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Identification of the So-called Paranuclear Vacuoles as the Processes of the Rumen Langerhans Cells in the Sheep

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Summary

The electron microscopic observation of the ovine ruminal epithelium revealed that the so-called "paranuclear vacuoles" of the epithelial cell cytoplasm are identical to the processes of the branching cells of Steven and Marshall (7). It was found that the "vacuoles" are surrounded by a definite double membrane. The plasma membrane and nuclear envelope of the basal cells containing such "vacuoles" are always present adjacent to the membrane of the "vacuole," showing that the "vacuoles" are processes of the branching cells. Birbeck granules were rarely observed in the branching cells. This suggested that the cells are rumen Langerhans cells, as recently reported by Gemmell (1). The presence of various organella of these cells, including centriole, lysosomes, phagosomes, and concentric rough-surfaced endoplasmic reticulum were described.

Peculair cytoplasmic vacuoles were present in the ruminal epithelium in the histological preparations of routinely treated tissues. The vacuoles were called as "paranuclear vacuoles" by Henrikson and Habel (2). The vacuoles, often in contact with basal cell nucleus, compressed the latter into a narrow crescent. Tamate *et al.* (8) reported that the vacuoles increased in number and in size when ruminal tissues were treated with distilled water in vitro, prior to fixation. They suggested that the paranuclear vacuoles were not artefacts, but were indicative of the absorptive properties of the rumen mucosa.

Electron microscopic study of Lavker *et al.* (5) showed that numerous paranuclear vacuoles were present in the basal cell cytoplasm of ruminal epithelium, fixed with care by glutaraldehyde and osmium tetroxide. The authors concluded, quoting the result of Tamate et al. (8), that the vacuoles possibly function in storage and transport of metabolites between the lumen and circulation system in ruminant body.

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Steven and Marshall (7) recently reported that presence of a second cell type in the ruminal epithelium. They suggested that the branching cell may be the structures described as "paranculear vacuoles" by Lavker et al. (5). No further evidence supporting this statement was presented by these authors.

It is puzzling that none of the recent works (1, 3, 4) on the ultrastructure of the ruminal epithelium reported the presence or absence of the "vacuoles". The aim of the present study is to clarify the relation between the "paranuclear vacuoles" and the branching cells, as the first step of studies on the ultrastructure of the ruminal mucosa in the ruminant.

Materials and Methods

Papillae from the anterior dorsal blind sacs of the rumens of five adult healthy sheep were used. The papillae were fixed either in 4% or in 5% glutaraldehyde in 0.1 M cacodylate buffer or in 0.1 M phosphate buffer, for two hours at pH 7.2 and 4°C. Post-fixation was done by 1% osmium tetroxide in veronal buffer at pH 7.2.

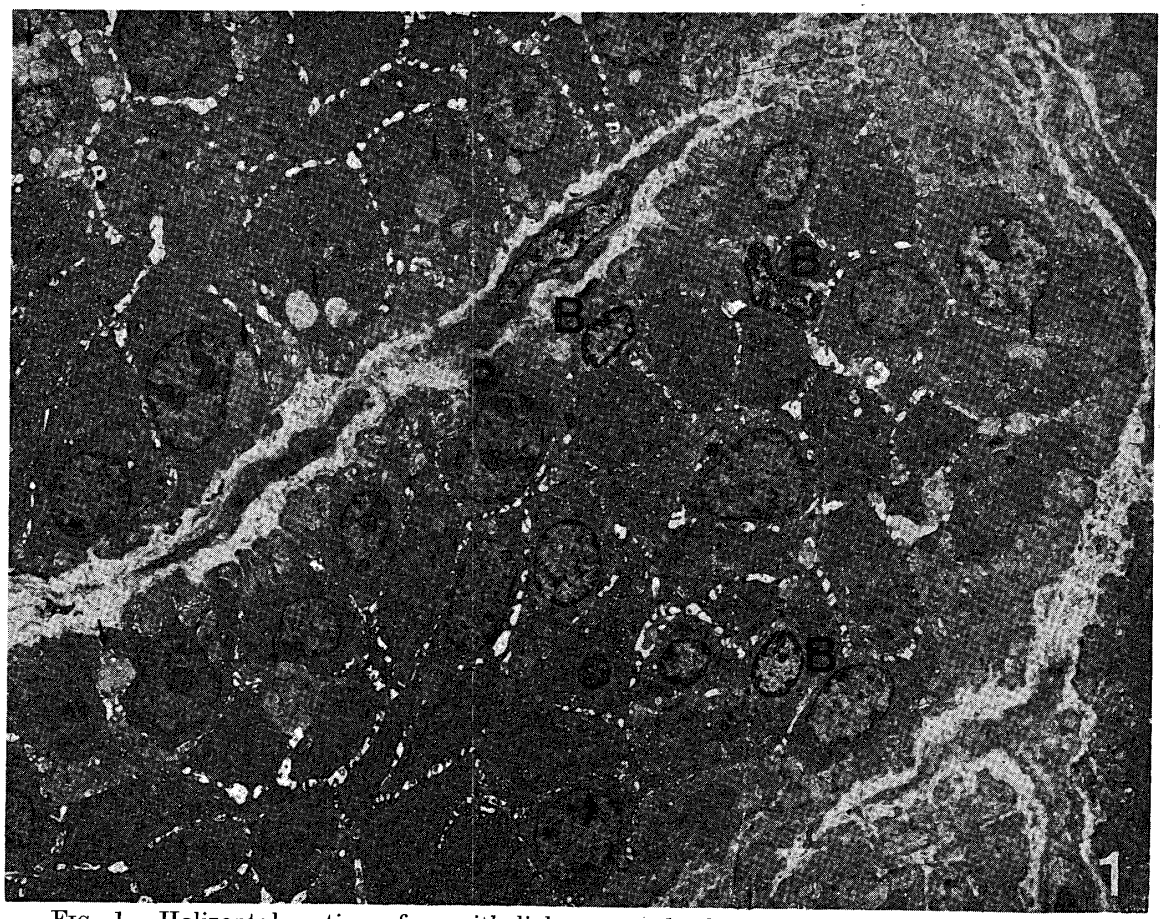
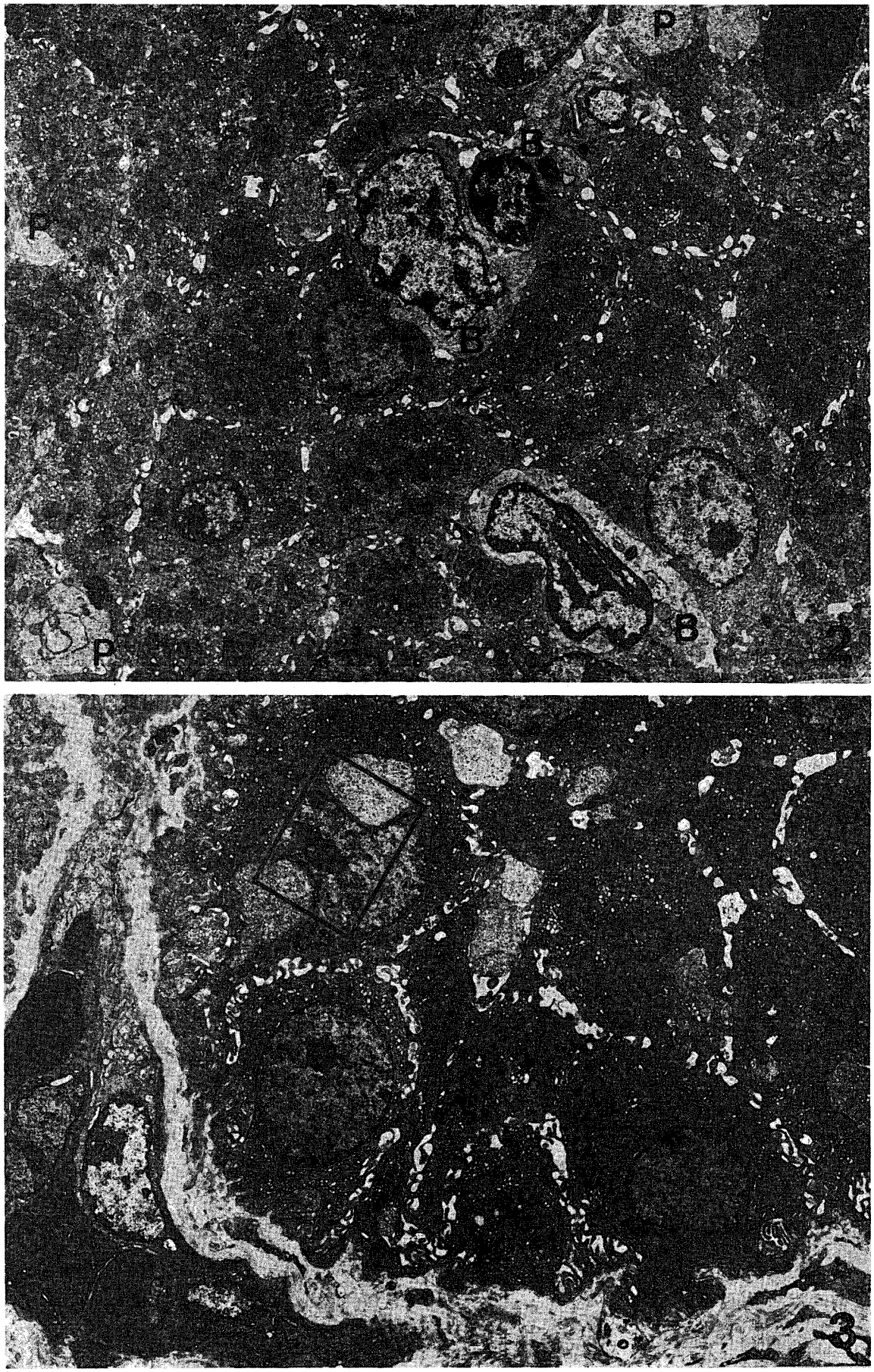


FIG. 1. Holizontal section of a epithelial peg at the level of stratum basale. Several "paranuclear vacuoles" (small arrows) and type A branching cells (B) are seen. ×2,500.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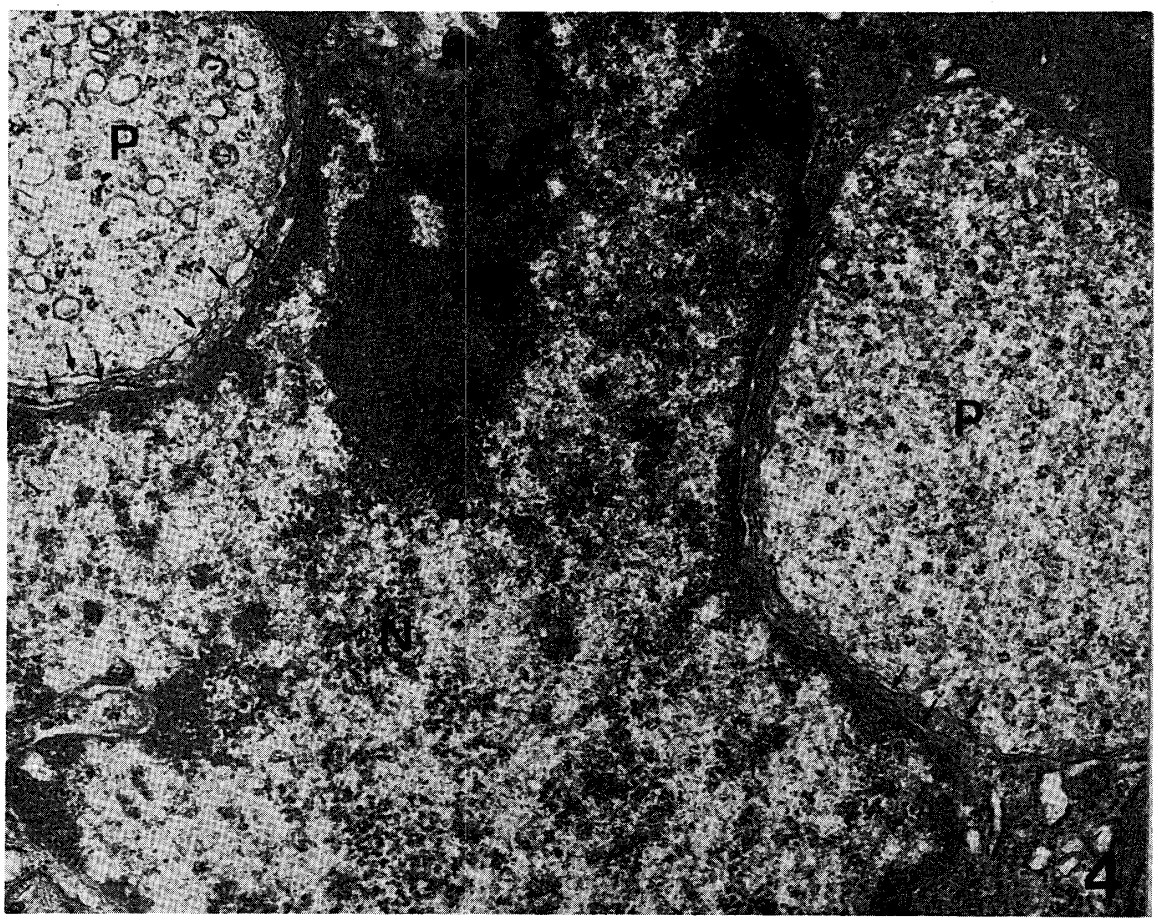


FIG. 4. Paranuclear vacuole-nucleus area at higher magnification (×25,000). The presence of two plasma membranes and outer membrane of nucler envelope (arrows) is noted between the "vacuoles" (P) and basal cell nucleus (N).

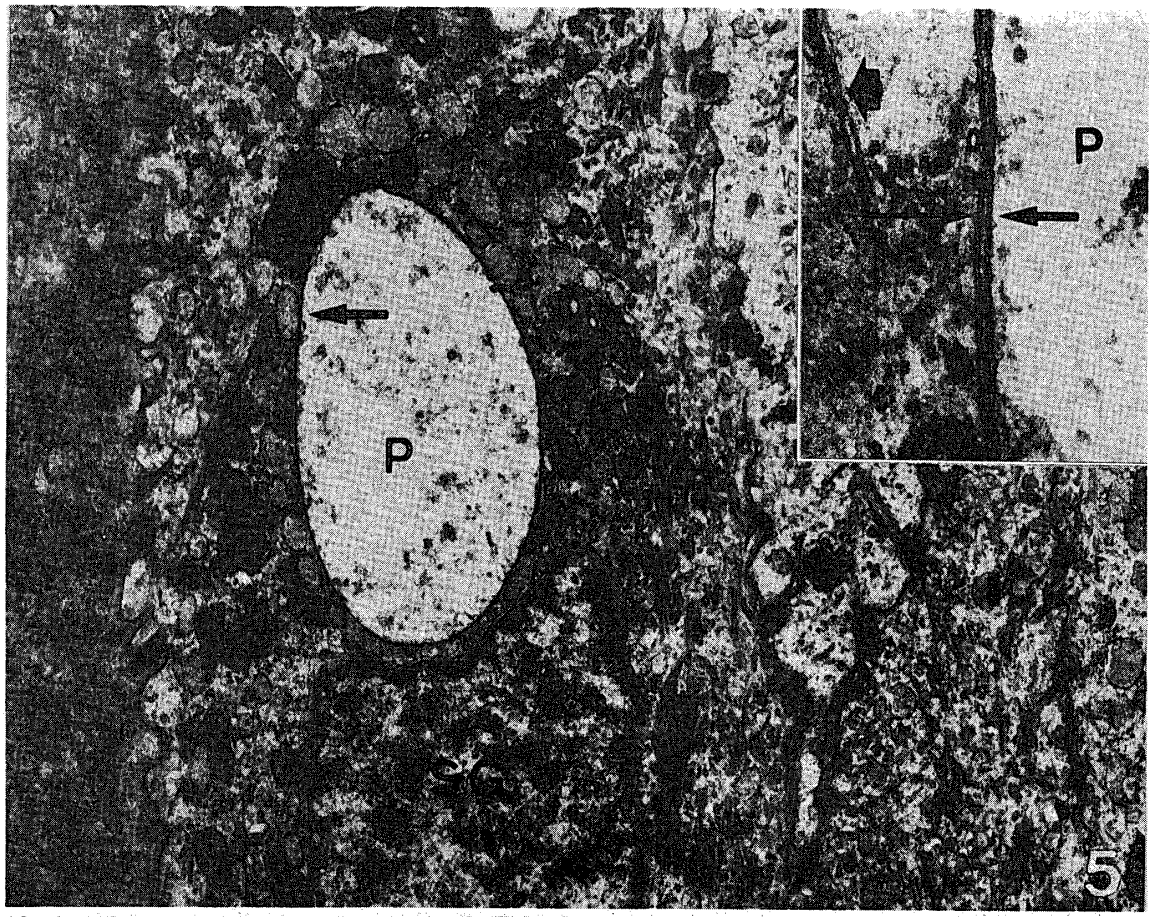
The tissues were then dehydrated in a graded series of a cold acetone (4°C) and embedded in Epon 812. Thin sections were cut by a Porter-Blum MT-2 ultramicrotome and were stained with uranyl acetate and lead citrate. The observations were carried out with a JEM-100B electron microscope.

Result and Discussion

The so-called "paranuclear vacuoles" were frequently observed in the cytoplasm of the ruminal basal cells. The size and content of these "vacuoles" differed from cell to cell (Figs. 1–3). When they were large, they more or less compressed the basal cell nucleus as described by Henrikson and Habel (2). The contents of the "vacuoles" appeared quite similar to the processes of adjacent branching cells containing ribosome-like granules and less dense amorphous

FIG. 2. Apposition of two branching cells (B). The processes of branching cells (P) appear quite similar to "paranuclear vacuoles" at Fig. 1. ×5,000.

FIG. 3. Cross section of a epithelial peg. A nucleus of a basal cell is compressed by two "paranuclear vacuoles." ×4,250.


P
P
5


6


7

FIG. 8. A branching cell with a prominent centriole (arrow). Golgi vesicles are dilated. ×7,500.

materials. Fig. 3 showed the presence of two "vacuoles" in one basal cell. The "vacuoles" were, however, encircled by a definite double membrane instead of a single one expected in the case of ordinary cytoplasmic vacuoles (Fig. 4). Fig. 5 showed a prominent "vacuole" in stratum spinosum. Again the "vacuole" was surrounded by a definite double membrane, and another double membrane was present between the former and the nuclear envelope. Above data indicated that the so-called paranuclear vacuoles of Henrikson and Habel (2) are the developed processes of the branching cells. The reason why the branching cell was misunderstood as cytoplasmic vacuoles may be that two plasma membranes are so close that they can be hardly distinguished in the electron photomicrographs of

FIG. 5. A large "paranuclear vacuole" in stratum spinosum. The vacuole compresses the spinous cell nucleus (SG). ×12,500. Insertion at right upper corner illustrates an area (arrow) of the vacuole perphery (×56,000). Two double membranes are clearly seen. The one (short arrow) is the plasma membrane of branching cell process, while the other (long arrow) is that of the spinous cell. Broad, short arrow indicates the mitochondrial membrane.

FIG. 6 and FIG. 7. Type B branching cells in stratum basale. Extensive Golgi apparatus (G) and dense phagosomes (arrow) are noted. Fig. 6, ×5,600. Fig. 7, ×7,500.

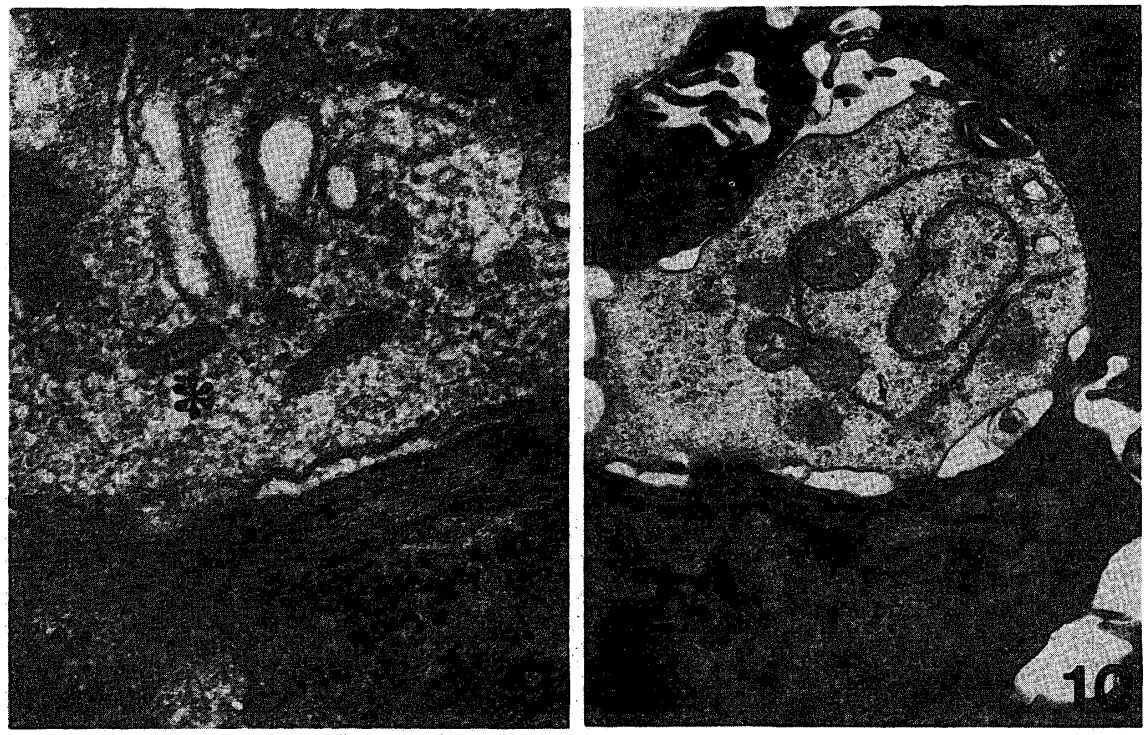


FIG. 9. A Birbeck granule (*) in a branching cell. ×5,6100.
FIG. 10. Concentric arrangement of rough-surfaced endoplasmic reticulum (arrow) in a process of a branching cell. ×8,500.

lower magnification.

Lysosome or phagosome-like large vacuoles with electron dense contents were frequently seen in the juxtanuclear cytoplasm of the branching cells (Figs. 6 and 7). Fig. 7 showed two large phagosomes which were in contact with wide areas of lighter cytoplasm. This suggested that the former were autophagosomes. Development of Golgi vesicles was also noted in these cells. The presence of a prominent centriole and of a Birbeck granule was showed in Figs. 8 and 9. Presence of rough-surfaced endoplasmic reticulum in concentric arrangment was frequently noted in processes of the branching cells (Fig. 10).

The presence of developed Golgi apparatus and phagosomes was already reproted (1, 5). Birbeck granules was found in ovine ruminal basal cells by Gemmell (1), and in bovine ones by Tamate (9). The result of this study together with those of foregoing ones (1, 9) indicated that the branching cell are the Langerhans cells in the ruminal epithelium. Gemmell (1) stated that the branching cells are capable of phagocytosis under certain conditions. Contrary to this statement, the present authors would suggest that the branching cells may be highly sensitive to the changes in the environment, and can undergo autophagocytosis under certain conditions.

Steven and Marshall (7) classified the branching cells into two types. In type A, the nucleus is darkly stained and deeply indented, its cytoplasm being pale and

contains few organella. In type B, the nucleus is smooth in outline and relatively pale, with larger cytoplasm containing abundant organella. In general, type A cells appeared quite similar to lymphocytes. These two types of the branching cells were frequently found in close apposition (Fig. 2). Silberberg (6) recently reported that such apposition of mononuclear cells to skin Langerhans cells takes place in contact allergic reactions in human epidermis. He suggested that some substance is secreted from Langerhans cells, and that such substance could be antibody formed by the cells. Further studies are needed to clarify the function of the branching cells, the Langerhans cells in the ruminal epithelium.

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