A STUDY OF THE FINE STRUCTURE OF THE EPIDERMIS OF *RANA PIPIENS*

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ABSTRACT

The epidermis of adult *Rana pipiens* has been studied by electron microscopy and histological and histochemical methods. It was found that the epidermis is engaged in the production of both keratin and mucus. The basal cells are mainly filled with tonofilaments, whereas the cells located in the mid-portion of the epidermis contain both tonofilaments and mucous granules. Golgi vesicles and endoplasmic reticulum are found in relative abundance in the mucus-producing cells and seem to be involved in the production of mucous granules. The mucus seen was partly retained within the cells and partly secreted into the intercellular spaces. The outermost keratinized cells contain mainly filaments and a few remnants of cell constituents.

INTRODUCTION

Most existing information about the integument of vertebrates has been obtained from land-dwelling mammals. It is generally known that mammalian epidermis is continuously renewed by the mitotic activity of the basal and spinous cells which form the two innermost layers of this tissue, and that cells derived from these layers are gradually differentiated into keratohyalin-containing granular cells and eventually into the horny cells which constitute the outer surface of the epidermis. In electron microscope studies of the epidermis of various mammals, including man (1–18), many fine structural details of different types of epidermal cells have been revealed. It has been found that the cells are tightly bound to one another at several points by desmosomes and that basal and spinous cells contain many filaments 60 to 80 A thick which often terminate at desmosomal contact points. Since these cells are not engaged in secretory activity, they contain little endoplasmic reticulum and few Golgi vesicles. The granular cells are filled with filaments and keratohyalin granules. The structure of the granules varies from species to species: in the epidermis of the rabbit (8) and man (17) the granules are irregular, whereas in the epidermis of the rat and mouse (17, 18) they are ovoid or spherical. The horny cells contain keratin filaments about 80 A wide, nuclear remnants, and small quantities of mitochondrial and other cytoplasmic debris.

Although amphibian epidermis has not been studied so intensively as mammalian epidermis, it is known that the epidermis of the frog differs both structurally and physiologically from that of mammals. The epidermis of the frog is not continuously renewed, but is replaced at regular intervals (19). Structurally, its germinative cells appear comparable to those of mammalian epidermis, but the ascending cells are very different. Frog epidermis contains no keratohyalin granules and no granular layer; furthermore, the horny layer is only one or two cells thick (20).

The investigation reported here was undertaken so that certain characteristic structures in the skin of land mammals and *Rana pipiens* might be compared. While the basal cells of *Rana pipiens* were found to be comparable to the basal cells of mammalian epidermis, the differentiating cells were markedly different in the two types of skin. The most profound difference noted was that mucus was formed in the amphibian epidermis at the stage during which keratohyalin granules are formed in mammalian epidermis. The morphologic manifestations of mucus production and other characteristics of the epidermis of the frog are described below.

RESULTS

The epidermis of *Rana pipiens* consists of 6 to 7 layers of cells (Fig. 1). The cytoplasm of the basal cells is basophilic and contains birefringent tonofibrils, which are moderately reactive to sulfhydryl. No PAS-positive material (mucus) is present in the basal cells, but many PAS-positive granules are scattered throughout the cytoplasm of the cells in the mid-portion of the epidermis. The fibrils of these granule-containing cells are similar to those of the basal cells. In the horny cells near the outer surface, occasionally some Feulgen-positive nuclear material is present, while the cytoplasm is strongly birefringent, shows an intense reaction to sulfhydryl groups, and contains PAS-positive material.

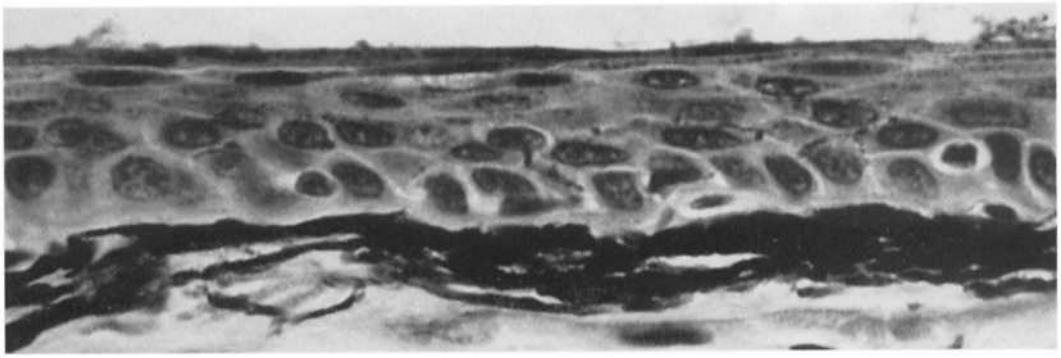


FIGURE 1 Photomicrograph of a cross-section of the epidermis from the thigh of *Rana pipiens*. The epidermis consists of 6 to 7 cell layers. There is no granular layer, and the horny layer is only 1 to 2 cells thick. About × 500.

MATERIALS AND METHODS

After skin from the thigh of adult *Rana pipiens* had been fixed in Bouin's solution, Helley's fluid, or 80 per cent alcohol containing 1 per cent trichloracetic acid, it was embedded in paraffin, cut into sections 8 micra thick, and stained with hematoxylin-eosin or toluidine blue. Some sections were also stained by the periodic acid–Schiff technic (PAS) for the identification of mucus; by the Feulgen method for DNA; and by the Barrnett-Seligman technic for protein-bound sulfhydryl groups. Double refraction was studied in deparaffinized sections.

For electron microscopy the specimens were fixed for 2 hours in 1 per cent osmium tetroxide buffered to pH 7.4 with veronal-acetate buffer (21). The skin was then dehydrated in ethanol and embedded in epoxy resin according to the method of Luft (22). Thin sections were cut with the Porter-Blum microtome and stained with lead hydroxide. They were studied in an RCA EMU-3F.

The electron microscope reveals still more structural detail in thin sections of the epidermis. The basal cells, which are attached to the basement membrane by half desmosomes, penetrate deeply into the dermis by long cytoplasmic extensions (Fig. 2). Both basal and spinous cells contain a large nucleus, and their cytoplasm is filled with filaments about 70 A thick which run in all directions. The filaments often form bundles, and some terminate at desmosomal contact points. There is only a moderate number of ribonucleoprotein particles (RNP) grouped into small islands between the filaments. The rough-surfaced endoplasmic reticulum is poorly developed and the cisternae appear empty. Golgi vesicles are seen only occasionally. Most of the mitochondria, which are quite numerous, contain dense bodies. The plasma

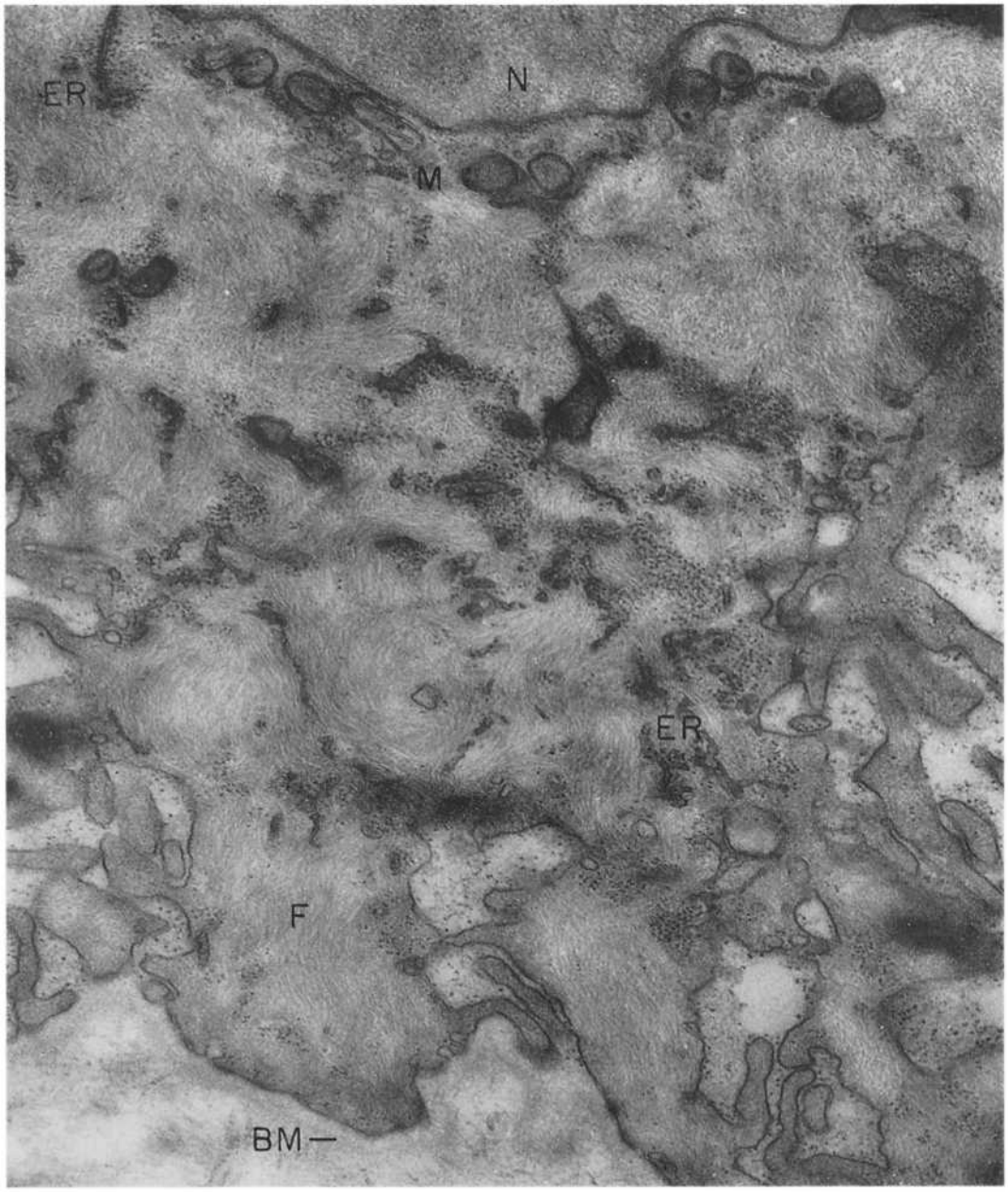


FIGURE 2 Electron micrograph of a portion of a basal cell. The cytoplasm is filled with filaments (*F*), and RNP particles occur in groups. Rough-surfaced endoplasmic reticulum (*ER*) is scanty, and the cisternae are fairly empty. Mitochondria (*M*) are numerous. The basement membrane (*BM*) appears as an amorphous structure. A portion of the nucleus (*N*) is seen at the top of the micrograph. × 28,000.

membrane is always highly convoluted and many desmosomes are visible along the membrane. The intercellular space around the basal cells is very prominent.

In electron micrographs the cells of the mid-portion of the epidermis appear in different stages of differentiation. The structures seen suggest that the most characteristic activities of these cells are

synthesis, storage, and excretion of mucus. The cells immediately above the basal layer are in an early stage of differentiation: their cytoplasm is filled with tonofilaments and a compact Golgi complex consisting of numerous, widely dispersed agranular vesicles and tubules (Fig. 3). The rough-surfaced endoplasmic reticulum is more abundant in these cells than in the undifferentiated basal cells, while their mitochondria appear unchanged, and their content of free RNP particles appears to be somewhat reduced. The cells are locked together by many desmosomes and the cell membranes appear convoluted. The intercellular spaces are clear; secreted material is not yet evident.

The cells nearer the outer surface of the epidermis are in a more advanced stage of differentiation than those in the mid-portion, as is shown by the many tonofilaments present in their cytoplasm and the abundance of rough-surfaced endoplasmic reticulum containing dilated cisternae which appear to be filled with a dense substance (Fig. 4). The agranular reticulum is also abundant in these highly differentiated cells; it consists of large, smooth-walled vesicles filled with an amorphous substance, the density of which is greater than that of the surrounding cytoplasmic matrix. Vesicles of this type, which apparently correspond to mucous granules, are scattered throughout the entire cytoplasm. Large ovoid or spherical granules of mucus are often present in the perinuclear region of the cells (Fig. 5), while smaller mucous granules accumulate at the periphery of the cells near their outer walls. The limiting membrane of the small granules is often closely attached to or fused with the cell wall (Fig. 4). While the mitochondria resemble those of lower levels, the free RNP particles seem to be less abundant than in the less differentiated cells. Mucus is secreted in large amounts by the cells near the horny layer. In this part of the epidermis the intercellular spaces contain numerous, small, dense droplets of mucus (Fig. 4).

The horny cells are almost entirely filled with keratin filaments between 70 and 80 A thick (Fig. 6). Endoplasmic reticulum, Golgi vesicles, and RNP particles cannot be seen in these cells; apparently these structures are completely eliminated during the transformation of mucus-producing cells into horny cells. Only a few nuclear remnants, a small amount of mitochondrial debris, and occasional mucous granules are visible. The membrane of most horny cells is straight; very slight convolutions occur only at the outer surface of the uppermost cells.

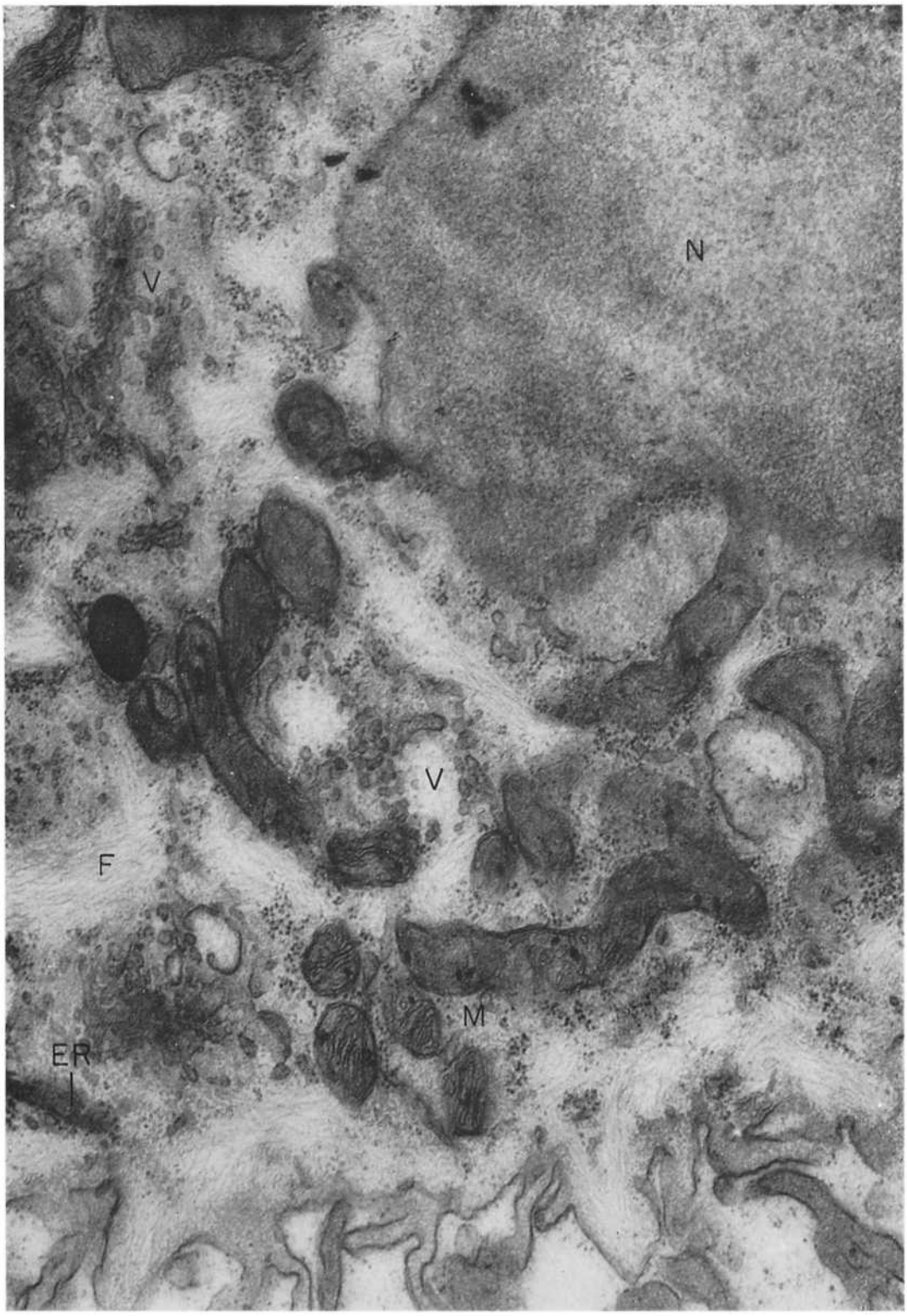
DISCUSSION

The basal cells of the epidermis of *Rana pipiens* are morphologically similar to those of land-dwelling mammals: both are filled with fine filaments, contain moderate numbers of mitochondria, and small islands of RNP particles scattered throughout the cytoplasm. Only small amounts of rough-surfaced endoplasmic reticulum are present and the Golgi zone is not well developed. Many, if not most, of the changes which take place in the amphibian cells during differentiation or keratinization resemble those which take place in mammalian cells (17, 23). For example, the prekeratin, which appears in the basal cells as birefringent, fine filaments containing sulfhydryl groups, does not change visibly during cell maturation, but eventually becomes the principal constituent, keratin, of the terminal horny cells. Nuclei, mitochondria, and certain other constituents, on the other hand, disintegrate and are partially or fully eliminated at corresponding stages in both types of epidermis as keratinization proceeds to the horny stage.

The most striking contrast between amphibian and mammalian epidermal cells appears in the middle layers of the epidermis where differentiation takes place. In this location mucous granules characteristically develop in the skin of *Rana pipiens*, whereas keratohyalin granules characteristically develop in the skin of land-dwelling mammals. These two types of granules differ structurally and chemically, but it is interesting to note that both are formed at corresponding levels of the epidermis and both disintegrate during the advanced stages of differentiation.

While the entire epidermis seems to participate in the process of keratinization, only the differentiating cells in the mid-portion of the epidermis appear to produce the mucus seen in the skin of

FIGURE 3 Electron micrograph of a part of a mucus-producing cell in an early stage of differentiation. Note the many vesicles (*V*) in the vicinity of the nucleus (*N*) and the relative abundance of endoplasmic reticulum (*ER*). The mitochondria (*M*) contain many dense bodies, and filaments (*F*) are numerous. × 36,000.



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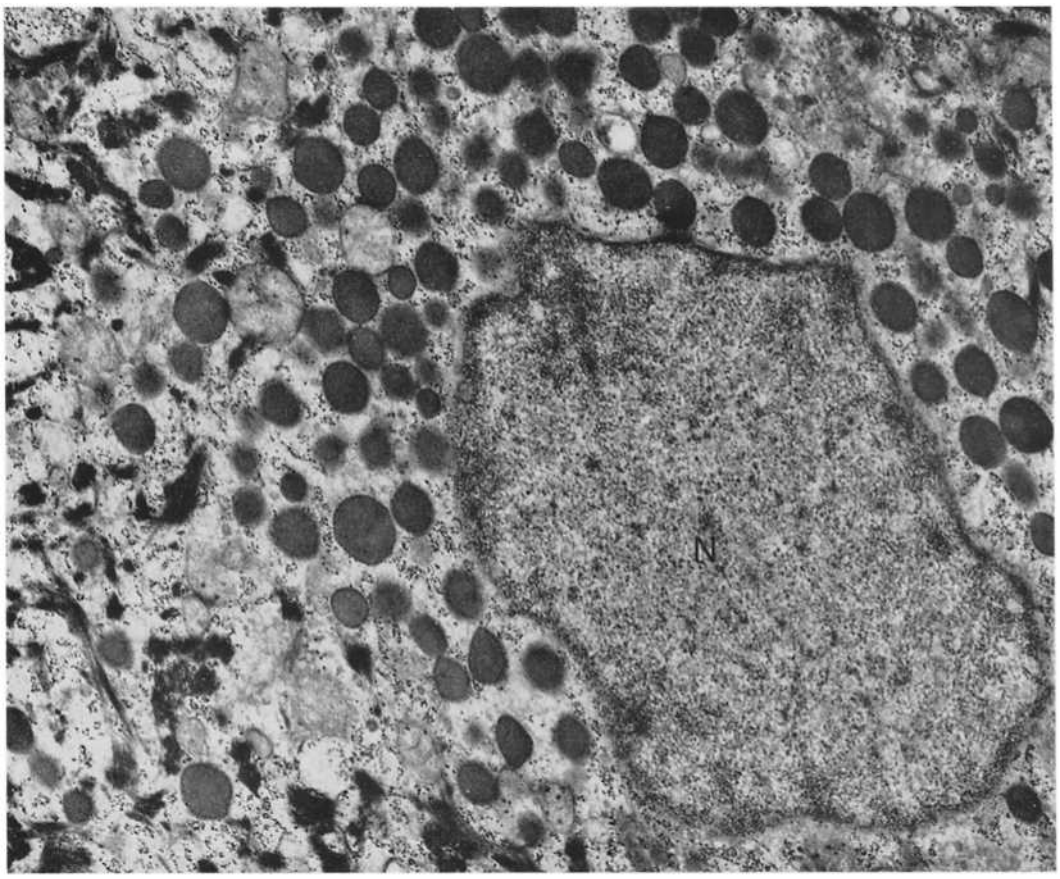

FIGURE 5 Electron micrograph of part of a differentiated mucus-producing cell. Note the large ovoid or spherical mucous granules around the nucleus (*N*). × 15,000.

Rana pipiens. Morphologic manifestations leave little doubt that the Golgi apparatus plays a basic role in the formation of mucous granules. Whether the Golgi vesicles synthesize or only store mucus remains to be determined, but, since the endoplasmic reticulum is profuse and its cisternae are often filled with a dense mucus-like substance (Fig. 4), it seems probable that synthesis takes place in the endoplasmic reticulum, and that the mucus there formed accumulates in the Golgi vesicles. It is interesting to note that the formation of secretory granules in goblet cells (24) and in the acinar cells of the pancreas (25) has been assumed to proceed in the same way.

The small droplets in the intercellular spaces (Fig. 4) are clear evidence that mucus is secreted by differentiating epidermal cells. The positive PAS reaction of the horny cells suggests, however, that mucus is also retained in the horny cells. Since intact mucous granules are rarely seen in the horny cells, it seems reasonable to suppose that the retained mucus is finely dispersed between the keratin filaments.

Electron micrographs suggest that mucus is re-

FIGURE 4 Electron micrograph showing mucus-producing cells located in the upper part of the epidermis. Membrane-bounded mucous granules (*MG*) occur near the cell membrane and scattered throughout the cytoplasm. The cells contain many filaments (*F*). The endoplasmic reticulum (*ER*) is relatively abundant. Note the dense droplets (*DD*) in the intercellular space. × 31,000.

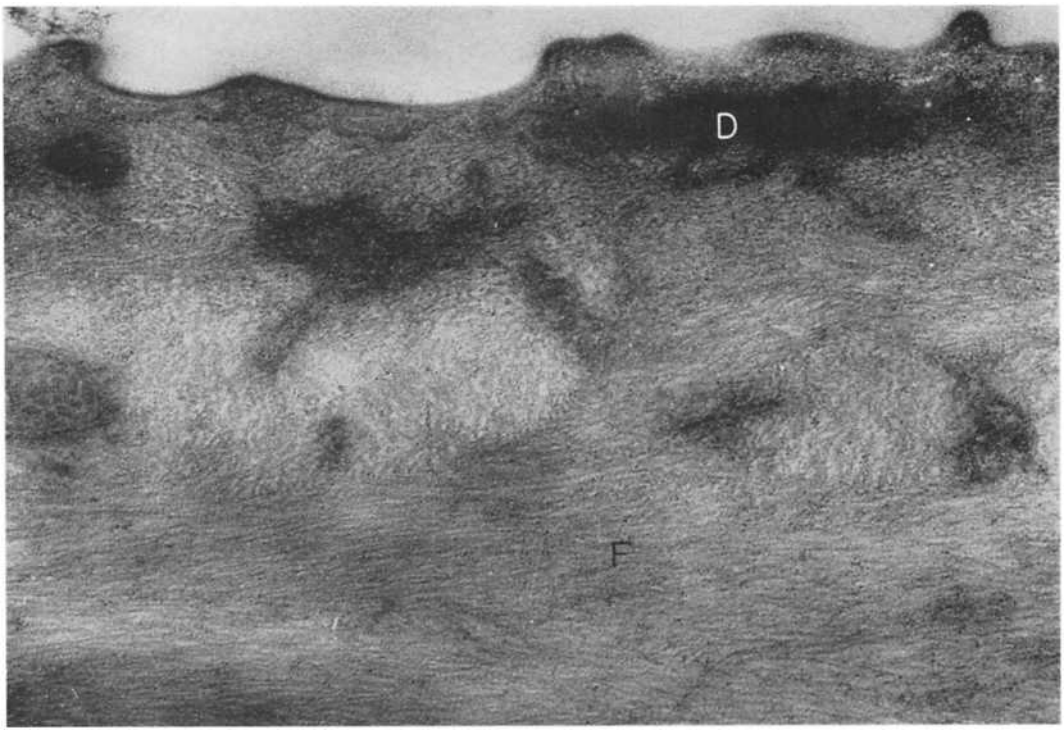

FIGURE 6 Electron micrograph of part of a horny cell located at the surface of the epidermis. It is filled mainly with filaments (*F*), but some debris (*D*) from other cell constituents also is present. × 42,500.

leased from small granules which lie near the cell membrane (Fig. 4). Because the membrane of the mucous granules often appears to be fused with the membrane of the cell, it seems logical to conclude that mucus leaves the cells through ruptures in the portion of the fused membrane which faces the intercellular space. It has been postulated elsewhere (25) that discharge of the content of zymogen granules is effected by a mechanism of this kind.

Mucus is capable of binding water in large quantities and, therefore, may serve to protect the frog from general dehydration and assume proper functioning of the cutaneous respiratory system. The production of mucus thus appears to be a vitally important function of amphibian epidermis.

The epidermis of frog larvae has previously been studied in detail with the electron microscope (26–29), and evidence has been obtained that it, also, is engaged in mucus production. In 3-mm larvae, the epidermis is only two cells thick, but secretory granules can be seen in the apical cells. In older larvae, filaments develop in the basal cells, whereas mucous granules develop in the fibrous cytoplasm of the more superficial cells (28). One may conclude, therefore, that the production of mucus is an inherent function of the epidermal cells of the frog, for it starts during early larval life and continues after metamorphosis has taken place.

Considered from a broad biological viewpoint, the findings reported here suggest that the well known bipotentiality (23) of the epidermal cells of "higher" vertebrates, *i.e.*, their capacity to produce either keratin or mucus, may be of phylogenetic origin. In such perspective, the mucous metaplasia induced by excess vitamin A or other means (30–34) may be interpreted as due to activation of a dormant mechanism that is present at all times in the epidermal cells of "higher" vertebrates.

This investigation was supported in whole by Public Health Service Research Grant A-5779 from the National Institute of Arthritis and Metabolic Diseases.

Received for publication, March 25, 1963.

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