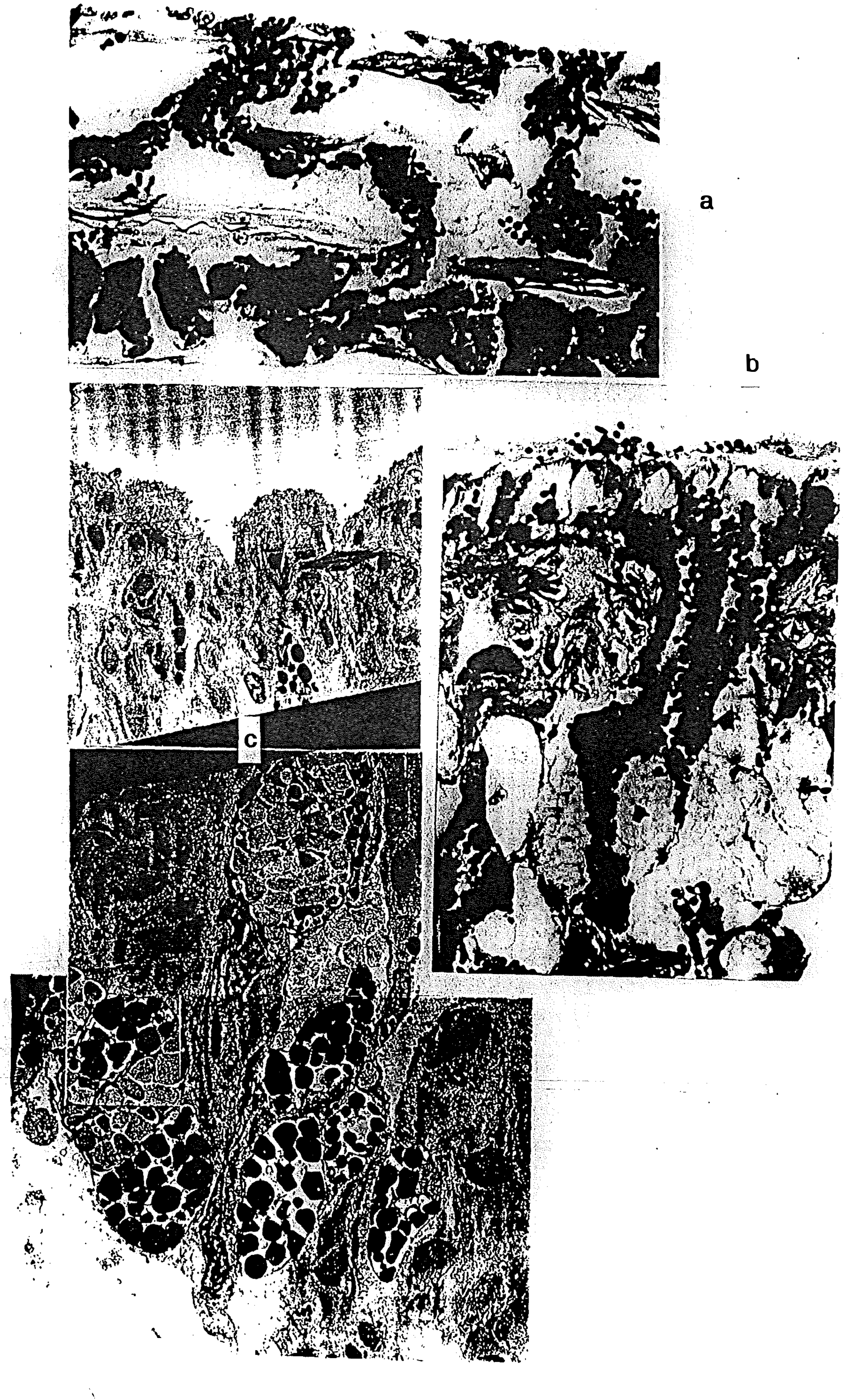
P.MARGARITIFERA

L.M.-T.E.M. EPITHELIUM AND SUBEPITHELIAL GLANDS
OF THE SHOULDER REGION

a. x 1000 Mallory"s . Three types of granular cytoplasm secretory glands in the Shoulder Region midway between the Isthmus and the Adductor Muscle.

b. x 1000 Mallory"s. Red and purple granules of granular cytoplasm secretory glands in the subepithelium of the Shoulder Region on the side of a sulcus near the base of the Isthmus.

c. x1.8K T.E.M. Different types of granular cytoplasm secretory glands and Turquoise Glands in the Subepithelium of the middle Shoulder Region.



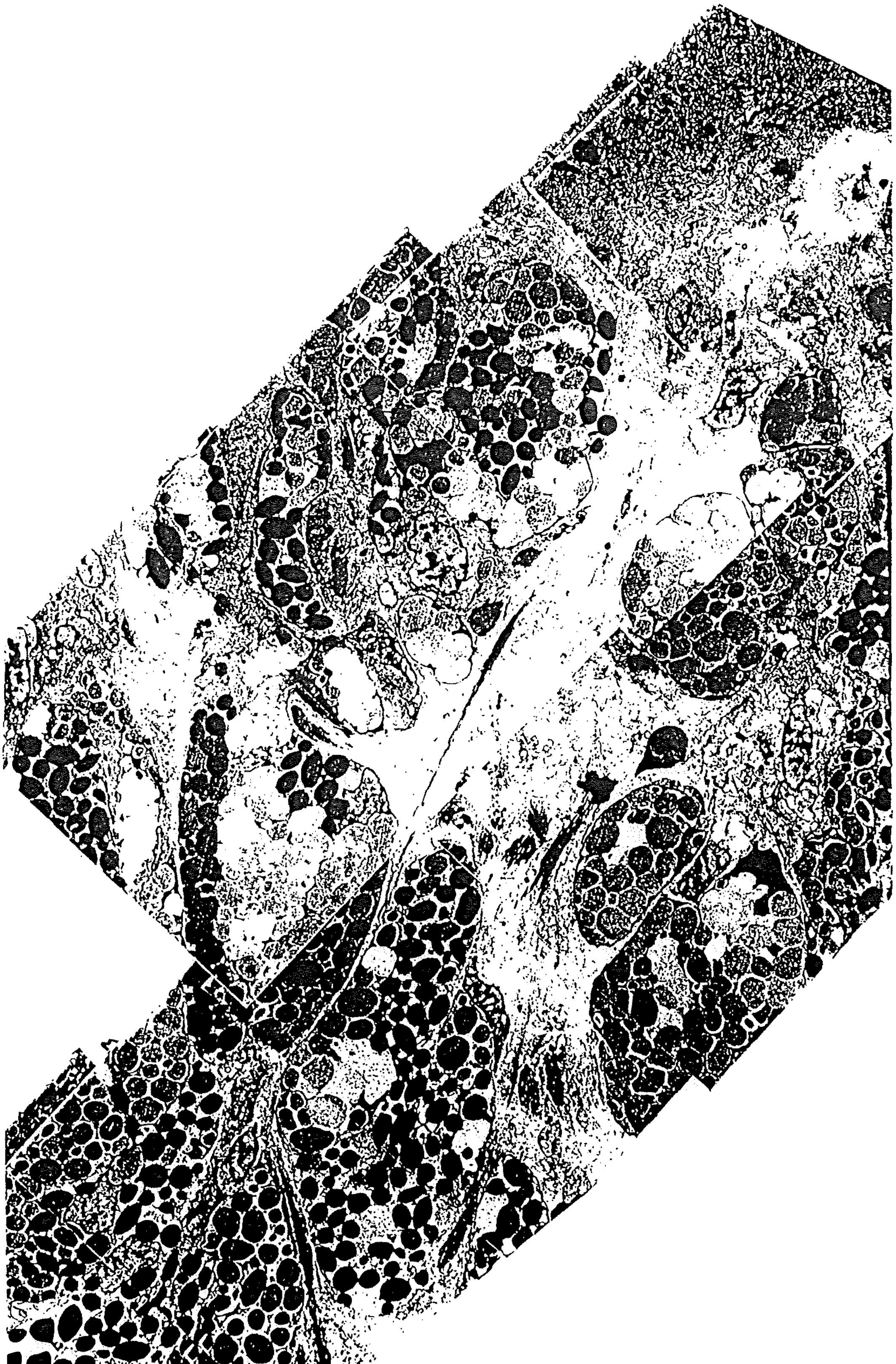
a

b

c

P.MARGARITIFERA.

x 1.75K T.E.M. Subepithelial Turquoise and granular cytoplasm glandular cells of the Pallial Gland Region.



P.MARGARITIFERA

T.E.M. PROXIMAL ANTERIOR PALLIAL REGION (a).

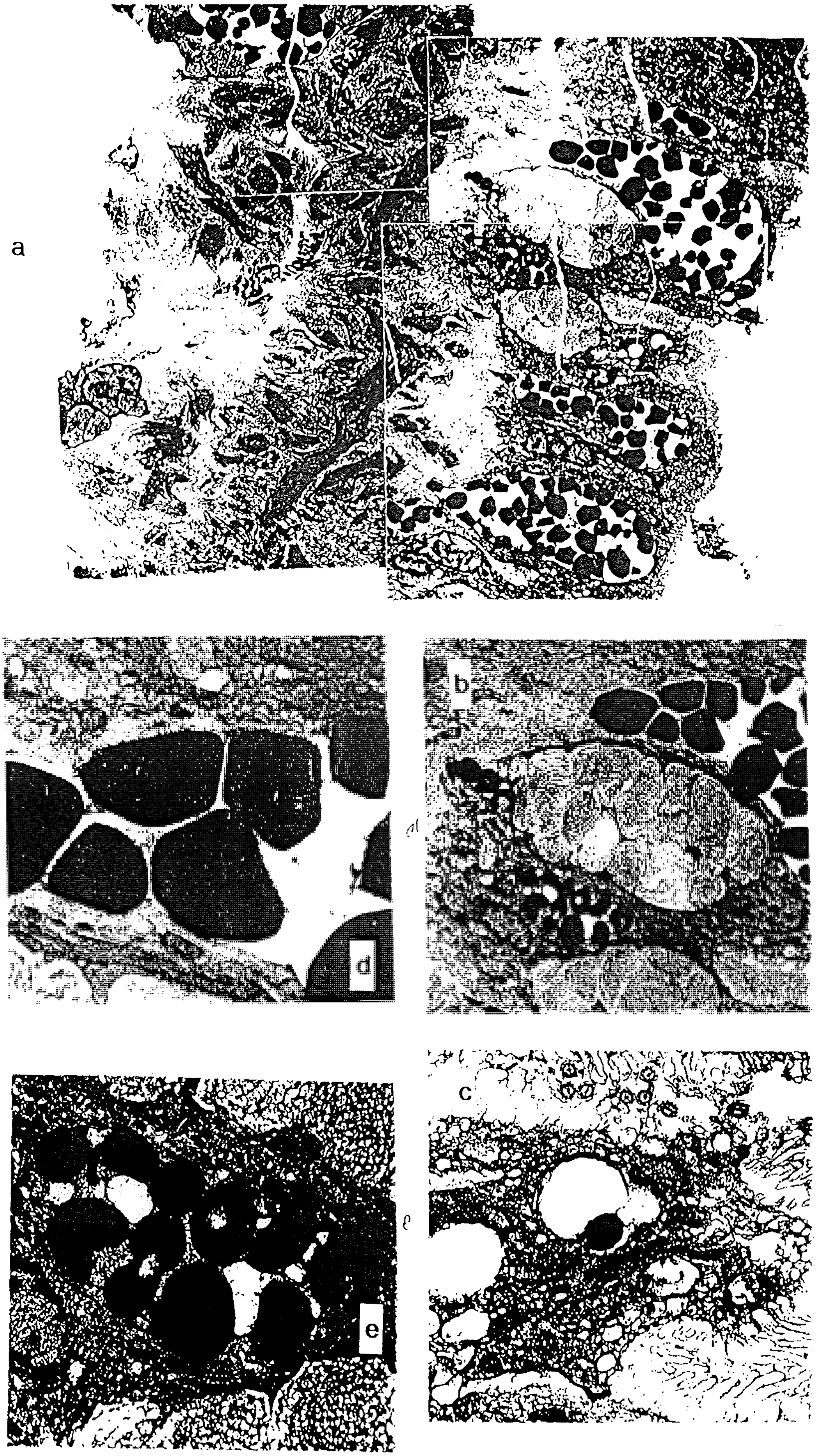
a. x 1.75K T.E.M. of Proximal Anterior Region epithelium and subepithelium, showing an array of granular cytoplasm secretory glands and Trabecular Turquoise Glands beneath surface epithelium on the right and the muscles, fibrous connective tissue and nerves of the subepithelium on the left. the surface epithelium bears apical microvilli.

b. x 3.5K T.E.M. Enlargement (from "a" above of intraepithelial granular cytoplasm secretory glands of two kinds and Turquoise Glands.

c. x 12K T.E.M. Epithelial surface showing microvilli and cilia.

d. x 12K T.E.M. Enlargement of polygonal granules in the top right of "b" above.

e. x 12K T.E.M. Enlargement of relatively small spherical granules in the gland between the two Turquoise Glands in "b" above.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P.MARGARITIFERA.

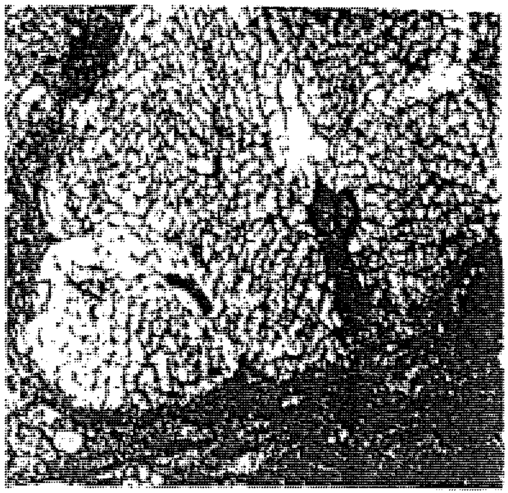
T.E.M. PROXIMAL ANTERIOR PALLIAL REGION (b)
-TURQUOISE GLAND AND NERVE.

a. x 12K T.E.M. Fine structure of one kind of Turquoise Gland in this location.

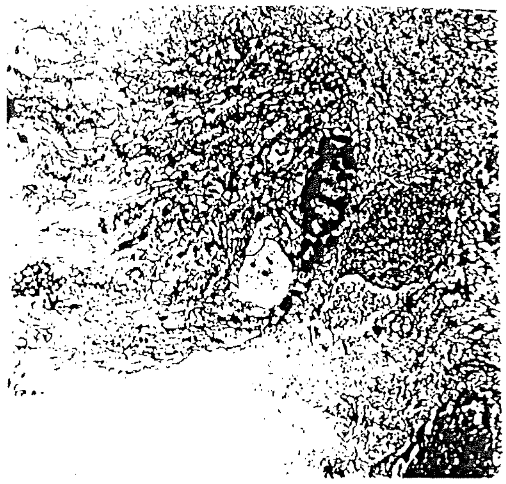
b. x 3.5K T.E.M. Nerve with what may be either the nucleus of a peripheral neuron or of a neural sheath cell and a variety of different neurosecretory granules in the subepithelium of this area.

c. x 12K T.E.M. Enlargement of a part of "b" above to show neurosecretory granules in a subepithelial nerve.

a



b



c

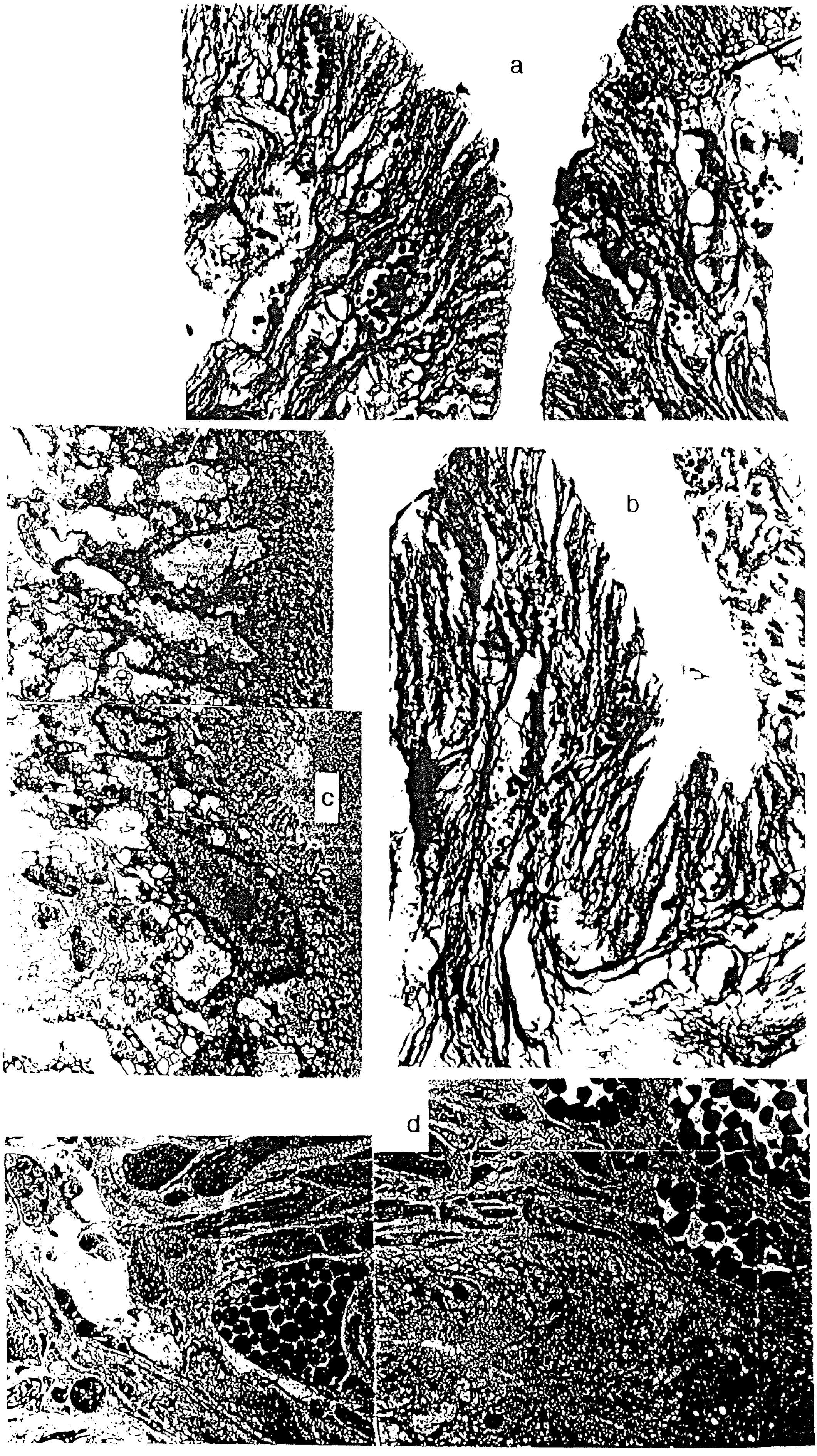
P.MARGARITIFERA.

L.M. - T.E.M. ANTERIOR DISTAL FOLDED REGION.

a.,b. 750 L.M. AB/MSB.Surface epithelium and subepithelium of this location with granular cytoplasm secretory glands, Turquoise Glands and sinus as well as subepithelial musculature and fibrous connective tissue.

c. x 1.75K T.E.M. Distal Folded Region outer epithelium with microvilli and numerous elongate radially directed sinus typical of this tissue.

d. x 5K T.E.M. of Distal folded Region showing apex of an infolding with outer epithelium bearing microvilli, two distinct types of subepithelial granular cytoplasm secretory glands and Turquoise Glands.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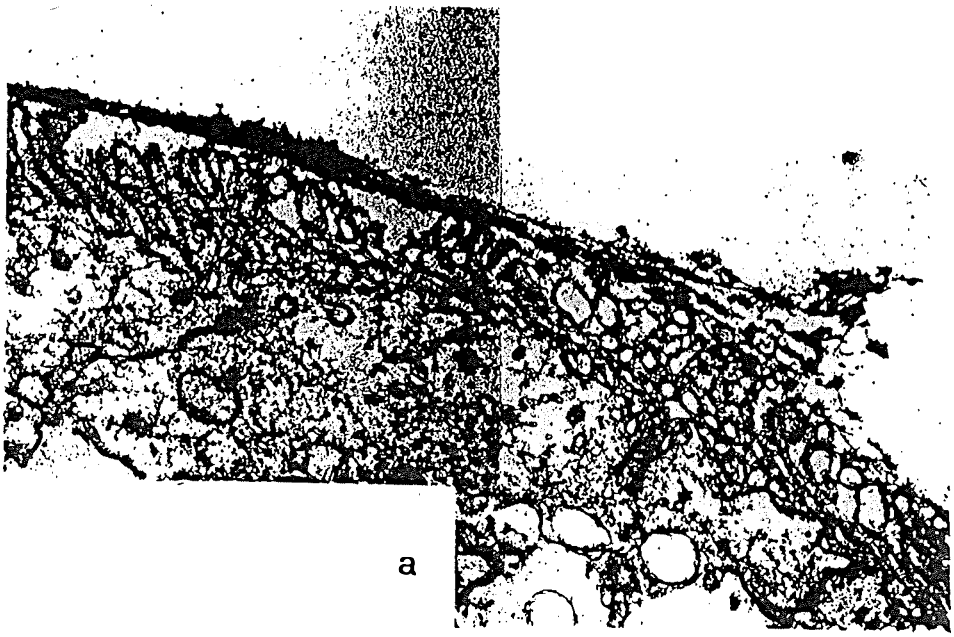
P. MARGARITIFERA.

L.M.-T.E.M. MANTLE EDGE GLAND-PROXIMAL AND DISTAL.

a. x 18K T.E.M. Surface of proximal Mantle Edge Gland showing surface microvilli and sheets of surface secretion.

b. x 750 AB/MSB. Distal Mantle Edge Gland showing elongate columnar epithelium and subepithelial sinus and granular cytoplasm secretory glands.



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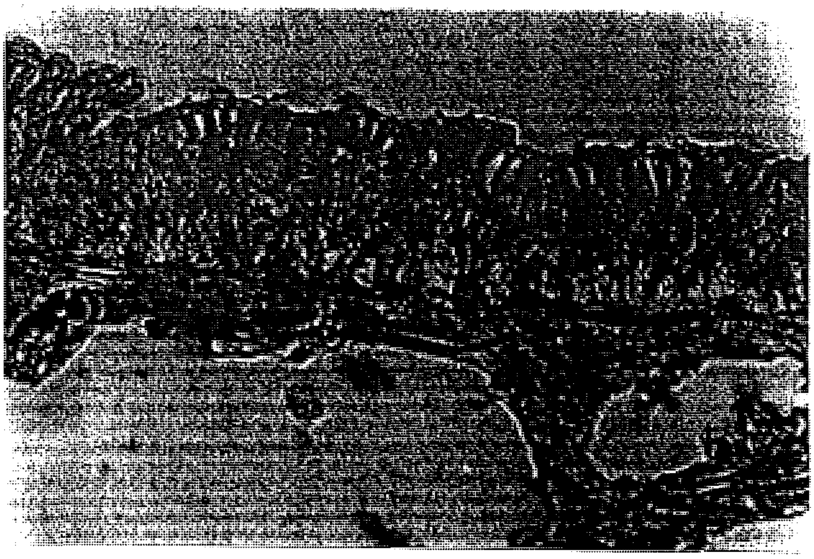
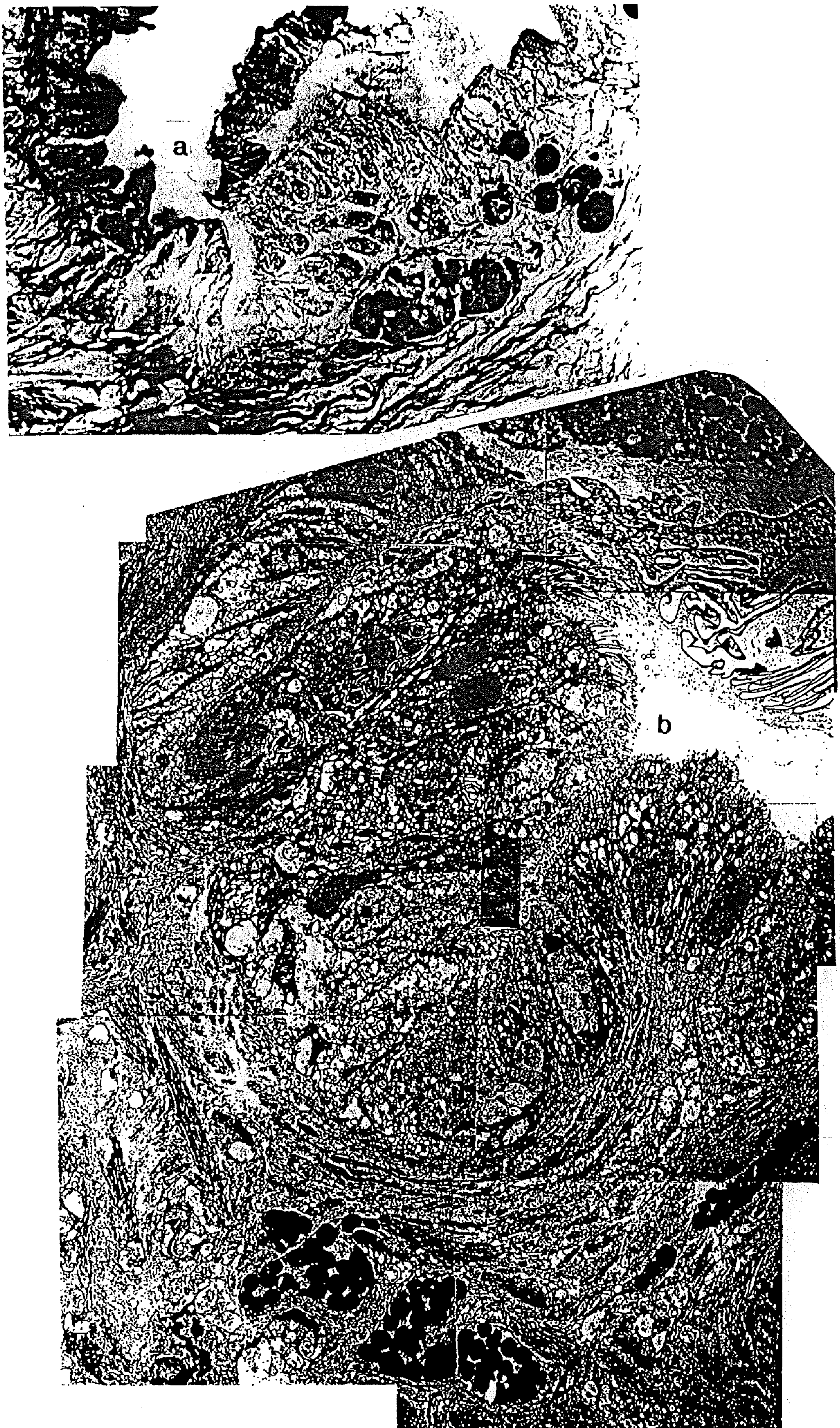


P. MARGARITIFERA

L.M. - T.E.M. ANTERIOR OMEGA GLAND AND DACTYLOCYTES - ORIGIN OF PLEATED SECRETION.

a. x 750 A.B.M.S.B. Apex of Anterior Groove F1 F2 showing Proximal Med. F1, Omega Gland and sub Omega Gland secretory glands, Dactylocytes, Black Granule Secretory cells and Proximal Epithelium of Lat F2.

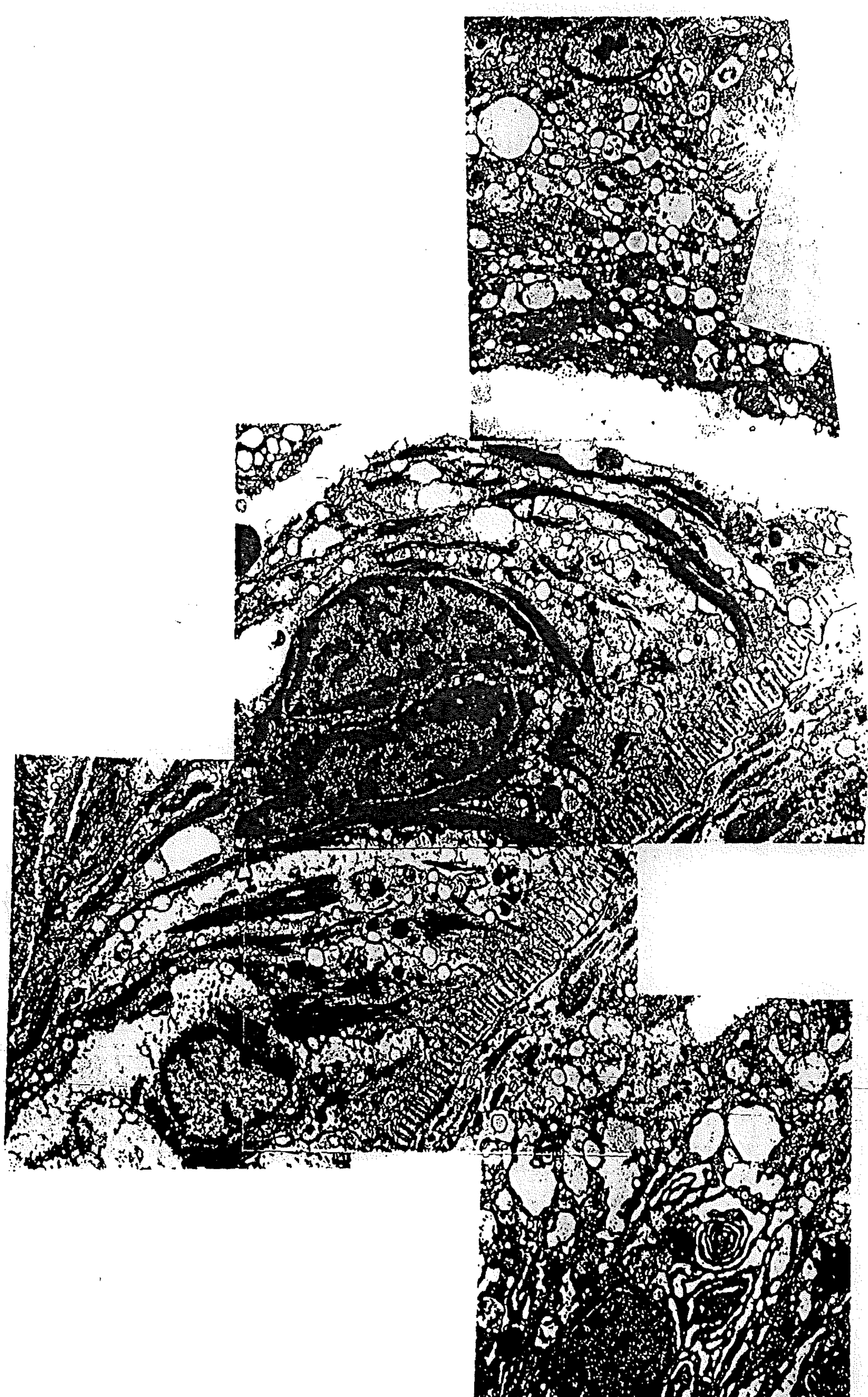
b. x 1.75K Collage showing Proximal Anterior Med F1 Epithelium with microvilli and intraepithelial Turquoise Glands and Subepithelial Granular Cytoplasm Secretory Glands, the Omega Gland with surface cells, Multivesiculate Cells Celtic Scroll Cells, the Multivesiculate Cell secretion, the Apical Cell, Dactylocytes with their microvilli lining the Apical Channel from which issues the Pleated Secretion, the Black Granule Secretory Cells which are the source of the Black Bodies on the Medial Secretion of Groove F1F2, the Pleated Secretion itself, and the Initial Receptacle Gland of Proximal Lat F2.


a
b

P. MARGARITIFERA

T.E.M. ANTERIOR OMEGA GLAND AND DACTYLOCYTES.

x 7K T.E.M. Showing the nucleus and distal part of the Celtic Scroll Cell of the Omega Gland and across the Apical Channel, the Dactylocytes whose microvilli line its medial surface. Lying in the Groove between the microvilli of the lateral surface of the Dactylocytes and the medial surface of the Omega Gland (here the Celtic Scroll Cell) is the simple sheet of secretory material which, more distally becomes the Pleated Secretion of Groove F1F2.



P. MARGARITIFERA

L.M. - T.E.M. CIRCUM-PALLIAL AND OTHER MANTLE NERVES. PERIPHERAL NEURONES AND OTHER CELLS OF THE CIRCUMPALLIAL NERVE AND TYPES OF NEUROSECRETORY GRANULES IN A NERVE IN THE SUBEPITHELIAL MUSCULATURE OF THE PALLIAL GLAND REGION.

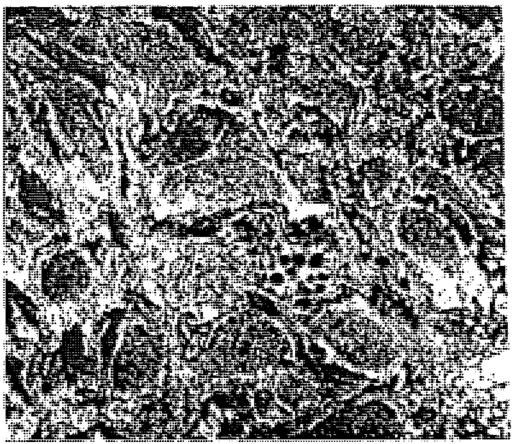
a. x 1100 — L.M. A.B./M.S.B. Large diffuse neuron like cells and other smaller cells amongst the nerve fibres in the Circum-pallial Nerve.

b. x 1.75K — T.E.M. Showing three morphological types of neurosecretion in a nerve trunk in the subepithelial musculature of the Pallial Gland region.

c. x 15K — T.E.M. As for "b" above at higher magnification to show large spherical, elongate ellipsoidal and small spherical neurosecretory granules.

a

b

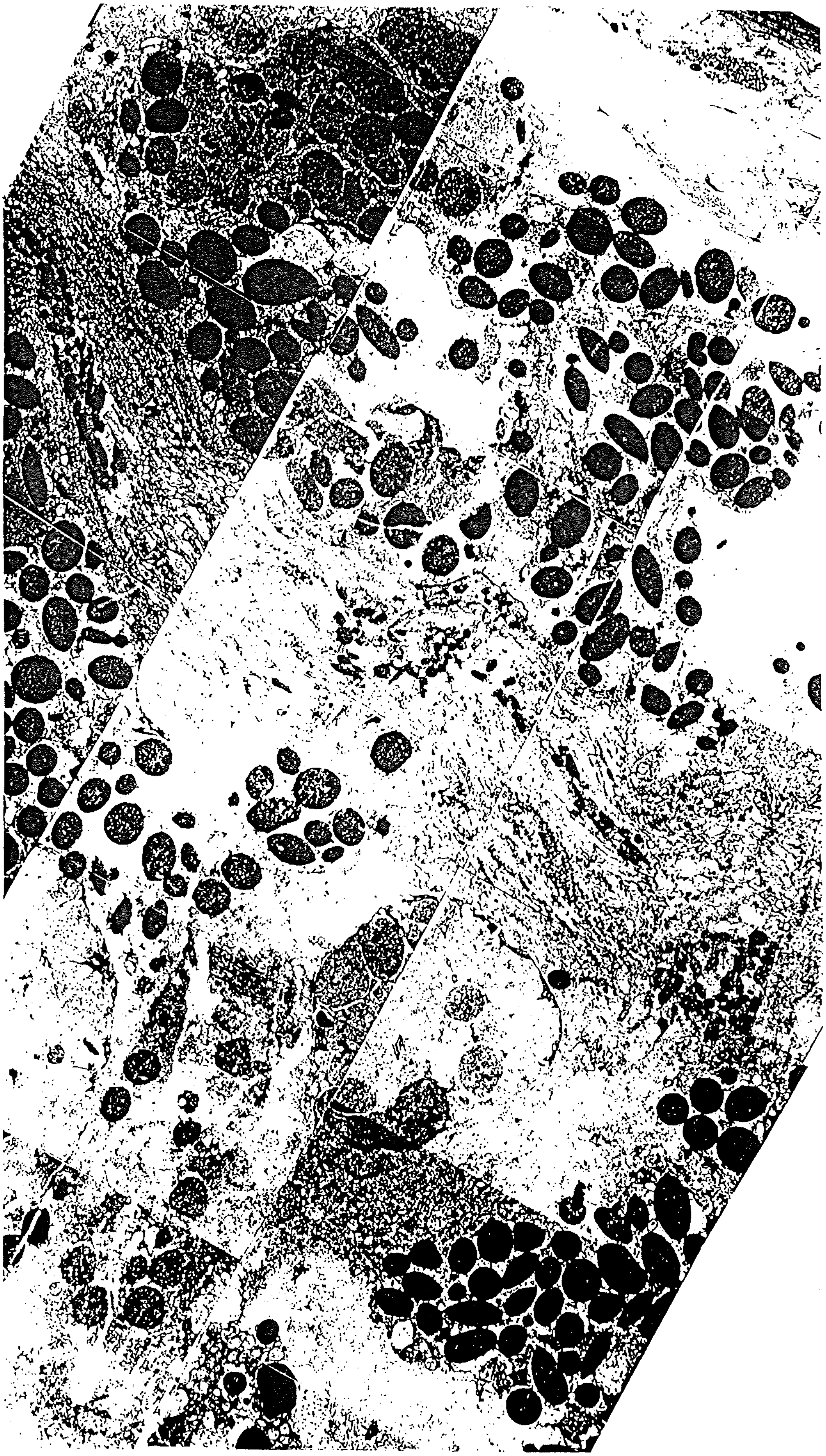


c

P. MARGARITFERA

T.E.M. NEURAL SUPPLY TO SECRETORY EPITHELIUM
IN PALLIAL GLAND REGION.

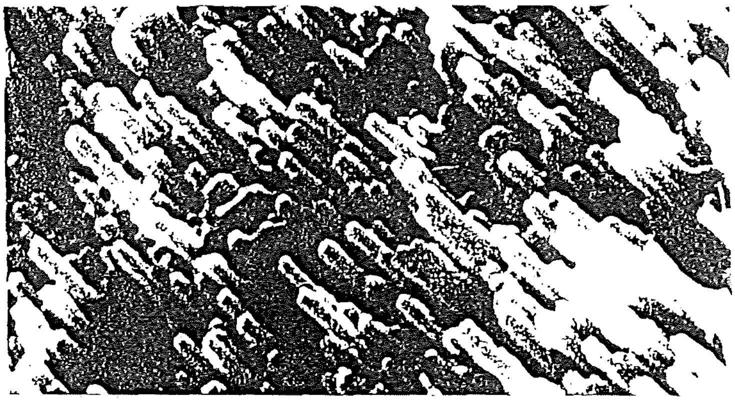
x 3.5K T.E.M. of neural supply to the granular cytoplasm secretory glands and the Turquoise Glands of the Pallial Gland. Elongate ellipsoid type meurosecretory granules are concentrated in the vicinity of granular cytoplasm secretory glands and minute spherical granules near the plasmalemma of the Turquoise Glands.

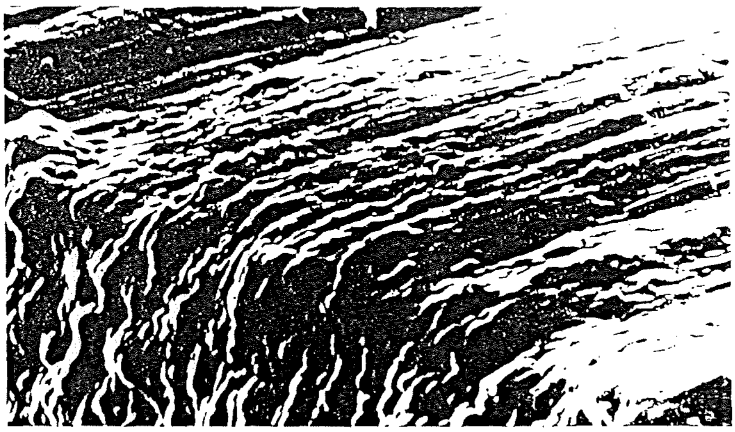


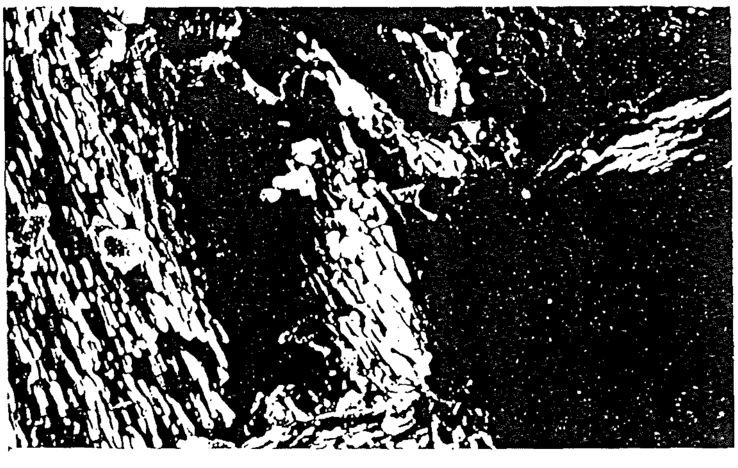
P. FUCATA

HINGE

a. x 200	Radially broken surface of enlarged middle part of hinge - fibrous layer.
b. x 25000	View of end of broken fibrous part of hinge.
c. x 13000	Broken end of "platy" layer of hinge.
d. x 1600	Hinge nacre junction showing multidirectional layering of nacreous layers in this area.
e. x 13000	Same centre at "d" above showing non uniform shape and thickness of Nacreous Tiles in this region.







P. FUCATA

DISTAL FOLDED EPITHELIUM.

a. x 700 — L.M. Radial section of Distal Folded Region showing numerous Turquoise Glands and granular cytoplasm secretory glands. A large local branch of the Circumpallial Nerve underlies the Epithelium and glands.

b. x 1.75k — A major fold of the Distal Folded Region showing the sinus directed normal to the epithelial surface. This surface is thickly covered with microvilli.

P. FUCATA

SECRETORY GLANDS OF THE DISTAL FOLDED REGION SUBEPITHELIUM.

a. x 1.75K T.E.M. Three types of subepithelial secretory gland in the Distal Folded Region. On left is an elongate Turquoise Gland, at centre a very large granular cytoplasm secretory gland with large polygonal granules. Note that many of the granules display a small circular area. (see Plate 4, "b", & "c"). To the right of this is a gland with relatively small spherical granules.

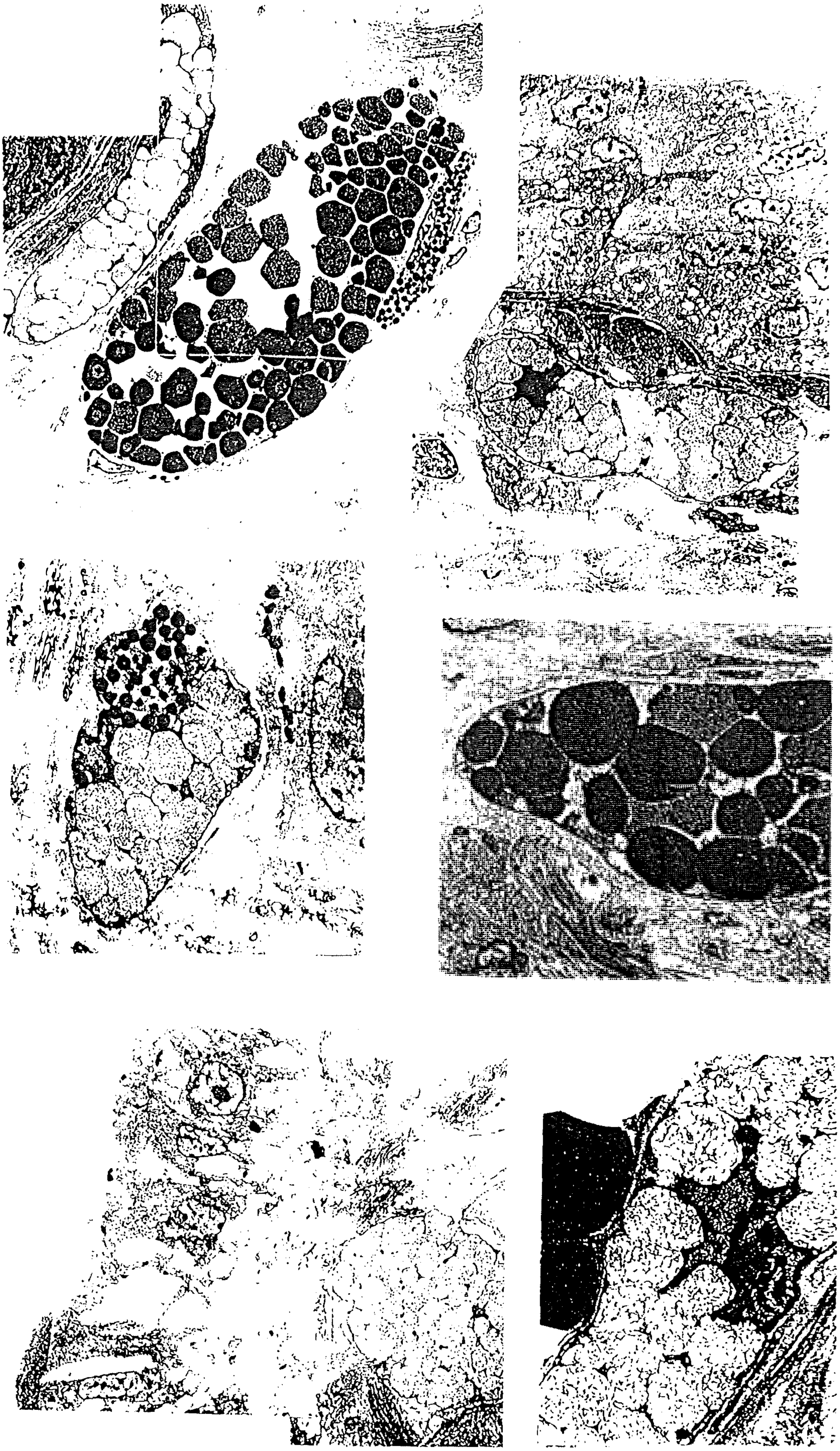
b. x 2.4K T.E.M. Nerve with nuclei in nerve trunk abutting a secretory gland adjacent to a Turquoise Gland.

c. x 4.8K T.E.M. Turquoise Gland and adjacent fine spherical granuled secretory gland.

d. x 4.8K T.E.M. Relatively large spherical secretory granules of a subepithelial gland.

e. x 1.75K T.E.M. Turquoise Gland in the subepithelium of the Distal Folded Region.

f. x 7K T.E.M. Turquoise Gland adjacent to large granule secretory gland.



P. FUCATA

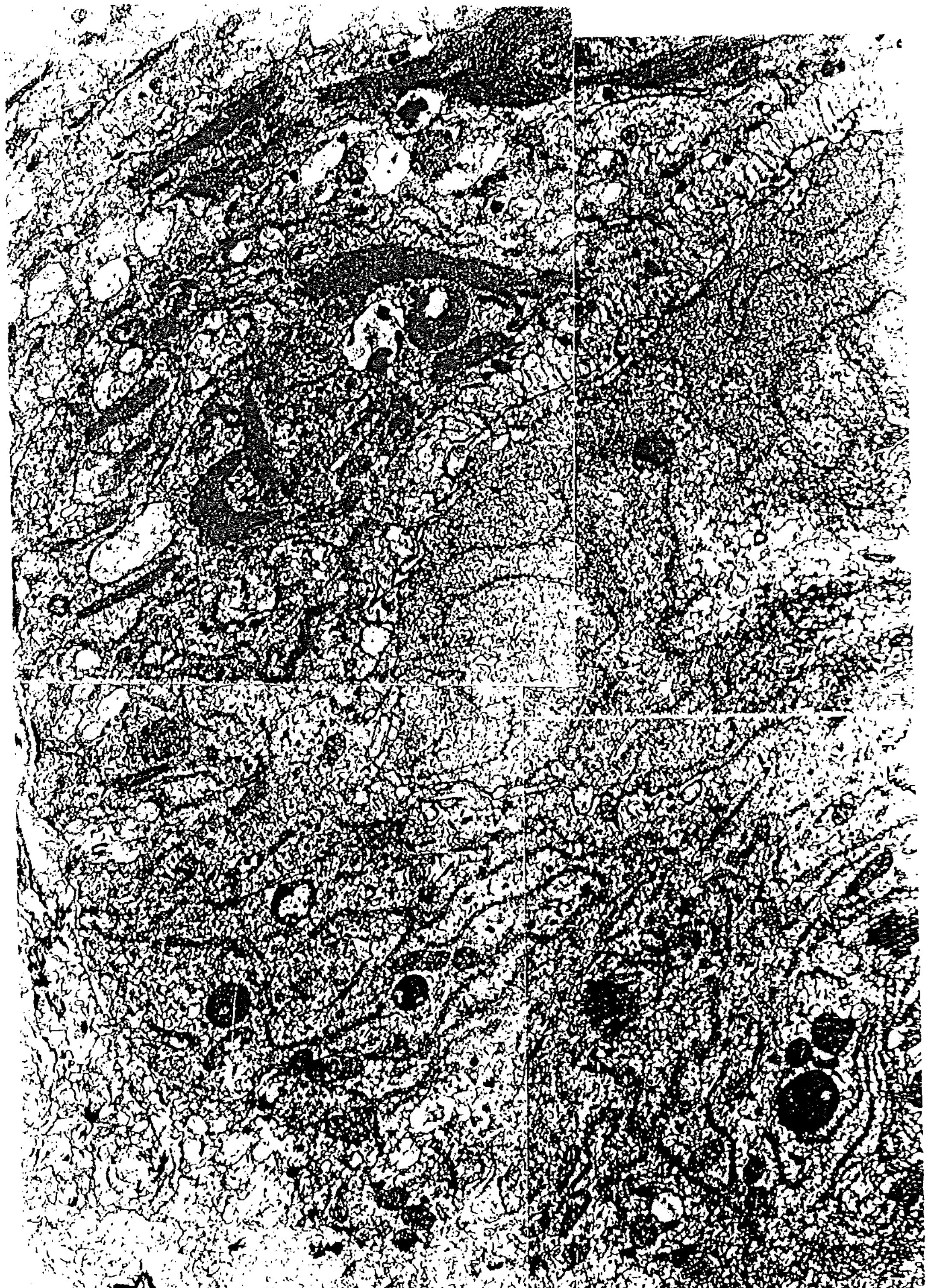
THREE MANTLE MARGIN FOLDS AND MANTLE EDGE GLAND.

a. x 75	A.B.M.S.B. Radial Section of Distal Mantle and Three Mantle Margin Folds.
b. x 750	A.B/M.S.B. Radial Section of Mantle Edge Gland of P.fucata.
c. x 1,75K	T.E.M. Of Mantle Edge Gland showing elongate columnar epithelium with dense covering of apical microvilli.
d. x 7K	T.E.M. Vesiculate apical cytoplasm of Mantle Edge Gland with numerous mitochondria and secretory tubules of small grained granular cytoplasm secretory gland.
e. x 3.5K	T.E.M. Apical cytoplasm of Mantle Edge Gland cells showing vesiculate ends of the surface microvilli and numerous vesicles, mitochondria and membranous organelles in the apical ystoplasm.

P. FUCATA.

ORIGIN OF PLEATED SECRETION
PROXIMAL END OF APICAL CHANNEL.

x 14K — Proximal end of the Apical Channel extends from just above the centre of the illustration to the top right corner.
Left and above its proximal end is the Multivesiculate Cell.
At the top of the illustration is part of the cytoplasm of the most proximal Dactylocyte.
Beneath and to the right of the proximal end of the Apical Channel is the apparently membrane bound amorphous secretion produced by the internal cells of the Omega Gland.



P. FUCATA.

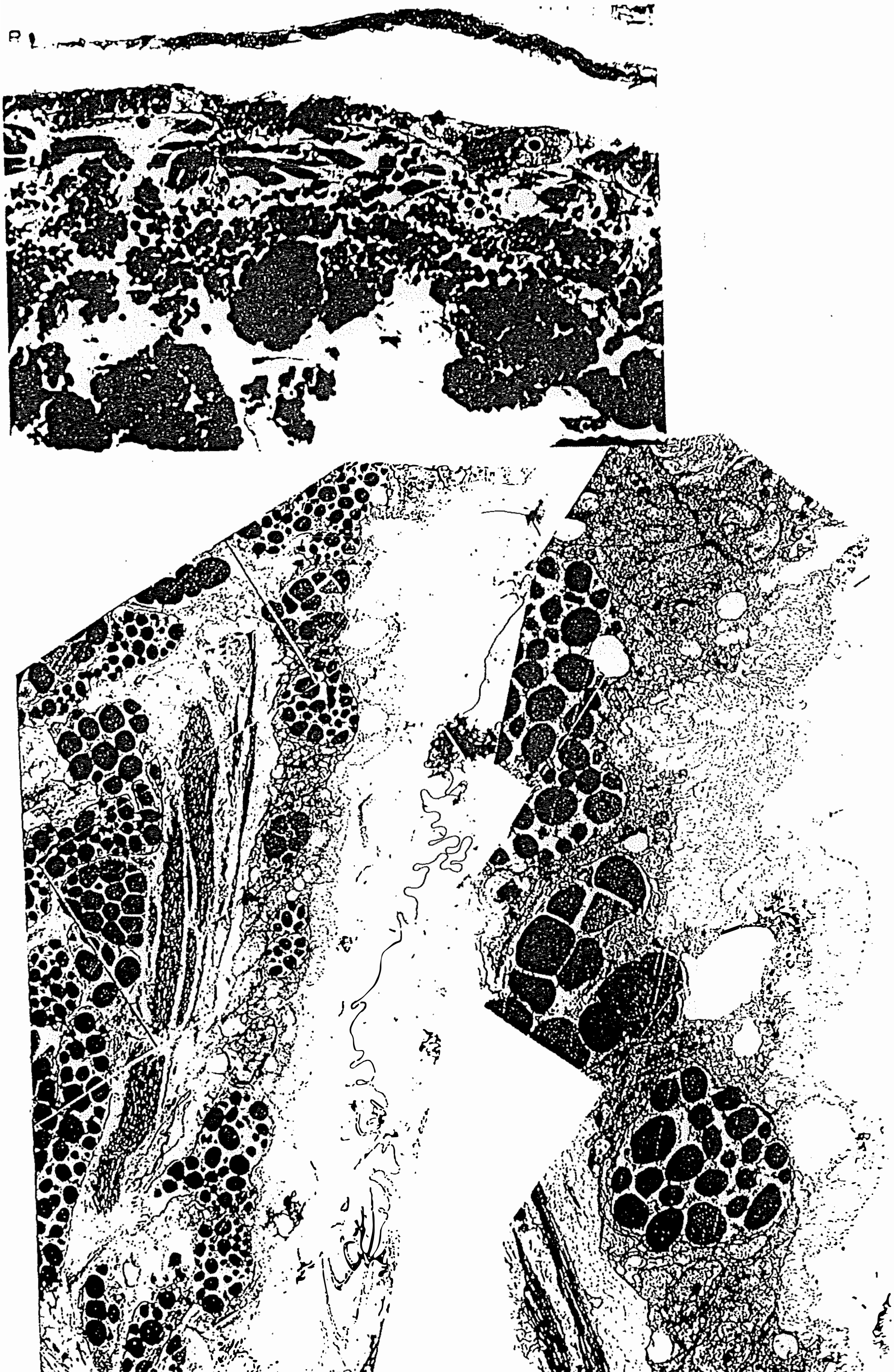
PROXIMAL LAT F2

SUBEPITHELIAL AND RECEPTACLE GLANDS.

a. x 750 AB/MSB. Proximal Lat.F2 showing the Pleated Secretion, surface epithelium with intraepithelial Receptacle Glands, subepithelial musculature and fibrous connective tissue, and beneath this, the subepithelial granular cytoplasm secretory glands of this location.

b. x 1.75K T.E.M. As for 'a' above. The types of granules in the Receptacle Glands reflect those in adjacent subepithelial granular cytoplasm secretory glands.

c. x 4.8K T.E.M. Enlargement of upper part of of the illustration in 'b' above showing distinctive granule types in each of the different Receptacle Glands.

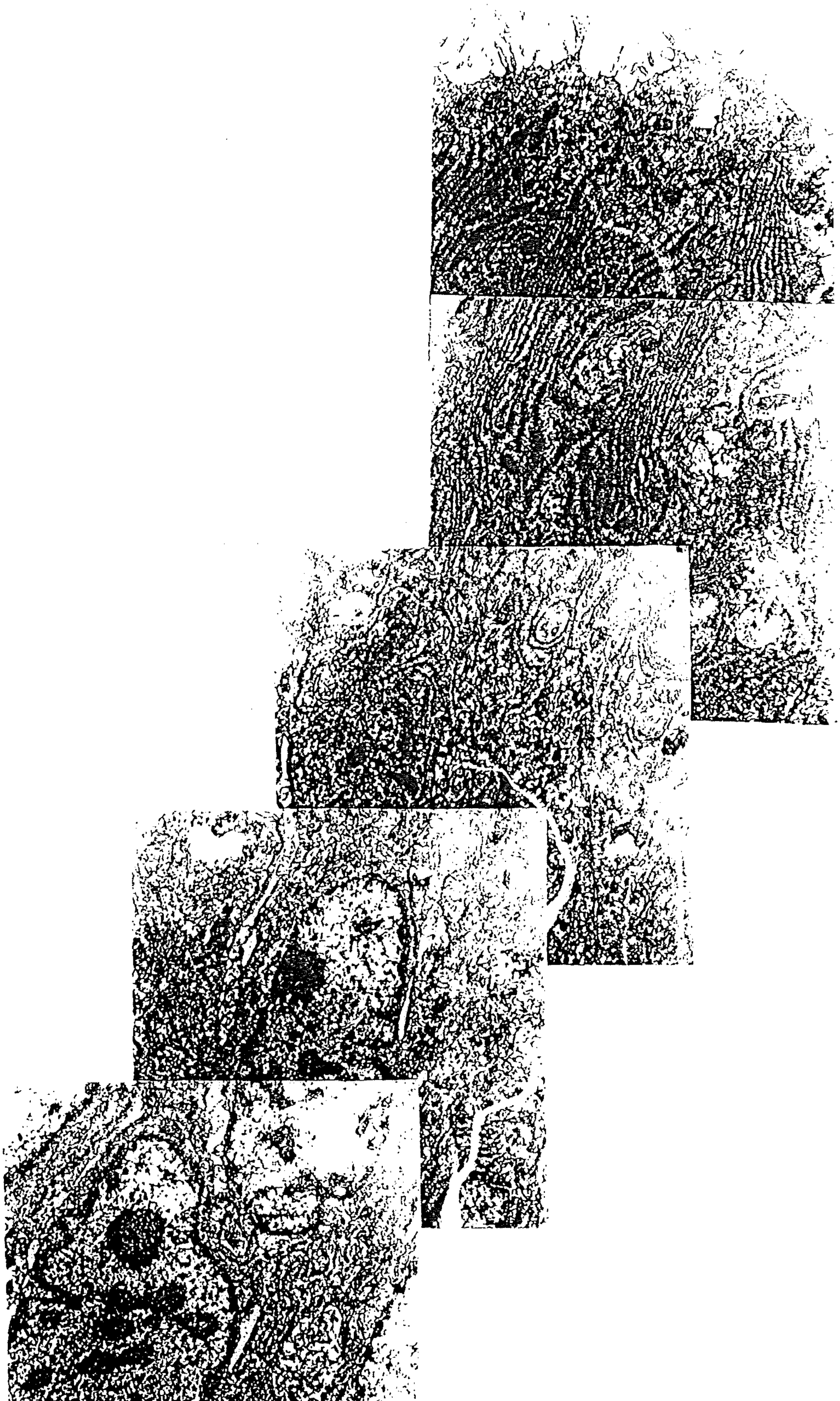


P. PENGUIN.

T.E.M. OF ISTHMUSISTIC EPITHELIUM.

x 7K

T.E.M. Ultrstructure of nuclei and apical cytoplasm and membrane of Isthmusistic epithelial cells showing especially vesicular thickening of the ends of the apical microvilli and the organelles of the apical cytoplasm.

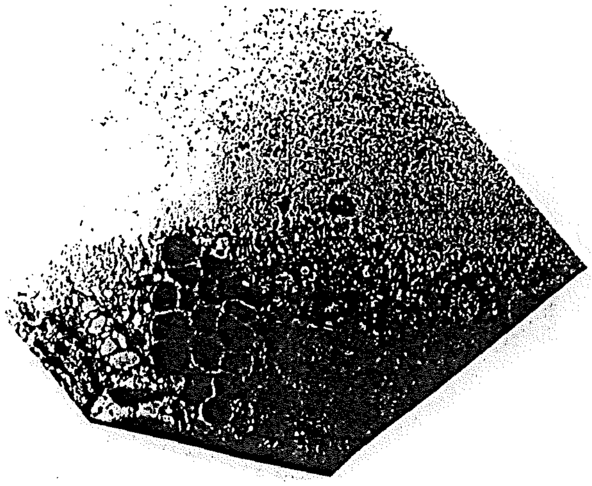


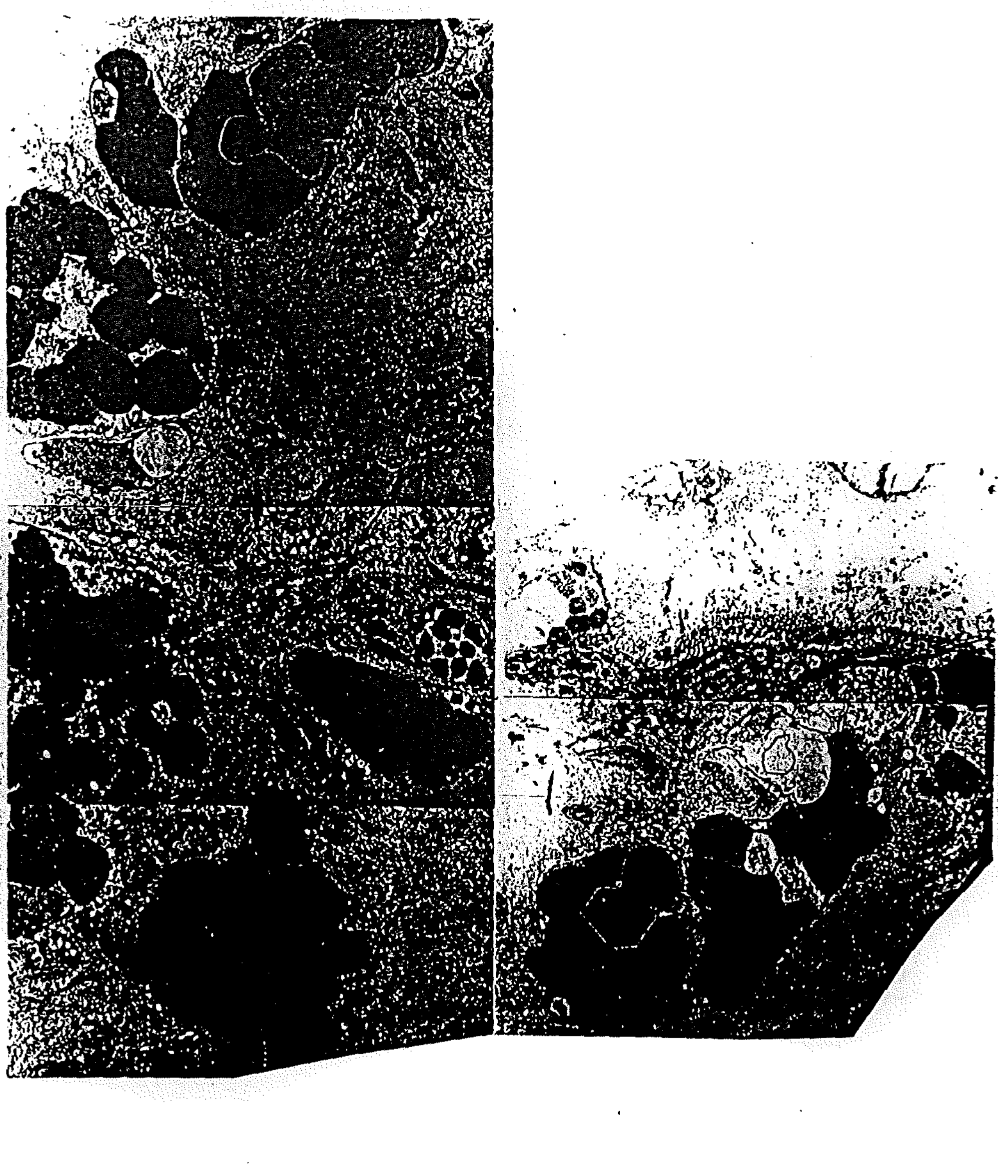
P.PENGUIN.

T.E.M. OF SHOULDER REGION

a. x 3.5K T.E.M. Intra-epithelial Granular Cytoplasm Secretory Gland.

b. x 3.5K T.E.M. Subepithelial glands of the Shoulder Region.





DISCUSSION OF RESULTS AND IMPLICATIONS AND RECOMMENDATIONS

At the commencement of this work, of whcih the electron microscopy of the external mantle and mantle margin was a part there were serious misconceptions related to shell production in pearl oysters. These can be listed as follows:-

Firstly the substance called in this work the Pleated Secretion of Groove FI, FII was called the "forming periostracum", and it was held that this material recurved around the distal extremity of Fold FI., and formed the outer layer of the shell.

Secondly, the Prismatic Layer was believed to be produced by the Mantle Edge Gland on the Lateral surface of Fold FI.

Thirdly the control of Shell Production was held to be under the control of a Growth Hormone - no direct involvement of the nervous system in shell growth was suggested.

These misconceptions had the following consequences:-

Firstly the sine qua non of understanding of production of a pearl shell by an animal like a pearl oyster was prohibited while ever the function of the pleated secretion was miisunderstood.

Secondly the entire array of glands and tissues which produce the Prismatic Layer of the pearl shell was allotted no function in shell production since it lies medial to the Pleated Secretion. If this latter after recurving was to form the exterior surface of the shell then anything medial to it on the Mantle Margin was excluded from participation.

Thirdly the gorwth hormone theory of Shell Growth control diverted attention from the remarkable network of branches of the circumpallial nerve which ramify through every secretory tissue on the External Mantle and Mantle Margin of a pearl oyster and well might act directly on each one of them.

Of probably more immediate economic consequence than the above is the finding that the Shoulder Gland is rapidly almost totally depleted of acid-mucopolysaccharide storage by inanition. Since this work indicates that the secretion by the Turquoise Glands is basic to nacre organic matrix production it well may be that nutrition lack, even for relatively short periods might have a major impact on pearl and pearl shell micromorphology and hence quality.

For this project, the aim of which is a knowledge back up to the Australian Pearling Industry, it is probably better to deal with "outcomes compared with objectives" and "implications and recommendations" simultancously.

The Australian pearling industry exists at the moment in a state of incipeint overproduction. For this reason it is not the intent that this project results directly in financial advantage but rather to provide the knowledge necessary for the Australian pearling induustry to be able to respond quickly and effectively to any change in the economic basis to the inudstry. By agreement with the Australian Pearl Producers Association we have planned to stay "one step back" from work directly related to production of money from pearl oysters.

Seen in this light, the first requirement was that the basic mechanisms of shell production be clarified. This work has,

1, demonstrated that the particularity of the histology of the various regions of the External Mantle and Mantle Margin of the species of pearl oysters studied seen with light microscopy is carried through to the ultrastructure level;

2, shown that the shell organic matrices of each region are formed from the combination of the secretions of the Turquoise Glands and the Granular Cytoplasm Secretory Glands of that region with perhaps the inclusion of the secreted terminal vesicles from the surface epithelial microvilli;

3, shown the physical nature at the ultrstructure level of the secretions which form nacreous organic matrices, prismatic layer organic matrices and the pleated secretion of Groove F1F2, which separates the secreted precursors of the prismatic layer from those of the nacreous layers;

4, in some instances, e.g. the roles of the various histological species of apical groove F1F2, defined their specific roles in shell formation.

The next array of problems are defined.

1. What part do the acid-mucopolysaccharides play in Shell Organic Matrix production?

2. What part do the granular cytoplasm secretory glands play?

3. What is the function of the secretions of the vesicular ends of the epithelial microvilli?

4. What is the mechanism of selerification in pearl shell organic matrix.?

5. What are the chemicals stored in the various types of neurosecretory granules seen, and what is their function in shell production?

6. What is the origin of the glandular contents necessary for high quality nacre production and what controls its production, storage, reabsorption or secretion?

7. What is the effect of nutrients or lack thereof on (6) above?

Some of these problems will be addressed by the ongoing work on electronmicroscopy of pearloysters and their shells; some others by chemical analyses of pearl oyster mantles, their secretions and the organic matrices of pearl shells.