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Histochemical and Ultrastructural Features of the Epidermis of the Land Planarian *Bipalium adventitium*

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ABSTRACT The epidermis of the land planarian *Bipalium adventitium* was examined by light and electron microscopy. In all regions, the epidermis consists of a simple columnar ciliated epithelium associated with a prominent basement membrane. The epithelial cells, possessing abundant microvilli and poorly developed terminal webs, are conjoined laterally at their apical ends by septate junctions. The epidermis of the creeping sole is distinguished from that of adjoining regions by an "insunken" condition of the epithelial cells, a greater number of cilia per cell, and an absence of glandular secretions other than mucus. The insunken cells of the sole possess large glycogen deposits and attributes of metabolically active cells. Unusual intranuclear inclusions of unknown significance are also found in many of the epidermal cells in all regions. The basement membrane lacks distinct layering and consists of fine fibrils displaying a beaded appearance but no obvious cross-banding. Histochemical tests indicate that the fibrils are collagenous. In addition to mucus, secretory material found in nonsole regions includes lamellated granules and rhabdites, both stained intensely by acidic dyes. Rhabdites and the basement membrane also contain disulfide-enriched proteins. In scanning electron micrographs, the sole appears as a faint, longitudinally oriented band extending along the entire length of the animal. In all regions except the sensory border of the head, the microvilli are generally obscured by the densely arranged cilia. The sensory border consists of a row of toothlike papillae and grooves covered almost exclusively by microvilli, small club-shaped structures, and larger spherical protrusions.

While only a few descriptions have been made of the ultrastructural features of the epidermis of land planarians (Storch and Abraham, '72; Storch and Welsch, '77; Bautz, '77), considerably more information is available on the fine structure of the epidermis of other turbellarians, including representatives of Orders Tricladida (Pedersen, '59, '61, '63, '76; Török and Röhlich, '59; Skaer, '61; MacRae, '67; Best et al., '68; Bedini and Papi, '74; Bowen and Ryder, '74; Hay and Coward, '75; Bautz, '77; Smales and Blankespoor, '78; Hori, '79), Polycladida (Pedersen. '66; Bedini and Papi, '74; Lanfranchi et al., '81), Rhabdocoela (Bedini and Papi, '74), Macrostomida (Bedini and Papi, '74; Reuter, '78; MacKinnon et al., '81; Tyler, '81), Catenulida (Bedini and Papi, '74), Proseriata (Bedini and Papi, '74; Bedini et al., '75; Reuter, '78), Acoela (Pedersen, '64; Dorey, '65; Bedini et al., '73; Bedini and Papi, '74), and Lecithoepitheliata (Bedini and Papi, '74). Unlike most other turbellarians, land planarians possess a ventrally located structure used in locomotion known as a "creeping sole" (von Graff, 1899; Hyman, '51). The epidermis of the sole is noteworthy in some species because the cells comprising it are sunken deeply into the underlying connective tissue and muscle instead of being arranged above a basement membrane, as in conventional epithelia (von Graff, 1899; Hyman, '51). Such inward sinking of cells has also been observed in the cephalic sensory area of the land planarian *Bipalium kewense* (Storch and Abraham, '72); in the epidermis of other turbellarians which

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lack creeping soles, such as the acoel flatworms *Convoluta* and *Mecynostomum* (Pedersen, '64; Dorey, '65; Bedini and Papi, '74) and representatives of the Order Lecithoepitheliata *(Prorhynchus)* (Bedini and Papi, '74); in the adhesive glands of a wide variety of both archoophoran and neophoran turbellarian species (Tyler, '76); and in the pharynx simplex of a number of archoophoran species (Doe, '81); but it appears to be absent in the sole of the land planarian *Rhynchodemus (Microplana) terrestris* (Bautz, '77).

The present study of the epidermis of the land planarian *Bipalium adventitium* was undertaken to obtain basic information on some of the histological and cytological features of the creeping sole and contiguous nonsole regions of this species. Various histochemical procedures for the demonstration of nucleic acids, proteins, and mucosubstances were used to analyze the secretions produced by the glandular cells associated with the epidermis; and both scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were used to observe cell-surface specializations and other ultrastructural features of the epidermal cells. The principal impetus for the study was a conviction that the accumulation of detailed information on the integument and other organs of turbellarians might be of heuristic value in future taxonomical and/or physiological and biochemical studies of these organisms.

MATERIALS AND METHODS

Specimens identified as *Bipalium adventitium* Hyman (Hyman, '43) were collected under flagstones in the back yard of one of the authors (J.D.M.). Tissues were taken from a total of seven animals, all believed to be from a single population. Very little could be determined about the behavior and ecology of the animals because of their unpredictable and infrequent occurrence. For examination by light microscopy, small pieces of the animals were fixed in Bouin's solution (Lillie and Fullmer, '76), ethanol-acetic acid (3:1), or 10% neutral formalin containing 1% mersalyl acid (Sippel, '80) and embedded in Ester Wax 1960 (BDH Chemicals Ltd.; Poole, England; distributed in the U.S.A. by Gallard-Schlesinger Chemical Mfg. Corp., Carle Place, NY) by a protocol recommended by Drury et al. ('67). The ester wax was selected because the resulting sections displayed less compression than those obtained after conventional paraffin embedding. Sections were cut at approximately 5 μm, mounted on gelatinized slides (Rogers, '73), and stained by a number of histochemical procedures. In all instances, staining of the sections of *Bipalium* was compared with that of control sections of mouse trachea, esophagus, intestine, and ovary. Generalized nuclear and cytoplasmic staining was obtained with Lillie's azure A-eosin B method (Lillie and Fullmer, '76). Two methods were used for the demonstration of collagenous fibers: a modified Masson procedure in which staining with hematoxylin was omitted; and the fast green-Van Gieson method (Lillie and Fullmer, '76). An orcinol-new fuchsin method (Lillie and Fullmer, '76) was used for the demonstration of elastic fibers. DNA and RNA were stained selectively by the pH 4 methylene blue procedure of Deitch ('66), performed with and without extraction with DNase or RNase (Swift, '66). Tests for mucosubstances included the periodic acid-Schiff (PAS) method for neutral mucosubstances, performed with and without diastase pretreatment (Pearse, '68); and the use of alcian blue 8GX at pH levels of 1.0 and 2.5 for the demonstration of strongly acidic and weakly acidic mucosubstances, respectively (Pearse, '68). Total basic groups of proteins were demonstrated by staining at pH 2.8 with the acidic fluorochrome brillant sulfaflavine (Leemann and Ruch, '72). Other tests included the phenanthrenequinone procedure of Magun and Kelly ('69) for arginine; the p-dimethylaminobenzaldehyde procedure of Adams ('57) for tryptophan; the carbodiimide-hydroxy-naphthoic acid hydrazide method of Curtis and Cowden ('75) for carboxyl groups; and the Morel-Sisley reaction for tyrosine (Ritter and Berman, '63). Sulfhydryl (SH) and disulfide (SS) groups were demonstrated by a fluorescent version (Curtis and Cowden, '80) of a maleimide procedure developed by Sippel ('80). The sections were observed with a Zeiss Photomicroscope II supplied with Kodak Plus-X film.

For inspection by TEM, small pieces of the animals were fixed in 3% phosphate-buffered glutaraldehyde, rinsed in buffer, postfixed in 1% phosphate-buffered osmium tetroxide, dehydrated through a graded series of ethanol solutions, cleared in propylene oxide, and embedded in Spurr low-viscosity embedding media (Spurr, '69). Semithin (ca. 1 μm thick) sections were mounted on glass slides and stained in a 1% solution of toluidine blue in 1% sodium tetraborate. Ultrathin sections

were doubly stained with uranyl acetate (Watson, '58) and lead citrate (Reynolds, '63). Specimens prepared for SEM were fixed as for TEM, dehydrated through a graded series of acetone solutions, dried in a critical-point apparatus (Parr Instrument Co.; Moline, IL), mounted on stubs, and coated with gold in a Denton Desk-1 Cold Sputter-Etch Unit (Denton Vacuum; Cherry Hill, NJ) or a Denton DV-502 High Vacuum Evaporator supplied with a tilting omni rotary fixture. Material on grids was examined with an Hitachi H-500 TEM supplied with Kodak Electron Image film. Gold-coated material was examined with an Hitachi S-430 SEM supplied with Kodak Commercial film 4127.

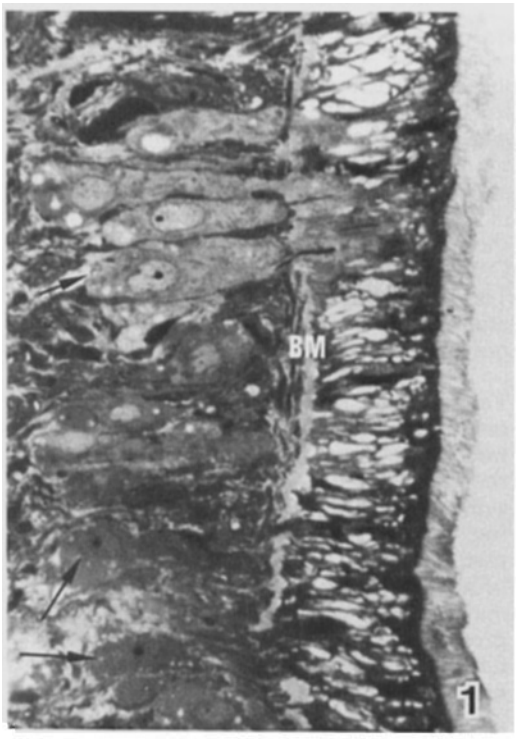
RESULTS

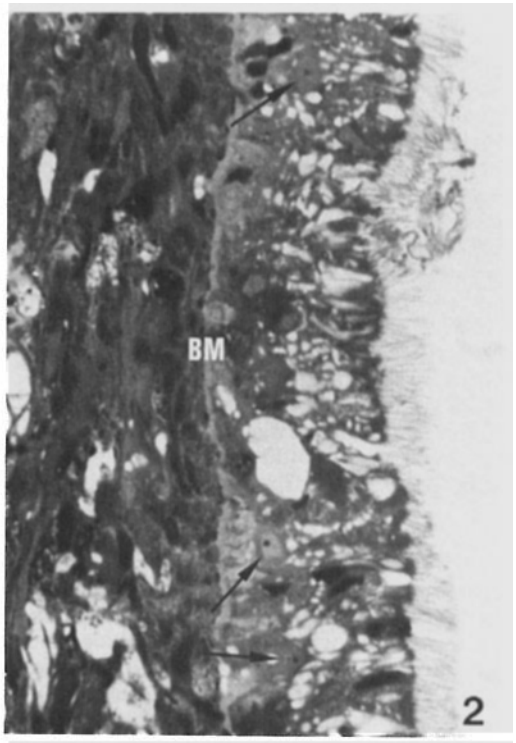
Light microscopy and histochemistry

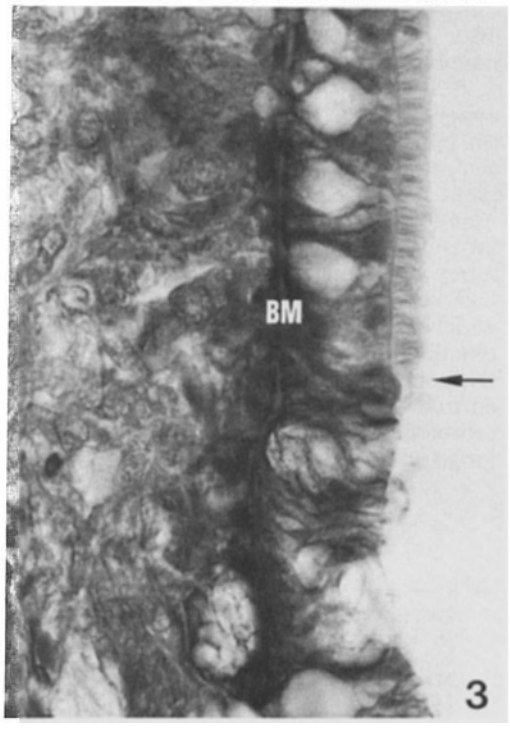
The epidermis of *Bipalium adventitium* consists of a simple columnar ciliated epithelium associated with a prominent basement membrane. In sections processed through any of the general staining routines, such as toluidine blue, azure A-eosin B, methylene blue at pH 4, the Masson procedure, or the fast green-Van Gieson sequence, the epidermis in the region of the sole can be distinguished easily from that of the remainder of the animal by the following features: an insunken appearance of the epithelial cells comprising the sole; a greater number of cilia per epithelial cell than in nonsole regions; an absence of rhabdites in the sole; and a larger number of mucous ("cyanophilous") gland cells in the sole (Figs. 1, 2). Transitions from the sole to contiguous nonsole regions are abrupt and easily perceived at histological levels (Fig. 3). After extraction with DNase and staining with methylene blue to demonstrate RNA, epithelial cells of the sole region display large quantities of coarse, heavily stained cytoplasmic material resembling Nissl substance (Fig. 4). Their nuclei usually contain single prominently rounded nucleoli. The cytoplasm of the insunken cells is stained very strongly by the PAS procedure (Fig. 5); and, since the staining almost disappears after pretreatment with diastase (Fig. 6), it is assumed that these cells contain large quantities of glycogen. Histochemical tests designed to reveal protein end-groups in all instances except that of the DACM procedure for SS groups yield generally distributed nuclear and cytoplasmic staining (Fig. 7). Staining with the DACM procedure for SS groups is confined almost entirely to extracellular locations, such as basement membranes and fibers associated with connective tissue in general, although rhabdites are also stained by this procedure (Fig. 8). Rhabdites are stained very strongly with acidic dyes used at low pH levels, such as biebrich scarlet in the Masson procedure, fast green in the Van Gieson routine, and brilliant sulfaflavine. The latter dye displays a rarely observed orange-red fluorescence metachromasia in the presence of rhabdites and thus is useful as an almost selective label for these structures. The phenanthrenequinone method of Magun and Kelly ('69) for arginine yields generalized nuclear and cytoplasmic staining, but the rhabdites are only very weakly fluorescent, if at all. Very weak or negligible staining of the rhabdites is also obtained with methods selective for tryptophan, carboxyl groups, tyrosine, and SH groups. Cilia are stained especially well with all protein end-group procedures except the DACM method for SS groups (Figs. 3, 7). The Lillie and Fullmer method for revealing elastin yields negative results; but procedures known to stain collagenous fibers (Masson, Van Gieson, PAS after diastase) reveal a prominent basement membrane underlying the epidermis (Figs. 3, 6). In the sole, the basement membrane appears to be interrupted at sites at which the epithelial cells have sunken inward from the surface. Immediately beneath the basement membrane, there is a layer of smooth muscle comprising the subepidermal musculature described by Hyman ('51). While in many turbellarian species this musculature is subdivided into outer and inner layers in which the fibers are arranged primarily in circular and longitudinal directions, respectively (Hyman, '51), in *Bipalium* the subepidermal musculature is very thin and composed predominantly of small bundles of longitudinally oriented fibers. The circularly

Abbreviations

BM, Basement membrane
G, Golgi complex
L, Lamellated inclusions
M, Mitochondrion
MC, Muscle cell
Mu, Mucus
N, Nucleus
NC, Nerve cell process
NS, Nonsole portion of epidermis
p, Club-shaped protrusion
RER, Rough-surfaced endoplasmic reticulum







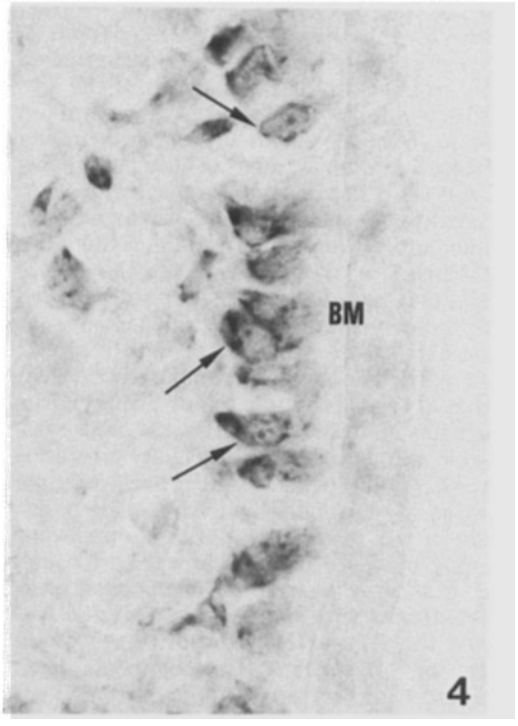


Fig. 1. Cross section of *Bipalium adventitum* sole showing insunken epithelial cells (arrows), basement membrane, and ciliated surface. Toluidine blue-stained plastic section. ×1,094.

Fig. 2. Cross section of dorsal surface of *Bipalium* showing epithelial cell nuclei (arrows) located above the basement membrane. Toluidine blue-stained plastic section. ×1,094.

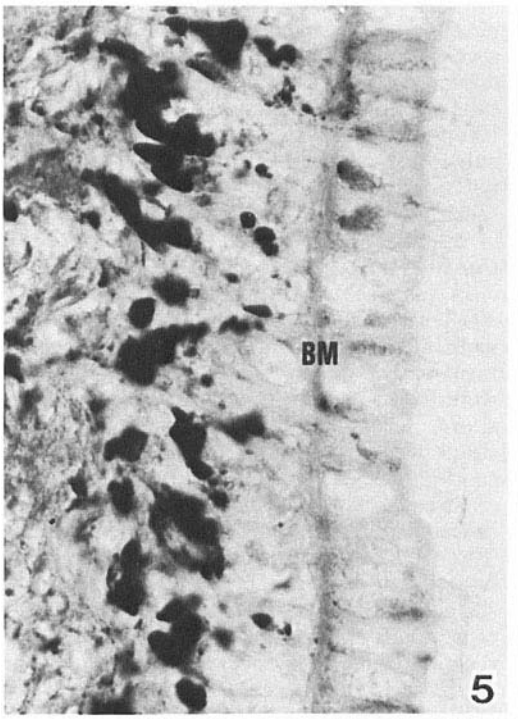
Fig. 3. Masson-stained ester wax cross section of junction between the sole (above the arrow) and nonsole regions. Cilia do not disappear in nonsole regions, but they are less conspicuous than in the sole. Note prominent staining of basement membrane. ×1,094.

Fig. 4. Ester wax cross section of sole stained with methylene blue after DNase extraction. Insunken epithelial cells (arrows) contain prominent nucleoli and clumps of cytoplasmic material resembling Nissl substance. ×1,094.

arranged components are seen with great difficulty, even in material stained by the Masson, Van Gieson, or DACM procedures (Fig. 9). The subepidermal musculature is underlain by a more highly developed parenchymal musculature (as defined by Hyman, '51), composed of a complex network of fibers which—in random sections through the animals—appear to be oriented in all directions—circularly, longitudinally, and obliquely. Apparently only one type of unicellular gland cell is associated with the sole: an insunken, roughly goblet-shaped cell whose apical cytoplasm, usually expanded, contains products reactive with the PAS procedure (with and without diastase) (Figs. 5, 6) but weakly stained with alcian blue at pH 1.0 and 2.5. Mucous cells with similar staining properties are found in nonsole regions of the epidermis; but they are fewer in number than in the sole.

Transmission electron microscopy

By transmission electron microscopy, it can be seen that the cells comprising the insunken epithelium are roughly flask-shaped in appearance, with the broad basal (proximal) portion containing the nucleus and the narrower apical (distal) region forming the free surface (Fig. 10). The nuclei of the cells are generally rounded in shape, although small folds of the nuclear membrane usually give them a rather scalloped outline. While the nuclei contain a predominance of very loosely organized chromatin, small and irregularly shaped patches of more highly condensed chromatin are scattered through the nucleoplasm and often are found in association with the inner surface of the porous nuclear membrane. Usually at least one nucleolus can be found in each nucleus (Figs. 10, 11). In addition, large inclusions—easily confused with nucleoli at the light microscopic level—are found in some, but not all of the nuclei (Figs. 12–14). These complex inclusions are variable in appearance and often contain regions which appear to resist infiltration with plastic. The inclusions can be found in the nuclei of both insunken and conventional epithelial cells, and they are frequently present in nuclei displaying ordinary nucleoli. At higher magnifications (e.g., ×100,000), viruses or other microorganisms have not been observed; and the identity and significance of the inclusions remain unknown at the present time. The basal portions of the cells immediately underlying the nuclei are typically filled with large deposits of glycogen. Small Golgi complexes, scattered mitochondria, and patches of free ribosomes as well as small stacked cisternae of the rough-surfaced endoplasmic reticulum (RER) are found frequently in the cytoplasm (Fig. 15). It appears likely that the latter comprise the Nissl-like substance visible by light microscopy. Slender microvilli and very numerous cilia, possessing the usual nine-plus-two organization of microtubules, project from the surface of the cells (Figs. 16, 17, 23). The rootlets of the cilia are not as prominent as those of other species of turbellarians, nor are the terminal webs of the cells well developed. In nonsole regions, the ciliary rootlets are visible occasionally in cross sections of the animals as short, cross-striated, vertically oriented structures (Figs. 16, 23), while in the sole the rootlets are cut transversely or obliquely in cross sections and thus appear to be oriented horizontally—i.e., parallel to the cell surface (Fig. 17). Cilia with longer than usual rootlets resembling those of receptor cells described by Storch and Abraham ('72) are seen occasionally (Figs. 16, 23); but the cilia of receptor cells are generally difficult to identify because of the very high density of conventional cilia. Numerous mitochondria are concentrated immediately beneath the free surfaces of the cells (Fig. 17). Junctions between adjacent epithelial cells are very difficult to see except at the free surfaces of the cells. Here it is evident that at least some of the cells are joined to one another by elaborate ladderlike septate junctions (Figs. 16–18). These junctions are most evident at points of contact between glandular and regular epithelial cells (Figs. 17, 18); but they can also be seen occasionally between adjacent regular epithelial cells. From inspection of ultrathin sections made randomly through the animals, it was not possible to determine whether or not all cells participate to some extent in junctions of this type. The basement membrane, visible by light microscopy, consists of fine fibrils resembling collagenous fibrils (Figs. 19, 20). The fibrils are oriented in all directions and do not display prominent cross-banding, although they have a slightly beaded appearance when they are examined at higher magnifications (Fig. 19). After conventional fixation, embedment, and staining of the kind used in the present study, a basal lamina is not visible beneath the epidermal cells; nor is there any evidence that the basement membrane is organized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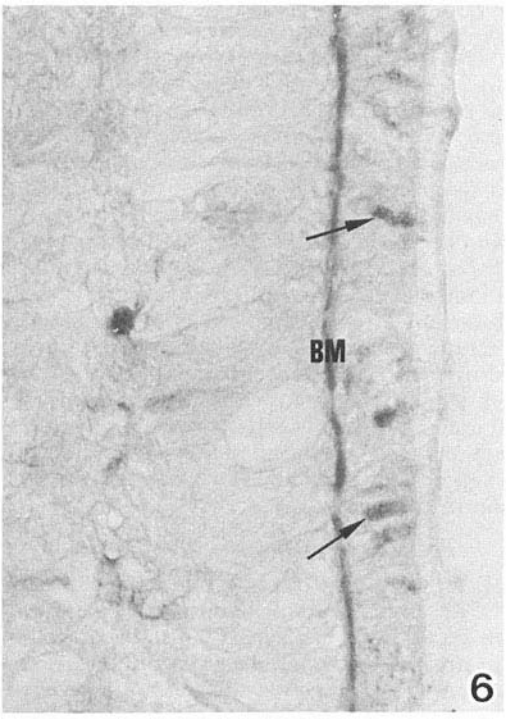
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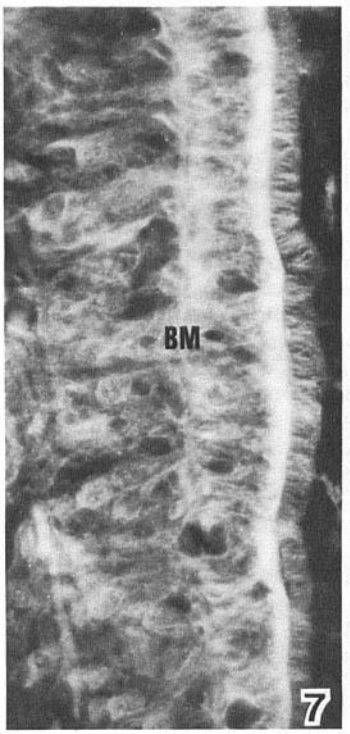
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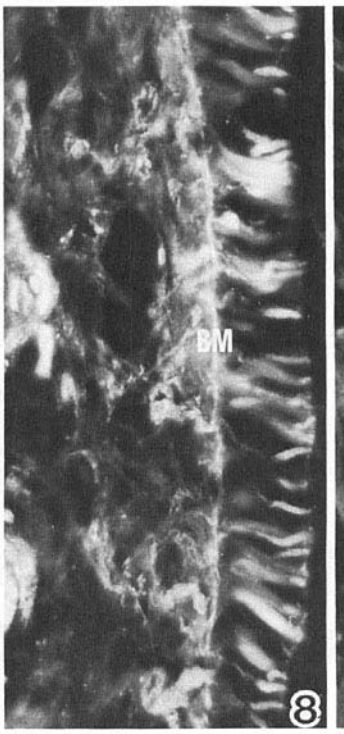
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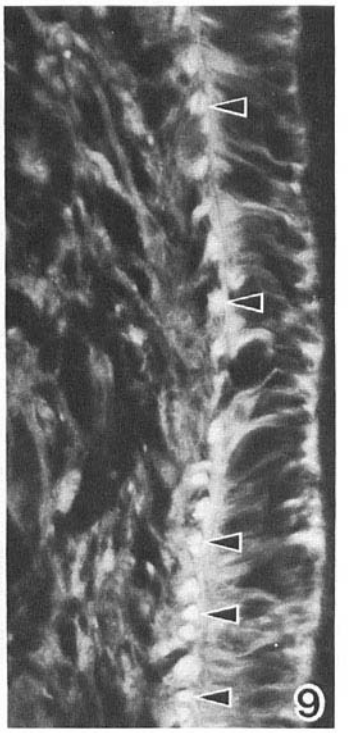
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into layers. Muscle cells comprising the subepidermal musculature possess thick and thin myofilaments and other attributes of invertebrate smooth muscle cells (Figs. 20–22). Processes of nerve cells, found frequently among muscle cells, contain two types of membrane-enclosed inclusions: small, clear vesicles and larger vesicles filled with an electron-dense material (Figs. 20–22). The processes closely approach the surfaces of muscle cells and appear to form functional endings (Figs. 21, 22), although such endings are small and seem to lack any special attributes other than the presence of vesicles. The presence of vesicles and the proximity of muscle cells and nerve processes strongly suggest that the processes are not merely passing through the tissue.

Nonsole regions possess epithelial cells resembling the insunken epithelial cells of the sole, but these are found in a conventional position above a basement membrane; and the glycogen granules of these cells are not aggregated into large deposits as in insunken cells of the sole. Although the plasma membranes of the cells are difficult to see except at the apical surfaces where the cells are conjoined by septate junctions (Fig. 23), the epithelium appears to be cellular, rather than syncytial, in all regions. In addition to mucus, two other types of secretory products are found in nonsole regions: rhabdites and small lamellated granules (Fig. 23). Both of the latter products can be considered "eosinophilous" because of their affinity for acidic dyes. Rhabdites are found in nearly every nonsole epithelial cell and appear to be produced by the epithelial cells themselves. The lamellated granules, whose size is nearly at the limit of resolution of the light microscope, closely resemble those found in the eosinophilous gland cells of *Dugesia* (Török and Röhlich, '59; Pedersen, '63) and *Bipalium kewense* (Storch and Abraham, '72). Clusters of cells containing lamellated granules in varying stages of development are found in the parenchyma beneath the epidermis and thus appear to be insunken epithelial cells. These cells have all of the attributes of very active secretory cells, including a prominent rough endoplasmic reticulum (RER) with distended cisternae, multiple Golgi complexes, and mitochondria (Fig. 24).

Scanning electron microscopy

At low magnifications, the sole can be seen as a faint, longitudinally oriented band approximately 450 μm in width extending along the entire length of the animal (Fig. 25). Because of the contraction of the body musculature at the time of fixation, transversely oriented folds and creases are produced, which, in turn, give the animals an almost segmented appearance. At present there seem to be no adequate methods for relaxing land-living planarians without simultaneously causing the discharge of glandular secretions. The dorsal surface closely resembles nonsole portions of the ventral surface (Fig. 26). All regions are covered to variable degrees with masses of extracellular material, assumed to be mucus and other glandular products combined with adherent debris of various kinds. Although dorsal and ventral surfaces can be distinguished at low magnifications by the presence of the sole in the latter, such a distinction becomes very difficult to make at higher magnifications because of the high density of cilia in all regions—dorsal and ventral, sole and nonsole (Figs. 27, 28). The differences between the sole and contiguous nonsole regions are much more subtle than might be expected from the results obtained by light and transmission electron microscopy. The cilia are approximately 0.2 μm in diameter and are tapered and slightly flattened at their free ends; and they do not possess notching of any kind (Figs. 27, 28). While the large numbers of cilia obscure such details as individual cell

Fig. 5. Ester wax cross section of sole stained by the PAS reaction. Insunken epithelial cells to left of the basement membrane are almost obscured by heavy staining. ×1,094.

Fig. 6. Ester wax cross section of sole stained by the PAS procedure after diastase treatment. Staining persists in basement membrane and cytoplasm of mucous cells (arrows) but is lacking in insunken epithelial cells. Compare with Figure 5. ×1,094

Fig. 7. Ester wax cross section of sole stained with brilliant sulfaflavine. Cilia and apical regions of the epithelial cells display strong fluorescence. ×1,094.

Fig. 8. Ester wax cross section of dorsal region stained with DACM for the demonstration of SS groups. Rodlike rhabdites and the basement membrane display prominent fluorescence. ×1,094.

Fig. 9. Ester wax cross section of dorsal region stained with DACM for the demonstration of SH groups. Small bundles of longitudinally oriented muscle fibers (arrowheads) can be seen in the subepidermal region. Circularly arranged components of the subepidermal musculature are seen with great difficulty. ×1,094.

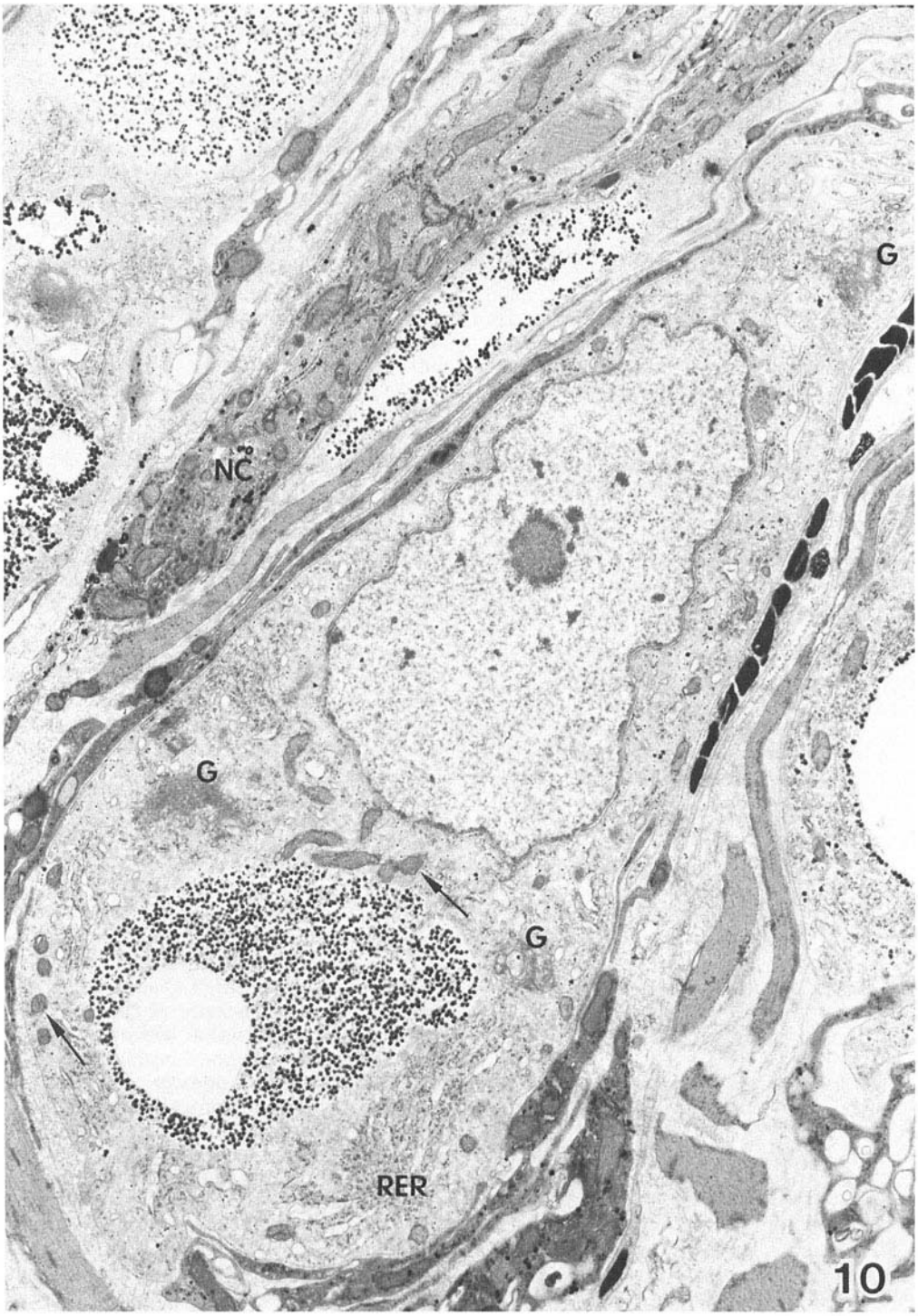
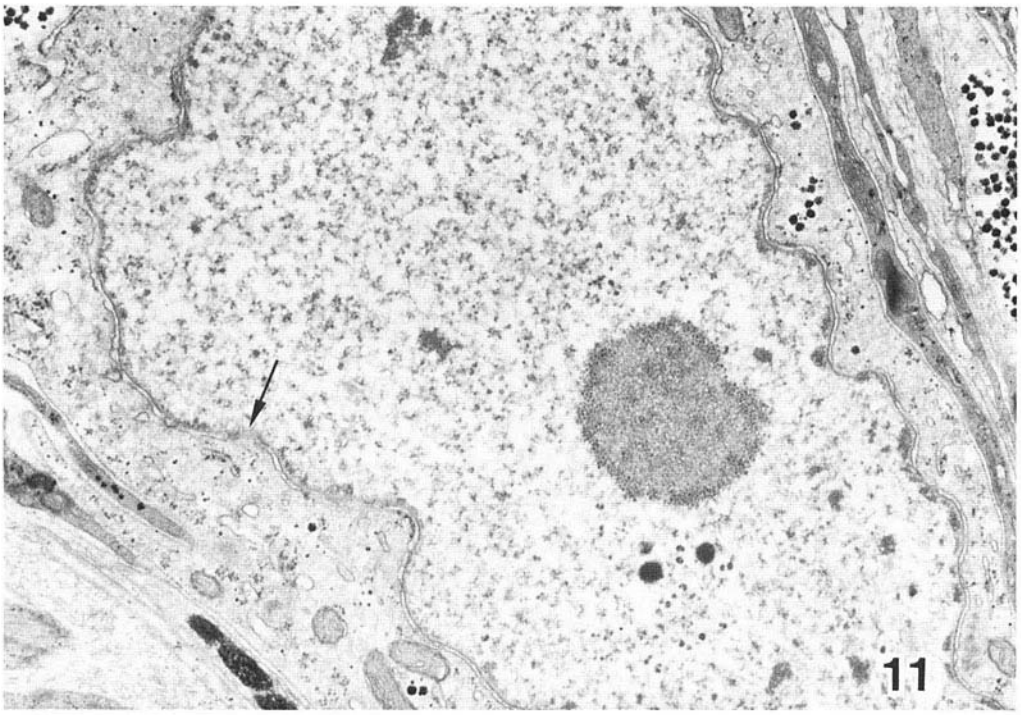
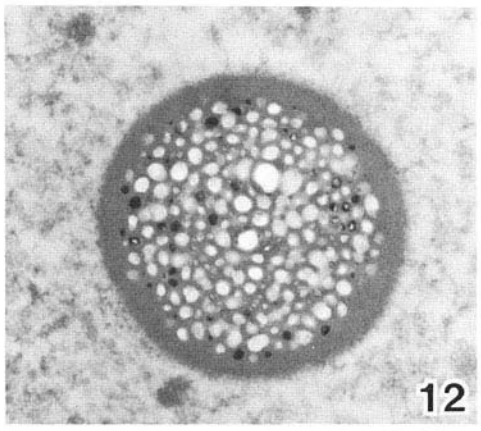
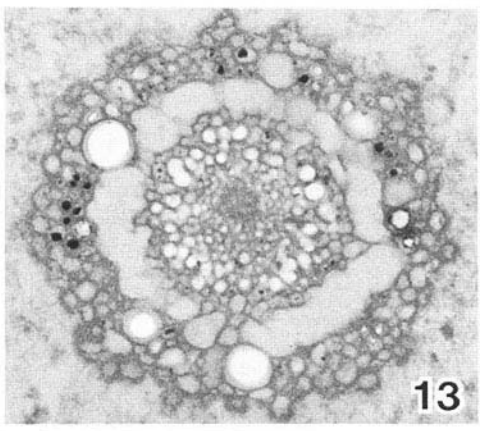


Fig. 10. Portions of three insunken epithelial cells of the sole. The cell in the center displays a prominent nucleus and nucleolus, Golgi complexes, RER, mitochondria (arrows), and a conspicuous subnuclear deposit of glycogen. Note nerve cell process containing vesicles and mitochondria. ×11,250.







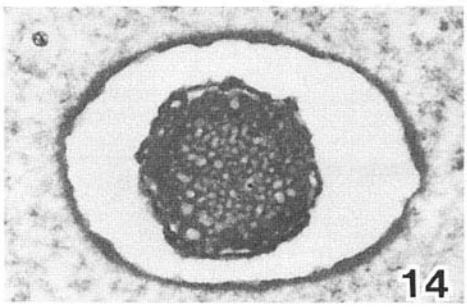
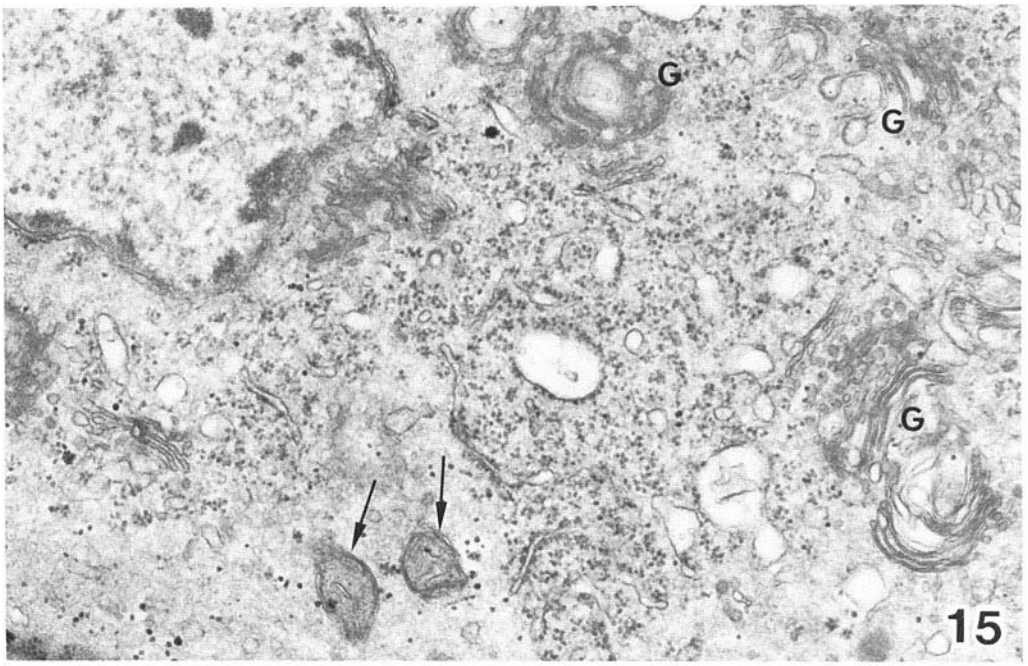


Fig. 11. Enlarged portion of a nucleus of an insunken epithelial cell of the sole. Note pore (arrow) and condensation of chromatin on inner surface of the nuclear membrane and in patches scattered throughout the nucleoplasm. ×20,000.

Figs. 12–14. Unidentified nuclear inclusions in insunken epithelial cells of the sole. Round areas in inclusions shown in Figures 12 and 13 appear to have resisted infiltration with plastic. ×30,000.



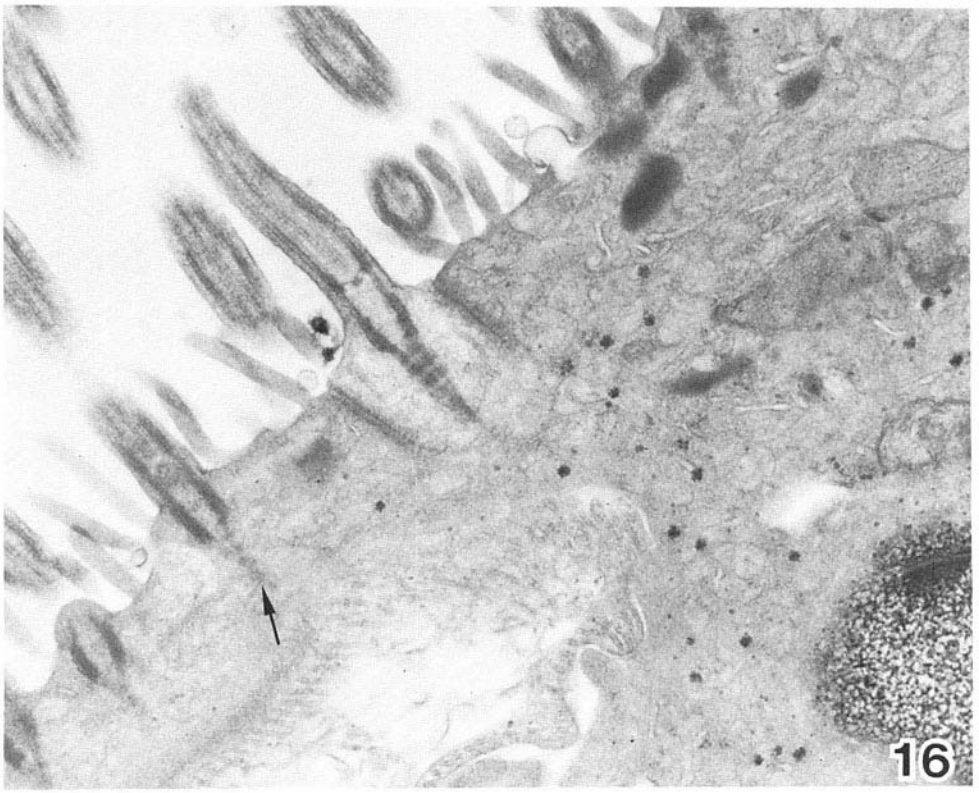


Fig. 15. Cytoplasm of insunken epithelial cell of sole containing Golgi complexes, mitochondria (arrows), RER, and free ribosomes. A small portion of the nucleus is in the upper left corner. ×30,000.

Fig. 16. Cilia and microvilli on surface of epithelial cells as seen in cross section of nonsole region. Cilium of a possible receptor cell displays a prominent striated rootlet. Rootlet of conventional cilium (arrow) is much less distinct. ×30,000.

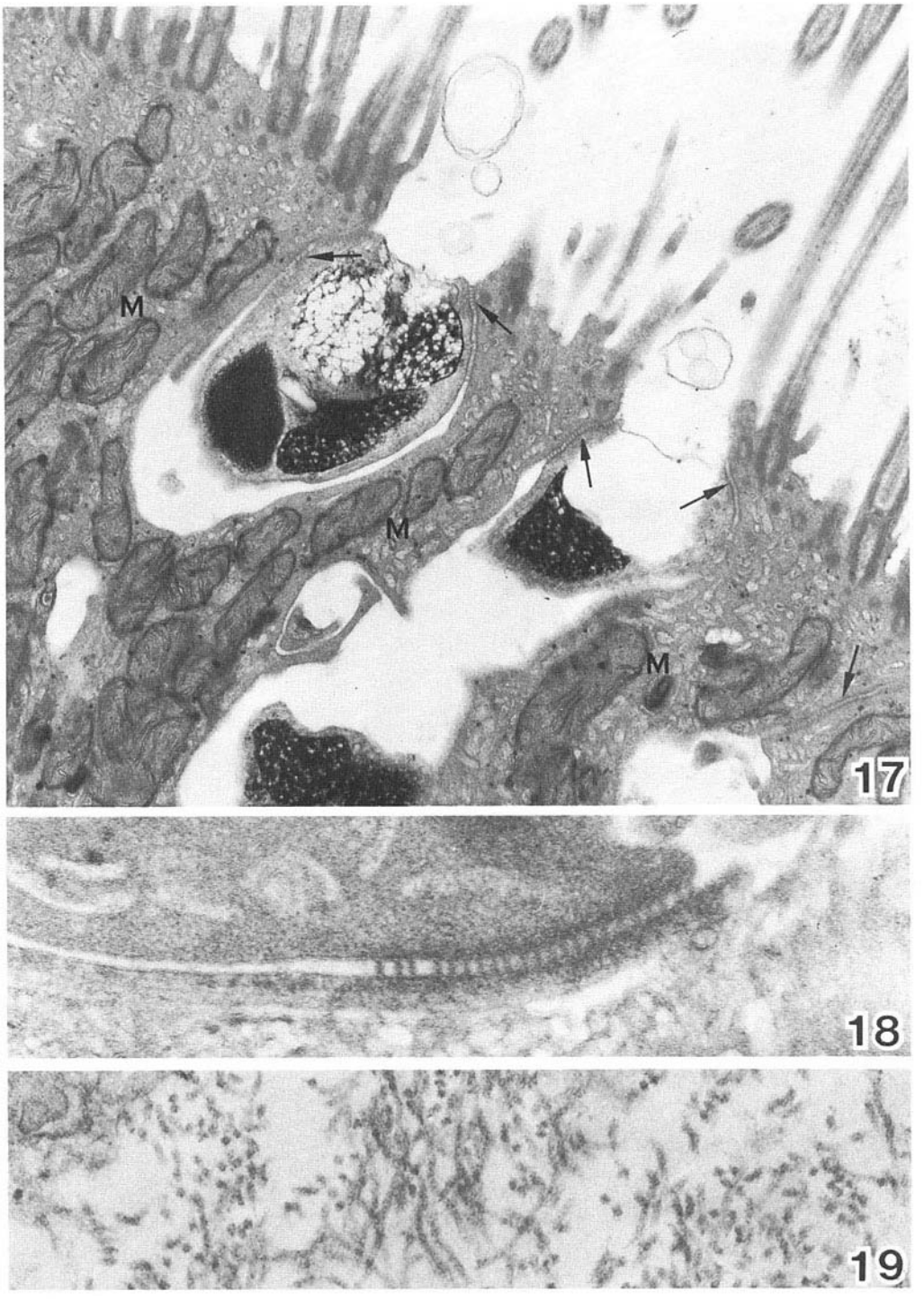
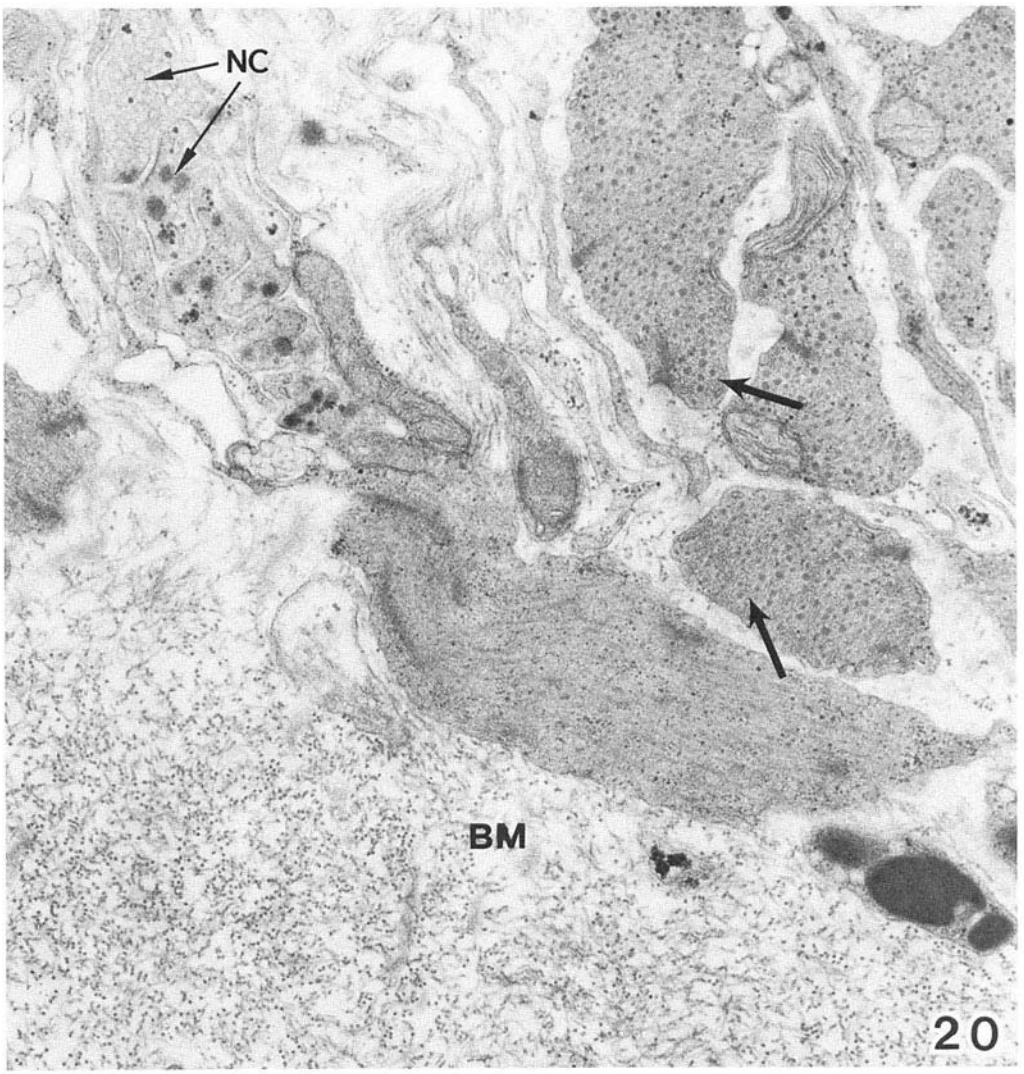
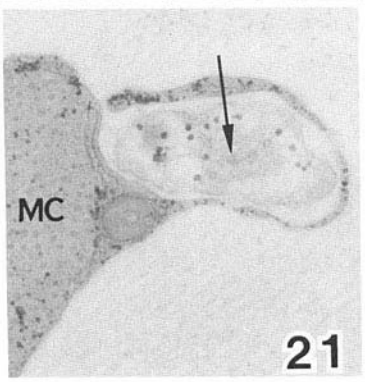


Fig. 17. Cilia and microvilli on surface of epithelial cells as seen in cross section of sole. Densely stained, weblike material is mucus in apical portions of mucous cells. Mucous and regular epithelial cells are joined by ladderlike septate junctions (arrows). Ciliary rootlets in cross section appear as small, rounded structures adjacent to the basal bodies of the cilia. Note large numbers of mitochondria. ×24,000.

Fig. 18. Septate junction between mucous cell (below) and regular epithelial cell of sole. ×105,000.

Fig. 19. Fibrils of basement membrane as seen in cross section of sole region. Note beaded appearance of fibrils but lack of distinct cross-banding. ×80,000.





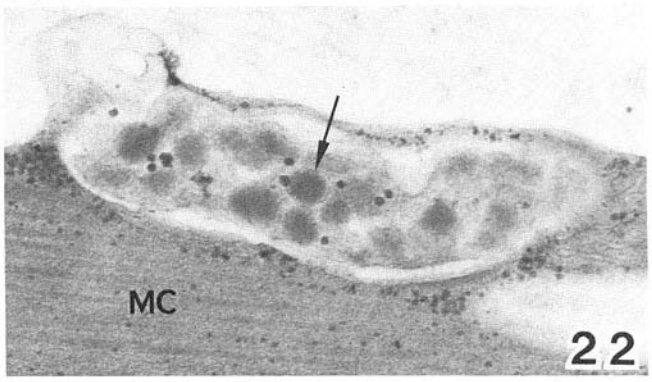


Fig. 20. Cross section of basement membrane region of sole showing orientation of fibrils in all directions and absence of layering. Smooth muscle cells in cross section display thick and thin filaments (arrows). Process of nerve cell contains small clear vesicles and larger, more electron-dense vesicles. ×30,000.

Fig. 21. Small process of a nerve cell (arrow) enveloped by a slender cytoplasmic process of a muscle cell. Note proximity of the plasmalemmas of the two kinds of cells. Glycogen granules of nerve cell process are much larger than those in the cytoplasm of the muscle cell. Myofilaments of muscle cell appear in cross section. ×37,500.

Fig. 22. Small process of nerve cell (arrow) closely associated with surface of muscle cell. Note large size of glycogen granules in process of nerve cell. Myofilaments of muscle cell appear in longitudinal section. ×52,500.

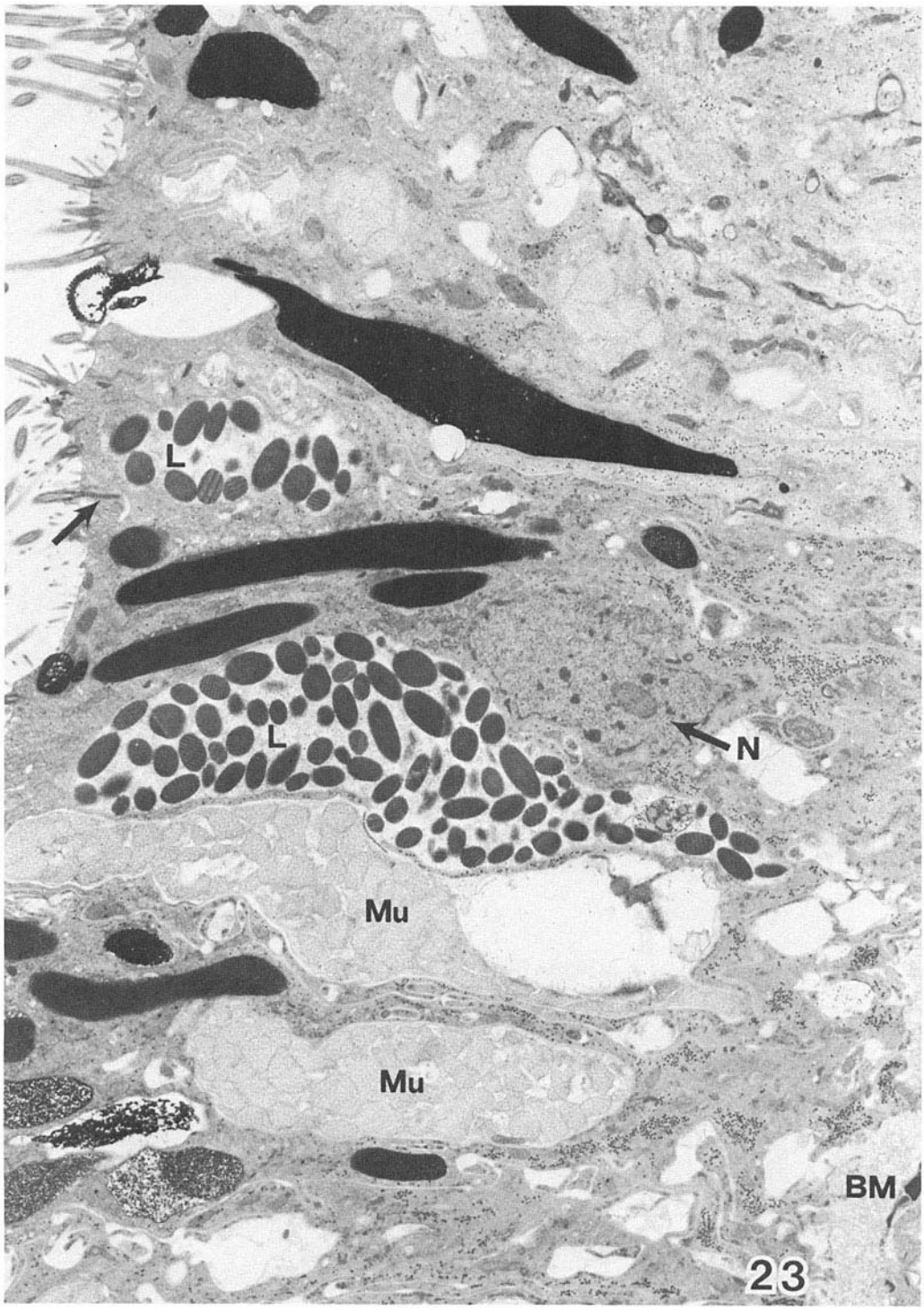


Fig. 23. Cross section of nonsole region showing general organization of the epidermis. Free surface is shown to the left, and a small portion of the basement membrane can be seen in the lower right corner. Note position of nucleus of one of the cells above the basement membrane, mucus-filled cytoplasm, lamellated granules, and densely stained rodlike rhabdites. Microvilli and cilia are visible on the free surfaces of the cells. The cilium of a possible receptor cell, recognized by its long rootlet (arrow), is visible. ×6,750.

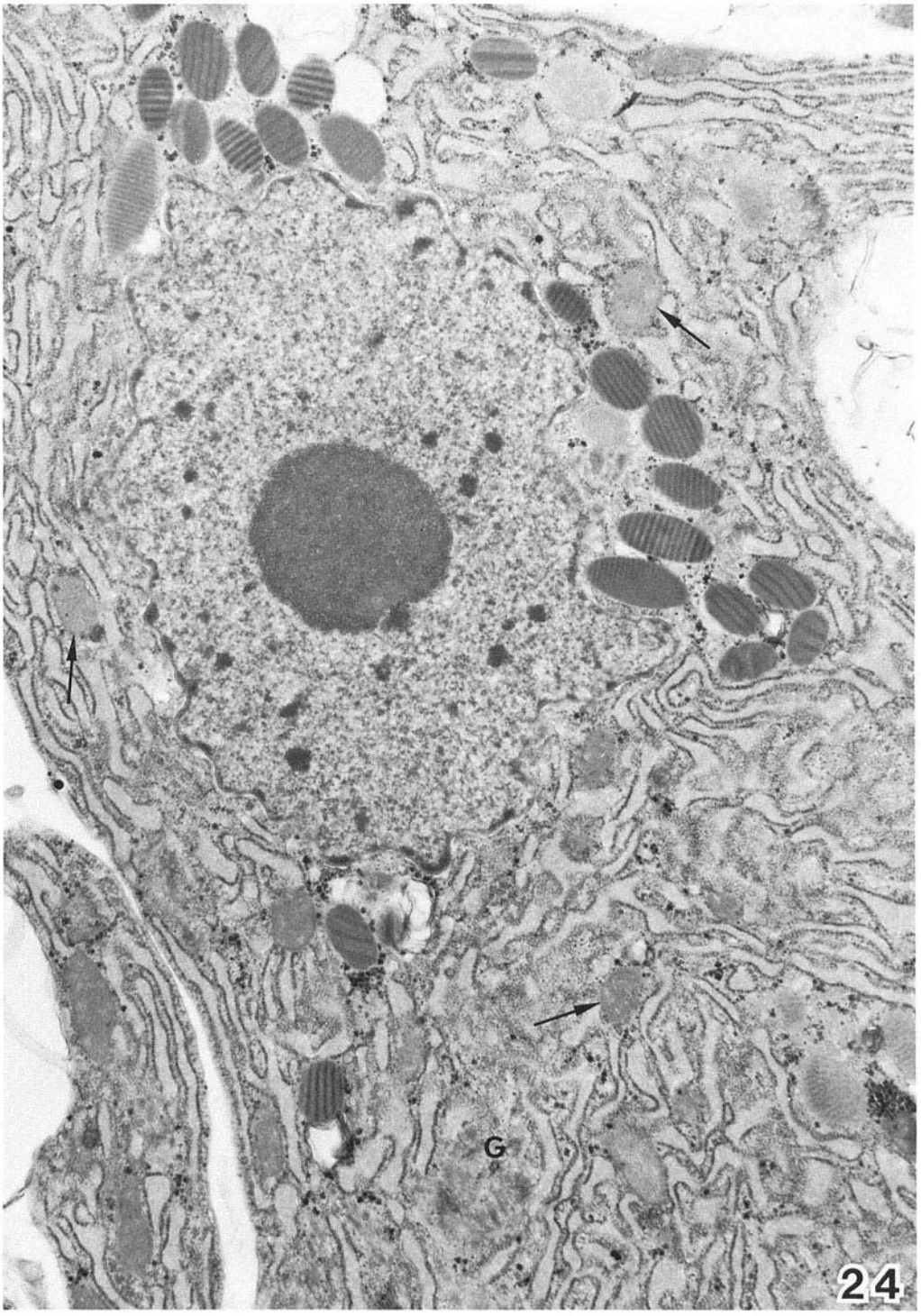


Fig. 24. Gland cell found in parenchyma underlying nonsole region containing lamellated cytoplasmic granules, scattered mitochondria (arrows), small Golgi complex, and extensive RER with distended cisternae. Note prominent nucleolus. ×18,000.

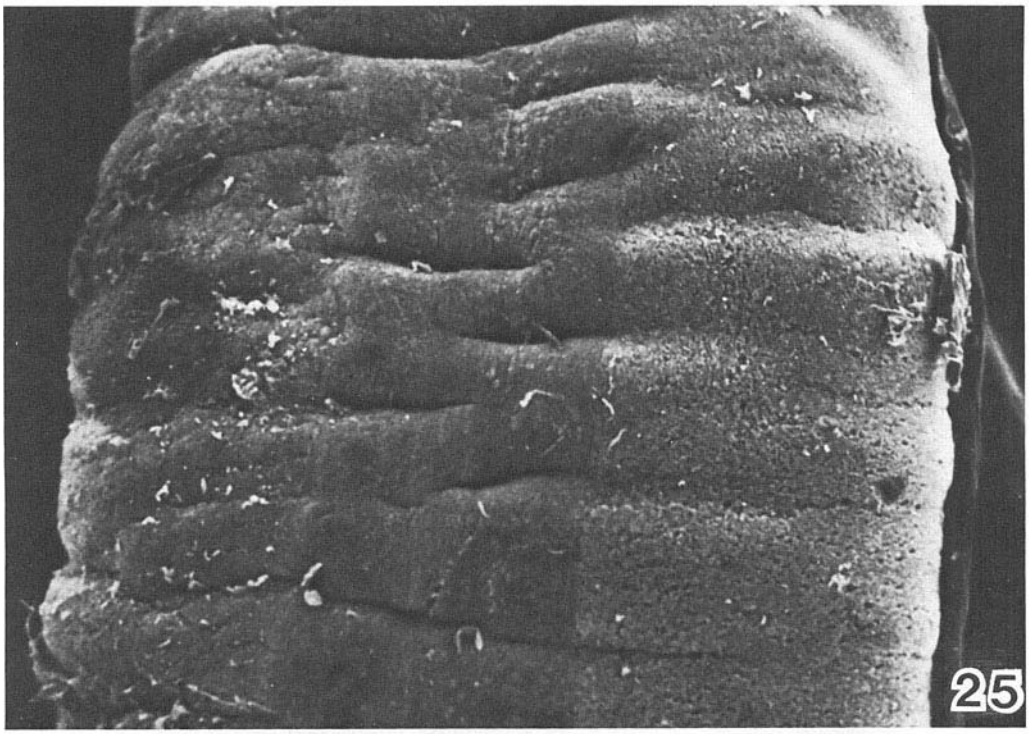


Fig. 25. Ventral view of *Bipalium* showing bandlike, longitudinally oriented creeping sole. Folds and creases are an artifact induced by fixation. ×87.5

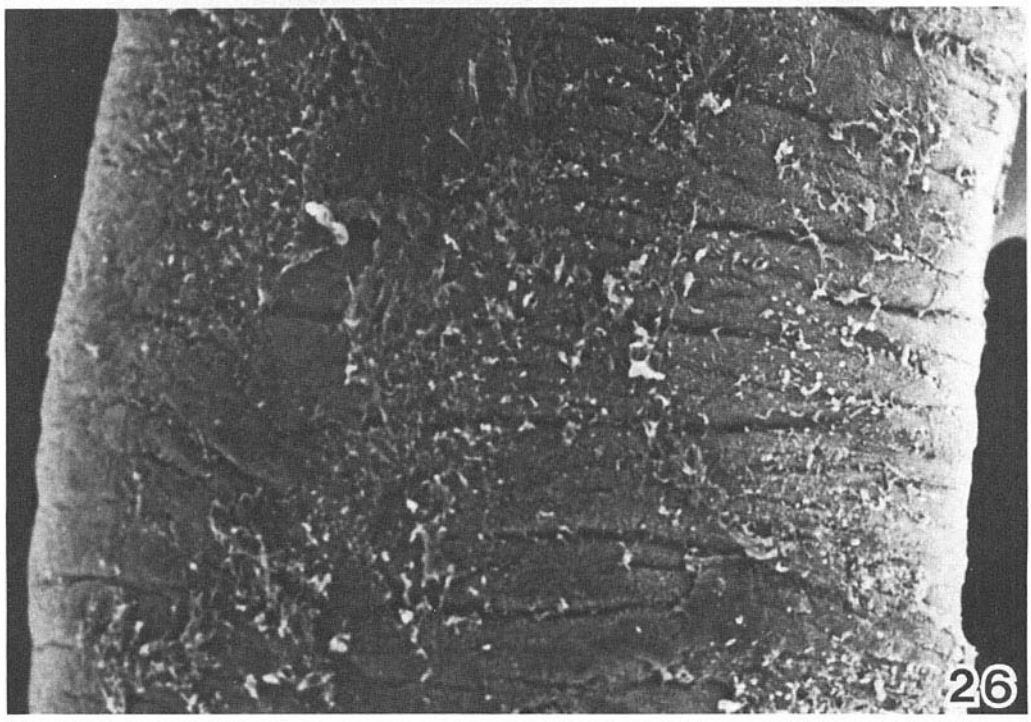


Fig. 26. Dorsal view of *Bipalium* showing irregular patches of mucus and adherent material. ×87.5

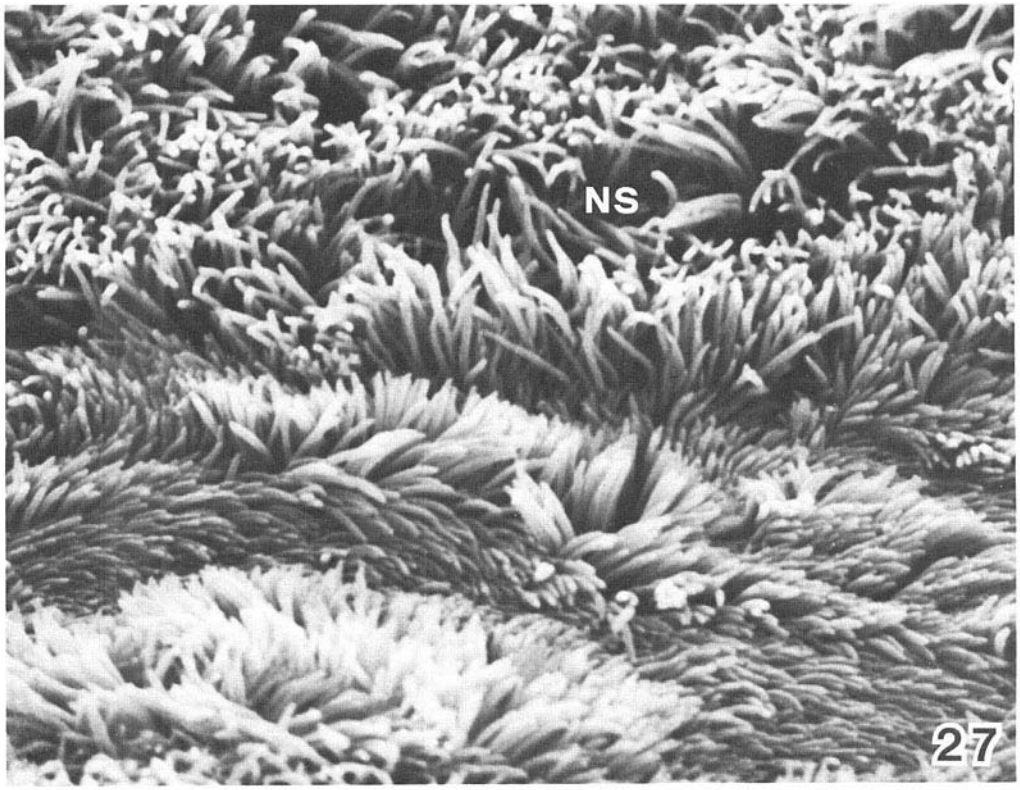


Fig. 27. Ventral view showing arrangements of cilia. Nonsole region occupies approximately the upper third of the micrograph, while the sole occupies the remainder. At this magnification, the organization of cilia of the sole into rows is seen with difficulty. ×5,600.

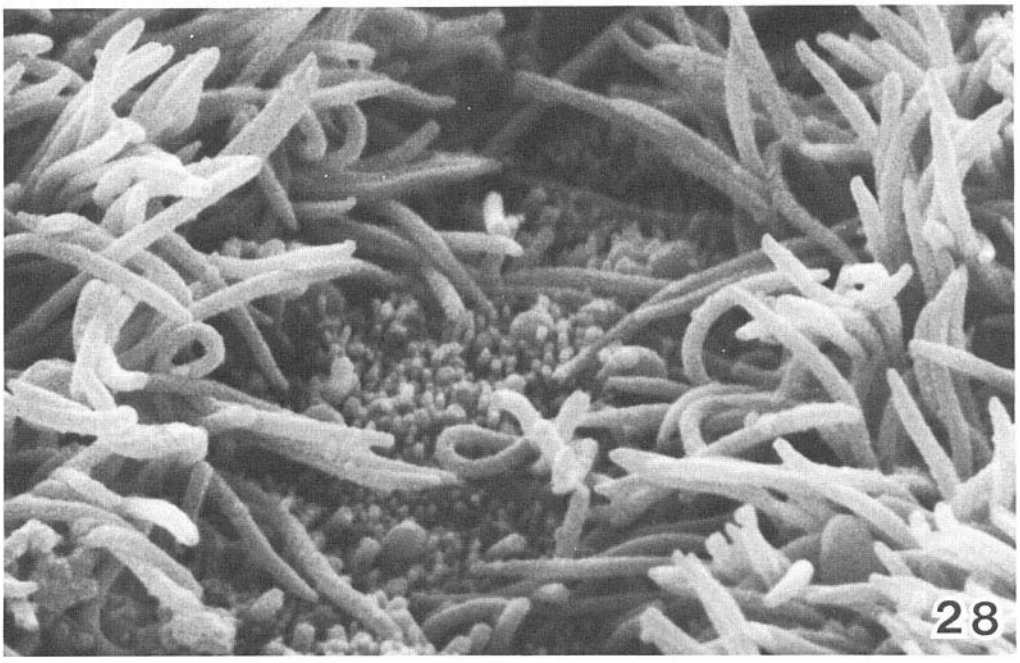


Fig. 28. Dorsal view showing cilia and microvilli. Cilia are slightly tapered and flattened at their tips, but they do not display irregularities of any kind. ×11,725.

boundaries, extrusions of glandular cells, and possible sensory cell processes, microvilli are visible occasionally, especially in nonsole and dorsal regions (Fig. 28). In general the cilia seem to be distributed randomly over the entire animal; but it is possible to discern in the sole a faint indication of an arrangement into longitudinally oriented rows (Fig. 29). These rows of cilia are less apparent in the center of the sole than in peripheral areas immediately adjacent to nonsole regions (Fig. 29). As the magnification is increased to a point at which individual cilia are visible, such distributions are much more difficult to perceive (Fig. 27).

The epidermis of the most anterior margin of the head differs from that found elsewhere since it is covered almost entirely by microvilli, rather than cilia. This portion of the head, comprising the sensory border described by Storch and Abraham ('72), consists of a series of toothlike papillae with intervening grooves (Fig. 30). The papillae are covered predominantly with microvilli, although somewhat larger, club-shaped projections and spherical structures of various sizes are also present (Figs. 31, 32). The latter are especially large and conspicuous in the ventral portions of the papillae (Fig. 31). Cilia extend from the dorsal surface into the grooves and to some extent over the lateral surfaces of the papillae (Fig. 31). The cilia in the grooves do not seem to differ in structure from those found elsewhere. Although *Bipalium adventitium* possesses numerous small eyespots along the margins of the head on both the dorsal and ventral surfaces (Hyman, '43, '51), these eyespots are not visible by SEM.

DISCUSSION

The ultrastructural characteristics of the epidermis of *Bipalium* conform in general to those presented by Rieger ('81a) as typical of the neoophoran epidermis, which is present in members of Orders Polycladida, Tricladida, Rhabdocoela, Lecithoepitheliata, Prolecithophora, and Proseriata. Some of the salient features of this epidermis include a thick basement membrane; a poorly developed terminal web in the epithelial cells; the presence of either single horizontally oriented (rostral) ciliary rootlets or of a combination of vertical and rostral rootlets; and an occasional sinking of the nuclei of the cells below the level of the basement membrane (Rieger, '81a). In *Bipalium*, however, the basement membrane does not display distinct layering of the kind described by Bowen and Ryder ('74) in *Polycelis tenuis* or by Hori ('79) in *Dugesia japonica*; and the ciliary rootlets in cells of the sole appear to be oriented horizontally in relation to the cell surface, while those in the cells of nonsole regions seem to be oriented vertically. Rieger ('81b) suggested that rostral ciliary rootlets might have an anchoring function, since they tend to extend in a direction opposite to that of the effective stroke of the cilia.

Sinking of the nuclei of the epidermal cells below the level of the basement membrane in *Bipalium* seems to be confined to two regions: the sole, as indicated by the results of the present study; and the sensory border of the head as described by Storch and Abraham ('72). In these regions, the nuclei of all cells appear to be involved—a condition by no means observed in the epidermis of all land planarians. As indicated by von Graff (1899), the epidermis of various species of land planarians may display considerable variability in the degree of insinking of the nuclei. Thus, the nuclei of all of the cells of a given regions may be located beneath the basement membrane; or the nuclei of only some of the cells in a given area may be in an insunken position while others are located in a conventional position above the basement membrane. The epidermal cells associated with the creeping soles of most land planarians are often in an insunken position; but this is not always the case (von Graff, 1899; Bautz, '77). An insunken condition is typical of the epidermis of members of Class Cestoda and Class Trematoda, although in these instances the epidermis is also truly syncytial: The nuclei of the cells, each enveloped by cytoplasm, are found beneath the basement membrane; but the apical portions of the cells are in confluence (Threadgold, '62, '63; Björkman and Thorsell, '64; Burton, '64; Lumsden, '66; Lyons, '77). While the epidermis of various turbellarians of the neoophoran orders may display a tendency to become syncytial through the development of extra basal infoldings (Rieger, '81a), a truly syncytial epidermis, as in the case of that of the rhabdocoel *Gyratrix hermaphroditus* (Bedini and Papi, '74), seems to be the exception rather than the rule.

The presence of an insunken epithelium in two regions of *Bipalium* which perform different functions—locomotion in the creeping sole and sensory reception in the margin of the head—suggests that an insunken condi-

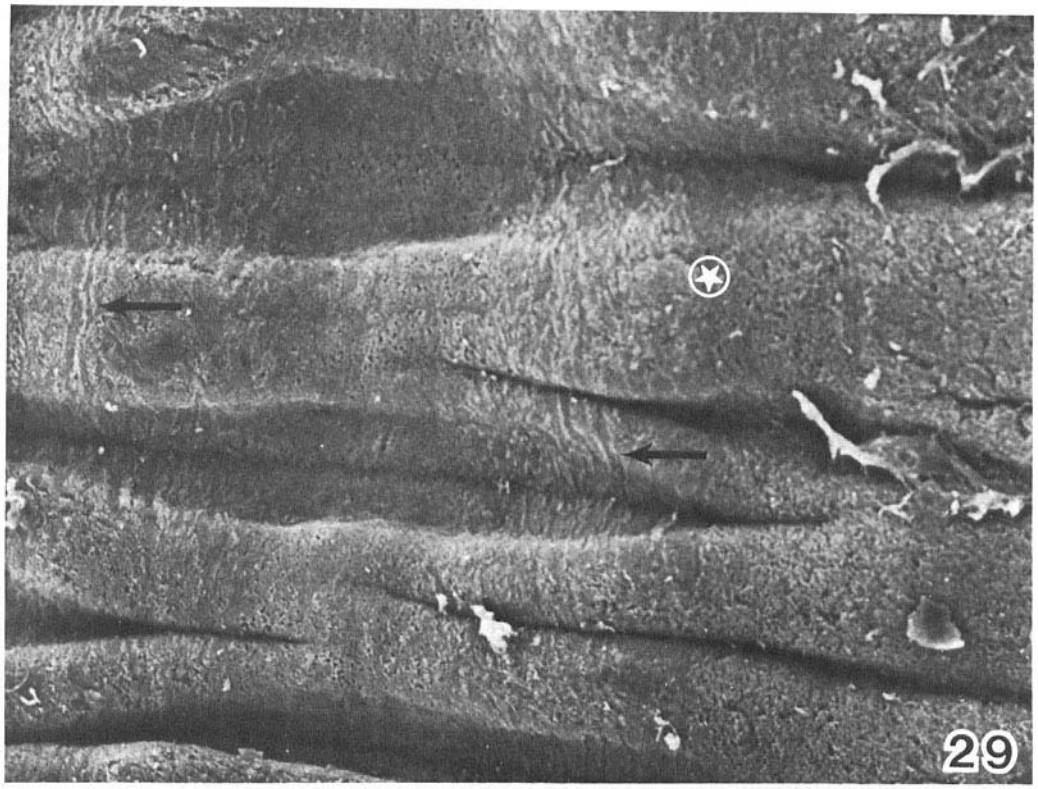


Fig. 29. Ventral view showing possible organization of cilia in longitudinally oriented rows in the peripheral portions of the sole (arrows). Note that center of the sole seems to lack such rows of cilia. Region to the right of the star is nonsole area. ×192.5

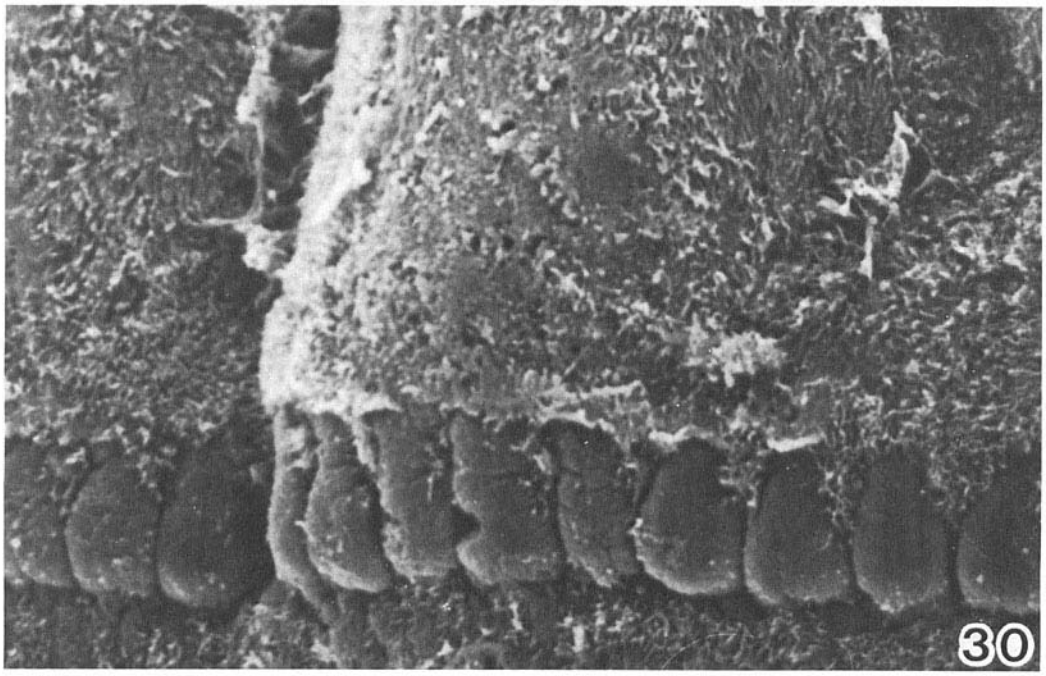


Fig. 30. Toothlike papillae and grooves of the sensory border of the head of *Bipalium*. ×900.

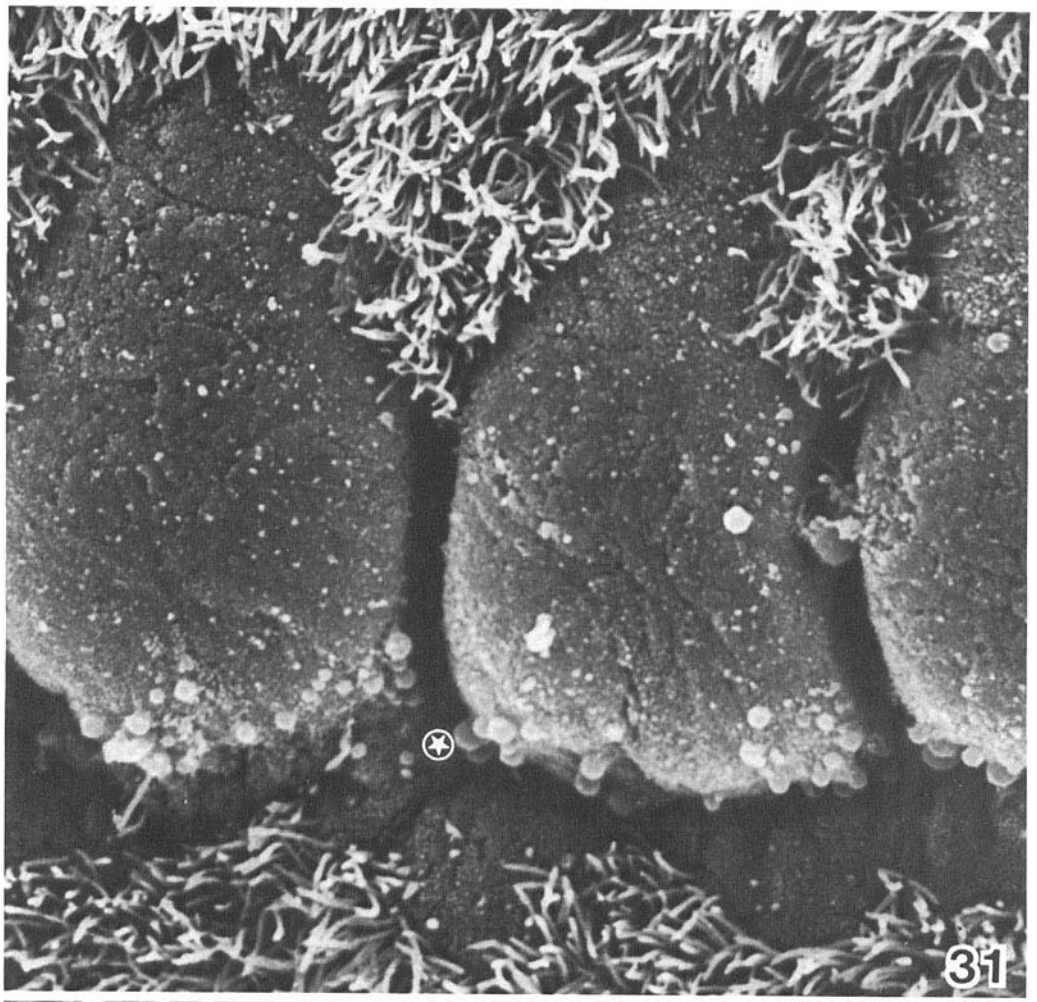


Fig. 31. Portions of three adjacent papillae with intervening grooves in the sensory border of the head. Note extension of cilia into the grooves between the papillae and to some extent over their lateral surfaces. Conventional cilia are absent on the surfaces of the papillae, and spherical protrusions (star) are most prominent in the ventral portions of the papillae. ×3,675.

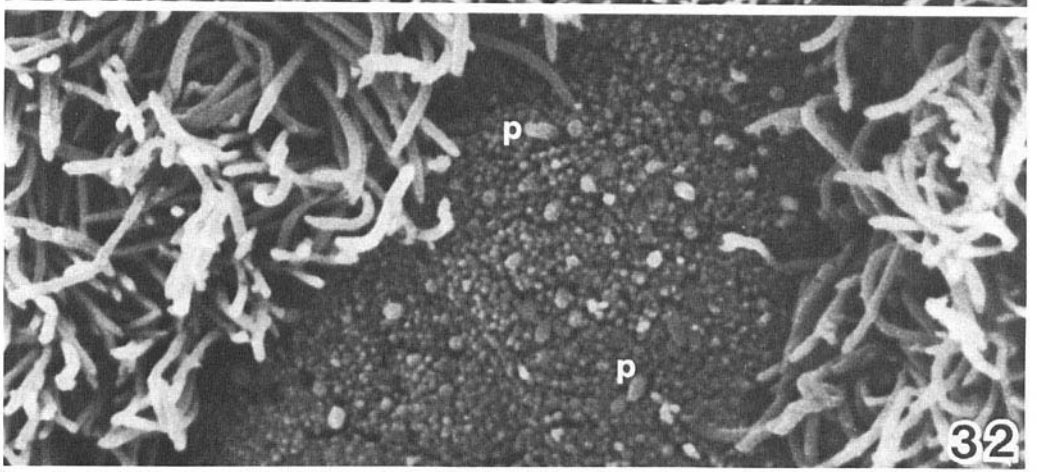


Fig. 32. Enlarged view of papilla to show pebblelike microvilli, larger clublike protrusions, and cilia of grooves. Note that the cilia resemble those found in nonsensory areas (Figs. 27, 28). ×9,000.

tion cannot be related directly to a single function. Nonetheless, the sole and the margin of the head seem to have at least one property in common: Both areas appear to be in contact with the substrate more closely than other regions of the body. Living specimens of *Bipalium* seem to glide smoothly over the substrate while rocking their heads from side to side, thereby continually touching the sensory portions of their heads to the substrate. In a study of the adhesive glands of a variety of turbellarians, Tyler ('76) suggested that under adverse conditions the insunken position of the nuclei of anchoring cells in these glands might protect the cells from total destruction: In response to stress, the cells would tend to lose only their outermost portions, which could then be regenerated from their insunken nucleated portions. It would also seem possible that cells conjoined only apically and not constrained by either a basement membrane or adjacent cells could move or undergo changes in position in accordance with functional demands more readily than regular epidermal cells situated above a basement membrane. Thus, they might be less susceptible to damage than regular epithelial cells. In addition, it seems likely that cells which are not bound tightly to adjoining cells could have more latitude in size than those of an epidermis in which the cells are located above a basement membrane. In the case of cells of the sole and sensory areas, considerable amounts of glycogen are accumulated in the cytoplasm. Conceivably the location of the cells permits a greater accumulation of glycogen—and thus a greater increase in size—than would be possible in cells which are located above a basement membrane.

Although an insunken epithelium and development of a syncytial condition are typical of the integuments of trematodes and cestodes, it is of interest that the integuments of parasitic turbellarians which have been studied to date do not display similar properties. Thus, in *Paravortex karlingi* and *P. cardii,* parasitic in the intestine and digestive gland, respectively, of the common cockle *Cerastoderma edule,* the epidermis does not display insinking; and the major adaptations to a parasitic mode of existence seem to be the presence of a greater number of microvilli per cell than in free-living forms and the absence of secretory products other than rhabdites (MacKinnon et al., '81).

In a study of the sensory border of the head of *Bipalium*, Storch and Abraham ('72) found two different types of ciliated receptor cells: one possessing a long cilium with a long, striated ciliary rootlet; and a second, branched variety with short, stumpy cilia lacking rootlets. Since the first type of receptor is found primarily in the grooves between adjacent papillae, while the second is located on the surfaces of the papillae, Storch and Abraham ('72) concluded that the two types of receptors might be chemoreceptors and mechanoreceptors, respectively. Although Bedini et al. ('75) found very similar types of receptors in the epidermis of representatives of the Order Proseriata *(Notocaryoturbella, Otoplana,* and *Parotoplanella),* they concluded on the basis of known reactions of these organisms to various types of stimuli that the long and clublike receptors are mechanoreceptors and chemoreceptors, respectively. While the identity and functions of the two types of receptors in the epidermis of *Bipalium* have not yet been clarified completely, it seems possible that the clublike projections on the surfaces of the papillae visible by SEM correspond to the short, stumpy cilia described by Storch and Abraham ('72), and that some of the cilia observed in the grooves between adjacent papillae correspond to the long receptors. It is of interest that the external appearance of cilia occupying the grooves does not seem to differ significantly from that of cilia found elsewhere. Because of the great density of conventional cilia in all regions other than the sensory areas, the cilia of receptor cells are much more difficult to identify in these areas by TEM or SEM. The clublike receptors of *Bipalium* resemble those found in the auricles of *Dugesia tigrina* (MacRae, '67; Smales and Blankespoor, '78), although the cilia lack the prominent, striated rootlets which are so typical of these receptors in *Dugesia.* The identity and significance of the larger spherical protrusions observed in the sensory areas of *Bipalium* by SEM are not clear, although similar protrusions have been found in the sensory areas of other turbellarians (Reuter, '78). It seems possible that some of the material might be extrusions of the mucous and eosinophilous glands known to be present in this area (Storch and Abraham, '72).

As indicated in the descriptions provided by Storch and Welsch ('77), the septate junctions of *Bipalium* appear to be of a pleated-

sheet, rather than a smooth variety. While much is left to be determined about the structure and function of septate junctions in various taxonomic groups of invertebrates, these structures appear to be analogous to zonulae occludens (tight junctions) of vertebrate cells (Noirot-Timothée and Noirot, '80; Green, '81; Graf et al., '82; Noirot-Timothée et al., '82). Thus, in *Bipalium* as in all other organisms they probably seal off the intercellular spaces from direct contact with the environment. Negative staining and freeze-fracture techniques are required for a better understanding of their heterogeneity in structure and function in *Bipalium* and other invertebrates.

Although the collagen of invertebrates has been studied much less extensively than that of vertebrates (Adams, '78, for review), available biochemical evidence indicates that the collagen of platyhelminthes does not differ fundamentally from that of lower vertebrates. Pikkarainen et al. ('68) reported that extracts obtained from the fish tapeworm *Diphyllobothrium*, when analyzed by starch-gel electrophoresis, yield a single α-component resembling that resulting from the collagen of lampreys. Similarly, in a study of the cattle liver fluke *Fasciola hepatica*, Nordwig and Hayduk ('69) found that the acid-soluble collagens extracted from this organism possess many properties in common with vertebrate collagens, including a similarity in amino acid composition, an ability to form cross-banded segments, a susceptibility to collagenase, and the presence of a tightly bound carbohydrate component. While the results of the Masson and fast green-Van Gieson procedures used in the present study of *Bipalium* suggest that the basement membrane is composed in part of collagenous fibers, further analyses are required for the biochemical characterization of this material, and for the determination of where and by what mechanisms it is synthesized and released. It seems reasonable to expect that the extracellular matrix of invertebrates contains materials analogous to such substances as laminin, fibronectin, and other glycoproteins and proteoglycans whose properties and functions are only now being actively investigated in vertebrates (Hay, '81, for review). While the Lillie and Fullmer procedure used in the present study did not reveal the presence of elastin, additional work is required to determine whether elastin is truly lacking in *Bipalium*, or if it is present either in very small amounts or in an unusual form. Regional differences and/or unusual properties of the extracellular matrix might play important roles in the remarkable regenerative capacities that *Bipalium* is known to possess (Morgan, 1900).

While intense staining of rhabdites with acidic dyes seems to be a general characteristic wherever they occur (Pedersen, '59, '63; Skaer, '61), as yet there is no complete explanation for its basis. Pedersen ('59, '63) concluded that arginine- and lysine-rich proteins are not likely to be the source of the acidophilia, since the Sakaguchi reaction for arginine yields nearly negative results, and both deamination and acetylation procedures do not reduce the amount of staining to any great extent. On the basis of the results of experiments involving uv absorption, extraction procedures, and paper chromatography, Skaer ('61) concluded that the intense acidophilia of the rhabdites of *Polycelis* might be attributable to their content of adenine (6-aminopurine). While Pedersen ('59, '63) found that the rhabdites of *Planaria vitta* and *Dugesia tigrina* are stained by the dihydroxydinaphthyl-disulfide (D.D.D.) method for SH groups, Skaer ('61) found that those of *Polycelis nigra* are stained by the Chèvremont and Frederic procedure for SS groups, but only after fixation in Bouin's solution. Because of the extremely sensitive and highly specific nature of the DACM method developed by Sippel ('80), there seems to be little doubt that the rhabdites of *Bipalium* are composed in part of SS-containing proteins.

To our knowledge, there have been no previous reports of the presence of *Bipalium adventitium* in Tennessee, although three other species of land planarians are known to inhabit the state: *Microplana (Geodesmus) atrocyaneus* (Hyman, '43, '54), *Diporodemus indigenus* (Klots, '60), and *Bipalium kewense* (Chandler, '74, '76). *Bipalium adventitium* was discovered by Hyman ('40) in a sample obtained from California and was named by her as a new species in 1943. It differs in two major respects from its close relative *Bipalium kewense*: It possesses only a single, longitudinally oriented stripe of pigment on its dorsal surface, and it is capable of reproducing sexually as well as asexually in temperate climates (Hyman, '40, '43, '54; Klots, '60). While natural populations of *B. kewense* have been found in various portions of the south-

eastern United States as far north as Tennessee (Hyman, '40, '43, '54; Dundee and Dundee, '63; Connella and Stern, '69; Barnwell, '69; Olewine, '72; Chandler, '74, '76), sexually reproducing individuals in the United States have been observed so far only in Louisiana (Connella and Stern, '69). Although the original source of members of the Family Bipaliidae is not known with certainty, it appears likely that they are natives of the Indo-Malay region and have been introduced into other regions of the world in soil samples accompanying ornamental plants (Hyman, '40; Winsor, '81). Natural populations are usually found in protected locations under such objects as stones, logs, or boards; they seem to require soil with a relatively high moisture content into which they can retreat during periods of adverse weather conditions; and they subsist almost entirely on a diet of earthworms (Hyman, '54; Klots, '60; Dundee and Dundee, '63; Connella and Stern, '69; Barnwell, '69; Olewine, '72; Chandler, '74, '76; Winsor, '81). *B. kewense* is known to be capable of producing a collagenase, presumably of use in the degradation of earthworm collagen (Phillips and Dresden, '73).

It is of special interest that the histological and cytological organization of these large planarians, aside from the creeping sole, does not differ significantly from that of their much smaller relatives, the freshwater triclads: The cells are small, the basement membrane does not display a great deal of complexity, and the collagenous fibrils are not distinctly cross-banded. While the microscopical features of *Bipalium* generally resemble those of *Rhynchodemus (Microplana) terrestris*, there are two major ways in which these two land planarians differ: The cells comprising the sole of *Bipalium* are in an insunken position, while those of *Microplana* are located in a conventional position (Bautz, '77); and the cilia are distributed over both dorsal and central surfaces in *Bipalium*, but they are considerably reduced dorsally in *Microplana* (Bautz, '77), as in most freshwater planarians (Hyman, '51; Hay and Coward, '75; Pedersen, '76; Bautz, '77; Smales and Blankespoor, '78). As more is discovered about the natural history of these organisms, it might be possible to arrive at an explanation of how, specifically, the histological and cytological attributes of the epidermis are related to the capacity of the animals to survive in a temperate climate on land.

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