Ultrastructural Changes in the Ruminal Epithelium after Fasting and Subsequent Refeeding in the Sheep

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Ultrastructural Changes in the Ruminal Epithelium after Fasting and Subsequent Refeeding in the Sheep

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Summary

The ultrastructural changes of the ruminal epithelium, especially of the basal cells after 3 days-fasting and subsequent refeeding were observed. Rumen papillae were collected from an adult sheep via fistula and fixed in 4% glutaraldehyde and 1% osmium tetroxide. Epon-embedded sections were observed by light and electron microscopes.

The most remarkable features of the ultrastructural changes were found in the proximal region of the basal cell cytoplasm. Fasting for 3 days decreased the volume of basal sinuses and the numbers of the proximal projections; both of which quickly recovered within 24 hours after refeeding. Morphological modifications of cell organella such as mitochondria, Golgi apparatus and ribosome clusters were observed during fasting and in subsequent refeeding. Rough-surfaced endoplasmic reticulum also disappeared during fasting, resulting in the formation of many smooth surfaced vesicles. The intercellular space, which became narrow after fasting for three days, was widened at six hours after refeeding.

The above data suggested that the proximal cytoplasm of the basal cells was the most reactive portion of the ruminal epithelium to the fasting and subsequent refeeding, i.e., to the physiological changes following the intake of feed and intraruminal fermentation. It is most likely that the volatile fatty acids passing through the epithelium may be most responsible to the ultrastructural changes observed. The time of sample collection of the ruminal tissues should be carefully chosen in connection with the feeding schedule of the experimental animals preceeding such collection.

The ultrastructure of the ruminal epithelium have been studied in details by several investigators. Most of this work are, however, done with animals under uncertain feeding conditions (2, 4~11).

Ample data have been accumulated on the keratinization process, (6, 7, 10), sodium transport system (3, 4), and osmotic barrier of the ruminal epithelium (3). The purpose of this study is to determine the structural changes of the epithelium associated with the food intake and subsequent physiological changes in the sheep. Fasting and subsequent refeeding were used as experimental condition.

Materials and Methods

A healthy adult sheep with a rumen fistula was used in this experiment. Prior to the experiment, the animal was fed a conventional feed once a day at 10.00 A.M. It was fasted for three days and then fed at 10 A.M. of the third day of the experiment. Rumen papillae were collected via fistula by a pair of modified Chevalier-Jackson type forceps, at 9.30 A.M. and at 4.00 P.M. of the third day, and at 9.00 A.M. of the fourth day. Other samples were collected from the same animal prior to the experiment and one month after the experiment at 9.30 A.M., while the animal was fed once a day at 10.00 A.M.

The collected specimens were fixed in 4% glutaraldehyde in 0.1 M cacodylate buffer for two hours at pH 7.2 and 4°C, followed by the postfixation in 1% osmium tetroxide in veronal buffer at pH 7.2. After dehydration through a series of cold acetone, they were embedded in Epon 812. Thin sections were cut by a Porter-Blum MT-2 ultramicrotome, and stained with uranyl acetate and lead citrate. The observations were made with a JEM 100B electron microscope.

Mitotic indexes and counts of rumen Langerhans cells were made with 1μ sections of the Epon-embedded specimens, stained by hematoxylin-eosin.

Results

1. *Intercellular Space and Basal Sinus*

In the regularly-fed condition, the proximal cytoplasm of the basal cells was characterized by the presence of numerous microvilli-like projections. Many of these proximal projections were anchored by half-desmosomes on the basement membrane which run smoothly with few infoldings (Fig. 1). The projections were present within a wide space formed by the proximal cytoplasm and basement membrane. This space was tenatively called as basal sinus. Desmosomes and tight junctions were occasionally seen between the projections and the body of the proximal cytoplasm (Fig. 2). These cell junctions were also present among the projections of lateral cytoplasm forming intercellular space (Fig. 1).

After fasting for three days, the proximal projection appeared to decrease in number, running parallel to each other and in close apposition. They were frequently connected by cell junctions. The basal sinus shrunk and became inconspicuous, resulting in the development of intensive infoldings of the basement membrane (Figs. 3 & 4). The intercellular space also became narrow. It was noted that cell junctions, especially the tight junctions or occluding ones, increased in number and tended towards the closure of the openings of the space and the sinuses (Fig. 4). At six hours after refeeding, the basal sinuses became swollen and contained increased numbers of the proximal projections with fewer cell junctions. The openings of the wider intercellular space and the sinuses were in most part open to lamina propria. The basement membrane showed relatively

smooth outline and fewer infoldings (Figs. 6–8).

At 24 hours after refeeding, the development of the basal sinuses and widening of the intercellular space were noted. The proximal projections became well-developed and cell junctions, specially the tight junctions, decreased in number. (Figs. 9–12).

2. *Cell Organella in the Basal Cells*

In regularly fed condition, rough-surfaced endoplasmic reticulum (rER) and Golgi apparatus were commonly seen in the basal cell cytoplasm. Free ribosomes were present in clusters (Figs. 1 & 2). Numerous mitochondria were present in the cytoplasm. Most of them were large with light matrix and typical cristae, and their outline in cross sections was irregular and more or less polygonal (Fig. 1). After fasting for three days, Golgi apparatus was seen only occasionally, rER disappeared almost completely, and smooth-sufaced vesicles were commonly seen. Free ribosomes were scattered in the cytoplasm with no clusters. It appeared that the mitochondria decreased in number and the matrix darkened. Their outline in cross sections was circular and smaller (Figs. 4 & 5). The swelling of cristae was seen.

At six hours after refeeding, clusters of free ribosomes were present in certain area of the basal cell cytoplasm. Rough-surfaced ER was not present, while numbers of smooth-surfaced vesicles decreased. Golgi apparatus was still seen only occasionally. The mitochondria became larger with lighter matrix. The swelling of cristae was not seen. Smaller type of mitochondria with darker matrix were, however, present in the same cytoplasm (Figs. 6 & 7). It was noted that large smooth-surfaced vesicles were present in the proximal cytoplasm where the basal sinus was present in regularly fed condition (Fig. 8).

At 24 hours after refeeding, rER was seen in limited numbers, whereas clusters of ribosomes increased in number. Relatively developed type of Golgi apparatus was present in most of the basal cells. The larger type of mitochondria further increased in number, though smaller ones with darker matrix were still seen (Fig. 11). The large vesicles in the proximal cytoplasm became obscure, due to the development of the basal sinuses in the same region (Figs. 9 & 11).

3. *The Basal Cell Nucleus*

After fasting for three days, the nuclei became closer to the basement membrane, due to the shrinkage of the basal sinuses mentioned in section 1 (Figs. 3 & 4). The ultrastructural changes of the nuclei during fasting and subsequent refeeding were otherwise not clear, though the nucleolus appeared to decrease its volume during fasting.

4. *Langerhans Cells*

It is well-known that the large processes of the rumen Langerhans cells are present in the juxtanuclear region of the basal cell cytoplasm. After fasting for three days, the Langerhans cells were still present in the intercellular space. Their processes were, however, rarely seen within the basal cell cytoplasm (Figs. 3 & 4). At six hours after refeeding, the processes were still absent within the cytoplasm. At 24 hours after refeeding, considerable numbers of processes were present within the cytoplasm (Figs. 9 & 10).

Counts of the numbers of the rumen Langerhans cells in stratum basale and stratum spinosum were made with 1 μ sections of Epon-embedded specimens. As seen in Table 1, the percentage of the rumen Langerhans cells in the stratum basale increased significantly within 24 hours after refeeding. In addition, it was noted that an aggregation of these cells in upper stratum spinosum was seen in the epithelium after fasting for three days.

TABLE 1. *Counts of Rumen Langerhans Cells in two Strata of Ruminal Epithelium*

Feeding condition	Cell numbers (%)		
	Stratum basale	Stratum spinosum	total
Daily fed (D)	23(41.8)	32(58.2)	55
Fasting for 3 days (F)	37(41.6)	52(58.4)	89
6 hours after refeeding (R–6)	52(51.0)	50(49.0)	102
24 hours after refeeding (R–24)	19(70.4)	8(29.6)	27

R–24>D,F, R–6 $0.02>p>0.01$

5. *The Subepithelial Capillaries*

The subepithelial capillaries located on the lateral sides of epithelial pegs or the tips of the proprial papillae were dilated in the regularly fed condition (Fig. 1). They showed extraordinary constriction after fasting for three days (Fig. 4), and became dilated at six hours after refeeding (Figs. 6 & 7). They were almost as large as those in a regularly fed condition at 24 hours after refeeding (Fig. 9). It was found that the development of the basal sinuses and the proximal projections were more or less parallel to the dilation of these capillaries.

6. *Mitotic Index of the Basal Cells*

The mitotic indexes of the basal cells were counted with 1 μ sections of Epon-embedded specimens. The value of the index was 1.23% at regularly fed condition, and was significantly lower after fasting for three days (0.11%), and at 6

TABLE 2. *Numbers of Mitotic and Non-mitotic Cells and Mitotic Index of Ruminal Basal Cells*

Feeding condition	Mitosis	Non-mitosis	Total	Mitotic Index (%)
Daily fed (D)	9	721	730	1.23
Fasting for 3 days (F)	1	654	655	0.11
6 hours after refeeding (R–6)	0	912	912	0.00
24 hours after refeeding (R–24)	4	488	492	0.81

D, R–24>F, R–6 $p \gg 0.01$

hours after refeeding. It was as high as 0.80% at 24 hours after refeeding, suggesting the recovery of mitotic rate in the basal cells (Table 2).

Discussion

In this paper, we reported a peculiar structure of the ruminal basal cells. This structure was tenatively called the basal sinus. It is surprising that little attentions have been paid for the existence of this structure, though it was repeatedly shown in photomicrographs of ruminal epithelium by various workers (2, 3, 4, 5, 6, 9, 10). The shrinkage and swelling of the basal sinus together with the decrease and increase of numbers of the proximal projections were noted during fasting and subsequent refeeding. We believe that such changes may be regarded as the reaction of the basal cell cytoplasm to the changes of the internal environment caused by the fasting and food intake. We are also inclined to believe that the volatile fatty acids absorbed via ruminal epithelium are most responsbile to these changes, and that the basal sinus is an extension of the proximal portion of the intercellular space, which develops between the proximal surface of the basal cell and the basement membrane.

The presence of the cell junctions in stratum basale was recently reported by Scott et al. (9). They called them occluding junctions, and were apparently identical to those we observed in this experiment. The present data suggested that these junctions may be regarded as gateways for the intercellular space and the basal sinuses. The development of such structures during fasting is probably promoted by the fall in the amount of the volatile fatty acids absorbed through stratum basale.

The ultrastructural changes of organella in the basal cells in this experiment may be regarded as the effect of fasting and subsequent recovery. The disappearance of rER and ribosome clusters was reported in the parechymal cells of rat liver after prolonged fasting (1). We believe that these changes probably are indications of a fall in the rate of protein synthesis during fasting.

The branching mononuclear cells of Steven and Marshall (10) have been recently identified as a special type of Langerhans cells with the presence of Birbeck granules (2, 8). The functional significance of this cell type is still unknown (2, 8). We may suggest, however, that the rumen Langerhans cells may be sensitive to the change of the intraruminal environment caused by food intake, because they migrated upward to upper stratum spinosum and lost contact with the juxtanuclear cytoplasm of the basal cells after fasting for three days.

The decline and rise of mitotic index of the basal cells during fasting and refeeding suggested that the proliferation rate of the ruminal epithelium probably depends on the feeding pattern in the ruminant. A further study is planned to clarify this problem, and will be reported in our next paper.

Acknowledgement

We wish to express our thanks to Dr. Y. Toyota of Kitasato University for providing the original model of the Chevalier-Jackson type forceps, and to Dr. Y. Tanabe of Research Institute for Scientific Measurements, Tohoku University for his help in reconstruction of the forceps for rumen biopsy. We also thank to Miss Y. Kamioka for preparing this manuscript.

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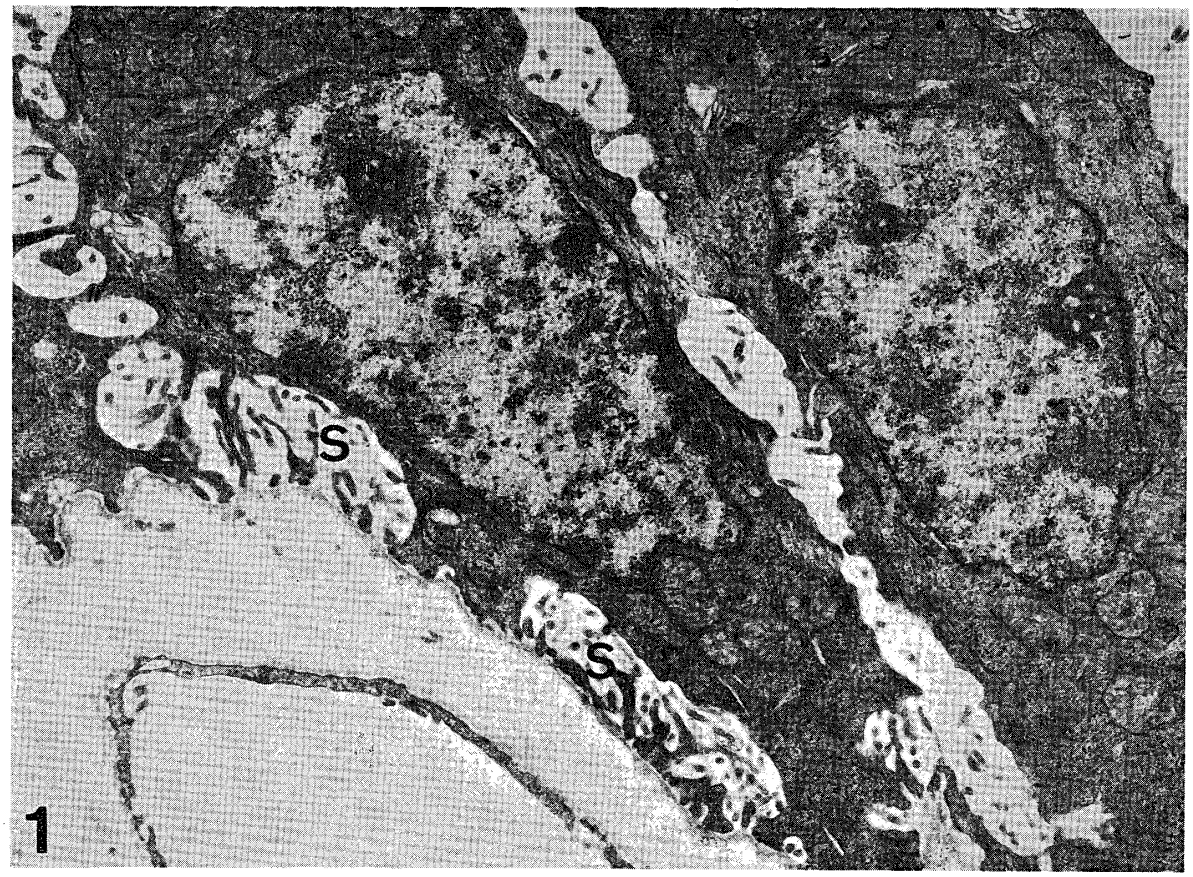
PLATE 1

Explanation of Figures

FIG. 1. Regularly fed condition. × 8250
Basal sinus(s), proximal projections, Golgi apparatus are well-developed. Note the wide intercellular space, smooth basement membrane, and dilated capillary.

FIG. 2. Regularly fed condition × 12500
Intercellular space and basal sinuses(s) are open to lamina propria. Cell junctions are few. Mitochondria are large and lighter.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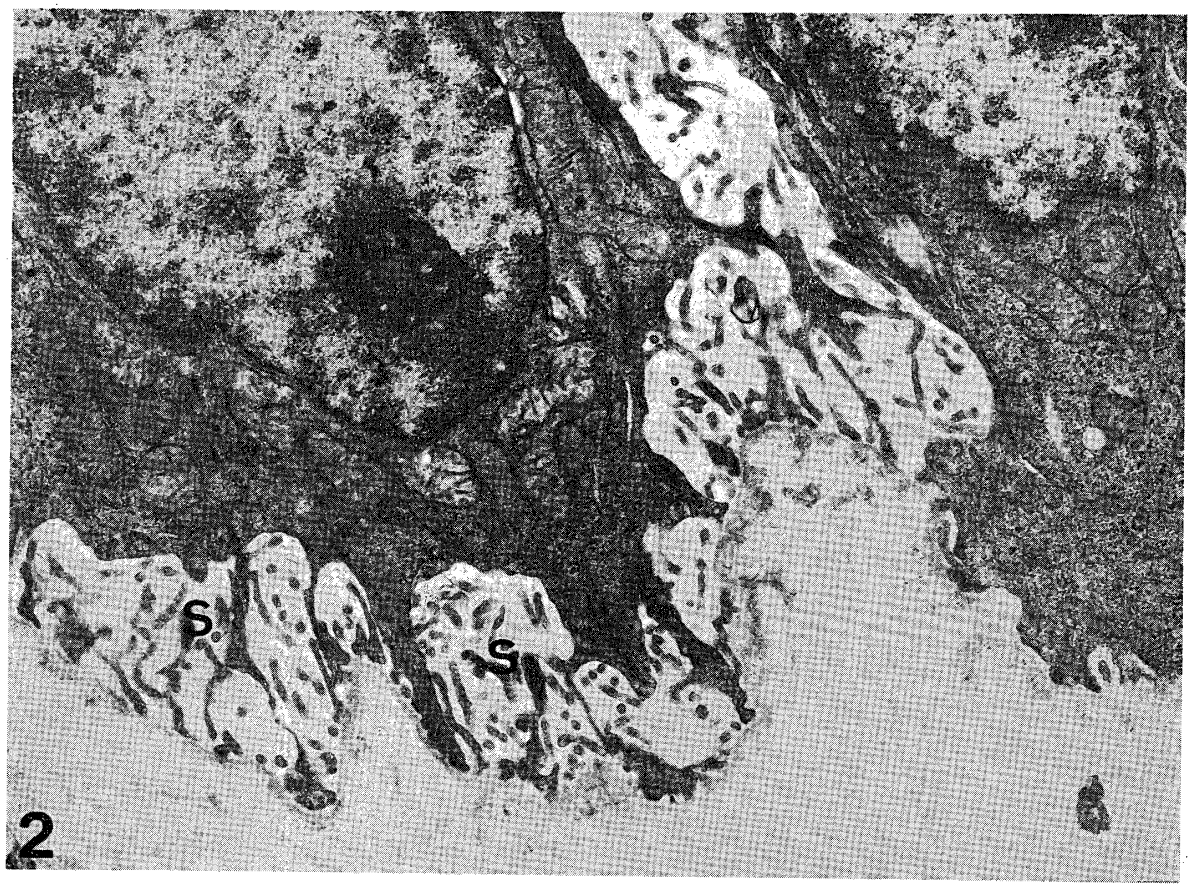
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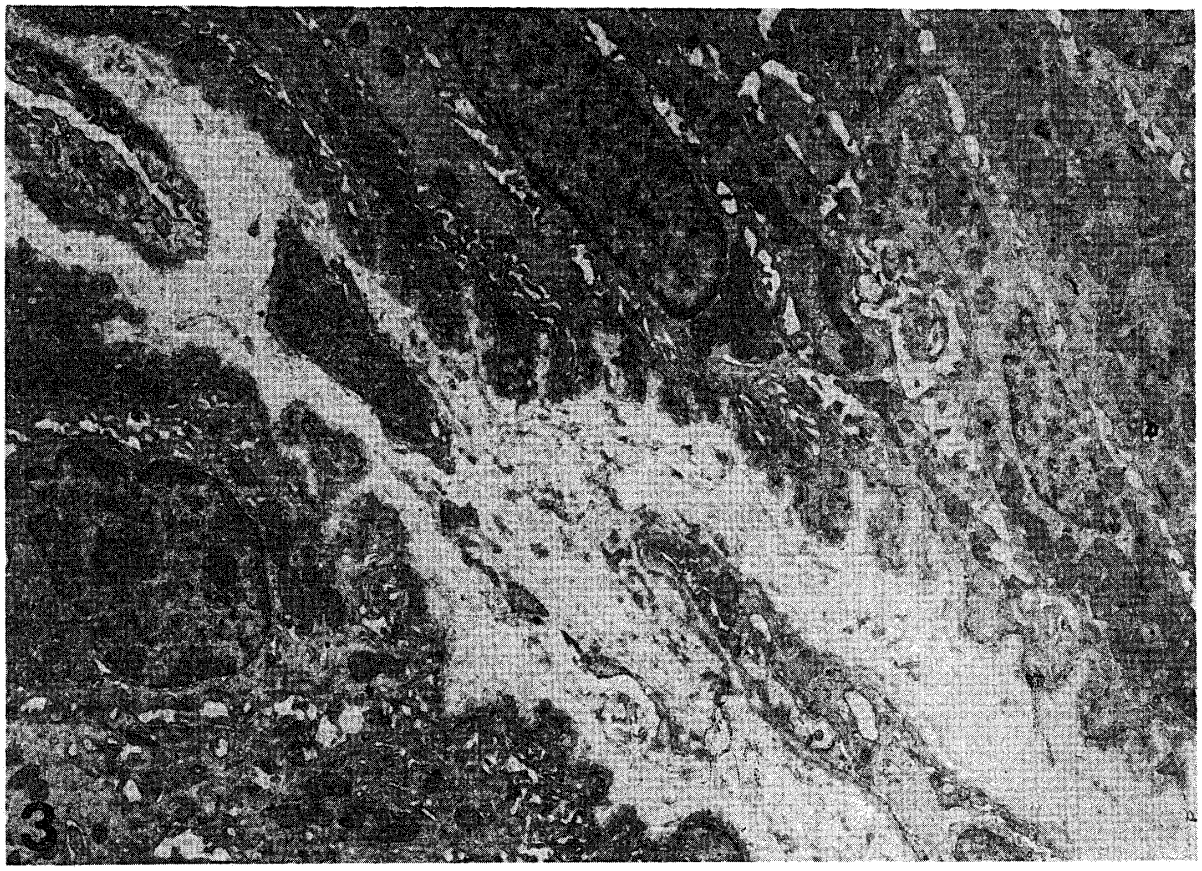


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PLATE 2

Explanation of Figures

FIG. 3. Fasting for 3 days × 6750
Note the infoldings of basement membrane and constriction of capillary.

FIG. 4. Fasting for 3 days × 8250
Intercellular space is narrow and partly closed by cell junctions (arrows). Basal sinus shrunk or not evident. Ribosomes are scattered with no clusters. Rough-surfaced ER disappears.

FIG. 5. Fasting for 3 days × 10000
Nucleus is located closer to basement membrane. Note the presence of small dark mitochondria(d) among large lighter ones(l).

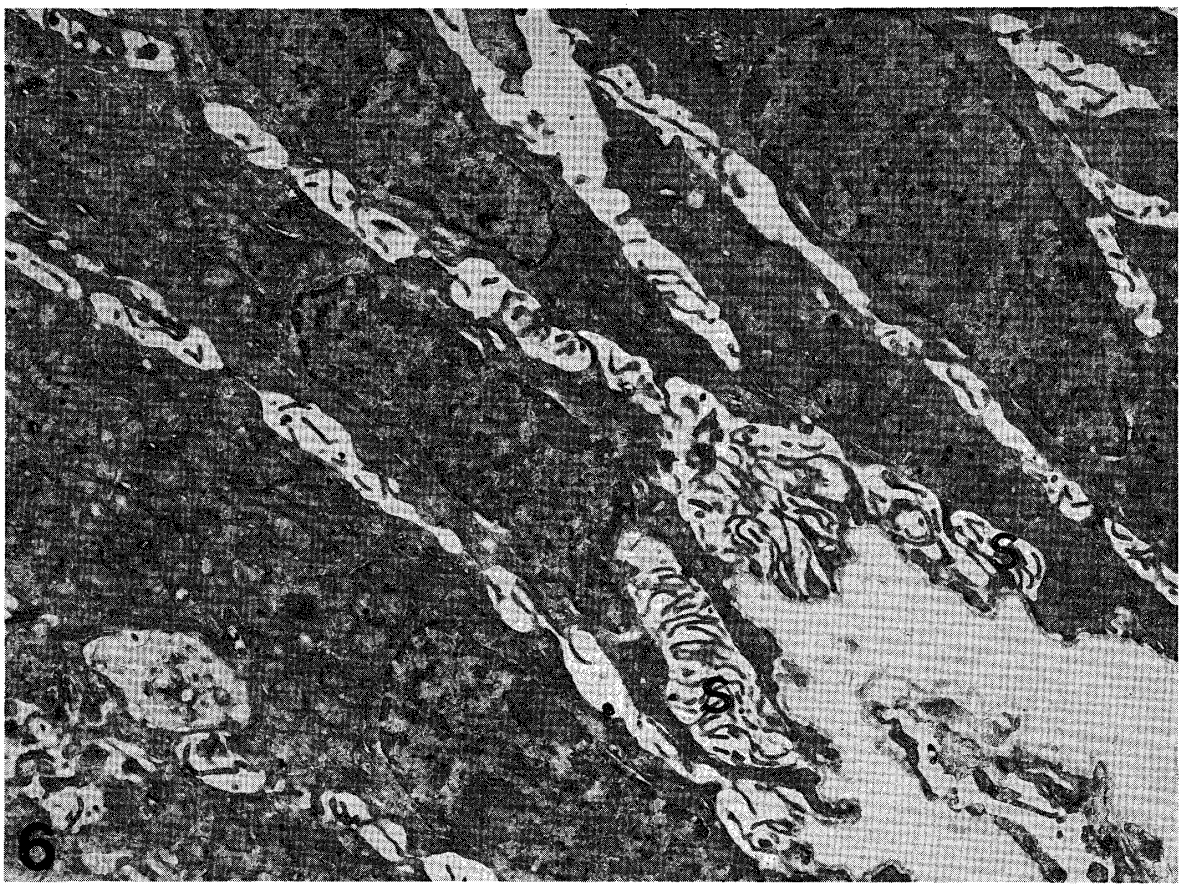
PLATE 3

Explanation of Figures

FIG. 6. Six hours after refeeding × 8250
Development of basal sinus(s) and proximal projections is noted.

FIG. 7. Six hours after refeeding × 10000
Dilation of capillary and proximal projections is seen. Wider intercellular space is partly closed by cell junctions (arrows).

FIG. 8. Six hours after refeeding × 7590
Golgi apparatus is seen, while some of the mitochondria are still small. Large vesicles (v) appear in the proximal cytoplasm where basal sinus is to appear. Ribosomes remain free.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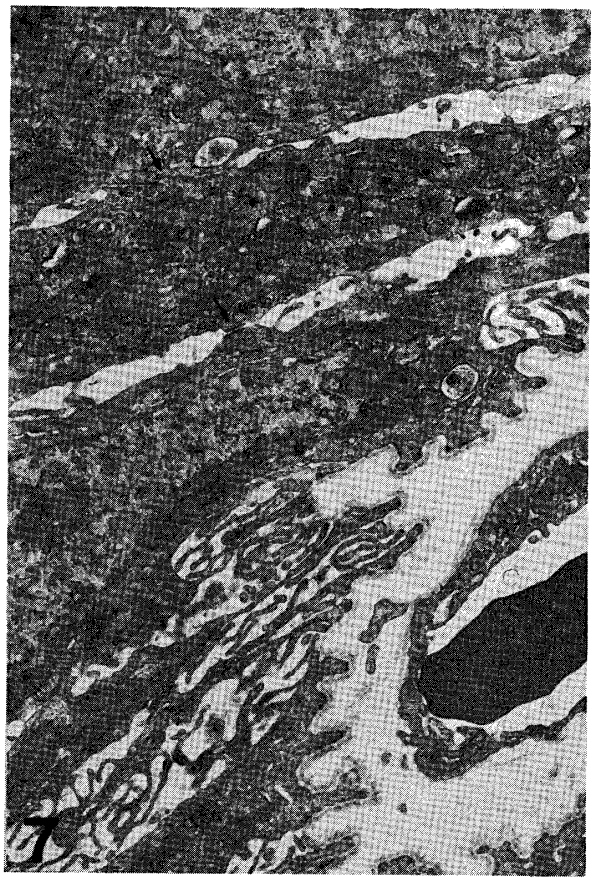
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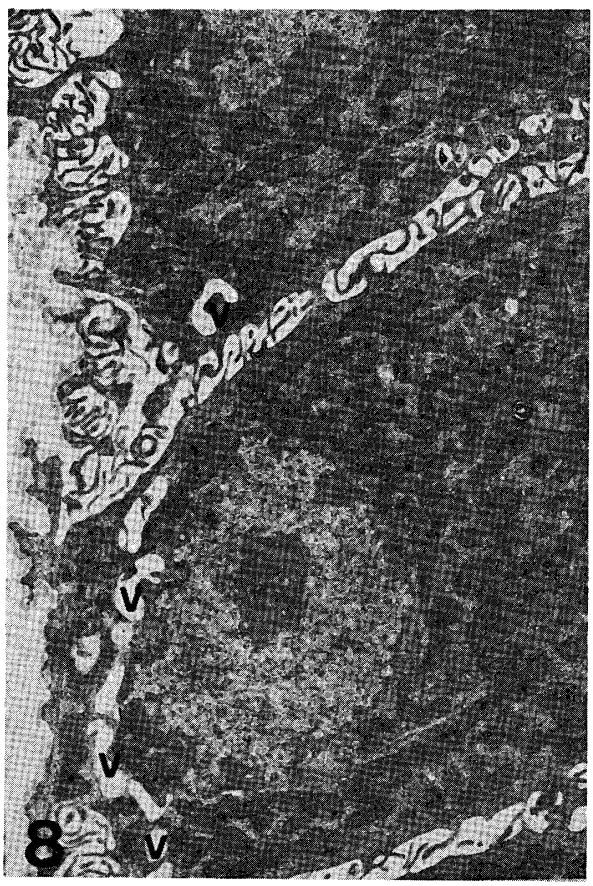
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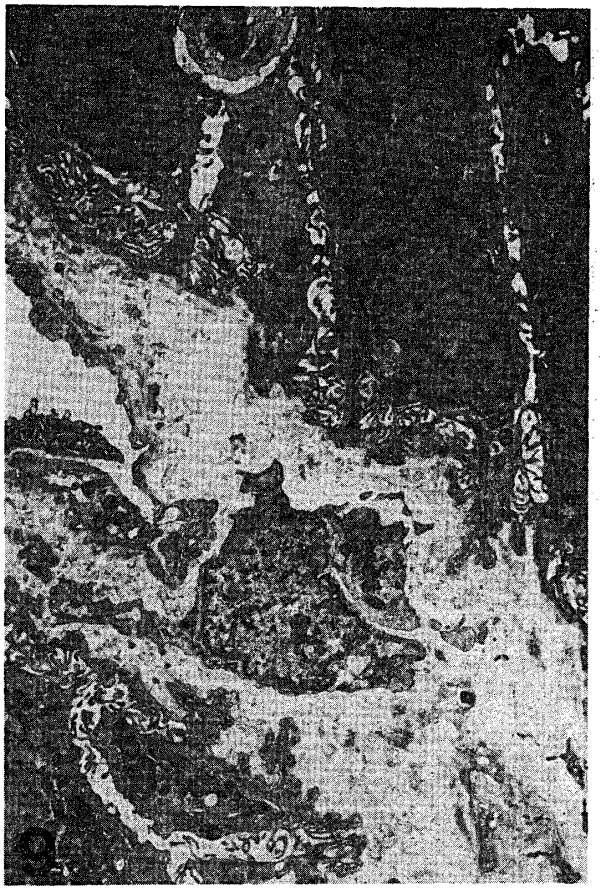

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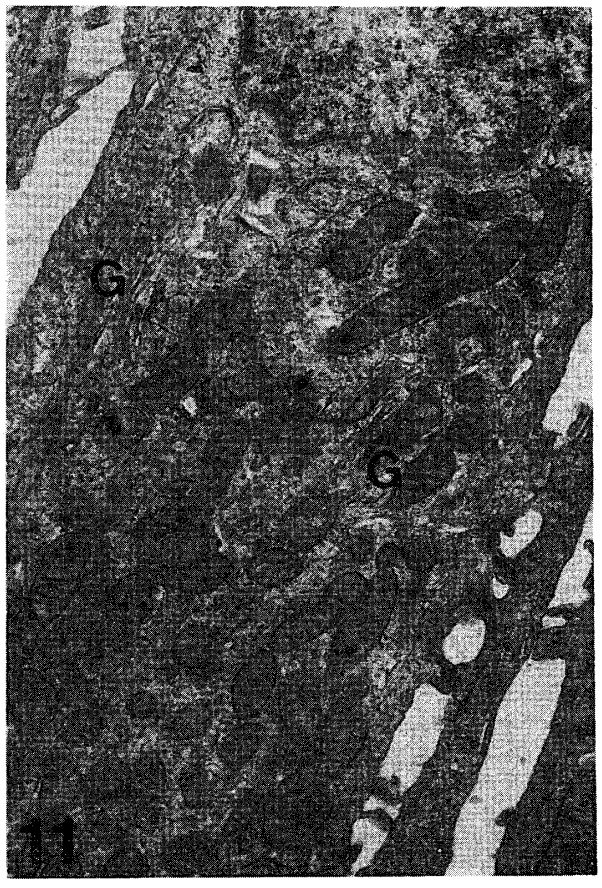

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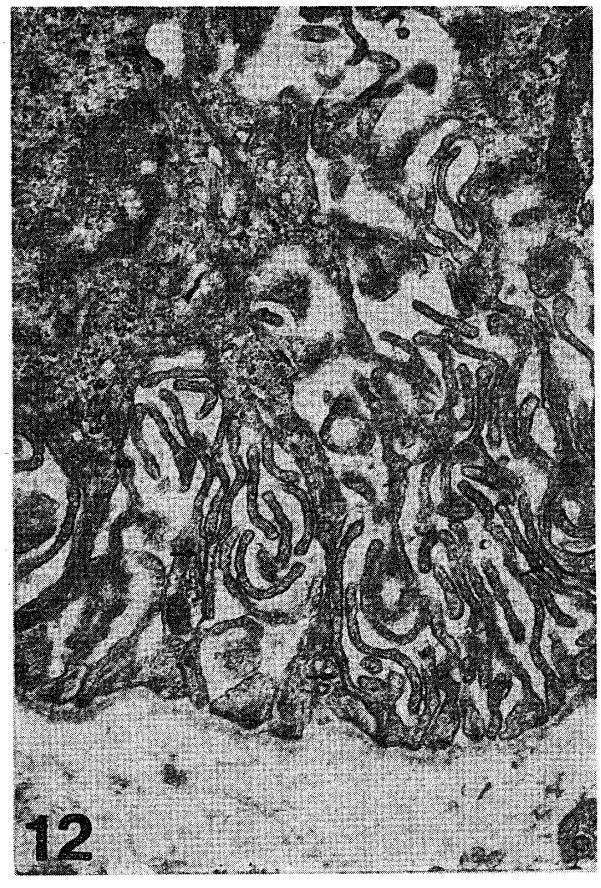

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Plate 4

Explanation of Figures

Fig. 9. Twenty-four hours after refeeding × 4000
Capillary is dilated and basal sinuses are evident.

Fig. 10. Twenty-four hours after refeeding × 7590
Nuclei are located more distal to basement membrane. Presence of processes (P) of rumen Langerhans cells (B) within the basal cell cytoplasm is noted. Mitochondria increase in size and become lighter.

Fig. 11. Twenty-four hours after refeeding × 12500
Development of Golgi apparatus (G) and clusters of ribosomes are noted.

Fig. 12. Twenty-four hours after refeeding × 20000
Extensive basal sinus is partly closed by cell junctions (arrows), while it is open to lamina propria in most areas. Development of proximal projections is noted.