FINE STRUCTURE OF THE LARVAL ANURAN EPIDERMIS, WITH SPECIAL REFERENCE TO THE FIGURES OF EBERTH

GEORGE B. CHAPMAN, Ph.D., and ALDEN B. DAWSON, Ph.D.

From the Department of Anatomy, Cornell University Medical College, New York, and the Biological Laboratories, Harvard University, Cambridge, Massachusetts

ABSTRACT

Small pieces of skin from 8 cm long *Rana clamitans* larvae were fixed in OsO_4, washed, dehydrated, and embedded in a methacrylate mixture. Ultrathin sections were cut on a Porter-Blum ultramicrotome and were examined in an RCA electron microscope, type EMU 2D. The sections showed that aggregates of fibrous material in the cells of the inner layer of epidermal cells are identical in disposition and size with the classical figures of Eberth. It is conclusively shown that these figures do not arise from an aggregation of mitochondrial filaments. The tendency of the fibrils to concentrate on attachment points, or thickenings of the basal plasma membrane, is noted. It is also observed that numerous mitochondria are located in the distal region of the cells of the outer layer of epidermis in association with the secretory vacuoles. Microvilli are seen occasionally on the free surface of the skin. Cisternae are found only in the cells of the outer epidermal layer, while vesicular endoplasmic reticulum is found in the cells of both epidermal layers.

INTRODUCTION

Conspicuous thread-like structures in the epidermal cells of middle and late tadpoles of the frog were discovered by Rudneff (1865). These same structures were described by Eberth, apparently independently, in 1866. Subsequent investigators have chosen to call the structures "mitochondrial threads" (Saguchi, 1913; Speidel, 1926), "figures of Eberth" (Weed, 1934), or "bodies of Eberth" (Cameron, 1936). Both the nature and the function of the structures have remained subject to dispute. Thus, Saguchi (1913) considered the structures to arise from the fusion of mitochondria and to penetrate the basal cell membranes to bind the dermis and epidermis together. Weed (1934) believed the structures to be fine fibrillar continuations of the connective tissue of the corium which supported the basal epidermal cells and strengthened the connection of the epidermal layer to the corium. Cameron (1936) regarded the bodies as reserve accumulations of secreted material which were used up in the formation of the dermal connective tissue.

Reviews of the possible functional significance of these bodies are found in Weed (1934) and in Cameron (1936).

Although the same or similar materials have been studied with the electron microscope by Weiss and Ferris (1954*a*, 1954*b*, 1956), Weiss (1957, 1958), Porter (1957), Eakin and Lehmann (1957), Salpeter and Singer (1959), Hay (1960), and Hama (1960), the apparent identity of skeins of fibrous material with the classical figures of Eberth has been unreported. This could be due to a restricted range of occurrence of the figures,

or to the fact that some authors have been interested only in very special regions of the epidermal cells. It may also be due to the fact that ultrathin sections excluded large areas of the figures, or to an unawareness of the classical observations.

It is, then, the main purpose of this report to point out the relationship between the aggregations of fibrillar material seen with the electron microscope in the epidermal cells of the tadpole and the classical figures of Eberth. Also included are observations on several other aspects of the fine structure of anuran skin.

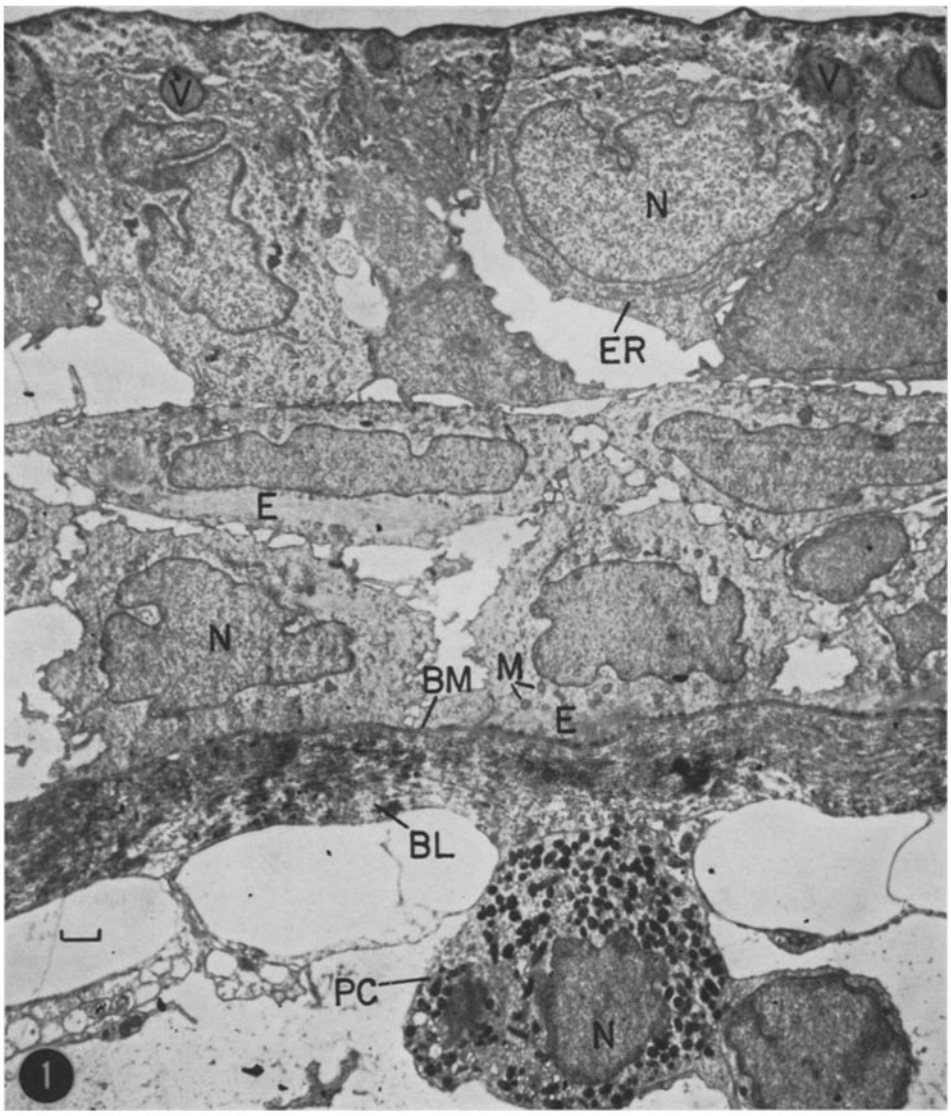
MATERIALS AND METHODS

Small pieces of skin, about 1.0 mm^3 in volume, were excised from the tails of 8 cm long *Rana clamitans* tadpoles. The pieces of tissue were fixed for 6 hours in a fluid of the following composition: 2.5 ml acetate veronal (9.714 gm sodium acetate + 14.714 gm sodium veronal made up to 500 ml in distilled water), 1.25 ml 10× Ringer's solution (0.1 gm $CaCl_2$, 7.5 gm NaCl, 0.1 gm KCl made up to 100 ml in distilled water), 2.0 ml 0.1 N HCl, 0.5 ml distilled water, 6.25 ml 2 per cent OsO_4. The pH of this fixative was 7.6. Fixation was carried out in an ice water bath at about 2°C. The tissues were then washed in a solution identical with the fixative except for the substitution of 6.25 ml of distilled water for the 6.25 ml of 2 per cent OsO_4. The tissues were dehydrated by a 2-hour passage through a graded ethyl alcohol series and were then suspended in a monomeric methacrylate mixture (60 parts normal butyl methacrylate, 40 parts ethyl methacrylate). Polymerization of the embedding material was carried out at 70°C in the presence of 1.5 per cent Luperco CDB. Sections were cut with a Porter-Blum ultramicrotome set at either 25 mμ or 50 mμ and were floated onto a surface of 40 per cent acetone in distilled water. The sections were picked up on collodion coated 200 mesh copper grids and were examined in an RCA electron microscope, type EMU 2D, which had been fitted with a 0.015 inch Canalco externally centerable platinum condenser aperture and a 50 μ copper aperture in the standard objective pole piece. Some sections were stained with uranyl acetate according to the method of Watson (1958).

OBSERVATIONS AND DISCUSSION

Fig. 1 is a low magnification survey micrograph showing the essential features of the skin of a larval *R. clamitans* of the size studied. The skin has an epidermis of two rather well defined cell layers. The outer layer is one cell thick; the inner layer is two cells thick. Beneath the epidermis, a distinct basement membrane, *BM*, may be seen, as well as the collagenous basement lamella, *BL*, and a pigmented cell, *PC*, of the derma or corium. Several of the nuclei are designated *N*. In this region, the outer surface of the skin is quite smooth and free from both microvilli and mucus. In the superficial cytoplasm, relatively large vacuoles, *V*, containing a mucous secretion may be seen.

Rather small, relatively sparse mitochondria, *M*, may be seen in most of the deeper lying cells. In the outer layer, the mitochondria are numerous and concentrated in a zone between the nucleus and the free surface of the epidermal cells. The rough surfaced endoplasmic reticulum, *ER*, appears as vesicles and as paired membranes in the cells of the outer epidermal layer and as vesicles in the cells of the inner epidermal layer. This observation thus illustrates, once again, the fact, pointed out by Palade (1956), that the type of endoplasmic reticulum may vary with the location or type of cell under study as well as with its physiological state. The cells of the outer layer are forming a secretory product, presumably mucus, apparently in vacuoles, *V*; the cells of the inner layer are apparently producing keratin which is retained within the cell. A similar relationship between the physiological state of a cell and the form of its endoplasmic reticulum has been dramatically illustrated by Slautterback and Fawcett (1959) in the case of the nematocyst-forming cnidoblast of Hydra. When this cell is at the height of secretory activity, its endoplasmic reticulum is characterized by the occurrence of numerous and extensive rough surfaced cisternal elements. In contrast to this, the undifferentiated interstitial cell, from which the cnidoblast arose, and the early cnidoblast essentially lack an endoplasmic reticulum, but do have many cytoplasmic granules which are assumed to be ribonucleoprotein. Again, when the nematocyst has been fully formed, the endoplasmic reticulum breaks up into small vesicles with relatively few adherent RNP particles. Thus, Slautterback and Fawcett (1959) point out that an endoplasmic reticulum represented essentially only by free RNP particles was found in cells concerned with the manufacture of material for local consumption (in the production of protoplasm for growth and division and for the maintenance of cellular integrity) while a rough



Abbreviations Used in the Figures

BL, basement lamella
BM, basement membrane
D, desmosome
E, figure of Eberth
ER, endoplasmic reticulum
F, outer dense zone
G, Golgi zone
M, mitochondria
MV, microvilli
N, nucleus
PC, pigmented cell
PM, basal plasma membrane
T, basal membrane thickening
V, mucous vacuole
Z, dense zone

FIGURE 1

Survey view of skin of larval *Rana clamitans*. The two cell layers of the epidermis, basement membrane (*BM*), basement lamella (*BL*), and a pigmented cell (*PC*) of the derma or corium may readily be distinguished. The inner layer of the epidermis is two cells thick. The figures of Eberth (*E*) are detectable only as indistinct regions in the cytoplasm of the cells of the inner epidermal layer. × 4,500.

surfaced cisternal endoplasmic reticulum was found in cells involved in the formation of a protein rich secretory product.

A Golgi zone does not appear in any of the cells of Fig. 1. When one is included in a section (*G*, Figs. 5 and 7), it is seen to consist of smooth surfaced paired membranes and vesicles. Terminal bars, desmosomes, and attachment points may be seen, but they are not prominent at this magnification.

At several loci in cells of the inner epidermal layer, skeins or tufts of fibrous material, *E*, may just be seen. Accepting the evidence cited by Porter (1957), these structures are considered to be aggregates of keratin fibrils. On comparison with the classical representations of the figures of Eberth (see Introduction for references), these structures are concluded to be identical with them because of their size relative to the cell size, their occurrence only in the cells of the inner epidermal layer, and their disposition within the cells.

Fig. 2 is largely occupied by a portion of the basal region of one of the inner cells of the inner layer of the epidermis and by basement lamella. This figure is particularly pertinent, for it reveals the presence, in the same cell, in close juxtaposition, of both figures of Eberth, *E*, and mitochondria, *M*. It is clear that the view that figures of Eberth result from an aggregation of mitochondrial filaments is untenable. The fibrous nature of the figures of Eberth is clearly shown. Examination of the basal plasma membrane, *PM*, reveals the association of fibrils from the figures of Eberth with the "dense thickenings," *T*, of the basal plasma membrane (Porter, 1957) or, as in Hama's figure (1960), the "attachment points." The configuration here more closely resembles a unilateral desmosome than it does the "bobbin" described by Weiss and Ferris (1954*a*, *b*). The two parallel densities, separated by about 350 A, described in this region by these authors and by Porter (1957) are rarely seen in this anuran material. Rather, one usually sees a quite dense layer, about 600 A thick, associated with the very dense thickenings (about 250 A) of the plasma membrane. The 600 A area seems to be due to the association of dense amorphous material with the fibrils in this region adjacent to the thickenings. These features may be more clearly seen in Fig. 4.

Fig. 3 reveals the intimate relationship often observed between the figures of Eberth, *E*, and the basal plasma membrane, *PM*. The basement membrane, *BM*, is also clearly shown in this figure, as is a region (single arrow) where this membrane penetrates deep into the basement lamella. These penetrating columns of basement membrane have been observed to pass completely through the basement lamella and to be in contact with the surface of a fibroblast of the corium as it lies closely apposed to the under surface of the basement lamella. Although the basement membrane material generally appears amorphous, fibrils may occasionally be seen in it. The double arrow indicates a collagen fibril which has been cut longitudinally for a short distance. It should be noted that fibrils of the alternating layers in the basement lamella do not here show the characteristic 90° direction shift between adjacent layers. A part of the nucleus, *N*, of an epidermal cell with its double-membraned envelope is also seen in this figure.

Fig. 4 also illustrates the fine structural aspects of the dermal-epidermal junction. An indication of the tendency for the keratin fibrils to loop from one attachment point to another may be seen at the double arrow. They may join adjacent points as minor loops, or as larger loops join more distant points. These observations are in agreement with the interpretation of Charles and Smiddy (1957) that both ends of tonofibrils are attached to the cell membrane at the desmosomes or attachment points. Such an arrangement, these authors believe, results in a linkage of the entire epidermal tonofibrillar network into an elastic system adapted to taking up the distortional strains which beset the epiderm. In this figure, the keratin fibrils measure about 60 A in diameter, the attachment points or basal plasma membrane thickenings, *T*, measure from 0.3 to 0.4 μ long by 250 A thick, and the dense zones, *Z*, just distal to the thickenings, measure 600 A thick. In the region between the basal plasma membrane and the basement membrane, small quantities of granular and fibrillar material may be seen. Occasionally, fibrils appear to span this region, as is indicated by the single arrow. It has not been possible to determine whether or not these fibrils penetrate the basal plasma membrane and/or the basement membrane. The granules 600 A in diameter reported by Weiss and Ferris (1954*a*, *b*) were not seen in this material. No convincing indications of a continuity of a substantial amount of the fibrous

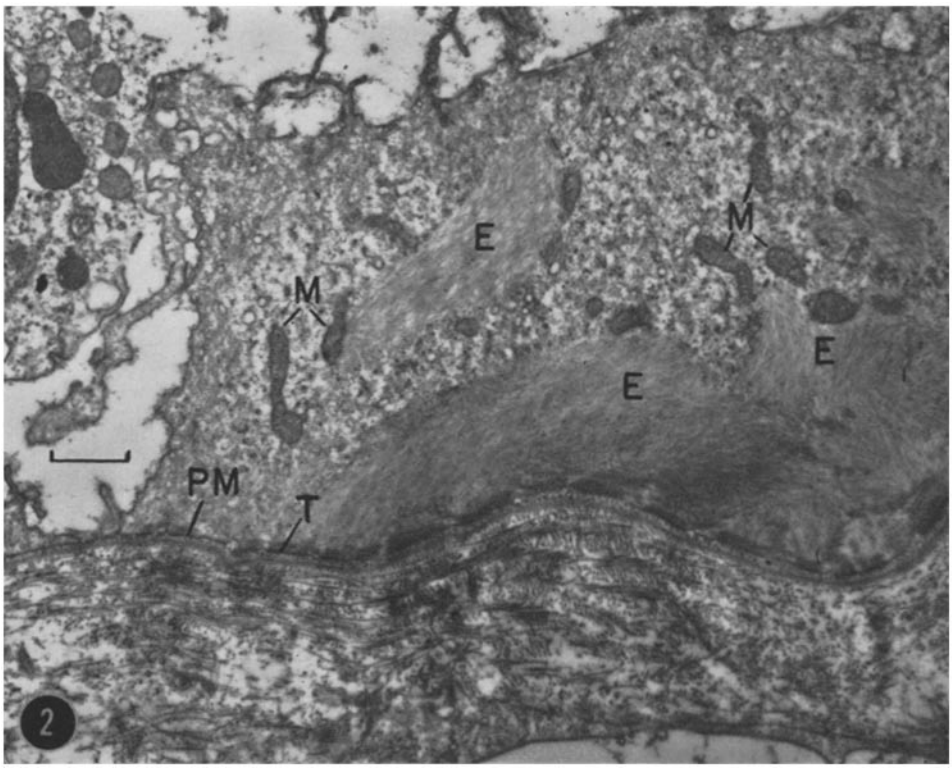


FIGURE 2

Part of the basal region of an inner cell of the inner layer of the epidermis, showing its relation to the basement lamella. The fibrous nature of the figures of Eberth is readily apparent, as is their relation to the dense thickenings (*T*) of the basal plasma membrane (*PM*). × 11,500.

material through the basal plasma membrane and basement membrane to the basement lamella, as reported by Weed (1934) and Cameron (1936), were ever seen. It has, thus, remained impossible to confirm or refute Weed's (1934) claim that the figures of Eberth serve to strengthen the basal epidermal cells or Cameron's (1936) claim that the figures are used up in the organization of the basement lamella. Within the basement lamella, several unidentified fibrils, oriented approximately perpendicular to the basement membrane, may be seen at *X*. Whether these fibrils represent continuations of the fibrils spanning the space between the basement membrane and the basal plasma membrane could not be ascertained. A group of vesicular elements of the endoplasmic reticulum is seen at *ER*.

The occurrence of a desmosome, *D*, in the same field with the attachment points in Fig. 4 renders appropriate several comments comparing these structures. As stated earlier, the attachment point resembles a unilateral desmosome, or, as Charles and Smiddy (1957) describe the appearance of the tonofibrillar tufts in cells of the dermal-epidermal junction in human skin, a half prickle. (In their terminology, a whole prickle is the equivalent of the paired structures constituting a desmosome.) Both structures reveal a thickened plasma membrane; both structures have aggregates of dense material associated with the plasma membrane thickenings. Perhaps the most significant similarity, however, between the desmosome and the attachment point is the fact that each serves as an anchor point for a system of cytoplasmic filaments, the individual elements of which appear basically identical. One does, however, get the feeling that a larger amount of filamentous material is associated with each attachment point than with each desmosome. If this somewhat subjective feeling is true, then it is

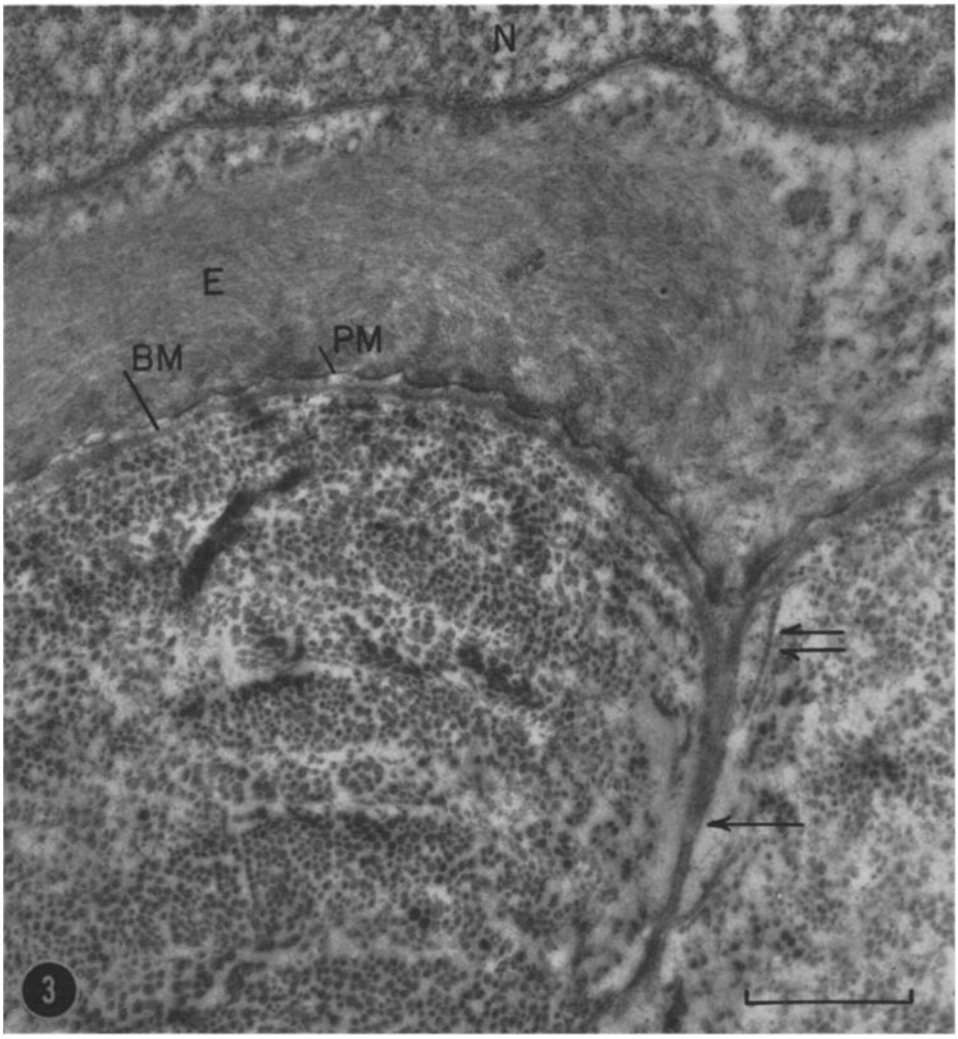


FIGURE 3

The intimate relationship often observed between the figures of Eberth and the basal plasma membrane is here clearly seen. The basement membrane and its occasional deep penetration (single arrow) into the basement lamella are also seen. The double arrow indicates a collagen fibril which lies in the plane of the section for nearly 1 μ. × 23,000.

suggested that the attachment point may be a region of greater cell rigidity than the desmosome. In any case, the high concentration of attachment points at the basal region of the cell suggests that this region is much more rigid than the lateral regions. Unless it can be shown that there is a continuity of fibrillar material across the desmosome or through the attachment point between epidermis and derma, the distortional strain-absorbing function proposed by Charles and Smiddy (1957) for the fibrillar material may be considered quite reasonable, and adhesion of adjacent cells would then, presumably, be mediated by an intercellular cement, as these authors suggest. (For a discussion of the relationship between desmosomes and intercalated discs in heart muscle, the work of Fawcett and Selby (1958) should be consulted. The fine structure of the desmosome is excellently presented by Odland (1958); a unique variation in the structure of

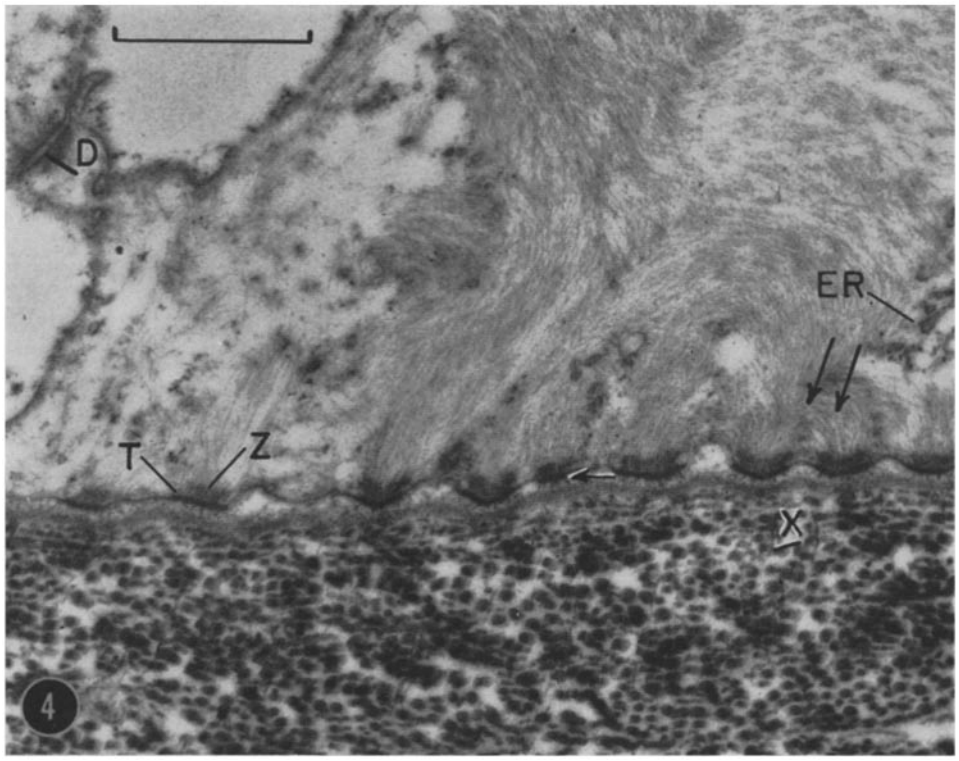


FIGURE 4

The fine structural aspects of the dermal-epidermal junction are seen. The double arrow indicates a region where the keratin fibrils are looping from one basal plasma membrane thickening (*T*) to another. The dense zones (*Z*) appear to result from the accumulation of amorphous material about the keratin fibrils as the latter approach the basal plasma membrane thickenings. The single arrow indicates fibrils, perpendicular to both the plasma membrane thickening and the basement membrane, which appear to span the region between these two structures. × 26,000.

desmosomes found in Hydra is described by Wood (1959).)

Fig. 5 includes parts of two cells of the outer epidermal layer. The main part of the figure is occupied by the nucleus, *N*, of the cell at the left. Numerous closely packed mitochondria, *M*, may be seen in the region between the nucleus and the free surface of the epidermal cell. Eakin and Lehman (1957) have assumed that these mitochondria are in some way related to the secretory function of the cells. Distal to the mitochondrial layer, aggregates of fibrous material, *F*, may be seen. These aggregates probably correspond to the "outer dense zone" noted in outer epidermal cells of amphibia by Eakin and Lehman (1957). Several ovoid secretory vacuoles, *V*, are quite prominent. It appears that the contents of these vacuoles, when released to the cell surface, form a delicate mucous coating of the skin. The secretory vacuole designated V_1 is particularly noteworthy because of its location close to the surface of the nucleus and adjacent to elements of the Golgi complex, *G*. This location suggests the participation of one or both of these structures in the elaboration of the secretory product. This observation and interpretation are in disagreement with the observations and interpretations of Schulz and DePaola (1958), who believe that a special type of cytoplasmic membrane, the delta-cytomembrane, is involved in the synthesis of mucous secretion in the axolotl. Although preparative treatments were quite similar, the mucus secreted by the axolotl epithelium appears rather different from that secreted by the larval frog epithelium. Furthermore, no structures corresponding to the delta-cytomembranes were ob-

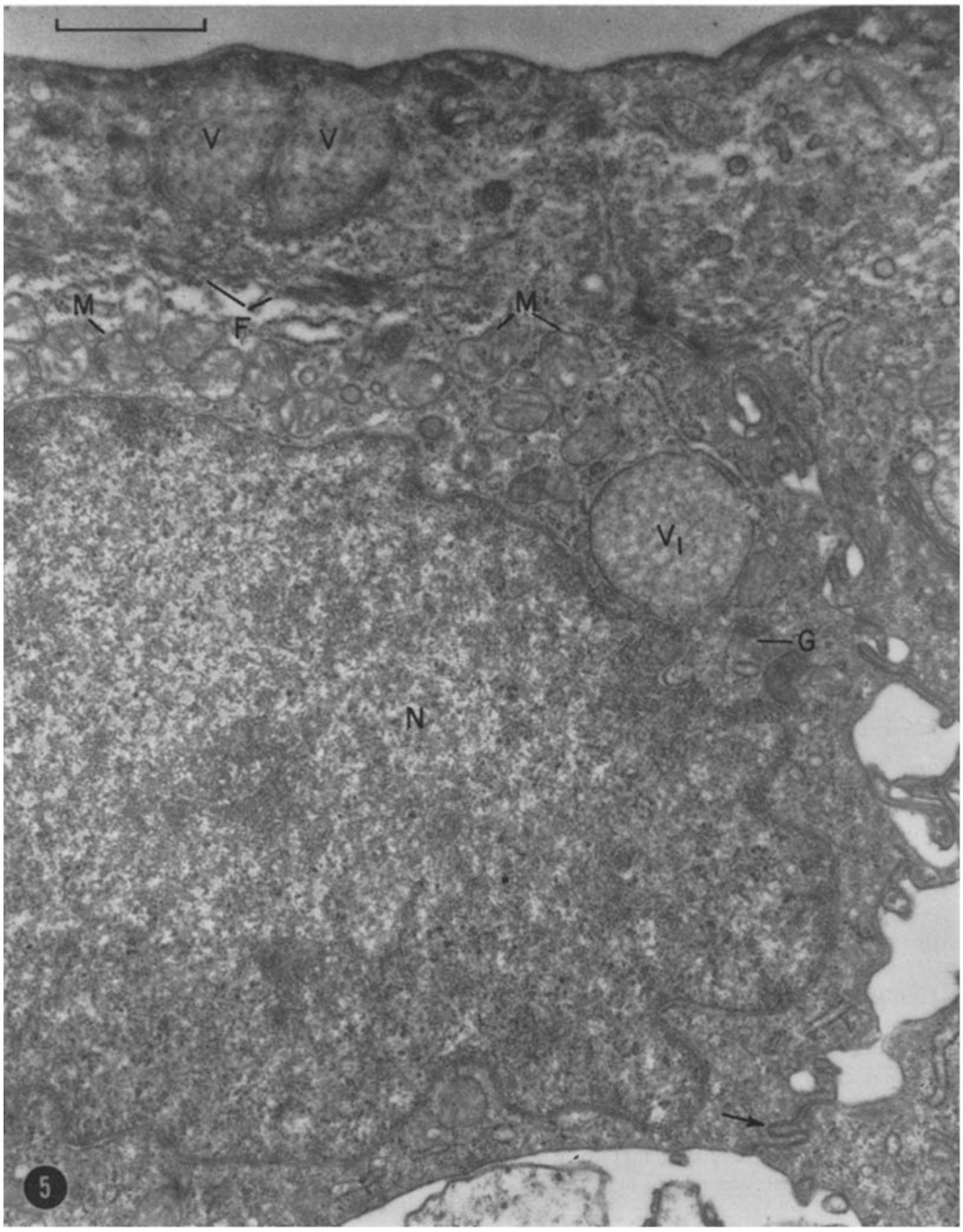


FIGURE 5

This figure indicates one appearance of the surface of the skin. Here the surface is relatively smooth. The densely packed mitochondria in this area are noteworthy. Their presence and concentration and the presence of the Golgi zone and the mucous vacuole (V_1) suggest that the mitochondria and Golgi material may be involved in the formation of the mucus. × 22,000.

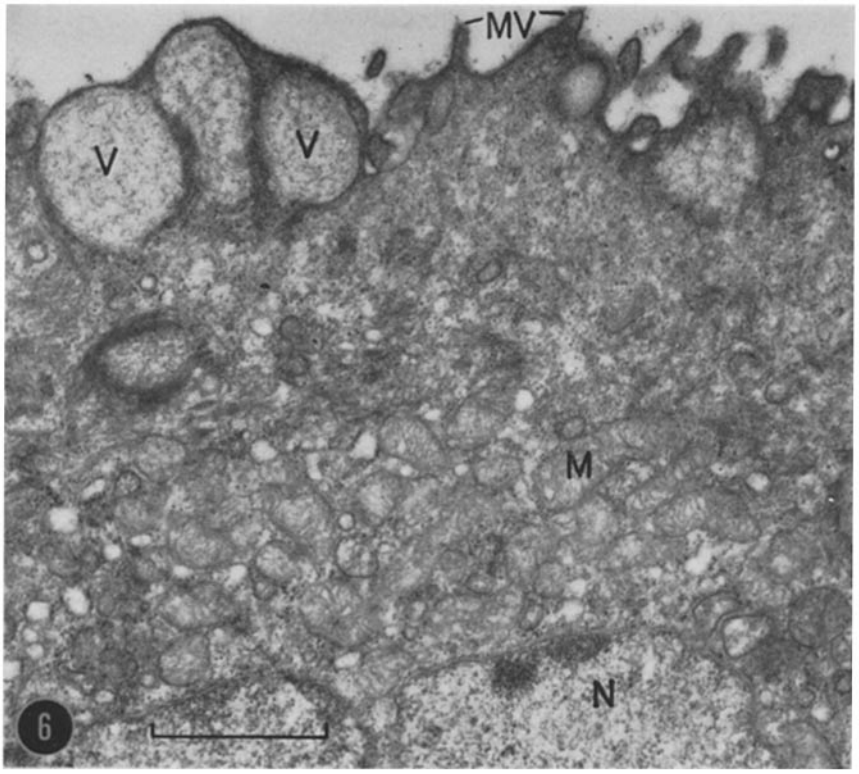


FIGURE 6

This figure reveals that microvilli (*MV*) may occur on the free surface of the tadpole skin epidermal cells. The group of three mucous vacuoles (*V*) and the accumulation of mitochondria (*M*) are also notable. × 24,000.

served in the present study. Additional work will be required to elucidate this interesting, though somewhat puzzling, situation.

The interdigitating plasma membranes of the two epidermal cells are particularly well shown at the arrow. It should be noted that in this figure the free surface of the epidermal cells is essentially quite smooth. This is in contrast to the appearance of this surface as seen in Fig. 6. Here, in addition to the structural features noted above, it seems that the epidermal cell surface may have a small number of widely spaced microvilli, *MV*, somewhat comparable to those described by Hay (1960) on the free surface of the epidermal cells of *Amblystoma*. It should be understood, however, that the apparent microvilli in the anuran epidermis may actually represent surface irregularities due to the breakdown or discharge of the secretory vacuoles on the cell surface.

Fig. 7 shows parts of two cells at the junction of the inner and outer layers of the epidermis and indicates the apparent change in the form of the endoplasmic reticulum between these two regions, as discussed earlier. In the cell from the outer layer (upper), both rough surfaced vesicular and elongated paired membrane (cisternal) types of endoplasmic reticulum may be seen. In the cell from the inner layer (lower), only vesicular elements of the endoplasmic reticulum may be seen. Whether or not this apparent change in the type of endoplasmic reticulum actually represents a relation to the kind of product formed and to the site of its utilization (vesicular-keratin-intracellular; elongated-mucus-extracellular) can only be definitively determined by further extensive study. Mitochondria, *M*, are visible in both cells,

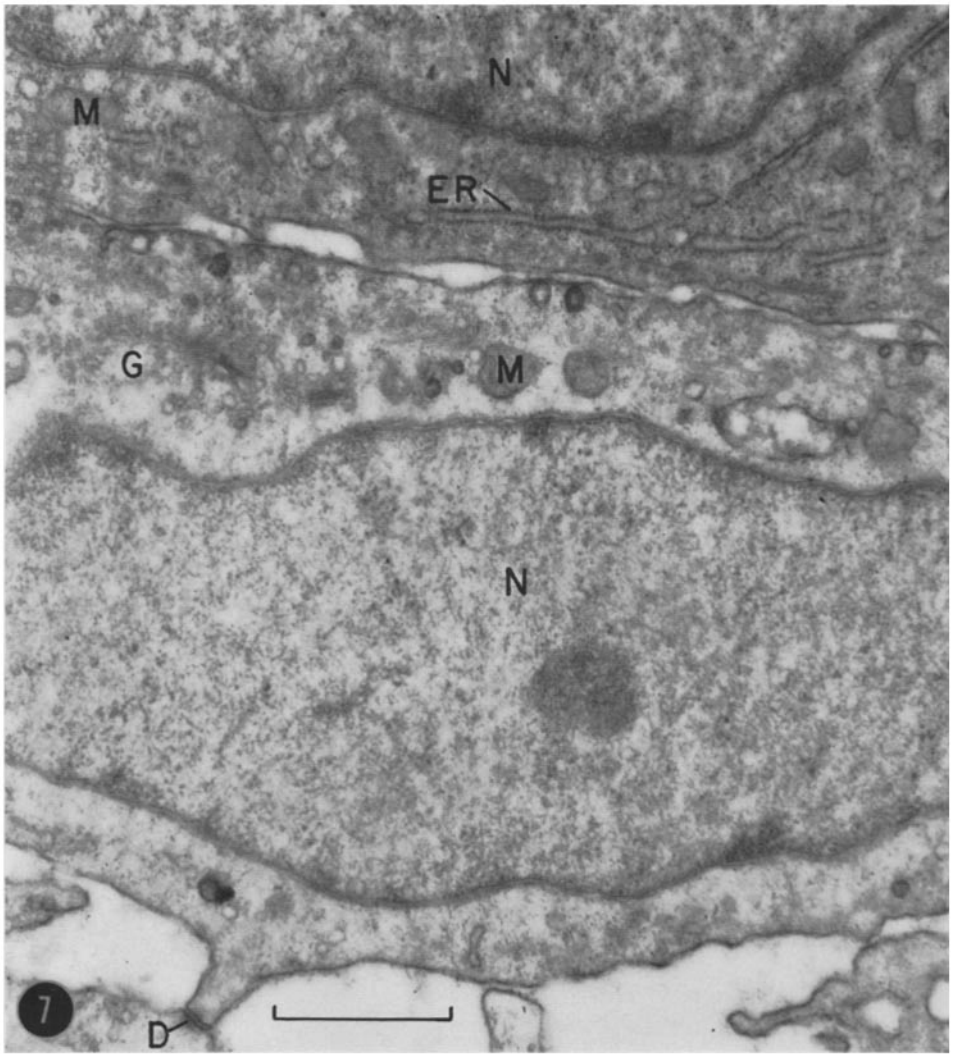


FIGURE 7

This figure reveals fine structural features of cells at the junction of the inner and outer epidermal layers. The endoplasmic reticulum of the outer layer cell is represented by several elongated profiles studded with ribonucleoprotein particles, as well as by vesicles. The inner layer cell shows only vesicular endoplasmic reticulum. A desmosome (*D*) also appears in the figure. × 24,000.

but a Golgi zone, *G*, appears only in the cell from the inner layer. (It should be noted that Golgi material regularly appears in cells of the outer layer.) A desmosome, *D*, is also apparent in the figure.

This investigation was supported by research grants (E-1720 and E-3517) from the National Institute of Allergy and Infectious Diseases, United States Public Health Service.

Received for publication, January 28, 1961.

BIBLIOGRAPHY

CAMERON, J. A., The bodies of Eberth as a source of connective tissue ground substance in *Rana catesbiana*, Shaw, *J. Morphol.*, 1936, **60**, 279.

CHARLES, A., and SMIDDY, F. G., The tonofibrils of the human epidermis, *J. Invest. Dermat.*, 1957, **29**, 327.

EAKIN, R. M., and LEHMANN, F. E., An electron microscopic study of developing amphibian ectoderm, *Wilhelm Roux' Arch. Entwcklngsmechn. Organ.*, 1957, **150**, 177.

EBERTH, C. J., Zur Entwicklung der Gewebe im Schwanze der Froschlarven, *Arch. mikr. Anat.*, 1866, **2**, 490.

FAWCETT, D. W., and SELBY, C. C., Observations on the fine structure of the turtle atrium, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 63.

HAMA, K., Fig. 17-1 *in* General Cytology, (De Robertis, Nowinski, and Saez), Philadelphia and London, Saunders, 1960.

HAY, E. D., The fine structure of nerves in the epidermis of regenerating salamander limbs, *Exp. Cell Research*, 1960, **19**, 299.

ODLAND, G. F., The fine structure of the interrelationship of cells in the human epidermis, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 529.

PALADE, G. E., The endoplasmic reticulum, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 85.

PORTER, K. R., Structure of cell and body walls. Observation on the fine structure of animal epidermis, *Proc. Internat. Conf. Electron Microscopy (1954)*, London, 1957, 539.

RUDNEFF, M., Ueber die epidermoidale Schicht der Froschhaut, *Arch. mikr. Anat.*, 1865, **1**, 295.

SAGUCHI, S., Ueber Mitochondrien (Chondriokonten) und mitochondriale Stränge (sag. Eberthsche intrazelluläre Gebilde) in den Epidermiszellen der Anurenlarven nebst Bemerkungen über die Frage der Epidermis-Cutisgrenze, *Arch. mikr. Anat.*, 1913, **83**, 177.

SALPETER, M. M., and SINGER, M., The fine structure of the adepidermal reticulum in the basal membrane of the skin of the newt, *Triturus*, *J. Biophysic. and Biochem. Cytol.*, 1959, **6**, 35.

SCHULZ, H., and DEPAOLA, D., Delta-Cytomembranen und Lamelläre Cytosomen. Ultrastruktur, Histochemie und ihre Beziehungen zur Schleimsekretion, *Z. Zellforsch.*, 1958, **49**, 125.

SLAUTTERBACK, D. B., and FAWCETT, D. W., The development of the cnidoblasts of hydra. An electron microscope study of cell differentiation, *J. Biophysic. and Biochem. Cytol.*, 1959, **5**, 441.

SPEIDEL, C. C., Studies of hyperthyroidism. IV. The behavior of the epidermal mitochondria and the pigment in frog tadpoles under conditions of thyroid-accelerated metamorphosis and of regeneration following wound infliction, *J. Morphol. and Physiol.*, 1926, **43**, 57.

WATSON, M. L., Staining of tissue sections for electron microscopy with heavy metals, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 475.

WEED, I. G., Cytological studies of the epidermis of *Rana pipiens* and *Rana clamitans* tadpoles, with special reference to the figures of Eberth, *J. Morphol.*, 1934, **56**, 213.

WEISS, P., Macromolecular fabrics and patterns, *J. Cellular and Comp. Physiol.*, 1957, **49**, Suppl. 1, 105.

WEISS, P., Cell contact, *Internat. Rev. Cytol.*, 1958, **7**, 391.

WEISS, P., and FERRIS, W., Electronmicrograms of larval amphibian epidermis, *Exp. Cell Research*, 1954*a*, **6**, 546.

WEISS, P., and FERRIS, W., Electron-microscopic study of the texture of the basement membrane of larval amphibian skin, *Proc. Nat. Acad. Sc.*, 1954*b*, **40**, 528.

WEISS, P., and FERRIS, W., The basement lamella of the amphibian skin. Its reconstruction after wounding, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 275.

WOOD, R. L., Intercellular attachment in the epithelium of *Hydra* as revealed by electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1959, **6**, 343.