Electron Microscopic Study on Parakeratotic Ruminal Epithelium in Beef Cattle

Hideo TAMATE and Tateki KIKUCHI

*Laboratory of Animal Morphology, Faculty of Agriculture,
Tohoku University, Sendai-shi, Miyagi 980*

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Abstract. The ultrastructure of the ruminal epithelium from the rumens of five fat-tened-beef cattle affected severely by gross parakeratosis was reported. In rumen parakeratosis the tonofibrillar bundles appeared in stratum basale, while rough-surfaced endoplasmic reticulum and ribosome clusters disappeared. The appearance of the bundles in stratum spinosum was more pronounced and it was suggested that the keratin synthesis might be complete in these strata and that the rate of post-mitotic aging was much faster in parakeratotic ruminal epithelium. The retention of nuclei and fibrous network in stratum corneum suggested that the disturbance of the activities of lysosomal enzymes might take place in rumen parakeratosis. Lipid droplets were present throughout the epithelium and they were large and abundant in stratum corneum. The development of extensive proximal projections of the basal cells indicated that the high intake of concentrates stimulated the increase in cell metabolism in stratum basale.

In a recent report we drew attention to the high incidence of rumen parakeratosis in cattle slaughtered in Miyagi Prefecture during the period May, 1970 to July, 1971 [17]. Fell et al. [6] reported that in calves fed on barley ruminal lesions including both hyperkeratosis and parakeratosis developed within 4 weeks after the change of diet. Similar lesions were described by Jensen and Mackey [10] in tissues of feedlot cattle.

The ultrastructural changes of parakeratotic epithelium in human psoriatic epidermis were investigated by Brody [1-3], and in palatal and buccal epithelia of zinc-deficient rats by Osmanski and Meyer [13]. In spite of several detailed studies on the ultrastructure of the ruminal epithelium in animals fed conventionally [9, 11, 15], so far as we are aware description of ruminal

parakeratosis have not been published. The aim of this paper is to report the ultrastructural changes of the ruminal epithelium in cattle found at slaughter to have macroscopic lesions of ruminal parakeratosis.

Materials and Methods

Rumens from five beef cattle killed at an abattoir in Sendai, Miyagi Prefecture, were used in this study. They were judged to be severely affected with gross parakeratosis, the main criterion used was the development of extensive clumping of rumen papillae [17].

Tissues from papillary clumps were selected for further study. Care was taken to choose individual papillae that appeared to be unaffected by inflammation or necrosis. They were collected within 40 minutes after death and were fixed in cold 4 to 5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2 and at 4°C, followed by postfixation with 1% osmium tetroxide in veronal buffer at pH 7.2. Thin sections were cut by a Porter-Blum MT-2

ultramicrotome, and stained with uranyl acetate and lead citrate. Observations were made with a JEM 100B electron microscope.

Results

1. Stratum basale

The basal cell contained a relatively small amount of rough-surfaced endoplasmic reticulum (rER) and few clusters of ribosomes. Free ribosomes were abundant in the cytoplasm. Mitochondria were small, rounded in cross-section, and usually had a darkly-stained matrix. Accumulation of tonofibrillar materials was often seen. Less frequently thick bundles of such fibrils were also present. Usually the basement membrane showed extensive infoldings due to the development of numerous projections of the proximal cytoplasm (Figs. 1, 2). Small lipid droplets were occasionally present.

2. Stratum spinosum

The lower layer of this stratum consisted of polygonal spinous cells. In some cells smooth-surfaced endoplasmic reticulum (sER) was abundant. Free ribosomes were abundant and the development of Golgi apparatus was noted in most cells. Numerous small bundles of fine tonofibrils were seen running at random throughout the cytoplasm, with no definite association with desmosomes. Small lipid droplets were commonly seen in the cytoplasm. The intercellular space was wide (Fig. 3).

The cells in upper layers of this stratum gradually became flattened toward the stratum granulosum. They contained relatively little rER. Mitochondria became fewer and much darker, irregular outline of which suggested degeneration. Free ribosomes were abundant. Numerous bundles of tonofilaments were present in the cytoplasm. Keratohyalin granules were present only occasionally. The intercellular

space was wide. Development of tonofibrillar bundles attached to desmosomes was rather poor. It was noted that lipid droplets of varying sizes were commonly seen in the cytoplasm (Fig. 4).

3. Stratum granulosum

Some granular cells contained a few typical keratohyalin granules of varying sizes, mostly small. The intercellular space was narrow and cell junctions including desmosomes and tight cell junctions were present, although they appeared more or less defective. Indeed it is possible that the presence of tight cell junctions rather than keratohyalin granules may be the most reliable criterion for the identification of granular cells (Fig. 5).

Swollen granular cells were occasionally present. It was observed that many granular cells retained a degenerating nucleus and also some organelles. Dark, membrane-bound bodies with the characteristic appearance of lysosomes were present. Otherwise the cytoplasm was occupied by the loose networks of tonofilament bundles. Large lipid droplets were commonly seen (Fig. 7).

4. Stratum corneum

This stratum consisted of several cell layers which showed a considerable variation in their cell constituents. Generally they contained heavy accumulation of thick tonofilament bundles. When the remnants of a nucleus was present, depositions of fine, electron-dense, keratohyalin-like granules and fibrillar materials were noted around it (Fig. 8). Lipid droplets were numerous.

The retention of desmosomal junctions throughout the entire stratum corneum resulted in the unusually narrow intercellular space. The junctions appeared, however, more or less defective. The luminal surface of this stratum showed a few cytoplasmic

projections (Fig. 8).

5. The cells in epithelial basal invaginations

The epithelium of rumens with severe parakeratosis was characterized by the development of extensive invagination towards the lamina propria or epithelial pegs. The invaginations were commonly associated with heavy sloughing of the covering horny cells, resulting in the infoldings of the papillary epithelium. The cells in the centre of the invaginations consisted of flat spinous cells which were continuous with those in upper stratum spinosum (Fig. 6).

Discussion

As in epidermis, the biosynthesis of the keratinous substances in the ruminal epithelium starts in the basal cells which initiate the post-mitotic differentiation or aging. The products of this synthesis, the tonofibrils, appear normally in upper spinous cells as a few keratohyalin granules [9, 11, 15]. The abrupt appearance of the keratohyalin granules is followed by the dispersion by lysosomal enzymes of the granules into fine, homogeneous cell contents of horny cells [12]. The presence of hydrolytic enzymes such as lipase and acid phosphatase was demonstrated histochemically [8].

Our present data suggested that, in severe rumen parakeratosis, the keratin synthesis took place much earlier than that in non-parakeratotic epithelia. The appearance of tonofibril bundles in basal and spinous cells together with the disappearance of rER and ribosome clusters indicated that the synthesis was complete in these cells, and that the rate of post-mitotic aging was much faster in parakeratotic epithelium. Sakata and Tamate [14] reported that the proliferation of the ruminal epithelium

was accelerated by the intermittent feeding in the sheep. We, therefore, are inclined to believe that the early synthesis of keratinous substances in parakeratotic epithelium possibly is the result of increased rate of proliferation in the same epithelium, as suggested by Bullough [4].

Parakeratosis without keratohyalin granules was reported in human psoriatic epidermis [1-3] and in the oral mucosa of zinc-deficient rats [13]. The rumen parakeratosis reported here appeared to more similar to the former type of parakeratosis, in which ultrastructural changes occurred in basal cells.

The second characteristic feature of the rumen parakeratosis is that the horny cells retained coarse fibrous network in the cell interior and also desmosomal junctions in the uppermost layers. This suggested that disturbances in the activities of lysosomal enzymes might take place in the rumen parakeratosis, as suggested by Osmani and Meyer [13] in the oral mucosa. Lavker and Matoltsy [12] reported the presence of a large number of lysosomes in the granular cells of normal rumen epithelium. It is not clear from the present investigation whether a disturbance of lysosomal enzymes synthesis occurs in parakeratotic ruminal epithelium, though we found only a limited number of lysosomes in the epithelium.

The third characteristic feature of the rumen parakeratosis is that the proximal cytoplasm of the basal cells possessed extensive projections associated with the increased volume of intercellular space. In view of our recent work on the same structures in fasted and refed lambs [16], we believe that this is an indication of increased cell metabolism stimulated by the high intake of concentrate in fattened beef cattle.

Lipid droplets are abundant in stratum corneum of normally keratinizing rumen

epithelia [7]. In the parakeratotic rumen epithelium, however, the basal and spinous cells also contained lipid droplets. At present we can advance no explanation for this change which, we believe, may be a characteristic of parakeratosis.

Structural as well as biochemical adaptation of the digestive tract could occur in a variety of animals under different feeding condition [5]. The tissues we observed were taken from animals of unknown feeding records. We do not know, therefore, whether ultrastructural characteristics we observed here were affected by the possible adaptation of the ruminal epithelium during protracted feeding. Further studies on the ultrastructure of rumen parakeratosis of animals fed experimentally for a few months may be most promising to clarify the exact mechanism of this type of parakeratosis.

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Explanation of Figures

All photomicrographs were taken from the sections of rumens grossly affected with severe parakeratosis except Figs. 2 and 6.

- Fig. 1. Stratum basale. Development of microvilli-like projections is noted. $\times 6,000$.
- Fig. 2. Stratum basale. Proximal cytoplasm of basal cell has numerous microvilli-like projections ($\blacktriangledown$). Basal membrane shows extensive infoldings ($\blacktriangledown$). The cytoplasm contains many free ribosomes. $\times 11,500$.
- Fig. 3. Lower stratum spinosum. Spinous cell cytoplasm contains numerous bundles of tonofibrils ($\blacktriangledown$) and lipid droplets (L). $\times 10,000$.
- Fig. 4. Upper stratum spinosum. Numerous tonofibrillar bundles ($\blacktriangledown$) and free ribosomes are present. Large lipid droplets (L) are commonly seen close to nucleus (N). Intercellular space is wide. $\times 10,000$.
- Fig. 5. Stratum granulosum. The granular cells contain few typical keratohyalin granules. They are identified by the presence of tight cell junctions ($\blacktriangledown$) and narrow intercellular space. Lipid droplets (L) are numerous. $\times 5,000$.
- Fig. 6. The spinous cells in epithelial basal invagination. The cells of basal invaginations, characteristic to parakeratotic ruminal epithelium, are similar to those in upper stratum spinosum. Processes of a Langerhans cell (L) are occasionally present close to nucleus (N). $\times 4,000$.
- Fig. 7. Stratum granulosum. A swollen granular cell retains relatively intact but degenerating nucleus (N), degenerating mitochondria (M), and large lipid droplets (L). A few keratohyalin granules (K) and lysosome-like dark bodies ($\downarrow$) are also present. Cell junctions ($\blacktriangledown$) are partly incomplete. $\times 10,000$.
- Fig. 8. Stratum corneum. Some cells in this stratum contain degenerating nucleus (N) and endoplasmic reticulum filled with fine keratinous particles (FK). Thick tonofibrillar filaments (TK) are also present. Partial retention of desmosomal cell junctions ($\blacktriangledown$) is commonly seen. $\times 6,600$.