A SMALL GRANULAR COMPONENT OF THE CYTOPLASM OF KERATINIZING EPITHELIA

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We have often observed small dense bodies in the cytoplasm of cells of the stratum granulosum during a study of the fine structure of normal and experimentally altered mouse ear epidermis. Odland (10) noted their presence in human epidermis, showed that they have an internal structure, and speculated that they may be degenerating mitochondria. Other authors seem to have overlooked them in their descriptions of the fine structure of skin, although the bodies appear in their micrographs (1, 6, 9, 11, 12). These small bodies appear to be a normal component of the cytoplasm of keratinizing epithelia, and may play some role in keratinization. We suggest that these bodies be referred to as *corpuscula.*

MATERIALS AND METHODS

Electron microscopic observations were made on the ear epidermis of eight normal adult male Swiss albino mice, and four adult male Swiss albino mice whose skin was made hyperplastic by topical applications of 5 per cent croton oil in liquid paraffin twice a week for 5 weeks. Specimens of ear epidermis were removed while the mice were under ether anesthesia. The specimens were fixed in either cold buffered 1 per cent isotonic osmium tetroxide (16) or cold buffered 2 per cent isotonic potassium permanganate (4) for periods ranging from 10 to 60 minutes, and were dehydrated and embedded in Epon by Luft's method (5). Sections were cut on either an LKB or a Porter-Blum microtome with a glass knife, mounted on carbonized Formvar-coated grids, stained with lead hydroxide for 15 minutes (14), and examined in an RCA EMU 3E microscope.

OBSERVATIONS

Despite minor modifications in techniques of fixation and embedding, the fine structure of the epidermis as seen in our preparations of the mouse

ear is similar to that previously described in the mouse skin (6, 11), human skin, (9), and keratinized mouse cornea (13). Epidermal cells of the deeper layers contain various components common to most epithelial cell types (mitochondria, rough and smooth surfaced components of the endoplasmic reticulum, and clusters of RNP particles). They also have the abundant filaments which are characteristic of the cells of integument. These tonofilaments, which are gathered in bundles (tonofibrils of light microscopy), appear to course through the cells and terminate in the desmosomes or nodes of Bizzozero. Dense oval or round bodies larger in diameter than mitochondria are seen especially well in the middle layers of the hyperplastic epithelium. These large bodies are the so called keratohyaline granules of light microscopy.

In addition to these components, electron micrographs of both potassium permanganate and osmium tetroxide–fixed skin show large numbers of small round or oval bodies which are smaller than the keratohyaline granules but larger than the RNP particles of the cytoplasm (Figs. 1 and 2). These are the bodies which we propose to call *corpuscula*. They are more easily seen in hyperplastic epidermis, where the various strata of the epithelium are better developed (Fig. 2), but they can be readily observed in normal epidermis (Fig. 1). No corpuscula appear in the stratum germinativum. In most cells of the deeper part of the stratum granulosum they are distributed throughout the cytoplasm, and often appear in the area close to the nucleus or near the Golgi apparatus (Fig. 2). Corpuscula do not appear in the superficial part of the stratum granulosum, which contains the large keratohyaline granules, nor do they appear in the stratum lucidum or corneum.

Corpuscula are round or oval in sections of tissue fixed with either osmium tetroxide or potassium permanganate; they are about 0.15 micron in diameter. Many corpuscula appear homogeneously dense and structureless, but some of them appear to have an internal structure (Figs. 1 to 5). Some appear to have a clear center. With both types of fixation after staining with lead hydroxide the particles appear to lie free in the cytoplasm rather than in particular topographic association with any other cytoplasmic component. There are, however, suggestions at the resolution achieved in the present study that corpuscula may be enclosed with a single smooth membrane (Figs. 2, 4, and 5). In some sections corpuscula appear to lie within invaginations of the cell surface membrane (Fig. 5) close to the space between two cells. Between cells of the superficial layers of the stratum granulosum (Fig. 6), which do not contain corpuscula, dense extracellular material is seen. A similar extracellular material between cells in this region was described by Brody (1). Our material does not provide any suggestion that corpuscula are related to mitochondria as was speculated by Odland (10).

DISCUSSION

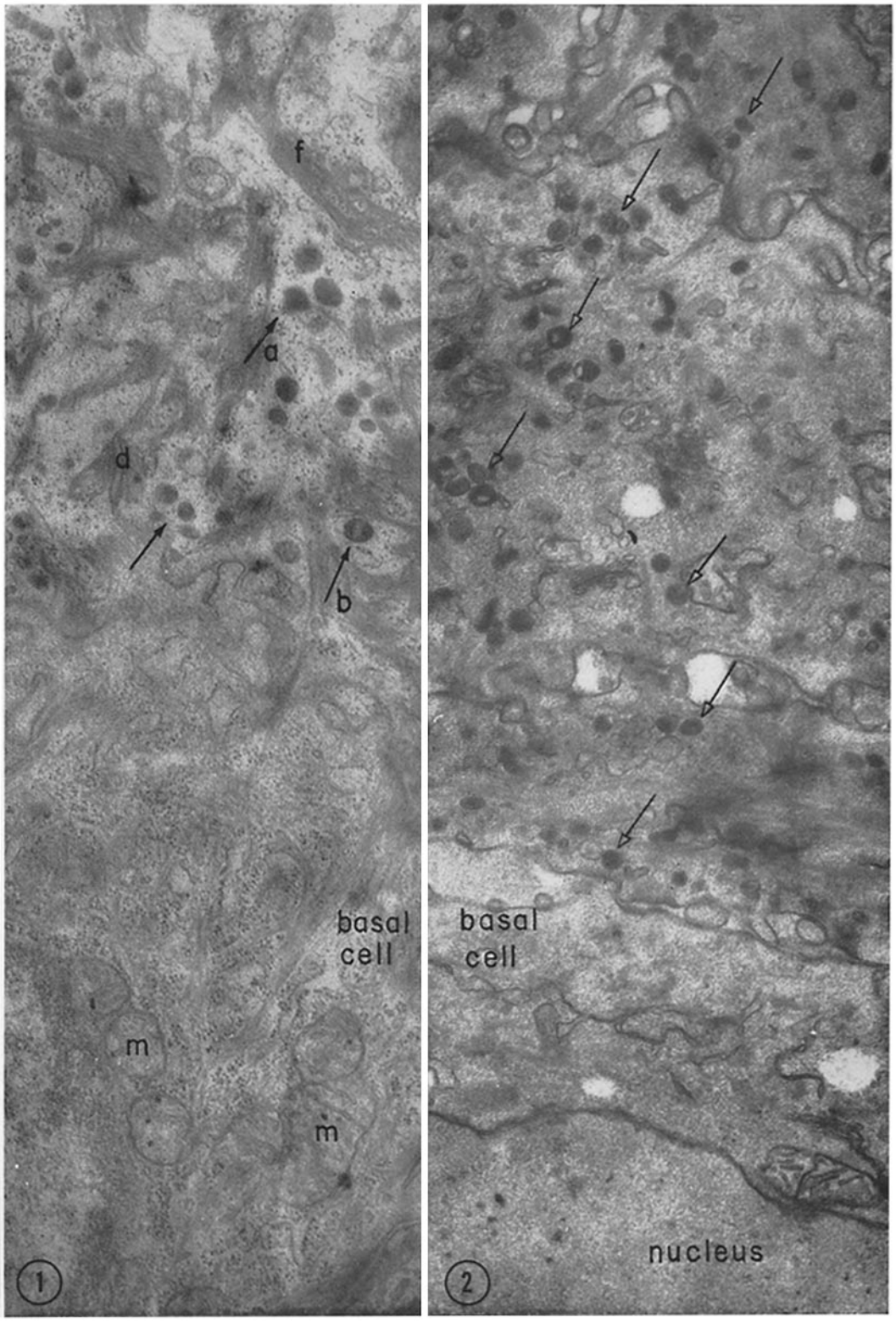
The size and the internal structure of the corpuscula are reminiscent of viruses. They are quite similar to the viral bodies observed by Dour-

FIGURE 1

This electron micrograph of osmium tetroxide–fixed mouse skin shows a part of two cells from a normal animal. The upper cell contains many small bodies (arrows) which we refer to as *corpuscula*. At arrow *a* there is a homogeneously dense corpusculum; at arrow *b* is a corpusculum with a translucent central part. Bundles of filaments (*f*) and mitochondria (*m*) lie in the cytoplasm. The basal cell (at the bottom) contains more RNP particles than the more superficial cell. A desmosome lies just under letter *d*. × 26,000.

FIGURE 2

This electron micrograph of potassium permanganate–fixed mouse skin shows parts of several cells from hyperplastic epithelium. Many corpuscula (arrows) are seen in the cells above the basal layer. The nucleus of a cell from the basal layer of the epidermis is at the bottom (*nucleus*). Neither the filaments within the cytoplasm nor the RNP particles are as readily apparent as in tissues fixed in osmium. × 21,000.



mashkin and Bernhard in molluscum contagiosum (2). In spite of the morphological similarity to this virus, however, the corpuscula of keratinizing epithelium are not seen within nuclei, in clusters, in crystalloid arrangements, or extracellularly in structurally intact form, as are viruses. Except for their presence, they do not appear to alter the morphology of the epidermal cells. The animals used for these observations were healthy and were not known to carry any viral infection. It is possible that corpuscula are previously unidentified and unsuspected viruses, such as those found by Karrer in otherwise normal chick embryos (3).

The corpuscula are also somewhat similar to granules observed by Watson in the cytoplasm of the ameloblast of the rat (15). He assumes that the granules in the ameloblast, which is a derivative of epidermis, represent secretory granules concerned with the production of enamel matrix. From our observations it would seem that the elaboration of corpuscula proceeds from within the cytoplasm, perhaps in relation to the Golgi apparatus. The contents of the corpuscula may be extruded into the intercellular space of the stratum granulosum, where we have observed dense material which no longer has the morphology of the corpuscula. Cells superficial to this zone are devoid of corpuscula.

The localization of corpuscula in the stratum granulosum and the presence of a substance which may be derived from them in the extracellular space correspond to a zone which Montagna has shown to be particularly rich in non-specific esterase (7). He has also described this same narrow band as rich in sulfhydryl groups (8).

SUMMARY

Corpuscula, cytoplasmic organelles of stratified squamous epithelium, are seen in the deeper part of the stratum granulosum of the normal and hyperplastic epidermis of the mouse. It is possible that their content is extruded into the intercellular spaces of the stratum granulosum. Their role in the process of keratinization is not clear.

Dr. Frei is a Life Insurance Medical Research Fund Fellow in Pathology. Dr. Sheldon is a Markle Scholar in Medical Science. This investigation was supported in part by Grant No. A-4422 from the United States Public Health Service, National Institute of Arthritis and Metabolic Diseases.

Received for publication, July 12, 1961.

FIGURE 3

This electron micrograph shows more corpuscula in permanganate-fixed hyperplastic epithelium and demonstrates some variations in their internal structure. At the arrow there is a corpusculum with a translucent center. Mitochondria, *m*. × 23,000.

FIGURE 4

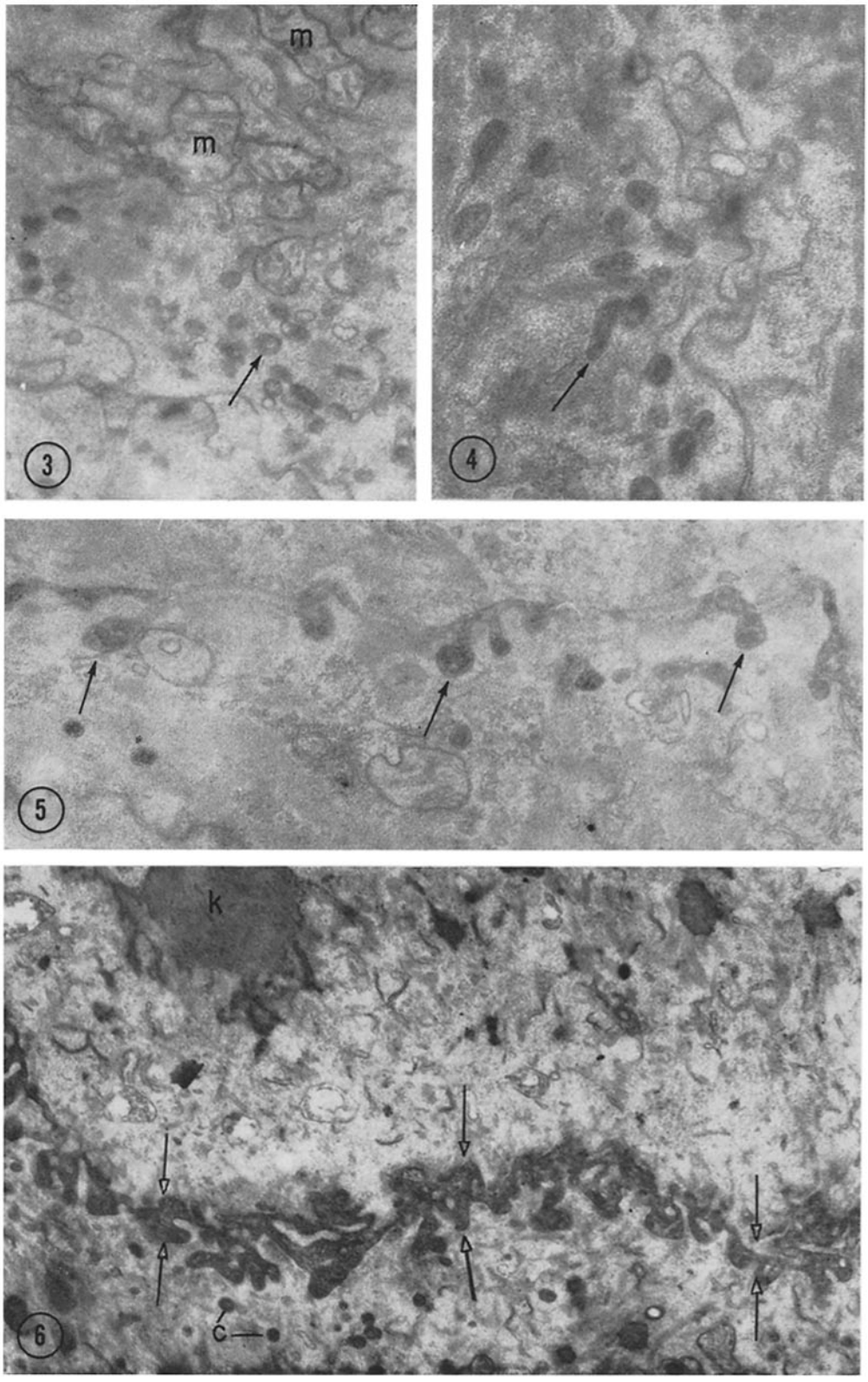
This electron micrograph shows the appearance of corpuscula near the surface of an epithelial cell from tissue fixed with potassium permanganate. At the arrow there is a corpusculum surrounded by a membrane. × 31,000.

FIGURE 5

This electron micrograph shows parts of two cells from the stratum granulosum of normal epidermis. Here corpuscula appear to lie within invaginations of the cell surface membrane (arrows), and material of a similar density appears between the cells. × 30,000.

FIGURE 6

This electron micrograph shows parts of two cells from the stratum granulosum of hyperplastic skin. The lowermost cell contains abundant corpuscula (*c*) but the uppermost cell, which contains a "keratohyaline granule" (*k*), is empty of corpuscula. The space between these two cells (arrows) contains a dense material. × 15,000.



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