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Scanning Electron Microscopic Observation of the Sheep Ruminal Mucosa at its Epithelium-Connective Tissue Interface

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Summary

The epithelium-connective interface of the sheep ruminal mucosa was observed by scanning electron microscopy. The interface was directly observed with separated epithelium and underlying lamina propria by PBS-EDTA incubation. The epithelial pegs appeared as bulbous downgrowth of epithelial cells which depressed the opposing connective tissue papillary bodies into irregular, low networks. This occurred in central area of developed broad papillae. In smaller papillae and in the peripheral area of the developed ones, the papillary bodies were seen as parallel ridges running regularly. Numerous finger-like projections were seen at the basal surface of the basal cells. The projections were identified as the microvilli-like projections in sections, but the former appeared shorter and wider than the latter. Their size and shape varied from cell to cell, and from papilla to papilla.

The mucosa of ruminant forestomachs is covered with stratified squamous epithelium. The main proliferative as well as metabolic zone of this epithelium is stratum basale. It is also characterized by the development of epithelial cell projections into lamina propria. The projections, the so-called epithelial pegs, undergo prominent hypertrophy in the parakeratotic ruminal mucosa of fattened beef (1) and in sheep ruminal mucosa stimulated by the intraruminal administration of butyric acid (2).

The epithelium-connective tissue (E-C) interface of mucosa provides the territory where active interaction takes place between epithelium and connective tissue. The structural configuration of this interface represents the role of activities played by the cell populations concerned. It is also genetically determined (3).

Klein-Szanto and Schroeder (3) recently gave us a scanning electron microscopic (SEM) view of the E-C interface of human oral mucosa and referred to the structural and functional significance of this interface. We applied their

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technic to adult sheep ruminal mucosa, and report here the results of the SEM observation of the same interface in the mucosa.

Materials and Methods

Rumen papillae were collected by biopsy via fistula by Chevalier-Jackson type forceps, from *atrium ruminis* and ventral sac (*Resessus ruminis*) of three adult sheep. Papillae were also collected from the two loci and cranial wall of anterior pillar of two adult sheep at slaughter. Papillae were incubated in a PBS-solution containing 20 mM EDTA (3) at 37°C for 90 minutes. After the incubation, the epithelium was separated from underlying connective tissue mechanically with fine forceps. The separated tissues were fixed with 3% glutaraldehyde and post-fixed with 1% osmium tetroxide. After hydration through a graded series of ethanol and an iso-amyl acetate bath, the tissues were dried in liquid CO₂ by the critical point method. They were coated with gold and observed by a FE-type scanning electron microscope (Hitachi S-700).

Some of the tissues observed by SEM were embedded in epon resin. Ultrathin sections cut from them were stained with uranyl acetate and lead citrate and observed by a transmission electron microscope (JEM 100B).

Results

The ruminal epithelium was easily separated from lamina propria after 90 min. -incubation in PBS-EDTA solution. Observation of ultrathin sections showed that the epithelium was separated at basal lamina and that basal cells retained their fine structure, specially the microvilli-like projections (Fig. 1). Fig. 2 showed the relief of lamina propria at the E-C interface of a tongue-like rumen papilla. The proprial surface of the interface was characterized by the presence of ridges of papillary bodies running parallel to the long axis of the papilla. At the tip of the papilla the ridges became individual finger-like projections. In a broad papilla (Fig. 3), low networks of the bodies occupied its central area, while the ridges seen in Fig. 2 were present in the periphery of the papilla.

The epithelial surface of the E-C interface, i.e., the basal aspect of stratum basale, was characterized by the development of epithelial 'pegs', which may be better called 'bulbs' (Figs. 4 and 5). The bulbs apparently corresponded to the shallow pits in the proprial surface (Fig. 3). In the periphery of the papilla, the bulbs were more or less flattened and ridge-like (Fig. 4).

In the small triangular papillae of the anterior pillar (Fig. 6), the proprial surface of the E-C interface was characterized by the presence of regularly running serrated ridges. The ridges were continuous with similar ones at the interpapillary area of the mucosa, where low networks were seen (Figs. 6 and 7). The epithelial bulbs inserted into pits of the networks which were smaller and less conspicuous than those in larger papilla (Fig. 8). At higher magnifications, the basal cells had

numerous finger-like projections which were identical to the microvilli-like projections in sections (Fig. 9). Generally the size and shape of the projections were consistent within the same cell, but varied from cell to cell and from papilla to papilla. The developed ones on the spherical basal cells were often tongue-like or rod-like (Figs. 9 and 10).

Discussion

The E-C interface of the sheep ruminal mucosa, as seen by SEM, appeared more specialized than those of human oral mucosae. On the epithelial surface, it was characterized by the development of epithelial bulbs which apparently depressed the proprial ridges into lower networks in certain areas. Such downgrowth of epithelial cells was not seen in human mucosae (3). Hamster forestomach mucosa seemed to lack such downgrowth (4). The bulbs were absent in sheep hard palate mucosa.*

The epithelial bulbs appeared to provide a larger cell population to the ruminal mucosa than other mucosa covered by stratified squamous epithelium. In the former, its epithelial cell population size can be quickly increased by the hypertrophy of the bulbs under certain proliferative stimuli (2, 4). This structure is also responsible to the extraordinary high adaptability of the ruminal mucosa to dietary factors (2, 5). The atrophy of the microvilli-like projections of the ruminal basal cells was reported by TEM in fasted sheep (6). Subsequent refeeding resulted in the recovery of the projections (6).

We are inclined to think that the peculiar configuration of the E-C interface of the ruminal mucosa may represent the genuine interactions played by epithelial and connective tissue cell populations at this interface. We will report the result of our further study on the structural configuration of this interface in rumens of sheep under altered dietary conditions.

Acknowledgement

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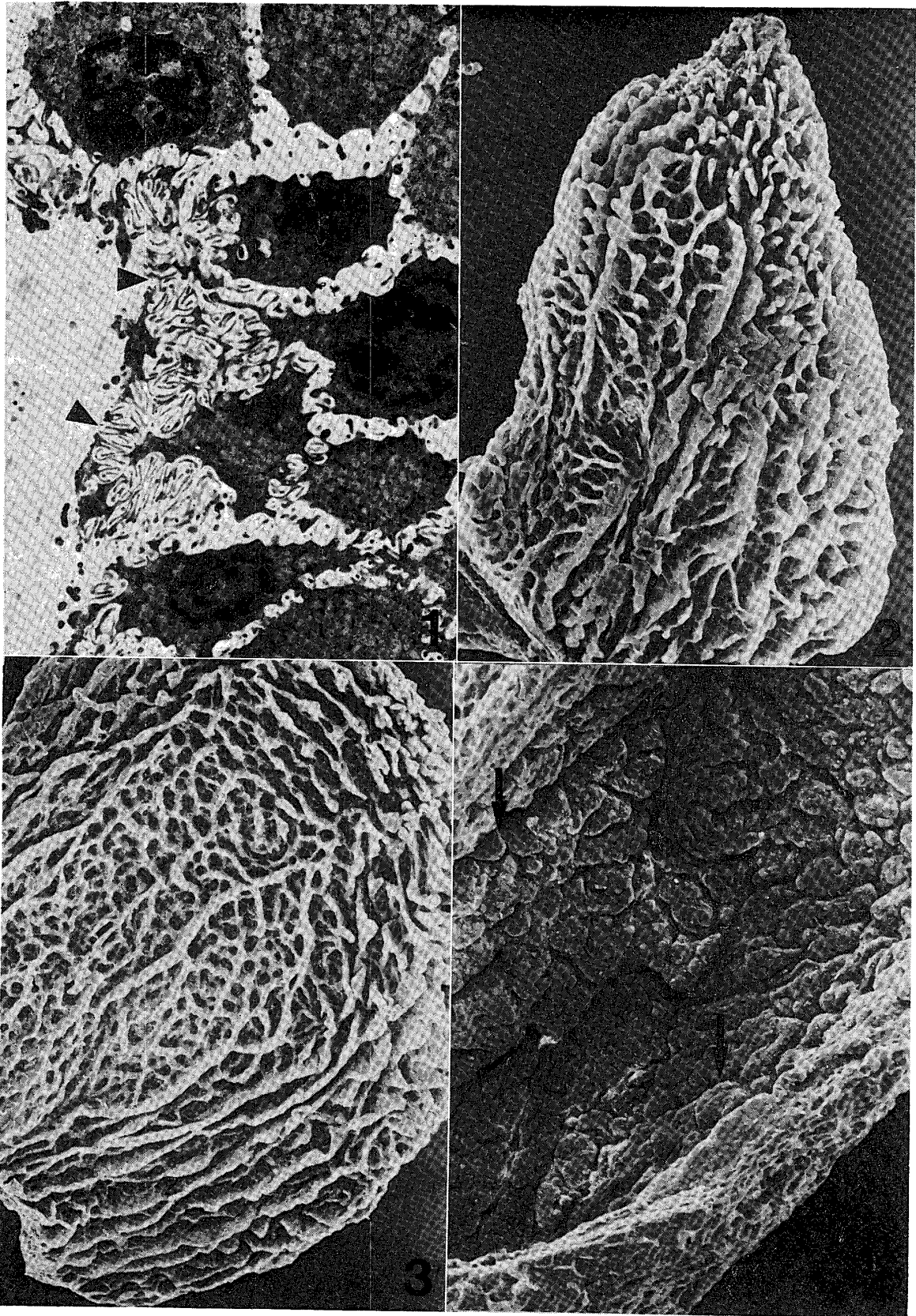
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PLATE 1

- FIG. 1. TEM view of the epithelium of a developed rumen papilla. The epithelium was separated from lamina propria at the basal lamina. Microvilli-like projections (▼) were well preserved after EDTA treatment. × 4000
- FIG. 2. Proprial surface of E-C interface of a tongue-like rumen papilla. Note parallel ridges of papillary bodies. × 84
- FIG. 3. Proprial surface of E-C interface of a broad rumen papilla. Low networks of papillary bodies were seen in central area. × 84
- FIG. 4. Epithelial surface of E-C interface of a developed rumen papilla. Epithelial downgrowth are seen as 'pegs'. At periphery they are flattened (↓). × 600



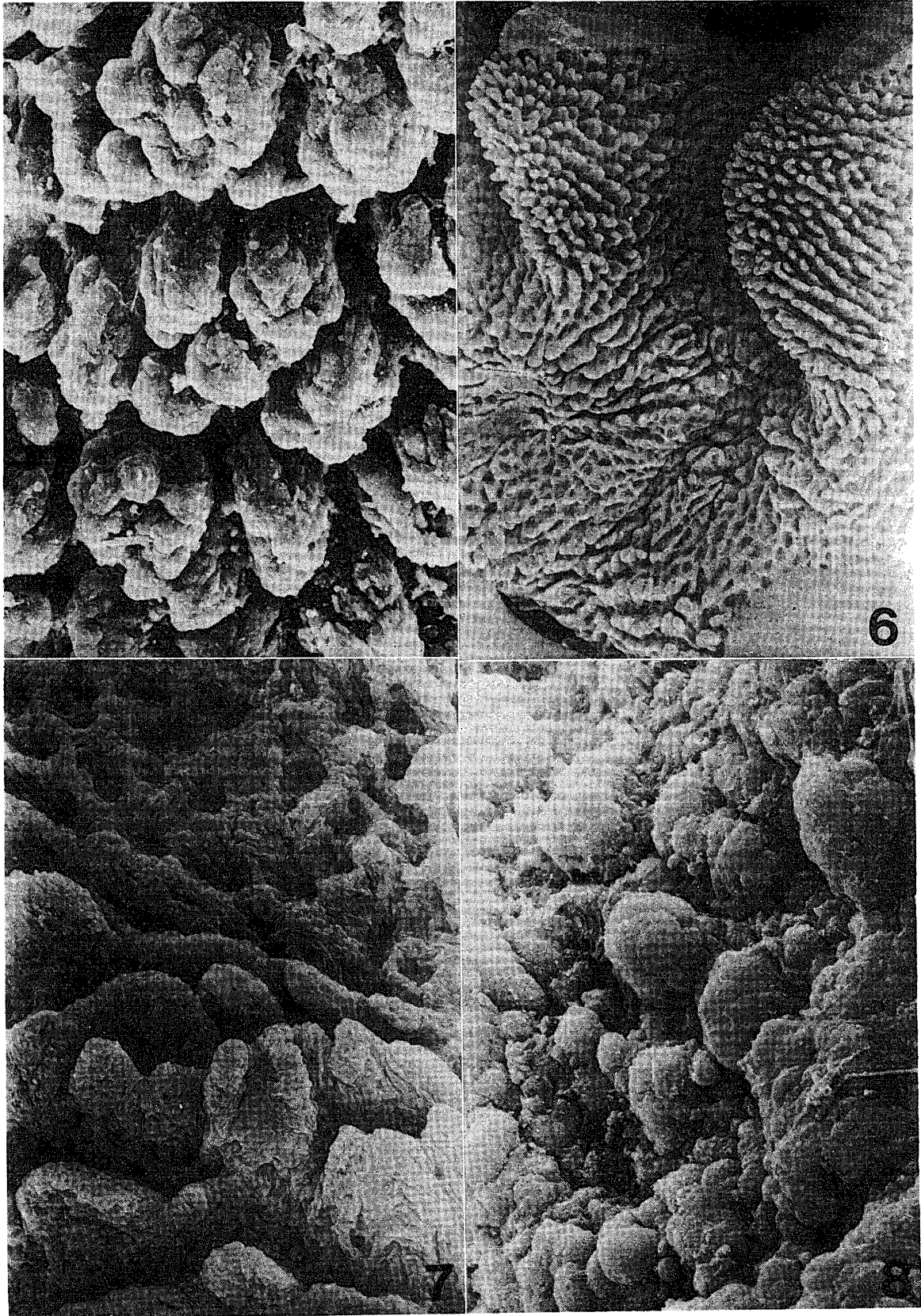


PLATE 2

- FIG. 5. Epithelial surface of E-C interface of a developed rumen papilla. Cell projections appeared as bulbs rather than 'pegs'. Small cells were present at tips of bulbs occasionally. $\times 72$
- FIG. 6. Proprial surface of E-C interface of cranial wall of anterior pillar. Low triangular papillae were composed of serrated ridges of papillary bodies. Low networks of the bodies were seen in interpapillary area. $\times 180$
- FIG. 7. Proprial surface of E-C interface at the base of a triangular papilla in Fig. 6. Shallow pits corresponded to small epithelial bulbs in Fig. 8. $\times 480$
- FIG. 8. Epithelial surface of E-C interface at anterior pillar. Low bulbs of epithelial cell downgrowth are seen. $\times 1200$

PLATE 3

- FIG. 9. Basal surface of a basal cell at tip of a bulb, in E-C interface of a developed rumen papilla. The spherical cell with numerous fine, finger-like projections. $\times 12000$
- FIGS. 10 and 11. Projections of basal cells at E-C interface. Some were tongue-like (\blacktriangledown) and rod-like (\downarrow). $\times 20000$ (Fig. 10); $\times 40000$ (Fig. 11)

