FORMATION OF HORNY CELLS

The Fate of Cell Organelles and Differentiation Products in Ruminal Epithelium

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ABSTRACT

Epithelial cells changing from the granular stage of differentiation to the horny stage are more numerous, and reveal sequential events of transformation in finer detail in the rumen epithelium than in other keratinizing epithelia thus far studied in the electron microscope. Studies of such cells indicate that transformation is initiated by the release of hydrolytic enzymes, as evidenced by the appearance of lysosomes. As lysosomes increase in number, the nucleus, ribosomes, mitochondria, Golgi apparatus, and mucous granules are gradually degraded. Furthermore, marked changes occur in permeability of the plasma membrane as voluminous amounts of the lysed cell components pass through and accumulate in the intercellular space in the form of an amorphous mass. Filaments, keratohyalin granules, and the content of the ER (ER-protein) are not lysed, revealing the action of released enzymes to be specific. During transformation, filaments become displaced toward the cell periphery and keratohyalin granules disperse and mix with the ER-protein in the cell center. Subsequently, the keratohyalin-ER-protein complex infiltrates the filament network whereby a fibrous-amorphous cell content is formed. Loss of fluids through the plasma membrane leads to reduction of cell volume and consolidation of the remaining cell content. The deep interdigitations formed between the cells ultimately interlock the outer part of the epithelium into a cohesive and protective stratum corneum.

INTRODUCTION

Mammalian epithelial cells pass through two distinct phases while they differentiate and change into horny cells. The first is the synthetic phase when the cell is making products that are necessary for keratinization. In epithelia, such as the epidermis, oral epithelium, esophageal epithelium, etc., the characteristic differentiation products are 60–80 A filaments, membrane-coating granules, and keratohyalin granules. After the epithelial cell has synthesized all of its differentiation products, the second or transformation phase begins. This phase involves the conversion of a fully developed granular cell into a horny cell consisting mainly of a filament-amorphous-matrix complex enveloped by a thickened plasma membrane (14, 17). For the most part, investigators studying the fine structure of keratinizing epithelia have concentrated on the synthetic phase of cell differentiation (2–4, 7, 9, 15, 17, 19, 21). Consequently, little attention has been focused on the transformation phase, e.g., the change of granular cells to horny cells. So far, only a few transition or T cells have been observed in the electron microscope (2, 4). Such cells were flattened and enveloped by a modified plasma membrane and contained keratohyalin

fused into large dense masses. The sequential events leading to the formation of the terminal filament-amorphous-matrix complex have not, as yet, been followed in detail.

T cells do not form a continuous layer below the stratum corneum of keratinizing epithelia such as the epidermis, oral, lingual, esophageal, and vaginal epithelia. Within these tissues, T cells are few and usually appear as single cells between the stratum granulosum and corneum (13). Furthermore, both granular and T cells adjacent to horny cells are generally poorly preserved (2, 3, 13, 16). Thus, these tissues are not favorable for detailed study of changes taking place during transformation of granular cells into horny cells.

During the course of our fine structural studies of bovine and sheep ruminal epithelium (9–11),

RESULTS

The rumen epithelium consists of four to five layers of nucleated cells and a relatively thin stratum corneum formed by two to three layers of horny cells. Between the stratum corneum and the granular layer, T cells are present in a relatively large number, revealing different stages of transformation (Fig. 1). Survey of T cells in the electron microscope reveals, in remarkable detail, the sequential events taking place while the cells change from the granular into the horny stage.

Granular Stage

The cytoplasm of the granular cell (Fig. 2) contains many small and large keratohyalin granules as well as mucous granules. Keratohyalin granules are predominantly located in
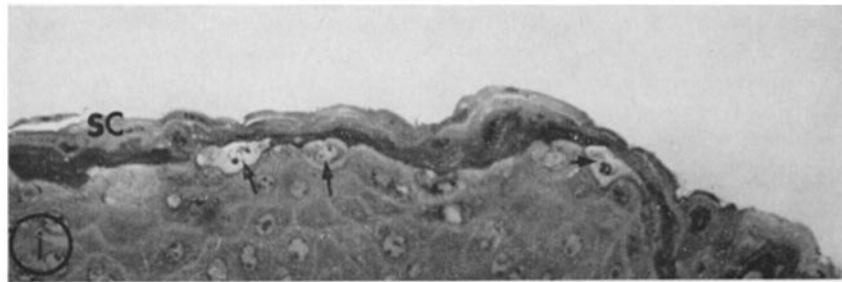

FIGURE 1 Photomicrograph of ovine ruminal epithelium. Note T cells (arrows) adjacent to stratum corneum (*SC*). × 300.

it was noted that in this keratinizing epithelium T cells were numerous, well preserved, and revealed sequential events in fine detail. Consequently, the ruminal epithelium was considered to be a suitable model for studying the transformation phase and has been used in this study.

METHODS

Tissue samples were obtained from sheep maintained on a diet of chopped coastal Bermuda grass hay fed *ad libitum*. Small tissue fragments were excised from the left lateral wall of the cranial sac of the ovine rumen, fixed in 2% osmium tetroxide buffered to pH 7.4 with veronal-acetate (18), and dehydrated in a graded series of cold (5°C) ethanol baths. The samples were embedded in Epon 812 (12) and sectioned with a Porter-Blum MT-2 microtome. Thin sections, stained with uranyl acetate and lead citrate (24), were examined in an RCA EMU 3F electron microscope.

the perinuclear region of the cell and are surrounded by ribosomes. Large membrane-bounded mucous granules appear in the perinuclear region of the cell, while the more abundant small membrane-bounded mucous granules are at the cell periphery. Filament bundles are distributed throughout the cytoplasm. Numerous cisternae of rough endoplasmic reticulum are scattered throughout the granular cell. The endoplasmic reticulum is dilated, and the cisternae are filled with material which reveals a fine filamentous structure (Fig. 3). This material will be referred to as ER-protein. In addition to these products, the granular cell contains abundant mitochondria, numerous free ribosomes, Golgi bodies, and a few lipid droplets. The plasma membrane is 60–80 A thick and in most places is closely apposed to adjacent cells.
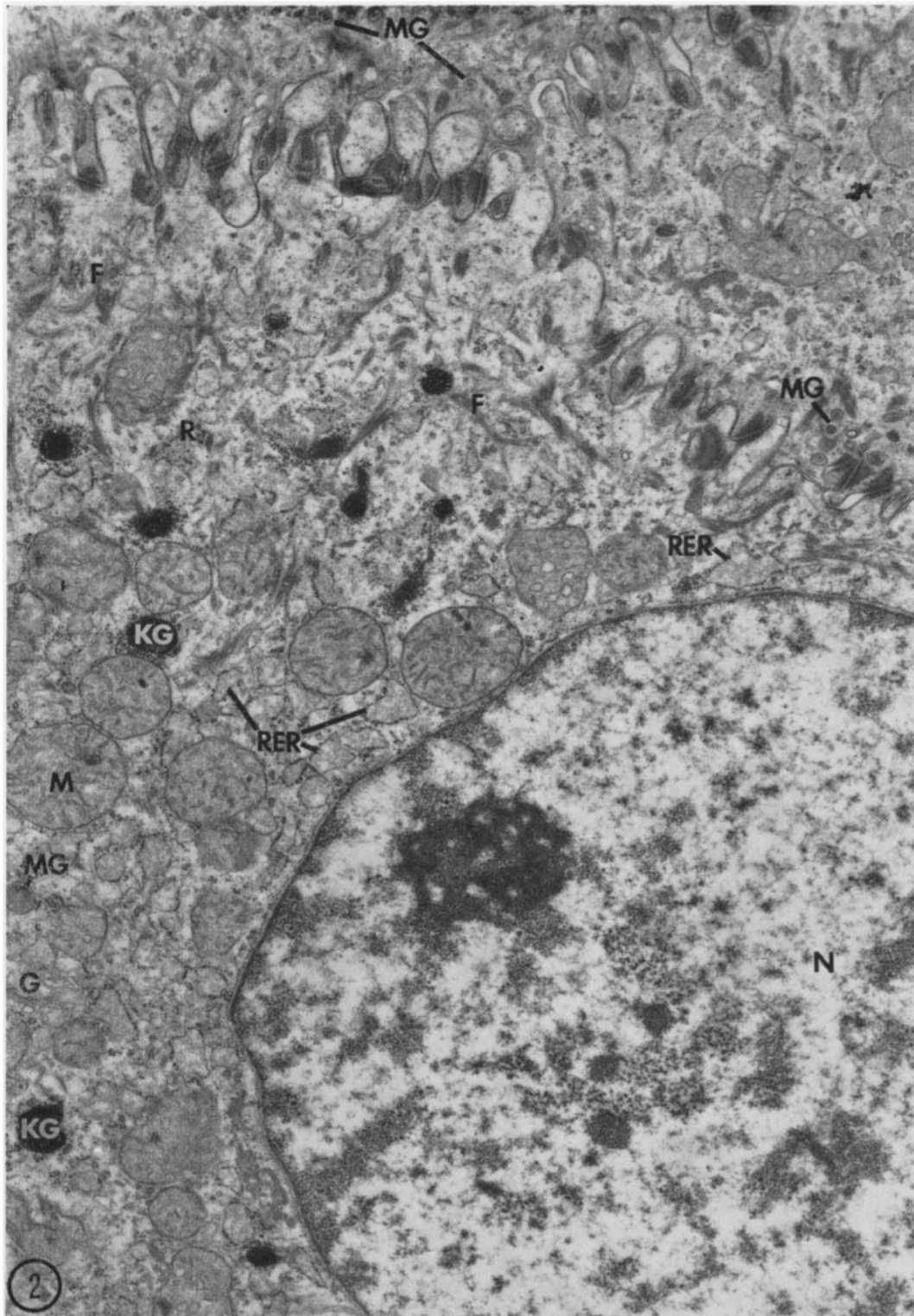
FIGURE 2 Part of granular cell showing keratohyalin granules (*KG*), large and small mucous granules (*MG*), dilated rough endoplasmic reticulum (*RER*), and filament bundles (*F*). Mitochondria (*M*), free ribosomes (*R*), and Golgi bodies (*G*) are scattered throughout the cytoplasm. *N*, nucleus. × 12,500.
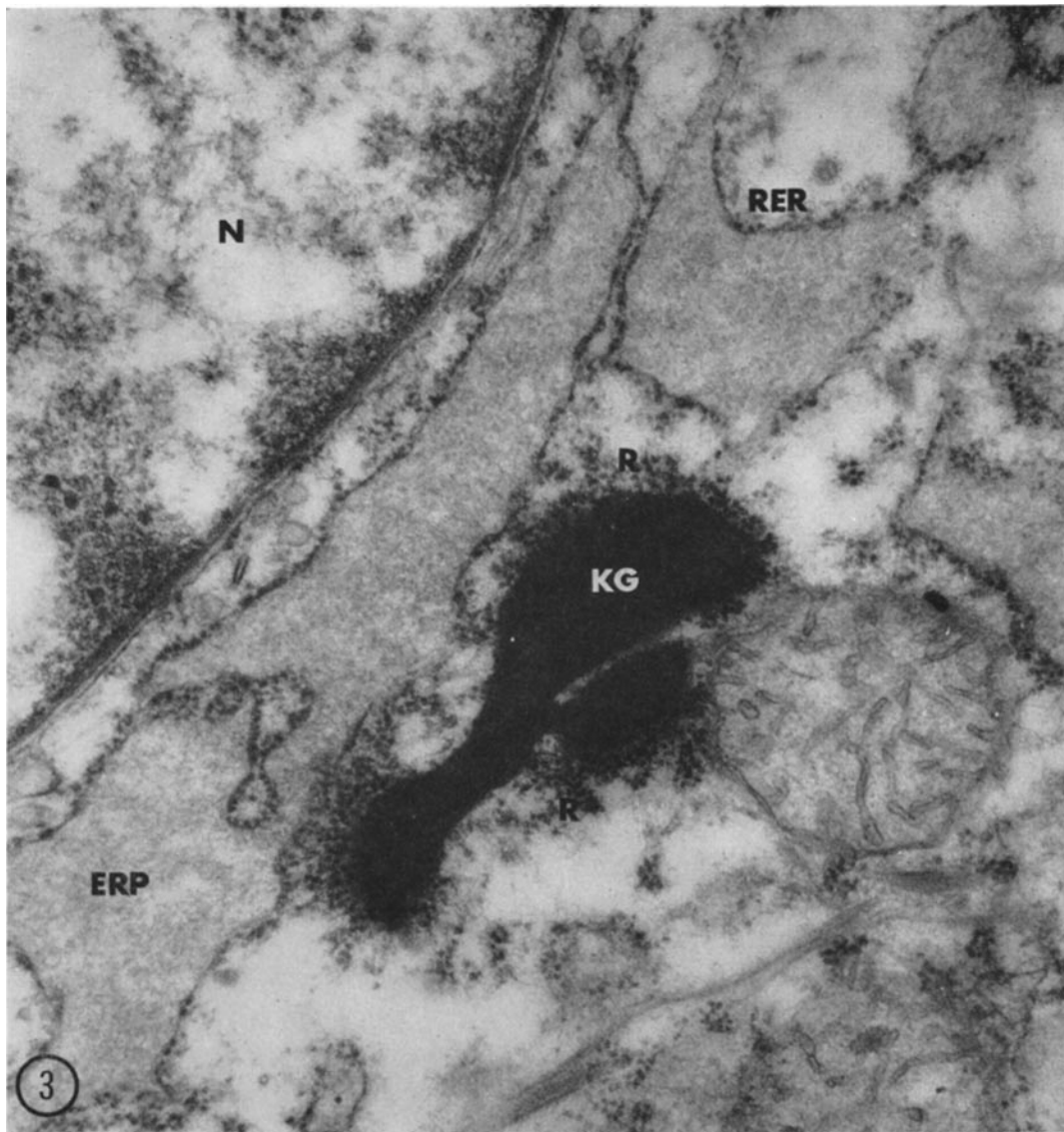
FIGURE 3 High magnification of the dilated rough endoplasmic reticulum (*RER*) showing the cisternae filled with the filamentous ER-protein (*ERP*). Note the large amount of ribosomes (*R*) which surround the keratohyalin granules (*KG*). *N*, nucleus. × 44,000.

Transformation Stages

During early stages of transformation (Fig. 4), the keratohyalin granules aggregate and form large masses, and filament bundles accumulate at the cell periphery where the small mucous granules are located. Cisternae of the rough endoplasmic reticulum are highly dilated and filled with ER-

protein. An occasional lysosome-like body can be seen in the cytoplasm. Aggregation and displacement of cell constituents toward the cell periphery result in a clearing around the nuclear region of the cell.

As lysosome-like bodies become more numerous, mitochondria, Golgi apparatus, mucous granules, ribosomes, and ER appear degraded (Fig. 5).
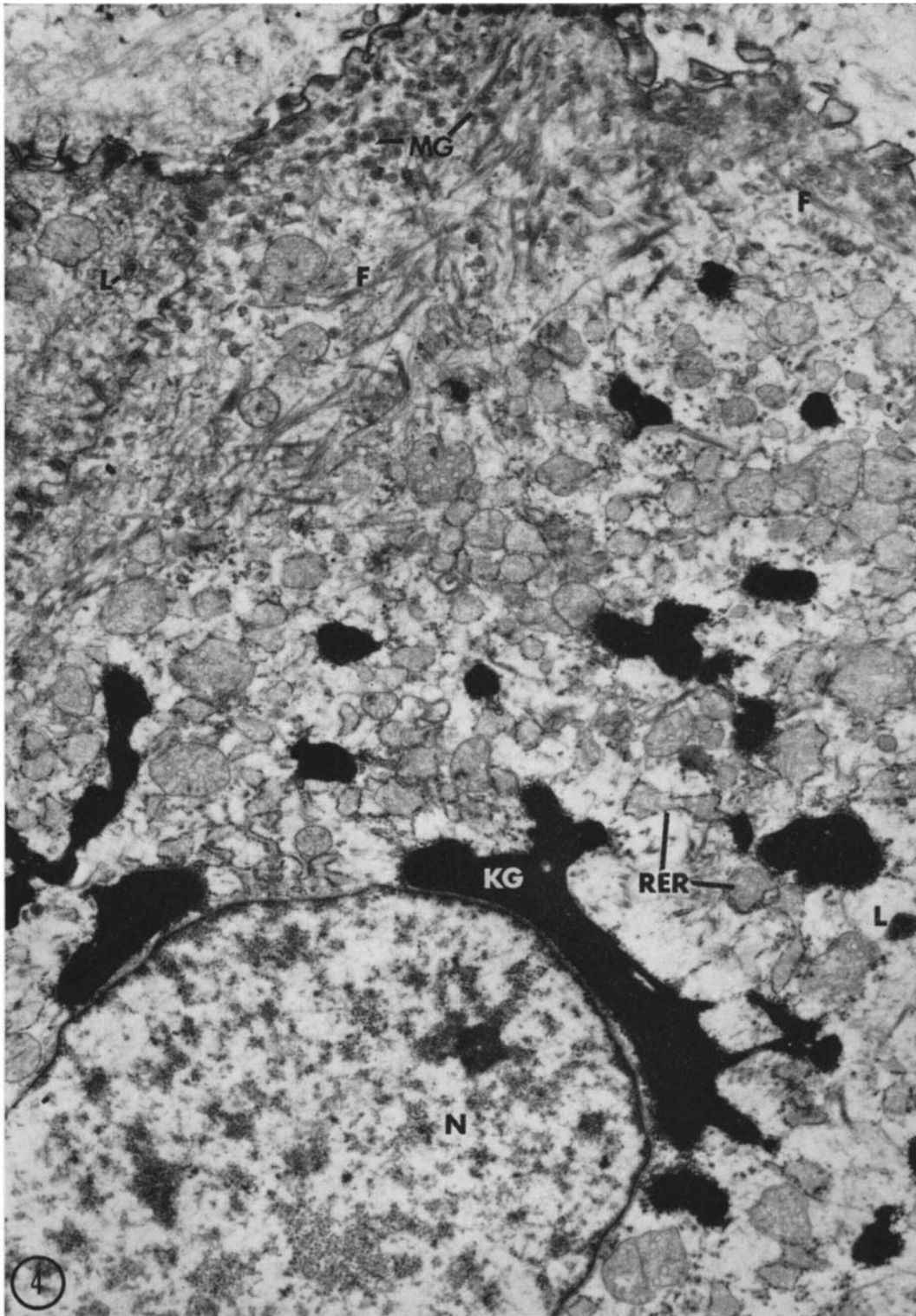
FIGURE 4 Aggregating keratohyalin granules (KG) and highly dilated rough endoplasmic reticulum (RER) are shown in a T cell in an early stage of transformation. Filament bundles (F) accumulate at the cell periphery, and lysosome-like bodies (L) appear scattered in the cytoplasm. N, nucleus; MG, mucous granules. × 12,500.
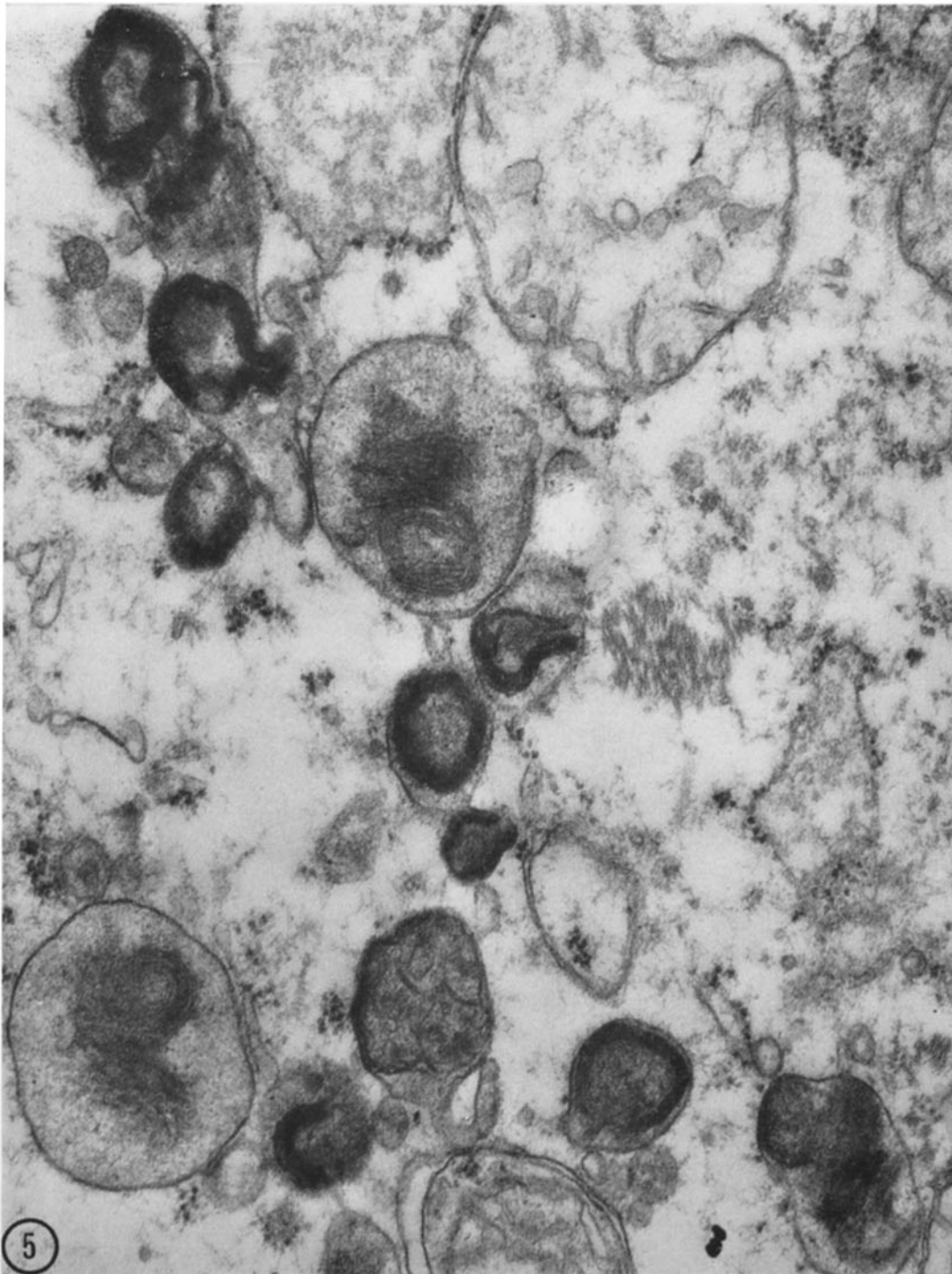
FIGURE 5 Various forms of lysosomal bodies in a T cell. × 46,500.

The keratohyalin granules are vacuolated and are noted spreading in the cytoplasm (Fig. 6). The ER-protein is free in the cytoplasm, and the membranes and ribosomes of the ER are not apparent. Filaments are not lysed; they are observed in increasing numbers at the cell periphery. Some of the lysed cell content passes through the plasma membrane and appears as a dense mass
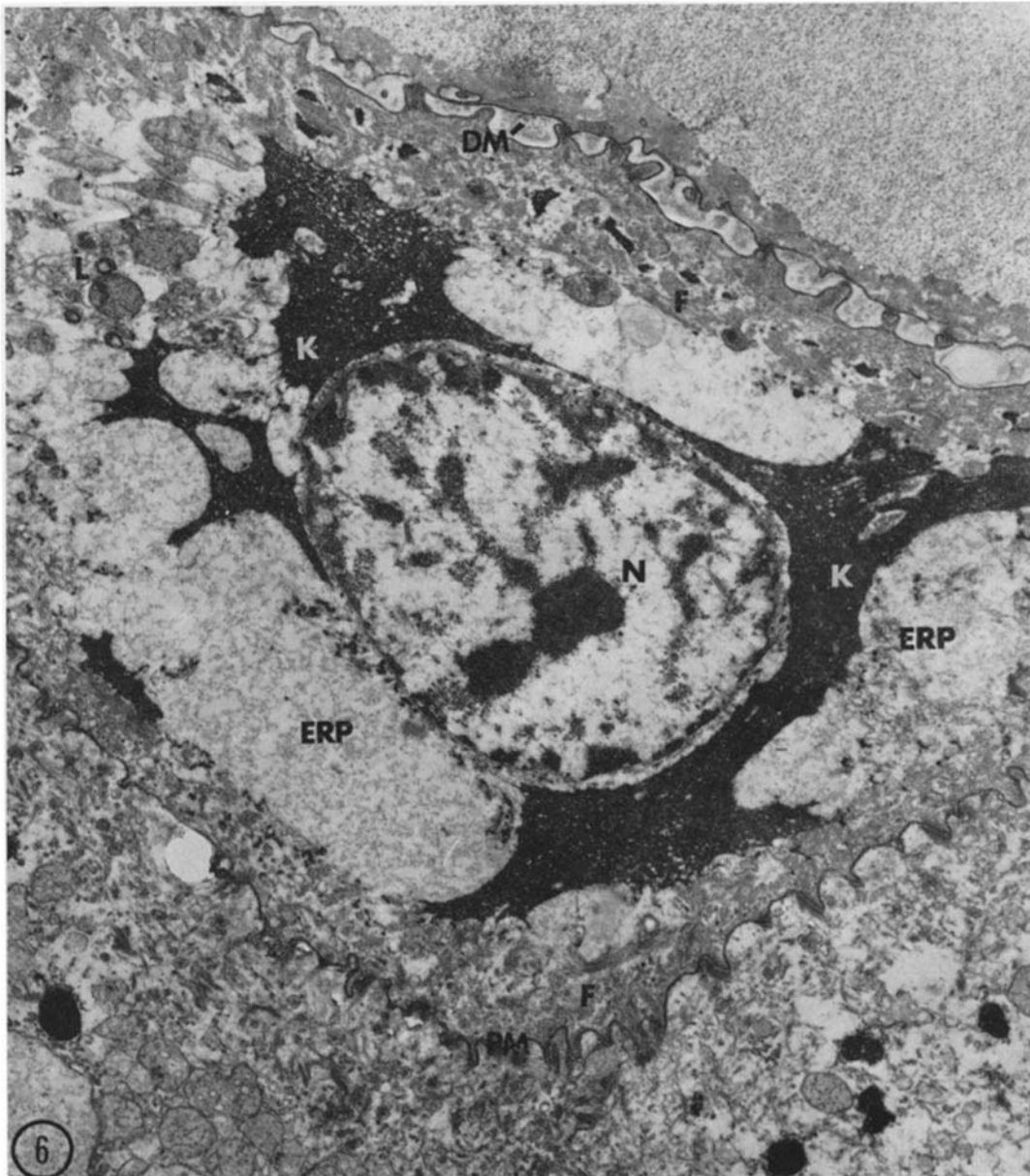

FIGURE 6 T cell in an advanced stage of transformation. Keratohyalin (K) is spreading in the cytoplasm along with the free filamentous ER-protein (ERP). Filaments (F) are numerous at the cell periphery. The thickened plasma membrane (PM) is closely attached to the adjacent granular cell. Widened intercellular spaces appear between the T cell and the neighboring horny cells filled with a dense material (DM). L, Lysosomes; N, degenerating nucleus. × 11,500.
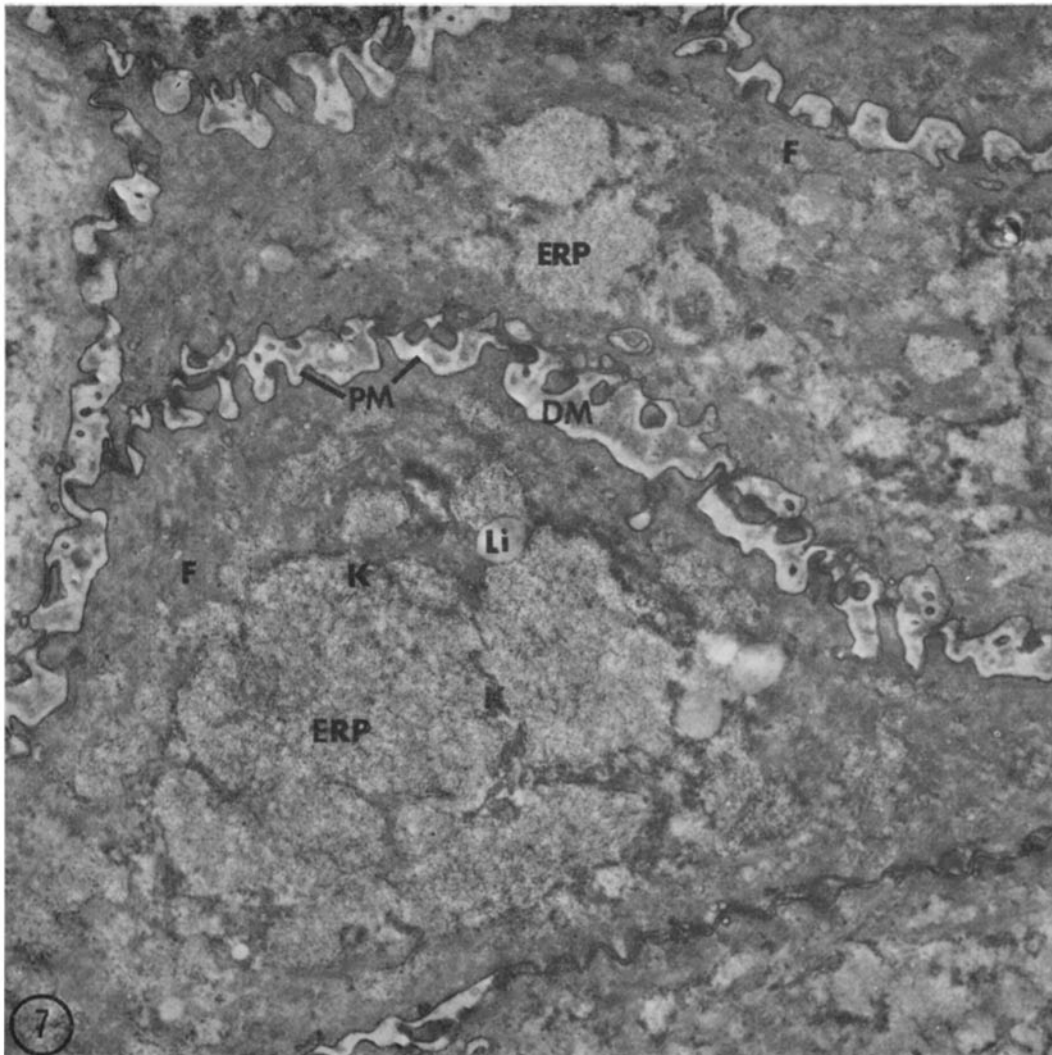
FIGURE 7 Portions of T cells in an advanced stage of transformation are shown. The cell content appears condensed and voluminous amounts of an amorphous material can be seen in the intracellular spaces *(DM)*. The central area of the cells is mainly filled with dispersed keratohyalin *(K)* and ER-protein *(ERP)*, keratohyalin appearing more dense than the ER-protein. The peripheral area mainly contains filaments *(F)*. The cells are enveloped by a thickened membrane *(PM)*. Lipid droplet, *Li*. × 10,500.

in the widened intercellular space (Fig. 6). Concomitant with loss of material, the T cells flatten and the plasma membrane shows deep folds and appears thicker.

During the subsequent phase (Fig. 7), cell organelles including the nucleus are further degraded. The cell volume decreases as more material is lost into the intracellular space, and the thickened plasma membrane forms deeper folds. Almost all of the filaments are, by now, displaced toward the cell periphery. In the central portion of the cell, dispersed keratohyalin is seen as a dense substance, as well as the translucent ER-protein and some remnants of other cell organelles (Fig. 7). In the peripheral area, the keratohyalin-ER-protein complex appears between the filaments (Fig. 8). Lipid droplets are scattered in the central part of the cell (Fig. 7).

Finally, a flattened horny cell with a condensed content is formed (Fig. 9). The horny cell
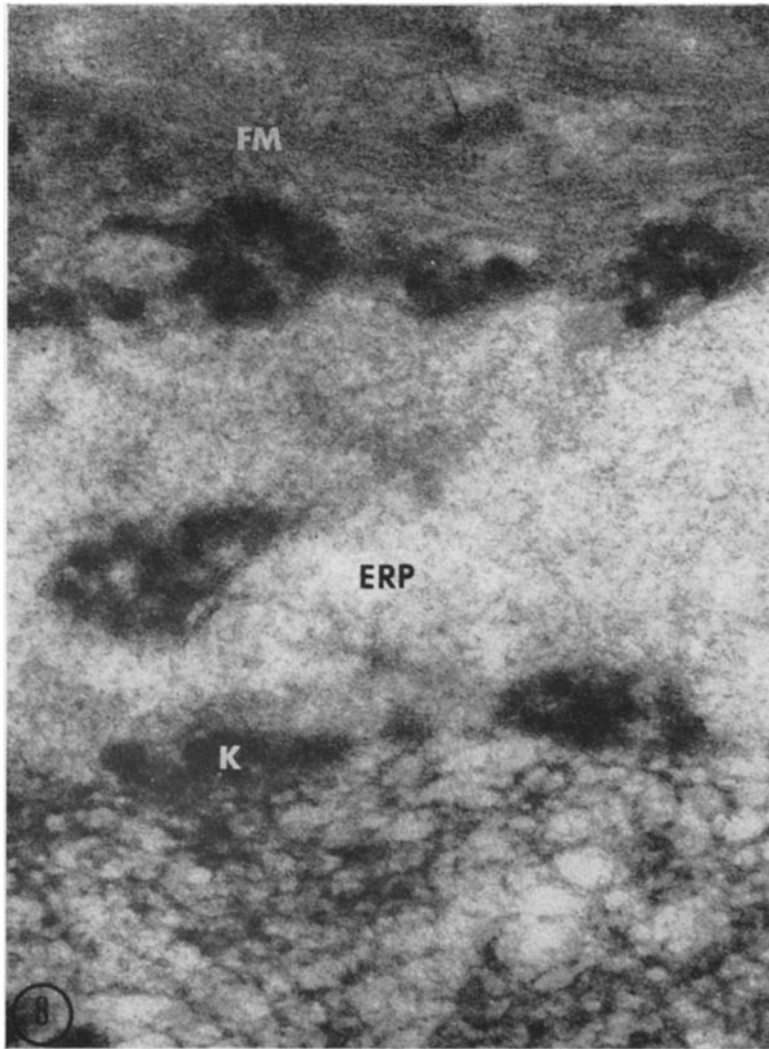
FIGURE 8   The terminal filament-matrix complex (*FM*), dispersed keratohyalin (*K*), and ER-protein (*ERP*) are shown at a high magnification in a T cell in an advanced stage of transformation. × 85,000.

is enveloped by a thickened plasma membrane which is thrown into microvillus extensions. Occasionally, lipid droplets and some cell debris are observed in the cytoplasm.

DISCUSSION

It is generally agreed that in keratinizing epithelia filaments are retained during transformation of epithelial cells into horny cells and will form the major part of the protective substance, keratin. While the decomposition of the nucleus is noted, retention or degradation of other cell com-

ponents is not adequately classified (2–4, 9, 14, 17, 21).

The appearance of lysosomes in ruminal epithelial cells, at a time when the cell is filled with differentiation products, indicates that hydrolytic enzymes play an important role in the transformation process. Although lysosomes have been noted, so far, only in a few keratinizing epithelia, substantial evidence supports the view that keratinizing epithelia synthesize a variety of enzymes necessary for the lysis of cell organelles. Lysosomes have been observed in normal (6)
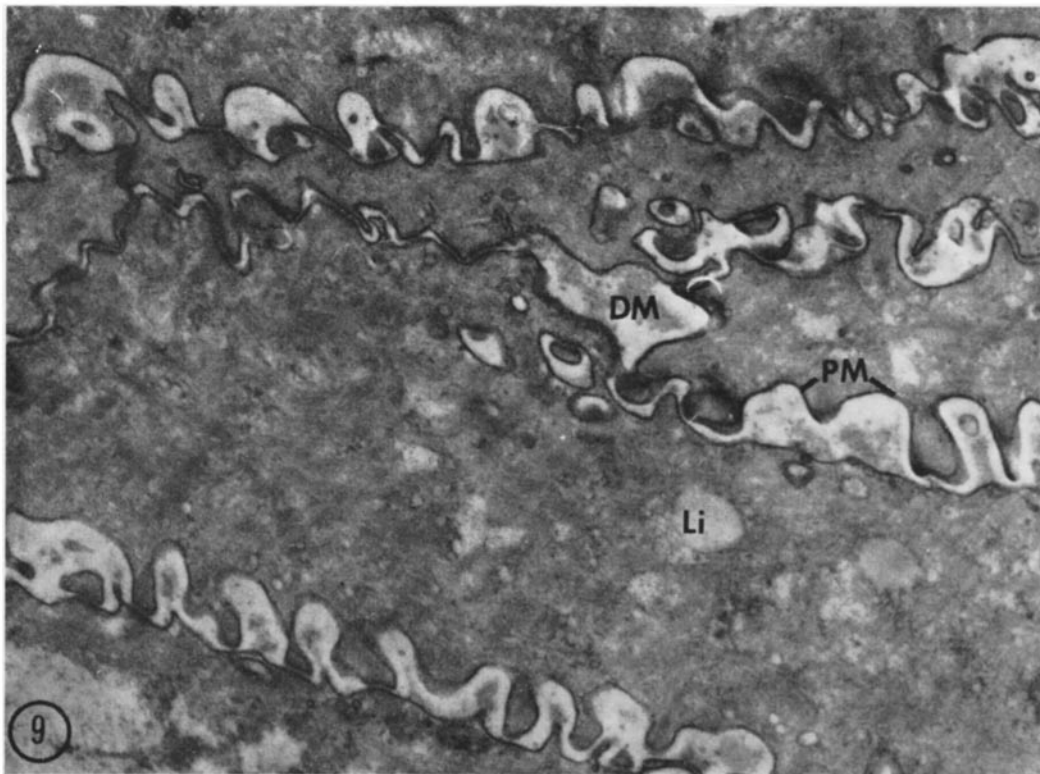
FIGURE 9 Portions of horny cells are shown. The thickened plasma membrane (*PM*) is thrown into microvillus extensions. Remnants of lipid droplets (*Li*) appear in the cells, and the intercellular spaces contain some dense material (*DM*). × 16,500.

and wounded human[1] and frog epidermis[2], experimental keratoacanthoma of the rabbit skin (20) and psoriatic epidermis (1). In the frog epidermis, Farquhar and Palade (6) noted that lysosomes increase in number in the stratum granulosum, and that remnants of lytic and autolytic vacuoles appear in the stratum corneum. In histochemical studies, a positive reaction was obtained for acid phosphatase in the upper part of the ruminal epithelium (25), oral epithelium (22, 23), and epidermis of various mammals (5, 8). Proteolytic enzyme activity was demonstrated in the granular layer of human epidermis by the silver protein method (26).

Degradation of organelles of T cells, at a time when lysosomes are numerous, suggests that the transformation process is initiated by the release

of hydrolytic enzymes. The action of the released enzymes seems to be specific. This study shows that nuclear materials, ribosomes, and membranous components of mitochondria, Golgi apparatus, ER, and mucous granules are degraded whereas filaments, keratohyalin, ER-protein, and lipid are saved. The plasma membrane is modified as it reveals marked changes in permeability. The degraded organelles pass through the modified plasma membrane and accumulate in the intercellular space. Thus, it would appear that selective degradation and elimination of cellular components is a major event during transformation.

As mentioned above, retention of filaments has previously been observed in various keratinizing epithelia. Brody (3) and Odland (16) postulated that half or more of the horny cell content is derived from keratohyalin and is seen as an amorphous matrix between filaments. However,

others (13, 14) believed that keratohyalin may not account fully for the horny matrix and assumed that some other protein may also participate in the formation of the matrix. This study provides support for the latter view. As shown above, keratohyalin granules first aggregate and form large dense masses in the cytoplasm. When hydrolytic enzymes degrade the synthetic organelles, the keratohyalin masses spread and mix with the degraded materials. The high electron opacity of keratohyalin allows one to follow its dispersion into small particles, and its mingling with the less electron-opaque filamentous ER-protein. Ultimate disposition of keratohyalin between filaments can be followed on the same basis.

Formation of ER-protein in large amounts during an advanced stage of differentiation is a new observation. Most investigators find that the ER is poorly developed in retaining cells, such as those of keratinizing epithelia, whereas it is well developed in secretory cells. The ER is less abundant in ruminal epithelial cells than in secretory cells; however, the cisternae become dilated at a time when keratohyalin formation begins and become larger as keratohyalin granules grow in size. The ER-protein is not released, as in secretory cells, nor transferred into Golgi vesicles; rather, it is stored and set free ultimately by hydrolytic enzymes. Probably the ER in keratinizing epithelia assumes a specific function and, during an advanced stage of differentiation, produces products for the horny matrix. Until recently, there have been no reports on dilated ER in differentiated cells of keratinizing epithelia. However, in this laboratory recent work with mouse esophageal epithelium, tongue epithelium, turtle epidermis, and human epidermis[3] has revealed that dilated cisternae of the endoplasmic reticulum, filled with a large amount of filamentous protein, are present in cells located next to the horny layer. This protein may be analogous to the ER-protein seen in the rumen epithelium.

Loss of fluids through the modified plasma membrane of transforming cells of the rumen epithelium seems to govern the process of consolidation of the remaining fibrous and amorphous substances. Disposition of a substantial part of cellular material into the intercellular space brings about changes in shape and size of the transforming cell. The desmosomal attachments prevent separation of T cells, and, therefore, deep interdigitations are formed while the cell content is desiccating. Formation of interdigitations may be regarded as a significant event since, thereby, the transforming cells will be permanently interlocked into a continuous protective sheet such as the stratum corneum.

The above interpretations lead to the conclusion that selective degradation of cell components and permeability changes of the plasma membrane play leading roles in the formation of horny cells. The nature of the enzymes which initiate the transformation process and degrade synthetic organelles is not known; only their action is apparent. Nor is the mechanism understood that saves the protective substances (keratin) from enzymatic degradation and brings about marked changes in plasma membrane permeability. Chemical aspects of the transformation process require clarification by further studies for better understanding of the complex mechanism whereby the protective layer of keratinizing epithelia is formed.

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