DNA Microphotometry on Ruminal Epithelial Cells in the Sheep

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DNA Microphotometry on Ruminal Epithelial Cells in the Sheep

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Summary

Using DNA microphotometry with conventional Feulgen staining, measurement of DNA content was carried out on the ruminal epithelium of adult male sheep. The spinous cells and most of intra-peg cells were made up of G1 population and not proliferative. The basal cells involved G1, S, G2 and M population and are the proliferative fraction of this epithelium. The relative number of S and G2 cells estimated from the histogram were about 12% and 17% in the two sheep, respectively. The presence of a few intra-peg cells with larger amounts of DNA than that of diploid cells may suggest the proliferative potential of this cell group.

Ruminal epithelium is a keratinizing stratified squamous epithelium and its thickness is directly related to the proliferative activity of the basal cells. Recently the cell kinetics of the ruminal epithelium have been studied with the mitotic index as a marker of the activity (1–7). The development of epithelial pegs was noted in connection with an increased mitotic rate promoted by the intraruminal administration of butyric acid (6, 8)

The autoradiographic analysis is an usual method for the investigation of the cell cycle parameters, but the technique has not been applied to ruminal forestomach except that of Hamada (9). In this connection, microphotometry and fluorometry are other useful methods for the examination of DNA distribution of the tissue cells. Thus in the present reprot, we studied the DNA content of sheep ruminal epithelium using a microspectrophotometer after Feulgen-Schiff reaction.

Materials and Methods

Two male Merino sheep, weighing 35.5 kg (Sheep 1) and 41.0 kg (Sheep 2), were used in this study. They were fed on the conventional diet of 400 g of concentrate and 600 g of orchard hay at 10:00 until one day before slaughter. They were sacrificed at 10:00 and pieces of the ruminal mucosa were obtained

from the atrium ruminis. The pieces were fixed in 10% phosphate-buffered formalin for 48 hrs, embedded in paraffin, and cut at 5 μ thickness.

The procedure for Feulgen staining for photometric measurement of DNA was performed according to the slightly modified method of Fujita (10). Hydrolysis was carried out in 5N HCl for 45 min. at 18°C. Schiff's reagent (Merk, Art. 9033) was diluted to 10% (0.05% of pararoseanilin) in glycine buffer adjusted to pH 2.3. Schiff's reaction was carried out at 18°C for 30 min. The sections were then transfered to sulfite rinse (glycine buffer, pH 2.3) and washed in running water. They were dehydrated and mounted in Entellan New (Merk).

The amount of Feulgen-DNA stain complex was measured with a micro-spectrophotometer (MMSP-TK, Olympus Optical Co. Ltd., Tokyo). Total extinction at 560 nm was used as a measure of the total amount of Feulgen-positive material in the nucleus. The measurements were carried out on nuclei of spinous, intra-peg and basal cells of the ruminal epithelium. Using the SD of the spinous cells in the same preparation, the mean G1–DNA content of the intra-peg and basal cell nuclei were determined as being within a range of $\pm$2SD from the G1 peak observed (11).

Results

The measuremant of Feulgen-DNA stain complex in arbitrary unit was made on the nucleus of three cell types in the ruminal epithelium of Sheep 1 and 2 (Fig. 1a-c). The histogram of the spinous cells exhibited only one peak in the diploid (2N) region, consisting of G1 cells only (Fig. 1a). The values of the intra-peg cells were also grouped in a single class, but a few nuclei showed a larger amount of DNA content than that of diploid nuclei (Fig. 1b).

The histogram of the basal cells showed a relatively broad peak representing cells with a diploid (2N) DNA content (G1 cells). Some cells located around the tetraploid (4N) DNA value represented G2 cells. The cells in S phase are found to be between the DNA values of 2N and 4N (Fig. 1c). It is obvious that most cells are located in G1 phase and that a relatively small number of cells are in the S and G2 compartments. From the histograms, it can be roughly estimated that the relative numbers of S and G2 cells at stratum basale are about 12% in Sheep 1 and 17% in Sheep 2. The coefficient of variation of the G1 peak were calculated to be 13.4% and 17.9% in Sheep 1 and 2, respectively.

Disccussion

Judging from the Feulgen-DNA contents, the spinous cells of sheep ruminal epithelium exhibited only diploid DNA contents consisting of G1 cells which are not proliferative. Some of the intra-peg cells with a larger amount of DNA content than that of diploid value may be in the S and G2 phases. The basal

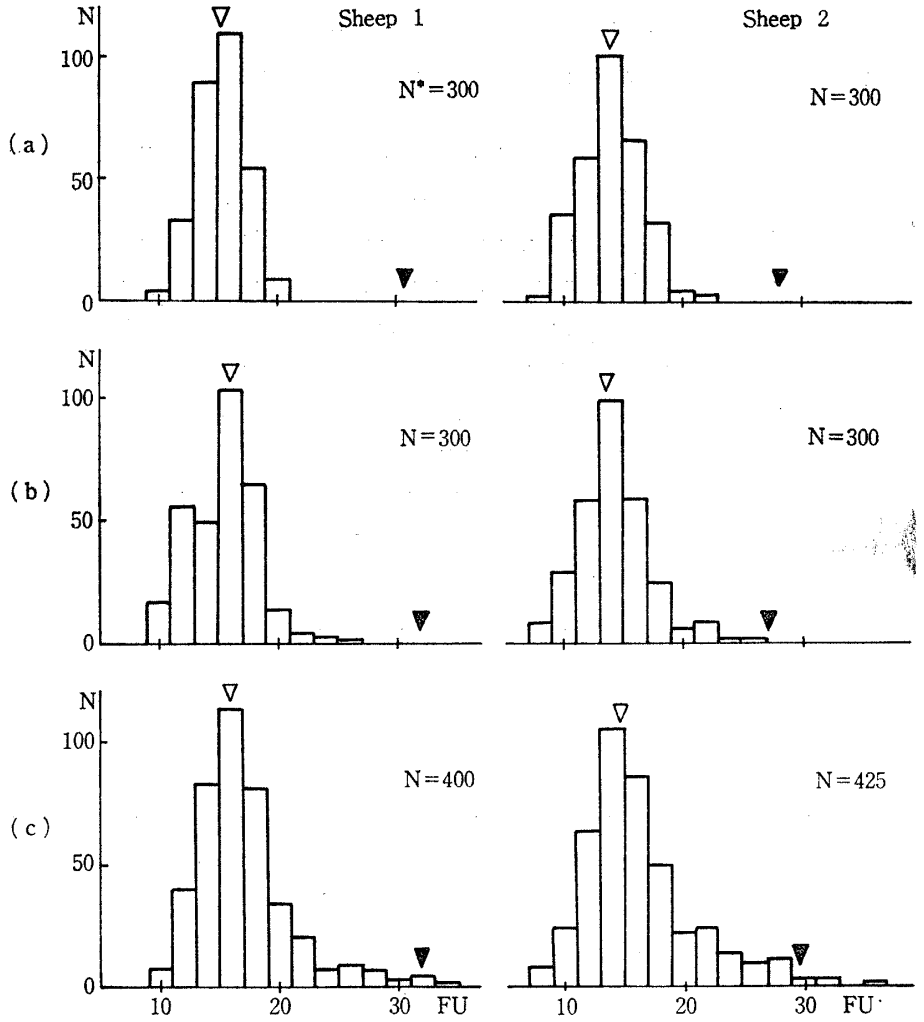

FIG. 1. Distribution of DNA-Feulgen content in spinous (a), intra-peg (b) and basal (c) cells of Sheep 1 and 2. *Abscissae*: Feulgen-DNA content/nucleus in arbitrary units; *Ordinates*: Number of nuclei measured; N*: Tatal number of nuclei measured; ▽: Mean value of diploid DNA content; ▼: Mean value of tetraploid DNA content.

cells are apparently made up of G1, S, G2 and M cells, indicating that the main germinal layer of the ruminal epithelium is stratum basale.

The relative number of S and G2 cells in basal cells of human epidermis were 5.9% (12) and that of adult mouse being 6.7±1.8% (13) with cytophotometry. In hyperplastic epidermis of newborn (13) and hairless mouse (11), they were 14.9% with microflow fluorometry. In this study, the relative number of S and G2 cells in basal cells, about 12% and 17% in two sheep are nealy equal to values of newborn and hairless mouse epidermis, but higher than that of normal human and mouse epidermis. This suggests that the ruminal epithelium of domestic ruminants are hyperplastic in nature, because the relative number of S and G2 cells in the basal cells are correlative.

Hamada (9) reported that the labeling index of ruminal epithelial basal cells

was 12% in a kid of 4 weeks of age. This value exhibits a relative number for the S phase and is close to our data.

The development of epithelial pegs in the rumens were related to the high proliferative activity (6, 8). The difference of relative number of S and G2 cells in two sheep may be correlated to the difference of the development of epithelial pegs in the two sheep.

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