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The Body Wall and Musculature of the Marine Triclad *Palombiella stephensoni* (Palombi, 1938)

Part One: General Tissue Structure as seen with the Light Microscope

by

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WITHDRAWN

The Body Wall and Musculature of the Marine Triclad *Palombiella stephensoni* (Palombi, 1938)

Part One: General Tissue Structure as seen with the Light Microscope

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ABSTRACT

THE morphology of the body wall and musculature of the marine triclad *Palombiella stephensoni* (Palombi, 1938) is described. The epidermis possesses a system of internal "channels"; these could be part of a mechanism protecting the epidermis from damage. It is suggested that the structure termed "basement membrane" by other workers is best called a "connective" layer. One type of subepidermal gland opens to the exterior by a system of branching ducts which penetrate epidermal cells, which indicates a complex "channel" system within these epidermal cells. The musculature conforms to the usual pattern for a marine triclad.

INTRODUCTION

This paper describes the morphology of the body wall and muscles of *Palombiella stephensoni* (Palombi, 1938), a marine triclad of the family Bdellouridae. It was first described by Palombi (1938) under the name of *Synsiphonium stephensoni*. More recently it has been assigned to the genus *Palombiella* by Westblad (1951). Specimens have been recorded from South Africa, Tristan da Cunha, Banks Peninsula, New Zealand (Nurse, 1955) and Wellington, New Zealand (the present study).

The Wellington material fits almost exactly the description given by Westblad (1951) for specimens from Tristan da Cunha. However, 3 differences are apparent. Firstly, the Wellington specimens have 28 testes, whereas those described by Westblad had 24. Nurse (1955) records specimens of *P. stephensoni* from Banks Peninsula, New Zealand with 36-38 testes. Secondly, the shape of the anterior end is blunt and not pointed, an observation also made by Nurse regarding the Banks Peninsula specimens. Thirdly, a variety of colour-patterns occurs. Most specimens are dark grey on the dorsal surface with irregular patches of pale cream as described by Nurse, but the colour varies from light grey to black, and there is also a form which is red-orange on the dorsal surface. Westblad described the Tristan da Cunha material as being mostly dark brown on the dorsal surface. Ciliated protozoans in the pharynx sheath and gut of the Wellington specimens are similar to those of the same regions in the animals from Tristan da Cunha and Banks Peninsula.

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MATERIALS AND METHODS

Specimens were collected from Island Bay and the western shore of Lyall Bay, Wellington, New Zealand. At low water the rock pools and rocky shore of the lower littoral zone were harvested for their green sea-lettuce (*Ulva lactuca*). This was transported to the laboratory and placed in large, shallow enamel trays with sufficient sea water to cover most of the sea weed. The *Ulva* was then examined and any *Palombiella* present were drawn into a pipette and squirted into bowls of sea water. The worms were kept alive and healthy in the laboratory in glass bowls 6 ins. in diameter and 2 ins. high. The water in the bowls was changed every two days, and the worms were fed finely chopped beef liver and muscle twice a week: for every 40 worms, 1 gm. of beef and liver mixture was placed in the bowls. After ½ hour the excess food in the bowls was discarded and the water was changed.

The worms proved difficult to narcotize. Substances commonly used for anaesthetisation of flat worms such as carbon dioxide, ether, coal gas and alcohol gave poor results and caused the worms to contract markedly. A 50:50 mixture of ground chloral hydrate and menthol crystals sprinkled onto the surface of the water finally gave the best results. However, the worms frequently extruded the pharynx, and some discharge from genital openings took place.

The specimens were fixed in bufferred 4% formaldehyde using the buffer mixture of Pease (Pease, 1964, p. 52); in Carnoy's 3:1 ethanol:acetic acid; in Lilies' ethanol-acetic acid -formaldhyde (Pearse, 1960); and in picroformaldehyde (4% formaldehyde 75cc. sat. aq. picric acid 25cc. calcium chloride 1gm.). In all cases fixation was for 18 hours after which the animals were washed and stored in 70% tertiary butyl alcohol.

Whole mounts stained in acetic acid-alum-carmine and in the copper sulphate-silver nitrate nerve fibre stain of Betchaku (1960) were prepared after bleaching of pigment with 100 Vols. hydrogen peroxide.

For paraffin embedding the animals were dehydrated in 95%, and in two baths of 100% TBA for one hour each. They were then infiltrated in a 50:50 mixture of TBA:paraffin wax for two hours in a paraffin oven, and finally placed in pure paraffin (M.P.54 C.) for two hours prior to embedding. Embedding troughs made from brass blocks and adhesive cellophane tape (Wigglesworth, 1959) were used, and the embedding process was carried out with the aid of a binocular microscope at a magnification of 16x. Serial transverse and sagittal sections were cut at 5μ thickness, extended on a water bath, and placed on standard microscope slides without the use of adhesive. Sections were pressed onto slides after the method of Gray (1953). When treated in this manner they adhered vigourously to slides and withstood treatment by most reagents. Sections were subjected to the following staining procedures: 1. Heidenhain's iron haematoxylin with a counter stain of Orange G; 2. Delafield's haematoxylin with Van Gieson's picrofuchsin mixture as counterstain; 3. The triple stain of Delafield's haematoxylin, eosin Y, and fast green FCF (Wineera, 1968), and a variation employing Heidenhain's haematoxylin in place of Delafield's; 4. Mallory's triple stain (Gray, 1953); 5. The sulphuric acid-haematoxylin stain for basement membranes (McManus and Mowry, 1952); 6. Gordon and Sweet's silver stain for reticulin (Pearse, 1960); 7. The orcinol-new fuchsin stain of Fullmer and Lillie for elastic fibres (Pearse, 1960); 8. Betchaku's (1960) copper sulphate-silver nitrate stain for nervous tissue; 9. The Falg technique (Gurr, 1965). Stained slides were dehydrated through an alcohol series, cleared in xylol, and mounted in D.P.X.

The embedding process of Pease (1964) was used for methacrylate embedding. Sections were cut at 0.2μ and 0.5μ on a sledge microtome; were mounted on slides, and stained using Mallory's triple stain.

REMARKS

It was found that bufferred 4% formaldehyde and picroformalin were unsuitable fixatives for preservation of the epidermis. In worms fixed in these substances the epidermis was frequently broken when seen in sections, and the cells were shrunken and distorted. Cellular detail was very difficult to see because of this distortion. By far the best preservation of form was given by Lillie's AAF. When this fixative was used the epidermis was seen as a fairly uniform layer of cells. Cilia were well preserved. Carnoy's fluid also gave good preservation of the epidermis, but not as good as the AAF of Lillie.

RESULTS

The Epidermis (Plate 1). The epidermis is a single, cellular layer. The cells are cuboidal to columnar, being more cuboidal on the ventral surface, and columnar on the dorsal. The entire epidermis is ciliated, but the ciliation is heavier on the ventral surface. The nuclei are spherical, drop-like, or oval structures. Unicellular basiphil glands occur scattered throughout the epidermis but especially on the dorsal and ventral surfaces at the head and tail ends of the body. These gland cells do not differ in appearance from other epidermal cells, except for the secretion which they contain.

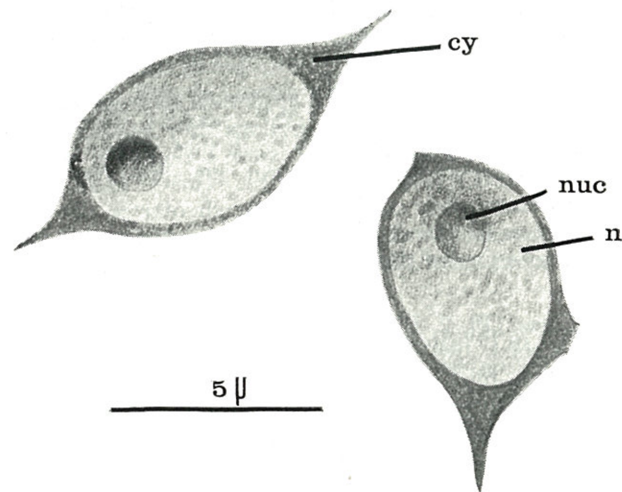
When seen in plan view, the cells of the epidermis fit together closely to give an approximately hexagonal arrangement. In sections they have a distinctive appearance which leads to the conclusion that the outer ⅓ region of the cells possesses a system of internal "channels" (Plate 1, Fig. 1). These "channels" are regularly spaced, are parallel to each other, and are perpendicular to the base of the cells. They are most prominent in the dorsal epidermis and are well demonstrated by sections tangential to the surface of the animal (Pl. 1, Fig. 2, 3).

The Basement Membrane (Pl. 2, Figs. 1, 2). Immediately below the epidermis and in contact with it is a thick (up to 2μ) non-cellular layer. It stains red with Van Gieson's picrofuchsin stain, blue with Mallory's triple stain and green with the triple stain of Wineera (1968). No reticular or elastin fibres could be demonstrated in this layer by the methods used. Sections of mouse and rabbit skin which were stained at the same time as those of *P. stephensoni* gave a positive result for both elastin and reticulin fibres. The sulphuric acid-haematoxylin method for basement membranes failed to stain this layer. The basement membrane has the same appearance when cut in transverse or sagittal section. At a magnification of x1250, fibres are recognizable in some areas, otherwise it is homogenous and non-cellular throughout. It is intruded between the epidermal cells at their bases (Pl. 2, Fig. 1).

Muscles (Pl. 2, figs. 1, 2). Immediately below the basement membrane of the epidermis is a thin layer of circular muscle. The fibres in this layer are roundish in transverse section and are approximately 1μ in diameter. Beneath the circular muscles are ribbon like longitudinal muscle fibres which are approximately 2.5μ wide and 1μ thick. Muscle fibres are separated by thin connective tissue fibres which are continuous with the basement membrane below the epidermis (Pl. 2, Fig. 2). At the anterior end of the animal the longitudinal muscles are often bipinnate in arrangement and very similar, when seen in transverse section, to the longitudinal muscles of the earthworm. Dorso-ventral muscle fibres of similar size to the longitudinal muscle fibres are abundant throughout the body, especially at the sides of the animal (pl. 2, Fig. 1, 2). No striations of any sort have been seen on the muscle fibres. The nuclei of muscle cells are smaller than those of the epidermal cells. They are ovoid to elongate, and are orientated with their long axis parallel to the muscle fibre.

Parenchyma (Pl. 2, Fig. 1, 2, 3, Pl. 3, Fig. 1, 2). Between the organs the body is filled with parenchymatous tissue. Numerous nuclei can be seen, but cell boundaries are indistinct except in the case of the neoblast cells. Hyman (1951, p. 78) defines this tissue as a syncytium.

The neoblast cells (Pedersen, 1959) are free cells with a characteristic morphology (Text Fig. 1). In sections fixed in Lillie's AAF they are seen as small to large cells with a round or oval nucleus invested with a thin layer of basiphil cytoplasm. The nucleus measures up to 5μ in diameter, and usually contains one or two large nucleoli. The whole cell measures approximately 7μ in diameter, with the cytoplasm being fairly evenly distributed around the nucleus. Sections treated with Mallory's Triple Stain show the nucleus as blue grey, with coarsely granular nucleoplasm. The nucleoli stain a vivid red-orange, and the cytoplasm, which is finely granular, colours a darker blue-grey. Neoblasts occur throughout the parenchyma, but they tend to be most numerous on the ventral side, and are scarce in the cephalic and caudal regions.



Text Fig. 1 cy., cytoplasm; n., nucleus; nuc., nucleolus.
Typical neoblast cells. For description see text.

Connective tissue fibres and muscle fibres are abundant in the parenchyma, and various vacuoles are also present. At least two types of subepidermal gland cell also occur in the parenchyma. One type of gland occurs as scattered single cells mainly in the ventral part of the animal, but it may be found anywhere in the parenchyma. In the head and tail regions these cells are aggregated into clusters. They are long, slender and are basiphil, staining with aniline blue in the Mallory technique and with haematoxylin. They open to the surface through long necks which penetrate between epidermal cells, mostly at the ventral surface, but also on the dorsal side. In the cephalic region of the worms these glands pour a profuse secretion to the ventral surface through large ducts.

A second type of gland cell is restricted mostly to the parenchyma of the lateral body margins. These cells are smaller than the basiphil gland cells but are also elongate. They open by way of long slender necks which usually penetrate epidermal cells at the ventral surface of the marginal area of the animal. However, they also open at other places on the ventral surface, and often extend from the lateral margins towards the dorsal midline. These cells are eosinophil and are shown very well by the Falg method of staining (Pl. 3, Figs. 2, 3, 4). With the Mallory technique they stain a distinct purple colour. When the necks of these cells penetrate epidermal cells they branch to form a fan-shaped system of ducts (Pl. 3, Fig. 2).

Pigment (Pl. 1, Fig. 1). Pigment, which is sub-epidermal, is confined mostly to the dorsal side of the parenchyma. On this side the pigment may be so dense as to completely obliterate other features of the anatomy (Pl. 1, Fig. 1). On the ventral side, however, pigment is present only as scattered granules. The pigment is laid down at the site of connective tissue fibres, particularly where these fibres course between the circular and longitudinal muscle fibres of the dorsal body wall. Other regions of the body are free of pigment. The pigment granules are very small, and are irregularly shaped. They can be bleached by hydrogen peroxide and by potassium permanganate solution.

DISCUSSION

The system of channels in the outer region of the epidermis could be part of a mechanism that functions to protect the animal from damage to its epidermis, as there is no cuticle. These animals are probably exposed to many kinds of environmental conditions such as the abrasive power of silt laden water, and the washing of the alga on which the worms are situated against rocks, which are potentially damaging. It seems reasonable to suppose that damage to the surface of an epidermis which has an internal system of channels forming "compartments" (such as that in *P. stephensoni*) could be repaired relatively easily by "partitioning off" the damaged "compartments". This "protective mechanism" could be especially effective in the case of unicellular epidermal gland cells, for if part of the surface of such a cell was damaged, the ease of isolation of the damaged region from the rest of the cell could allow the cell to continue functioning as a secretory structure. If such a mechanism did not exist, it is probable that any damage done to the surface of epidermal cells, and in particular to the surface of epidermal gland cells would result in a great loss of cell contents. This, in turn, would probably lead to the death of the cells concerned.

It must be noted that the precise morphology of these channels in the outer regions of epidermal cells could not be determined in the present study. The above theory, therefore, is largely conjecture, and must wait until the structures upon which it is based are examined by electron microscopy before it can be fully determined.

There is evidence which suggests that the structure in marine triclads which is termed a basement membrane (Hyman, 1951, Gamble, 1896, Grassé, 1961) should not be called by this name until its ultrastructure can be elaborated. Fawcett (1966, p. 353) states that the basement membrane as seen with the electron microscope (which he terms "basal lamina" to avoid the connotations of the word "membrane") is a moderately dense band 500-700A thick. It is separated from the basal cell membrane by a light zone about 400A wide. This relatively thin layer would not be visible

with the light microscope (Bloom & Fawcett, 1966, p. 5). The thickness of the basal lamina varies with different types of epithelia. Fawcett suggests that the layer between an epithelium and the underlying connective tissue to which light microscopists originally assigned the term "basement membrane" included some of the underlying collagenous fibres of the connective tissue. In the present study this "basement membrane" layer is very thick and has the staining properties of collagen, that is to say, it stains red with Van Gieson's picrofuchsin stain, and is deeply coloured by the acid dyes aniline blue and fast green FCF which Lillie (1945) considers to be excellent collagen stains. Moreover, it is continuous with fine similarly staining fibres which course throughout the body parenchyma and between muscle fibres, and which enclose specialised organs such as the gut and the reproductive organs. This suggests that it has the same functions as the connective tissue of higher animals. However, with the techniques used in this study no cellular material was demonstrated in this layer, and because of the difficulty of defining cell boundaries within the parenchyma, the relationships between the cells of the parenchyma and (a) the fibres of the basement membrane and (b) the connective tissue fibres deep in the parenchyma, could not be evaluated. Therefore, the term "connective tissue", which implies cellular material as well as extracellular fibres and other substances, is best avoided for the present. The terms "connective layer" and "connective fibres" seem appropriate.

Fibrous proteins belonging to the collagen class have been identified from the skeletal tissues of most invertebrate groups of animals (Rudall, 1955; Gross, Sokal, and Rougvie, 1956). Ruddall shows that among the various groups there generally is an inverse relation between α chitin and collagen production. That is, where collagen is reduced to a minimum as for example in hydrozoan polyps and in insects, chitin usually forms a high proportion of the total bulk of the animal; and conversely, where collagen forms the bulk of the skeletal system α chitin is absent or present in only small amounts. There are no records of the presence of collagen in turbellarians. However, the work of Rudall and of Gross et al suggests that it probably would be present, because it occurs widely in other lower metazoan groups, and because chitinous material is generally lacking in turbellarians except in the reproductive organs of some species.

According to Hyman (1951), the basiphil gland cells in triclads are for the secretion of "slime" to aid in locomotion, and the eosinophil ones are for the secretion of adhesive substances. The position of these gland cells in *P. stephensoni* appears to confirm this conclusion. The former type are most abundant in the ventral parenchyma of the anterior end of the animal, while the latter occur as a zone extending around the lateral body margin. Hyman adds that both secretions probably also serve to protect the surface of the animal from harmful environmental substances and to entangle prey. The present study supports the views of Hyman (1951, p. 74) that the fine necks of the subepidermal eosinophil glands pierce the epidermal cells as they open to the surface (Pl. 3), and also that these fine gland cell necks terminate in "papillae" which extend beyond the epidermis (Pl. 3, Figs. 1, 2). Once again, it must be noted that the detailed structure of these cells could not be determined. It is clear, however, that for the gland cell necks to open through the epidermal cells, a system of internal membranes must be present in the epidermal cells to enclose the secretory substance of the gland cells and to "pipe" it to the surface. It was mentioned earlier that the epidermis over the general body surface possesses a system of internal "channels" (Pl. 1, Fig. 1), but it is not clear whether this existing system is utilised by the subepidermal glands or whether the fine ducts of the glands remain separate from these internal epidermal "channels". It is possible that the epidermal cells through which these glands open are specialised for this task, but in the present study no morphological differences were observed betwen these and other epidermal cells.

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Plate 1

Fig. 1 Transverse section of the dorsal epidermis stained by the Falg technique and photographed by Nomarski interference microscopy. The nuclei of the cells are large oval structures. The outer $\frac{1}{3}$ region of the cells possesses a system of internal "channels" which are perpendicular to the base of the cells.

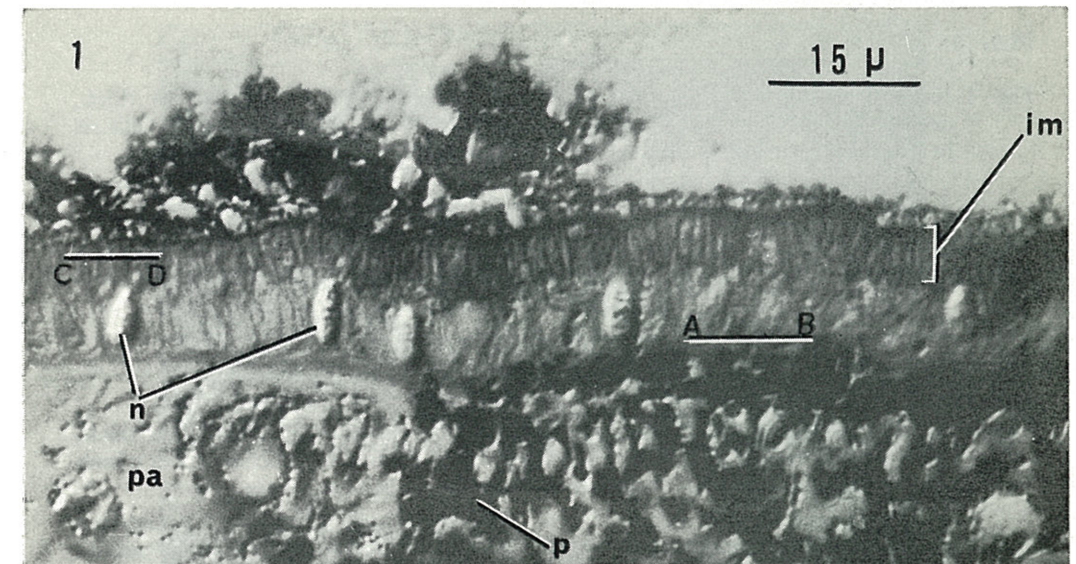
i.m., region of internal channels; n., nuclei; p., subepidermal pigment; pa., parenchyma.

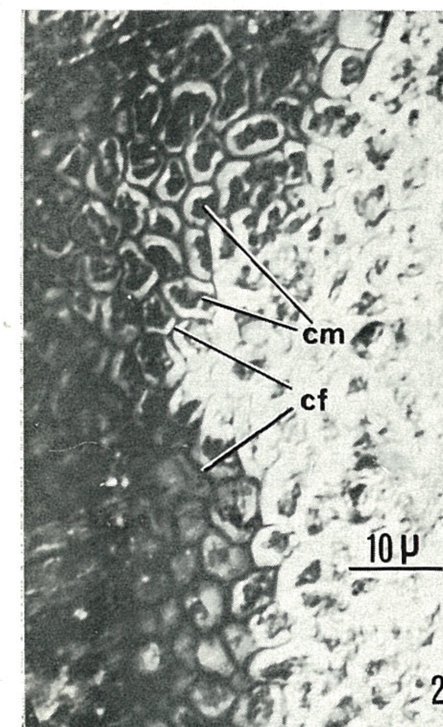
Fig. 2 Photomicrograph of a portion of dorsal epidermis sectioned tangentially. The section is at a level corresponding to A-B in Fig. 1, and passes through the bases of epidermal cells. Connective fibres enclose the cell bases, giving a polygonal pattern, in which each cell is mostly surrounded by 6 other cells. Stained by Mallory's technique.

cf., connective fibres; cm., cellular material.

Fig. 3 Photomicrograph of a portion of dorsal epidermis sectioned tangentially. The section is at a level corresponding to C-D in Fig. 1. The system of internal "channels" is cut transversely and appears as many small tubes. Secretions from 3 subepidermal eosinophil glands are present. Stained by Mallory's technique and photographed by Nomarski interference microscopy.

im., the region of internal "channels" cut transversely; es., secretion from eosinophil glands.





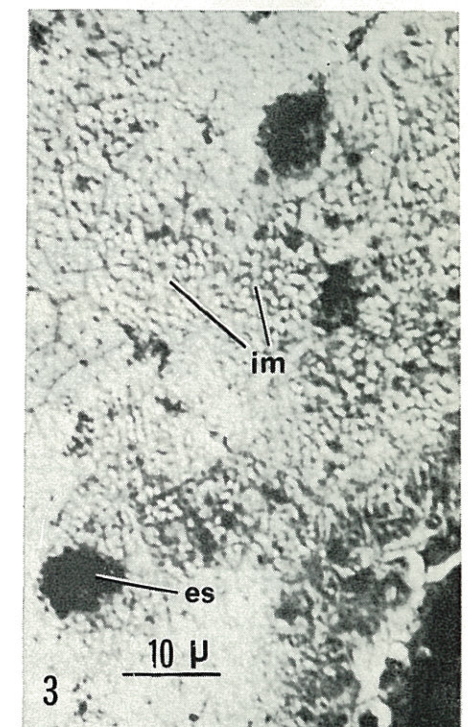


Plate 1. The Epidermis

Plate 2

Fig. 1 Sagittal section of dorsal body wall. The "basement membrane" is thick, densely stained and sends "septa" between epidermal cells so that the cells sit in cup-like depressions in the "basement membrane". Mallory Stain.

bm., basement membrane; d., dorsoventral muscle fibres; e., epidermis; pa., parenchyma; s., septum.

Fig. 2 Sagittal section of ventral body wall. The "basement membrane" is a thick, dense line. Circular muscle fibres are separated by fine connective fibres which are continuous with the "basement membrane". Mallory stain.

bm., basement membrane; cm., circular muscle fibres; cf., connective fibres; d., dorso-ventral muscle fibres; e., epidermis; lm., longitudinal muscle fibres; pa., parenchyma.

Fig. 3 Sagittal section of anterior region of the parenchyma. Basiphil and eosinophil glands are present. Both types are long and slender. Mallory stain.

bg., basiphil glands; eg., eosinophil glands; e., epidermis; pa., parenchyma.

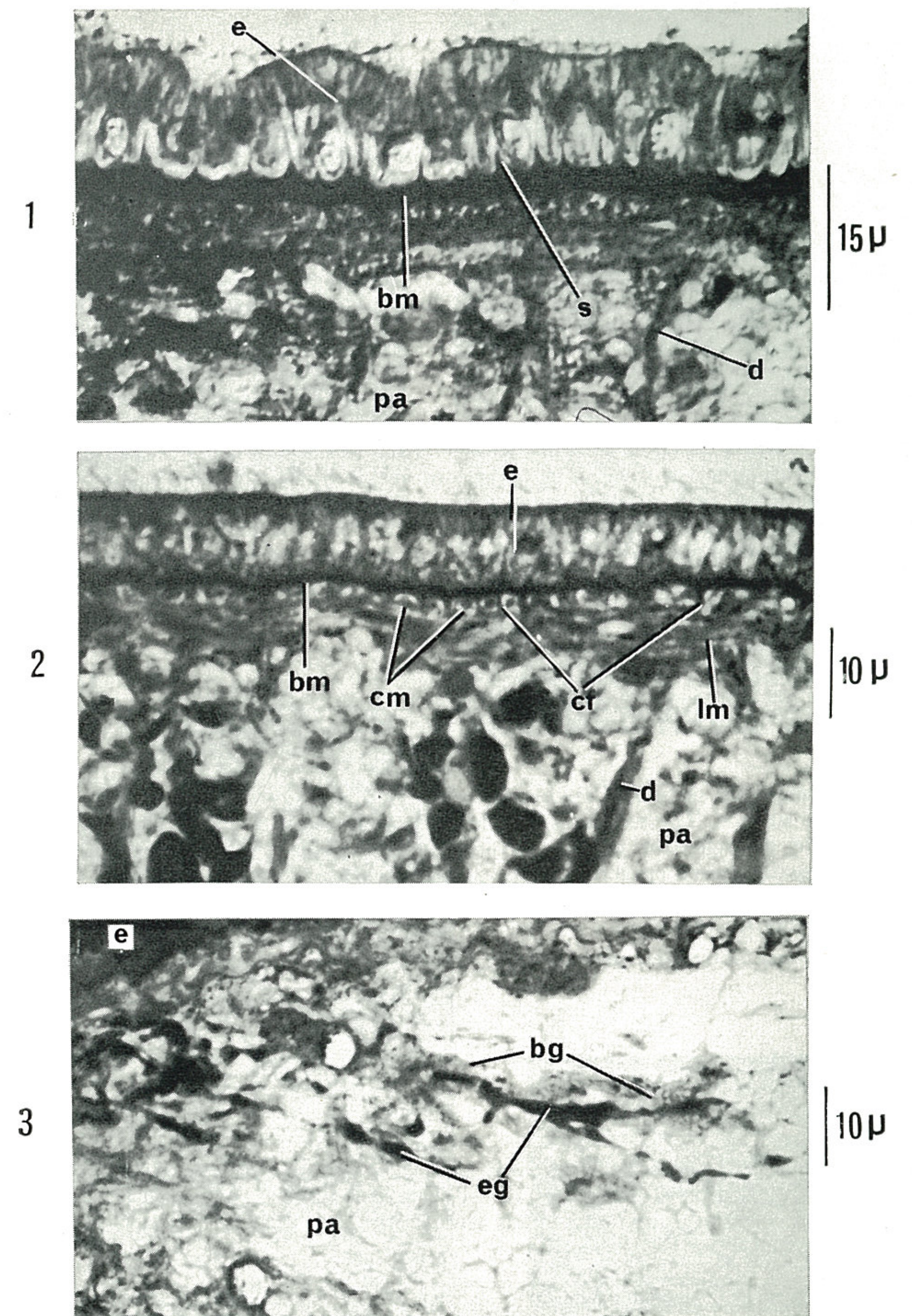


Plate 2. The Basement Membrane, Muscles, and subepidermal glands

Plate 3

Fig. 1 Transverse section of the dorso-lateral epidermis. Eosinophil secretions within the parenchyma are entering the bases of 2 epidermal cells. At the surface of the epidermal cells is a row of "papillae". Stained with Delafield's haematoxylin and eosin, photographed by Nomarski interference microscopy.

es., eosinophil secretion; n., nuclei of epidermal cells; pa., parenchyma; p., papillae.

Fig. 2 Transverse section showing subepidermal eosinophil glands opening to the ventro-lateral surface. The secretions reach the surface by passing through epidermal cells. Fan-shaped systems of ducts are formed within the epidermal cells. "Papillae" are seen where secretions reach the surface. Stained with the Falg technique and photographed by Nomarski interference microscopy.

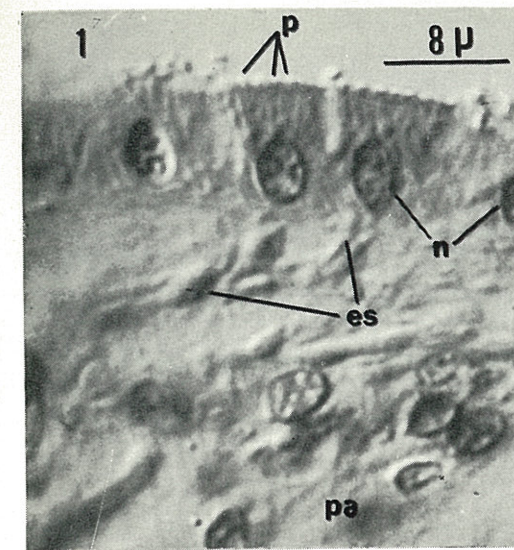
e., epidermis; es., eosinophil secretion; p., papillae; pa., parenchyma.

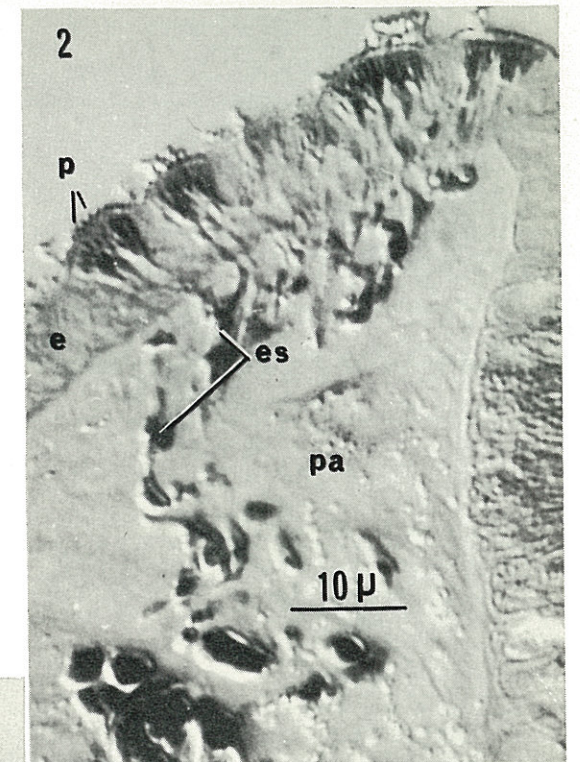
Fig. 3 Tangential section of epidermis at a region where eosinophil secretions reach the surface. The section is at a level corresponding to C-D in Plate 1, Fig. 1. The arrangement of the secretion masses is evidence that the secretions actually pierce epidermal cells, and do not reach the surface by penetrating between epidermal cells. Falg staining, photographed by Nomarski interference microscopy.

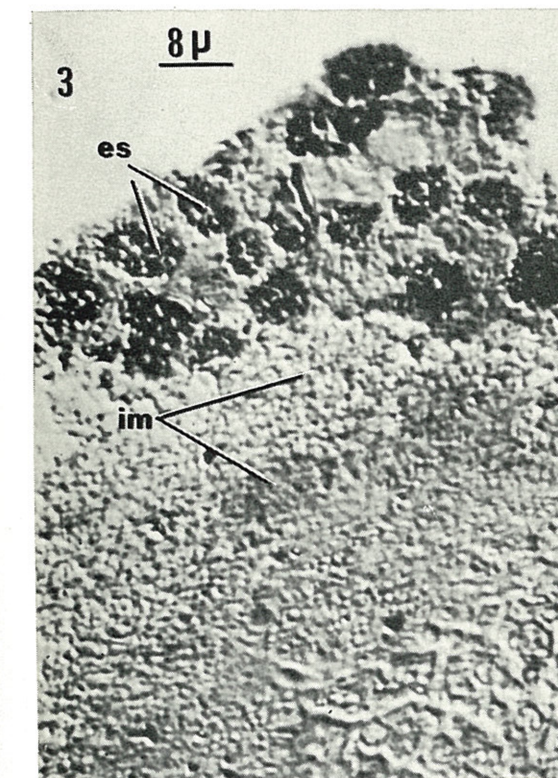
es., eosinophil secretion; im., region of internal channels of epidermis.

Fig. 4 Tangential section of epidermis at a level corresponding to A-B in Plate 1, Fig. 1. The polygonal patern of cells is apparent. One cell (arrowed) contains large eosinophil secretory ducts from underlying glands. Stained and photographed as for Fig. 3.

p., pigment.







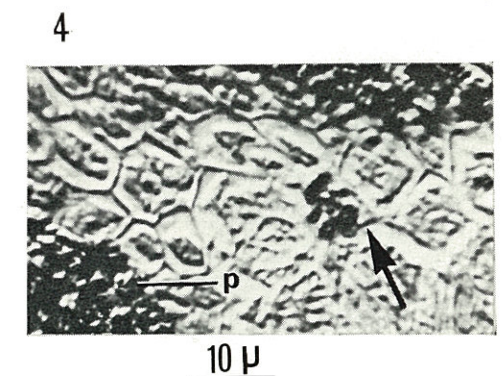


Plate 3. The Eosinophil Glands