Light and Electron Microscopic Observation of the Forestomach Mucosa in the Golden Hamster

Tohoku Journal of Agricultural Research
Vol. 27, No. 1, August 1976
Printed in Japan

Light and Electron Microscopic Observation of the Forestomach Mucosa in the Golden Hamster

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(Received May 31, 1976)

Summary

The histology and ultrastructure of the forestomach mucosa of the golden hamster was investigated in comparison with the ruminal mucosa. The histological organization of the former showed a general resemblance to that of the latter, though muscularis mucosa is absent in the ruminal mucosa. In ultrastructure, the epithelium of the hamster forestomach showed a typical keratinizing stratified epithelium. In the stratum basale, however, the epithelial pegs and proximal finger-like projections which are characteristic in the ruminal epithelium are poorly developed. The ultrastructural features of the upper strata of the hamster epithelium suggest that the rate of cell proliferation and postmitotic aging are probably much slower in the hamster forestomach epithelium than in the ruminal epithelium.

Langerhans cells with typical Birbeck granules are commonly found among the keratinocytes of the hamster forestomach.

Golden hamster is known to possess a forestomach which contains microorganisms (1). On account of this organ, this animal has been studied nutritionally as a pilot animal for domestic ruminants. The histology of its forestomach is little known except for the work of Hoover *et al.* (2). The similarity in the histology of the organ to that of the rumen, therefore, remains obscure.

We made the following observations in order to compare the histology and the ultrastructure of the forestomach mucosa of the golden hamster with those of the rumen, which probably is a most intensively investigated type among the forestomach in mammals.

Experimental Procedures

Conventionally fed adult golden hamsters were sacrificed by exsanguination. The forestomachs were immediately removed and fixed as follows. For light microscopy, the organ was fixed with Carnoy's solution by immersion or by injecting the solution into the lumen of the organ. Samples were cut out at the middle portion of the organ and embedded in paraffin. Five micron-thick sections

were stained with hematoxylin and eosin. For electron microscopy, the samples were cut out at the middle portion of the organ along the greater curvature. They were fixed with glutaraldehyde and osmium tetroxide, dehydrated with a graded series of ethanol, and embedded in Quetol-651 (3) through n-Butyl-Glycidyl-Ether (4). Ultrathin sections were stained with Reynold's lead solution and uranyl acetate. They were observed with a JEM–100B electron microscope.

Results and Discussion

Light Microscopic Observation

The cross sections of the organ show that the mucous membrane consisted of epithelium, lamina propria, muscularis mucosa, submucosa, muscularis externa, and serosa (Figs. 1–3). The upper part of the mucosa of this organ is highly stretched when it is filled with food (Fig. 1), whereas it is extensively folded when the organ is vacant (Fig. 2). The epithelial peg seen in the ruminal epithelium is not observed, indicating that the folding may be due to the contraction of the muscularis externa as Hoover *et al.* pointed out (2).

The epithelium is a typical keratinizing stratified squamous epithelium. It is divided into four cell layers, stratum basale, stratum spinosum, stratum granulosum, and stratum corneum (Fig. 3). The cells in the stratum basale are small and columnar to cuboidal in shape, having small and darkly stained nucleus. These cells are germinating cells (Fig. 3). The cells in the stratum spinosum are rather large and somewhat flattened. They have large and oval shaped nuclei. Some nuclei contain one or two nucleoli (Fig. 3). The flattened cells in the stratum granulosum are stacked and contain larger and paler nucleus and characteristically oval keratohyalin granules (Figs. 1 & 3).

The cells in the stratum corneum are strongly stained with eosin and are lacking nuclei. To the luminal surface of this layer, some amounts of food, bacteria, and protozoa are attached (Figs. 2 & 3).

Another type of cell exists among the epithelial cells. They have extremely pale cytoplasm and strongly stained nucleus (Fig. 1). They localize from stratum basale to stratum granulosum. Some of them protrude cytoplasmic projections into the intercellular spaces between the epithelial cells.

Apparent muscularis mucosa separates the lamina propria and the submucosa (Figs. 1–3). Only small blood vessels exist in the lamina propria, while relatively large ones are found in the submucosa. In contrast, the ruminal mucosa which has developed blood vessels has no apparent muscularis mucosa, while relatively large blood vessels are present close to the epithelium, especially lateral to the epithelial pegs.

Electron Microscopic Observation

1. *Stratum basale*

The cells of the stratum basale are attached to the basement membrane by hemidesmosomes, which are scattered along the bases of the cells. Most of the cells in this layer are columner or cuboidal in shape (Figs. 4 & 5).

Many mitochondria with smooth and straight cristae are present above and below the nuclei (Fig. 5). These nuclei have darkly stained chromatin and distinct nuclear pores. Some cells show mitotic figures. Between the mitochondria there are numerous free ribosomes and a scattered distribution of rough endoplasmic reticulum. Most cells have Golgi apparatus around the nuclei.

Finger-like cytoplasmic processes commonly extend into the wide lateral intercellular spaces and are connected with those of the neighbouring cells by desmosomes (Figs. 4, 5 & 10). Desmosomes are least numerous and simplest in this layer. In some cases, the basal cells are connected with each other by tight junctions. From the desmosomes tonofilaments extend into the cytoplasm forming small bundles arranged parallel to the cell membrane (Fig. 5). Proximal projections protruding toward the basement membrane, which are common in the ruminal epithelium, are scarce (Figs. 4, 5 & 10). At the boundary between the stratum basale and the stratum spinosum the desmosomes increase in number and complexity (Figs. 4–6).

2. *Stratum spinosum*

In the stratum spinosum, the lower cells are oval in shape and serrated in outline. This appearance results from the subdivision of the intercellular spaces by radially arranged cytoplasmic processes which are attached by obliquely placed desmosomes to similar process from neighbouring cells (Figs. 4 & 6).

The tonofibrils attain their maximum length in this region (Fig. 6). The mitochondria are dispersed, the number of which are almost the same as that of the basal cells. Some mitochondria begin degeneration. As the process of the differentiation through the stratum spinosum, the epithelial cells in the upper portion of this layer become larger and flattened (Fig. 4). Consequently the cytoplasm become larger but not so large as the spinous cells of the ruminal epithelium. The nuclei also become flattened. Mitochondria undergo further degeneration, while the intercellular spaces become much narrower than those in the stratum basale.

It is noted that the cells of the stratum basale and spinosum in the golden hamster's forestomach contain a smaller number of the mitochondria than that of the ruminal epithelium. This suggests that the forestomach epithelium of the golden hamster may have a lower metabolic ability than that of the ruminat forestomach.

3. *Stratum granulosum*

The cells lie parallel to the luminal surface. Their nuclei are flattened showing signs of degeneration (Figs. 4 & 7). Most of them contain a few characteristic intranuclear granules (Figs. 7 & 8). Cytoplasm of these cells is filled with fine fibrils (Figs. 7 & 8). Toward the upper limit of this layer, the intercellular spaces are largely obliterated by the development of the desmosomes (Figs. 7 & 8). Close apposition of cell membranes adjacent to desmosomes are observed.

Most of mitochondria are degenerating in this layer, while free ribosomes are still present in the cytoplasm. Characteristic electron dense and oval-shaped keratohyalin granules are observed, which show little contact with fibrils and ribosomes. This type of keratohyalin granules are stated to be characteristic for the slowly differentiating stratified squamous epithelium (5).

The electron dense intermediate lines of the desmosomes come to thicken in the upper layers (Fig. 8). The swollen type of granular cells commonly seen in the ruminal epithelium were not observed. At the upper limit of this layer many vacuoles are present just inside the cell membrane (Fig. 8).

4. *Stratum corneum*

The transition to the stratum corneum is abrupt (Fig. 8). The stratum consists of 15–25 layers of flat, elongated cornified cells. In this layer all nuclear and cytoplasmic structures have disappeared except the densely and uniformly packed fine keratin fibrils and some amount of interfibrilar materials (Figs. 8 & 9). The cornified cells lost their electron density and developed homogenity along the uprising. The keratohyalin granules which are apparent in the stratum granulosum now entirely disappear. The cell membrane is thickened, making interlockings with neighbouring cells (Figs. 8 & 9). Between these cells, intercellular fuzzy materials are seen (Figs. 8 & 9). Desmosomes are changing their form into the so-called squamosomes in the epidermis (6).

5. *Langerhans cells*

Throughout the stratum basale and stratum spinosum, there is a second cell type of the epithelium. They are readily distinguished from the surrounding epithelial cells by their smaller size and electron lucent cytoplasm (Figs. 4 & 11). This is further characterized by a complete lack of desmosomes or other points of attachment to neighbouring cells, and the presence of cytoplasmic processes which thread their way through the intercellular spaces (Fig. 4). The nucleus of the cells is darkly stained. Mitochondria, rough endoplasmic reticulum, and free ribosomes are usually sparsely distributed in the cytoplasm, while the Golgi apparatus and dark lysosome-like bodies are in general limited to the paranuclear

region (Fig. 11). Some cells of this type contain the characteristic Birbeck granules in their cytoplasm (Fig. 11), by which we identify them as a kind of epithelial Langerhans cell found in the ruminal epithelium (7 & 8).

6. *Subepithelial space and the blood vessels*

The subepithelial space is bounded on the luminal side by the basement membrane of the epithelium, and on the other by the inner layer of smooth muscle fibers of the muscularis externa. Most of the space is loosely filled with collagen fibers, among which pass the long irregular processes of fibroblast-like cells, occasional extravascular leucocytes, and blood vessels (Figs. 4 & 10). Smooth muscle fibers of the muscularis mucosa are distributed between the lamina propria and submucosa (Fig. 10).

As is observed by light microscope, only small blood vessels are distributed in the lamina propria. All of them are of the non-fenestrated type (Figs. 4, 10 & 12).

7. *General discussion*

As is described above, the forestomach of the golden hamster has a general resemblance in histology and ultrastructure to those of the rumen. There are, however, a smaller number of mitochondria in the cells of the stratum basale and the stratum spinosum, and only small blood vessels in the lamina propria when compared with the ruminal epithelium, suggesting that there exists some difference in the physiological properties of this organ.

The epithelium of this organ may be equal to the phase 1 type epidermis (9) by the absence of the epithelial pegs and by the oval shaped keratohyalin granules. This type of the epidermis is characterized by a lower mitotic rate and a slower postmitotic aging process. On the other hand the phase 2 type epidermis, which has some resemblance with the ruminal epithelium by the presence of developed epithelial pegs, is characterized by a higher mitotic rate and a faster postmitotic aging process.

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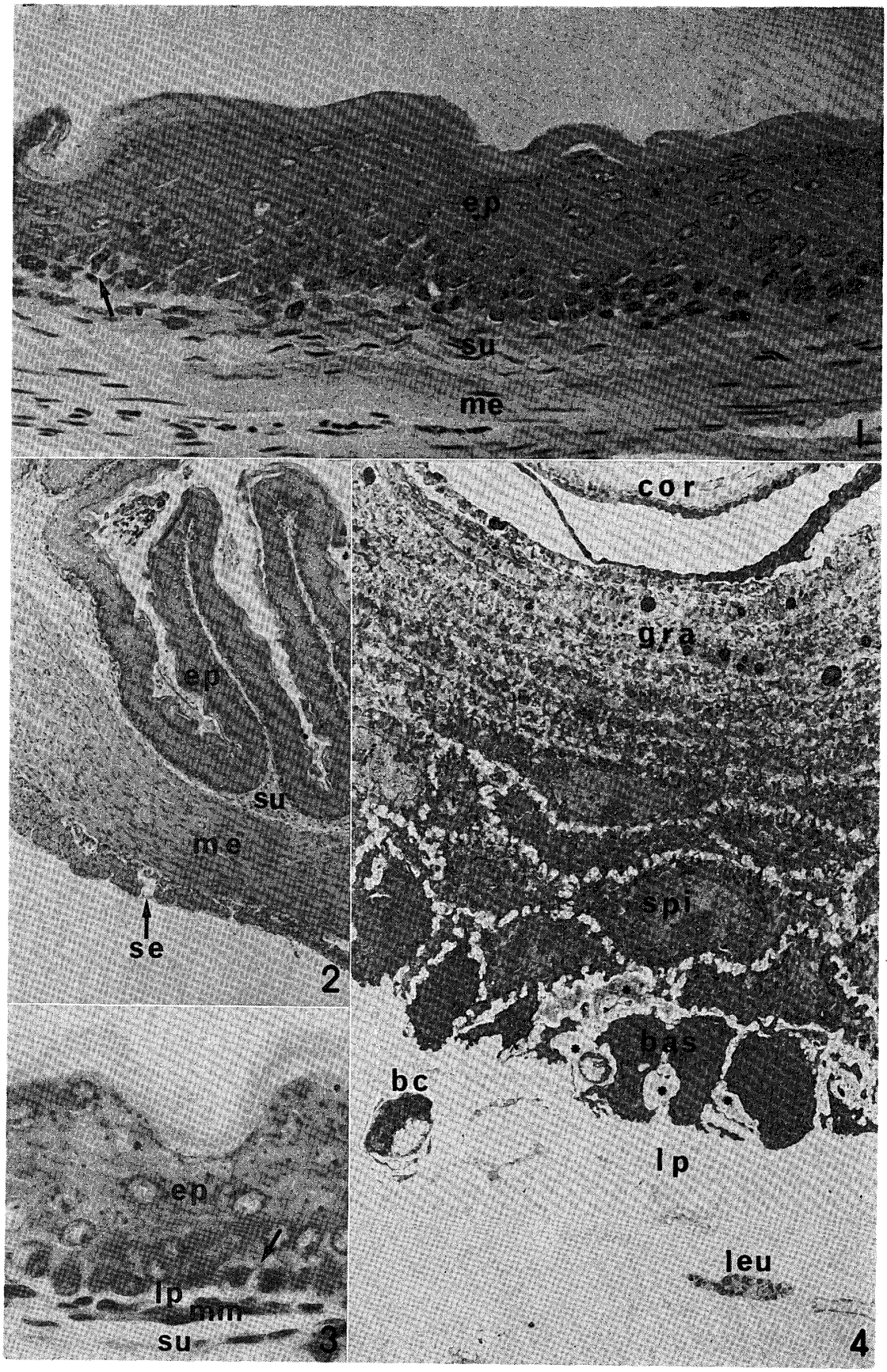
Plate I

Fig. 1. Cross section of stretched mucosa. × 740
epithelium (ep), submucosa (su), muscularis externa (me), Langerhans cell (arrow)

Fig. 2. Cross Section of the forestomach. × 88
infolded epithelium (ep), submucosa (su), muscularis externa (me), serosa (se)

Fig. 3. Higher magnification of the mucosa. × 880
epithelium (ep), lamina propria (lp), muscularis mucosa (mm), submucosa (su), basal cell in mitosis (arrow)

Fig. 4. Electronmicrograph of the epithelium and lamina propria ×2630
epithelium (ep), lamina propria (lp), stratum basale (bas), stratum spinosum (spi), stratum granulosum (gra), stratum corneum (cor), Langerhans cells (*), leucocyte (leu), blood capillary (bc)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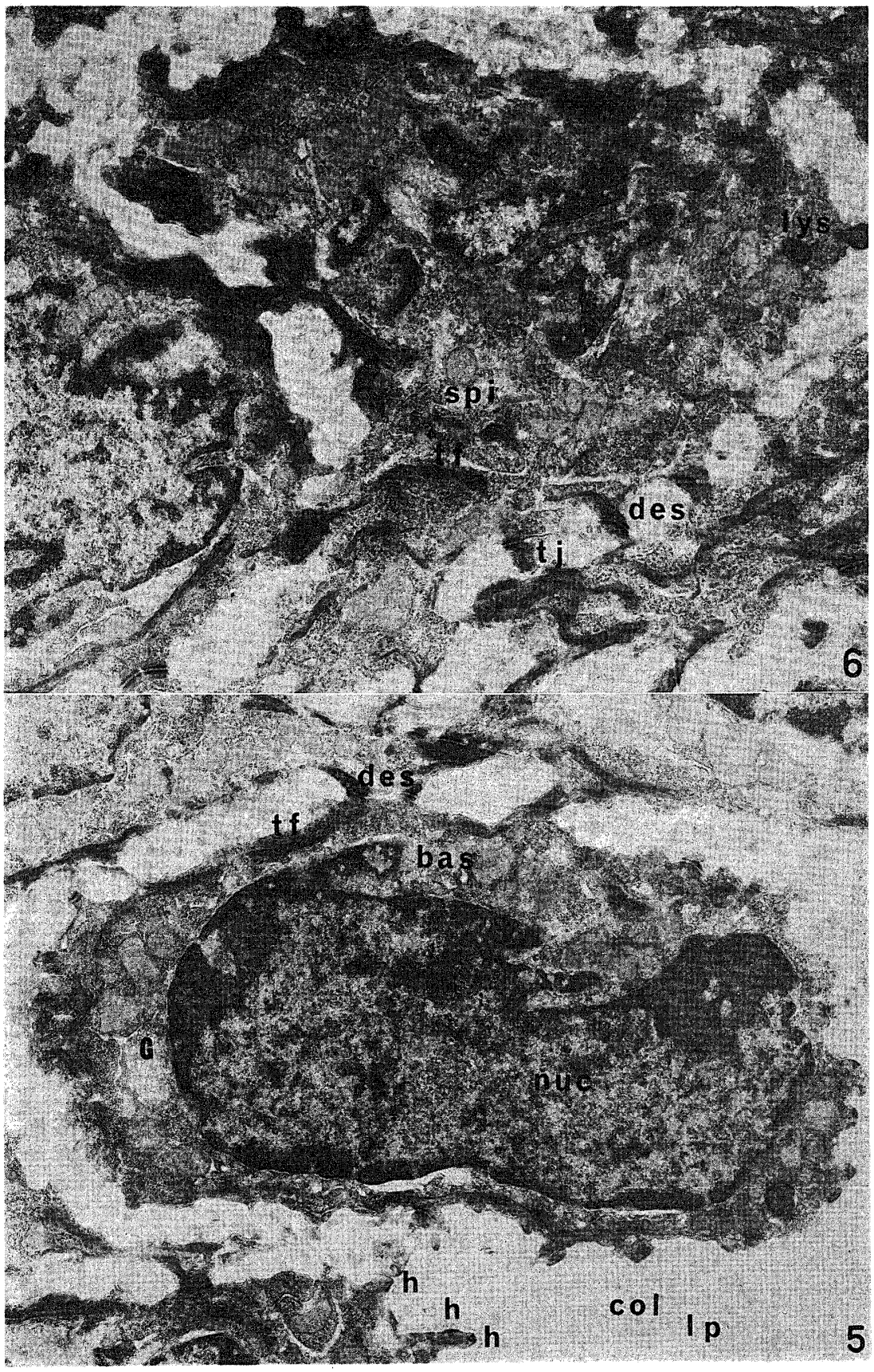
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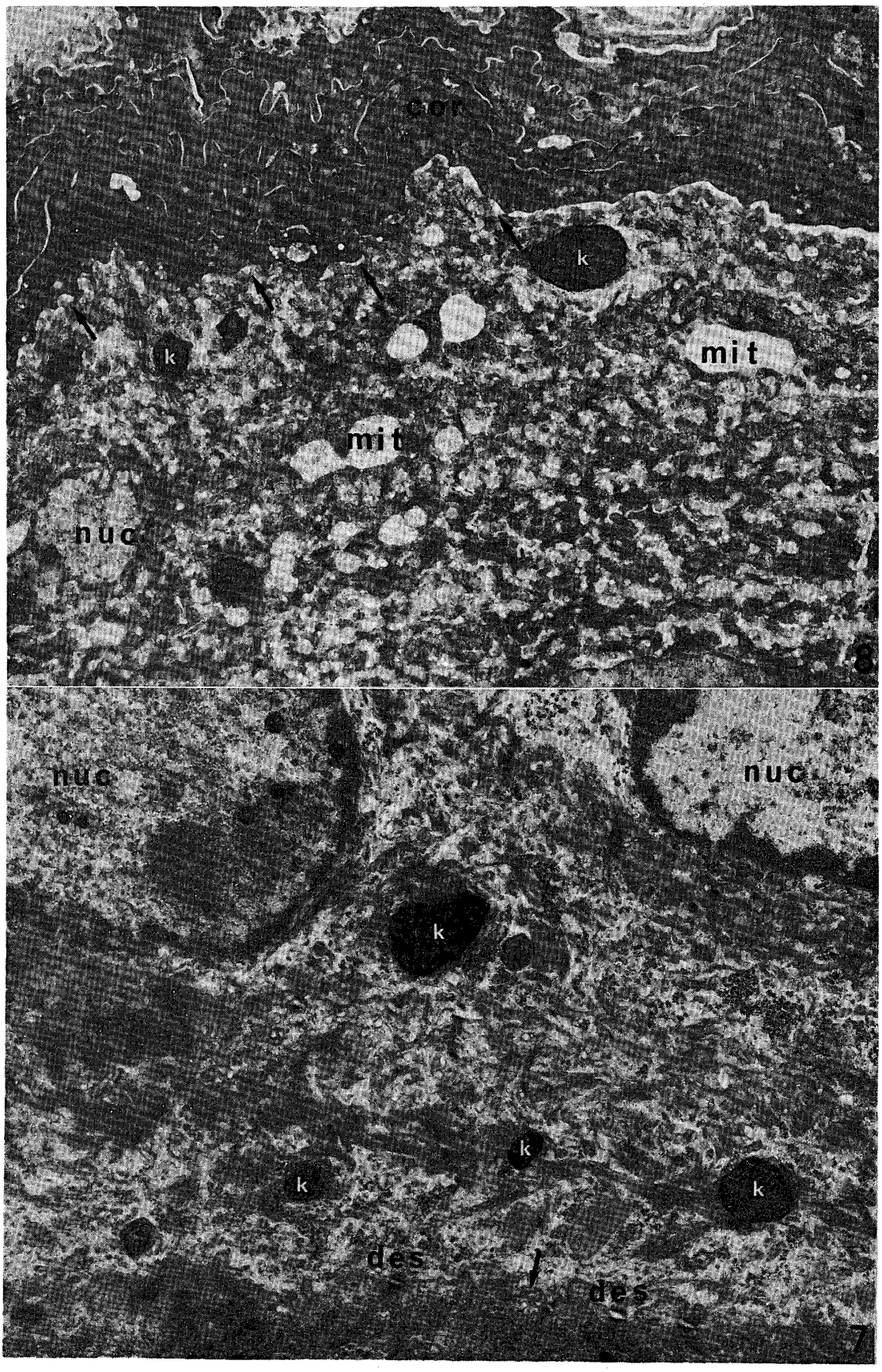
PLATE II

Fig. 5. Basal cell. ×14830
lamina propria (lp), hemidesmosome (h), collagen fiber (col), nucleus (nuc), Golgi apparatus (G), tonofilaments (tf), desmosome (des)
Fig. 6. Spinous cell. ×14830
lysosome-like body (lys), tonofilaments (tf), tight junction (tj), desmosome (des)

Plate III

Fig. 7. Lower granular cells. ×14830
nucleus (nuc) containing intranuclear granules, keratohyalin granule (k), desmosome (des), narrow intercellular space (arrow)

Fig. 8. Upper granular cells and lower cornified cells. ×8020
flattened nucleus with intranuclear granule (nuc), keratohyalin granule (k), degenerating mitochondria (mit), vacuoles (arrows)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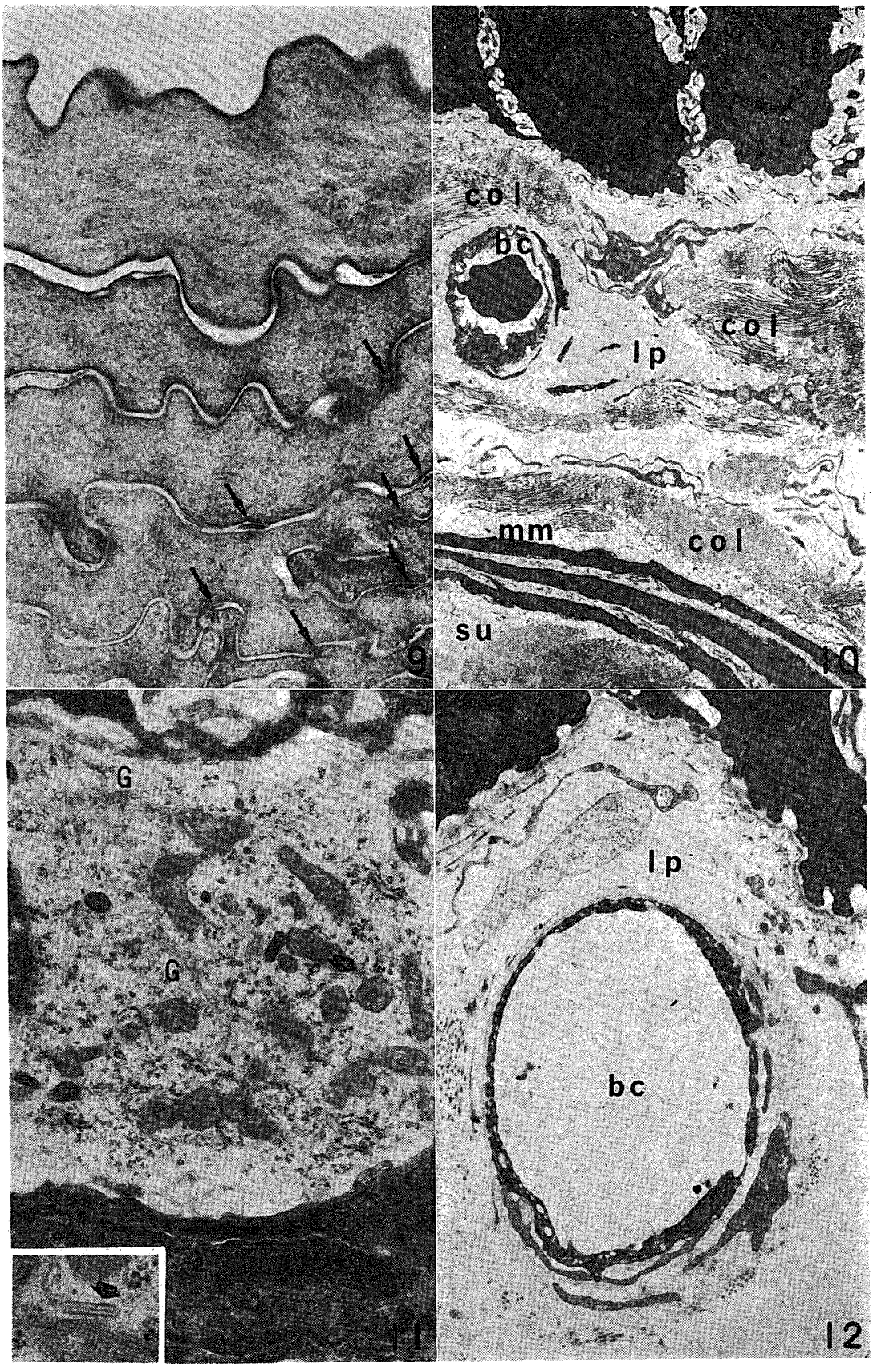
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PLATE IV

Fig. 9. Upper cornified cells. ×33200
squamosome (arrows)
Fig. 10. Lamina propria and muscularis mucosa. ×6000
muscularis mucosa (mm), blood capillary (bc), collagen fiber (col), submucosa (su)
Fig. 11. Langerhans cell. ×21000
Birbeck granule (arrow), Inlet: higher magnification of the Birbeck granule.
Fig. 12. Blood capillary in the lamina propria. ×15000
non-fenestrated type of blood capillary (bc), lamina propria (lp)