SCANNING ELECTRON MICROSCOPY OF THE MUCOSAL SURFACE OF THE FORESTOMACHS AND ABOMASA OF GREY, WHITE AND BLACK KARAKUL LAMBS

H. B. GROENEWALD, Department of Anatomy, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110

ABSTRACT

GROENEWALD, H. B., 1992. Scanning electron microscopy of the mucosal surface of the forestomachs and abomasa of grey, white and black Karakul lambs. *Onderstepoort Journal* of *Veterinary Research*, 59, 167–174 (1992)

Homozygous grey and white Karakul lambs suffer from a lethal genetic factor causing death after weaning. Previous studies revealed large milk-filled rumens in the grey and white lambs which was attributed to a significant decrease in the number of myenteric ganglia and neurons in the rumen wall. This study was undertaken to determine the effect of milk on the epithelial lining of the forestomachs of affected grey and white lambs. In the forestomachs of the black lambs the polygonal epithelial cells were tightly packed, seemed to overlie one another and cytoplasmic projections occurred on the cell surfaces. In the grey and white lambs the epithelium had an eroded appearance due to sloughing of the surface cells and the cytoplasmic projections were lower and had a weathered appearance compared to the black lambs. No obvious differences could be detected in the abomasa of grey, white and black lambs. It is concluded that the milk in the forestomachs of the grey and white lambs is responsible for the epithelial changes.

INTRODUCTION

Mortalities in grey lambs of various sheep breeds have been described by a number of authors since the early part of this century. Contescu & Epureanu (1939) reported a mortality rate of 30,3 % in grey Zurkana lambs under the age of ten months. Most of the latter died at 5 to 6 months of age due to digestive disturbances (Contescu & Leagu, 1941). Mortalities in grey Karakul lambs have been reported by Nel & Louw (1953) and Nel (1965). They described the condition as the "Lethal factor" and Nel (1965) proved it to be a genetic disorder.

Groenewald & Booth (1989) indicated a significant difference in the size of the rumens of the grey, white and black Karakul lambs respectively. Grey lambs have the largest and black lambs the smallest rumens. Furthermore, the presence of milk in the rumen and reticulum of affected grey and white lambs is a constant finding (Groenewald & Booth, 1989). Recent studies indicated a decrease in the thickness of the tunica muscularis as well as a reduction in the number and size of the myenteric ganglia and neurones in the rumens of affected grey and white lambs compared to normal black lambs (Groenewald & Booth, 1990).

When put on a high roughage diet after weaning affected lambs develop a pot belly, become emaciated and die (Nel & Louw, 1953). On post-mortem examination these lambs display distended thinwalled rumens and sand impacted abomasa (Nel, 1965). Only homozygous grey lambs are affected and they can be identified at birth by the lack of pigmentation of the tongue, palate and ears (Nel & Louw, 1953). Homozygous white Karakul lambs are born with the same lethal factor, also develop pot bellies, are generally "poor doers" and eventually die. However, they survive for a longer period compared to the grey lambs.

The development of ruminal papillae depends on the nature of the ration (McGavin & Morrill, 1976; Arias, Cabrera & Valencia, 1978; Kamel, Ali, Saber & Hassan, 1984). Friction and the resulting desquamation of cells result in the loss of the outermost layer of the ruminal epithelium (Scott & Gardner, 1973). Scanning electron microscopical (SEM) evidence of sloughing of surface cells in the forestomachs of sheep has been presented by Tamate, Kikuchi, Onodera & Nagatani (1971), Scott & Gardner (1973) and Amasaki & Daigo (1988). Differences in the morphology and arrangement of cytoplasmic projections of epithelial cells have also been noted (Tamate et al., 1971; Scott & Gardner, 1973).

The mucosa of the abomasum consists of hillocks which gradually enlarge forming intervening grooves. In some cases the hillocks fuse forming a deep hollow between them, which seem to show the onset of the gastric pits (Asari, Fukaya, Yamamoto, Eguchi & Kano, 1981).

No information could be found in the literature on the surface structure of the mucosa of the forestomachs of 24-hour-old sheep lambs, and it was therefore decided to use 24-hour-old black Karakul lambs as controls.

The present study was undertaken to determine whether the presence of the milk found in the forestomachs of grey and white lambs affected the structure of the epithelial surface.

MATERIALS AND METHODS

Five grey and white lambs respectively with unpigmented tongues, palates and ears and five black Karakul lambs which were randomly selected as controls were slaughtered 24 hours after birth. Two additional grey lambs were slaughtered immediately after birth, before they suckled.

One square centimetre samples were taken from analogous areas of the rumen, reticulum, omasum and abomasum of all the lambs. They were rinsed with phosphate buffered saline (PBS), pH 7,4 and abomasa specimens were vigorously shaken in Ringers solution to remove the mucous from the mucosal surface.

The samples were pinned with insect pins to wax squares to prevent them from curling and fixed with





FIG. 1 Mucosal surface of the reticulum of a black lamb displaying tongue-shaped papillae (TP). 36×10^{-2} FIG. 2 Mucosal surface of the omasum of a white lamb displaying conical papillae (P). 60×10^{-2}

4 % glutaraldehyde in Millonig's phosphate buffer. After fixation the samples were dehydrated through a graded ethanol series, and dried in a Polaron critical-point drier with liquid carbon dioxide. The dried samples were gold coated in a Polaron E5100 Coating Unit and studied with a Phillips XL20 scanning electron microscope operated at 10 kV.

RESULTS

The mucosa of the rumen and reticulum in all the lambs displayed numerous tongue-shaped papillae

(Fig. 1), and the omasum conical papillae (Fig. 2). The papillae surface were generally grooved. In the black lambs the rumen, reticulum and omasum was covered with flattened, polygonal epithelial cells (Fig. 3) with distinct borders. No sloughing of the surface cells was evident. Numerous cytoplasmic projections were noted on the cell surfaces (Fig. 4). In the rumen and reticulum the projections appeared globular (Fig. 4) and more flap-like with a contorted arrangement in the omasum (Fig. 5).

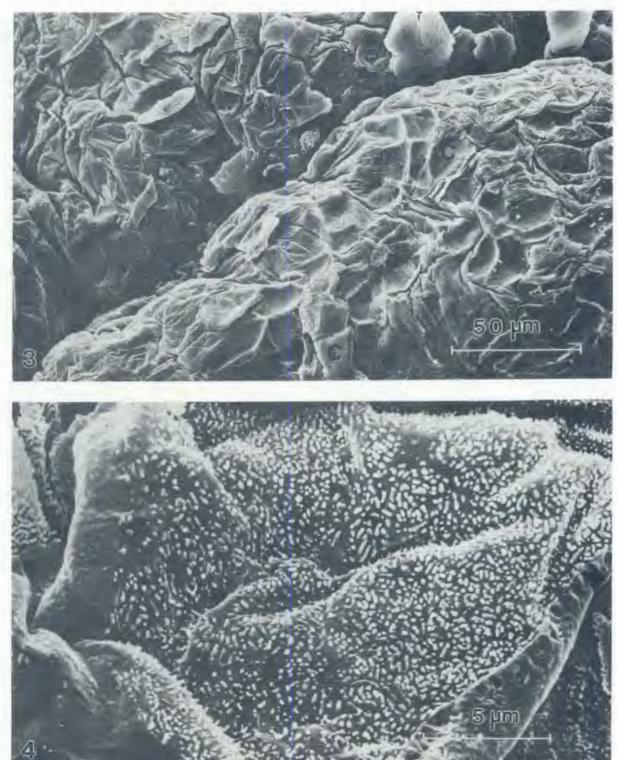


FIG. 3 Ruminal surface epithelium of a black lamb. The cells (C) have distinct borders and seem to overlie each other. 600×10^{-2} FIG. 4 A surface epithelial cell of the reticulum of a black lamb. Note the globular cytoplasmic projections on the cell surface. 6000×10^{-2}

The morphology of the rumen, reticulum and omasum of the two grey lambs that did not suckle were similar to that of the black lambs (Fig. 6 & 7). However, the mucosal surface of the rumen, reticu-

lum and omasum of the grey and white lambs that did suckle differed significantly from the black lambs. Although the shape of the reticulum papillae appeared similar to those of the black lambs (Fig. 8)

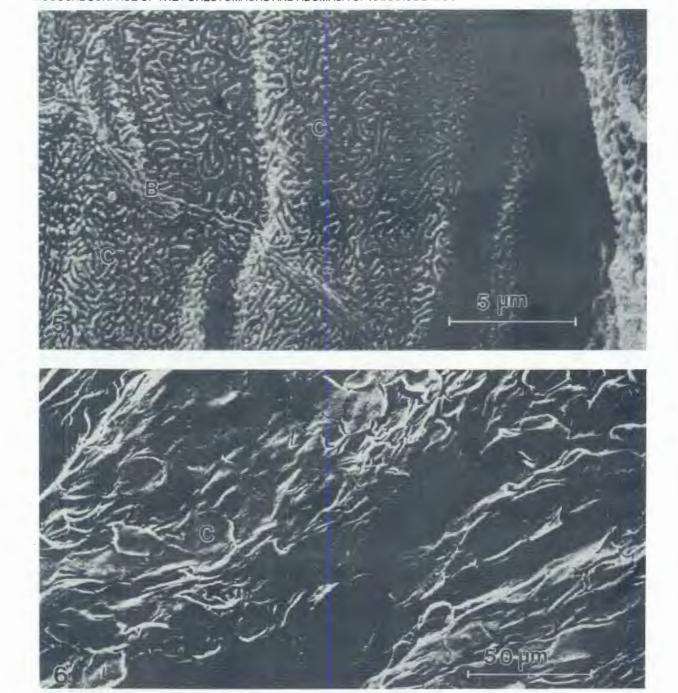


FIG. 5 Surface epithelial cells (C) of the omasum of a black lamb with flap-like, contorted cytoplasmic projections on the cell surfaces. Note the clear borders (B) between the cells. 6 000 ×

FIG. 6 Surface epithelium of the rumen of a grey lamb that did not suckle. The general appearance of the cells (C) is similar to that of the black lambs. $600 \times$

the surface epithelium had an eroded appearance due to sloughing of surface cells (Fig. 9). The cytoplasmic projections on the cells were lower than those of the control lambs and had a weathered appearance (Fig. 10). Large numbers of bacteria were present on the surface of the cells of the affected lambs (Fig. 11).

In the black lambs the mucosal surface of the abomasum consisted of hillocks fused to a greater

or lesser degree to form deep intervening hollows (Fig. 12). The surface cells of the hillocks had a granular appearance due to the presence of small, squat, cytoplasmic projections (Fig. 13). No obvious differences were evident in the morphology of the mucosal surface of the abomasa of the grey, white and black lambs.

DISCUSSION

Papillae of various shapes and sizes have been



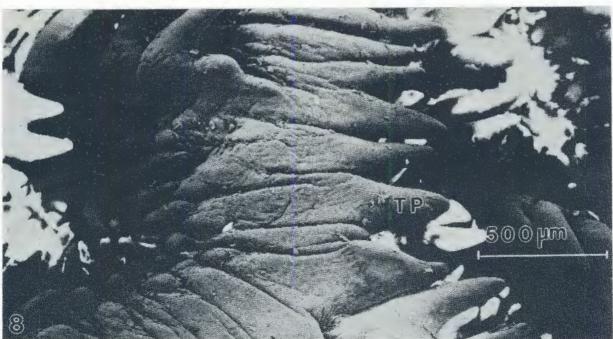


FIG. 7 Surface epithelial cells of the omasum of a grey lamb that did not suckle. The cells reveal flap-like projections similar to those seen in the omasa of black lambs. $3\,600\,\times$

FIG. 8 Mucosal surface of the reticulum of a white lamb with tongue-shaped papillae (TP). $60 \times$

described in the different compartments of the ruminant stomach (Gardner & Scott, 1971; Tamate *et al.*, 1971; McGavin & Morrill, 1976; Arias *et al.*, 1978; Asari *et al.*, 1981; Kamel *et al.*, 1984). The present study confirmed these results. In the rumen and reticulum the typical tongue-shaped papillae was evident as described by Kamel *et al.* (1984) in goats. The morphology of the papillae in the omasum were similar to the conical-shaped papillae

described in the bovine omasum by Gardner & Scott (1971). Very little information could be found on the surface structure of the mucosa of the forestomachs of newborn lambs.

It should be borne in mind that because of the swallowing reflex in newborn lambs, milk bypasses the forestomachs and goes directly to the abomasum via the oesophageal groove (Habel, 1956; Swenson, 1984). No milk or ingesta was therefore

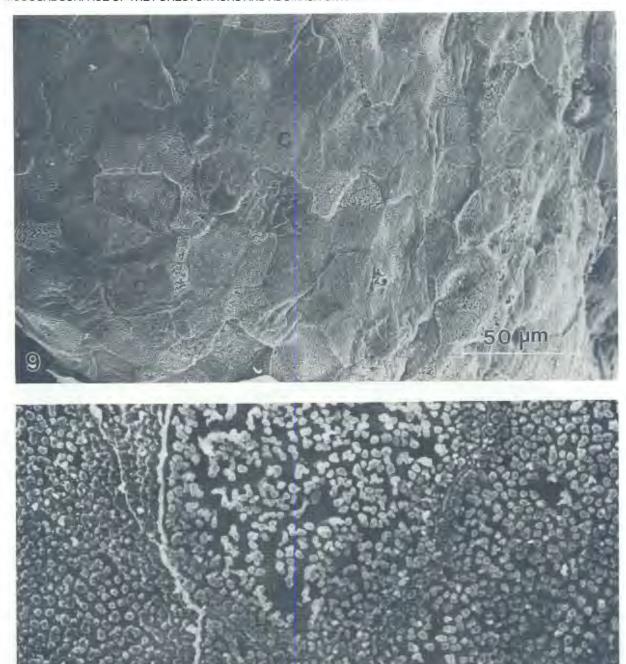


FIG. 9 Surface epithelium of the rumen of a grey lamb that had suckled. Note the eroded appearance resulting from the sloughing of surface cells. Underlying cells (C) are visible. 600 ×

FIG. 10 Surface epithelial cells of the rumen of a grey lamb that had suckled. The cytoplasmic projections have a weathered appearance $6000 \times$

present in the forestomachs of the control newborn black lambs. Scanning electron microscopical studies in newborn black lambs revealed smaller papillae with little or no differences in shape when compared to older animals. Furthermore, no evidence of sloughing of surface cells was observed in the forestomachs of these lambs. This contrasts sharply with the situation in older animals in which

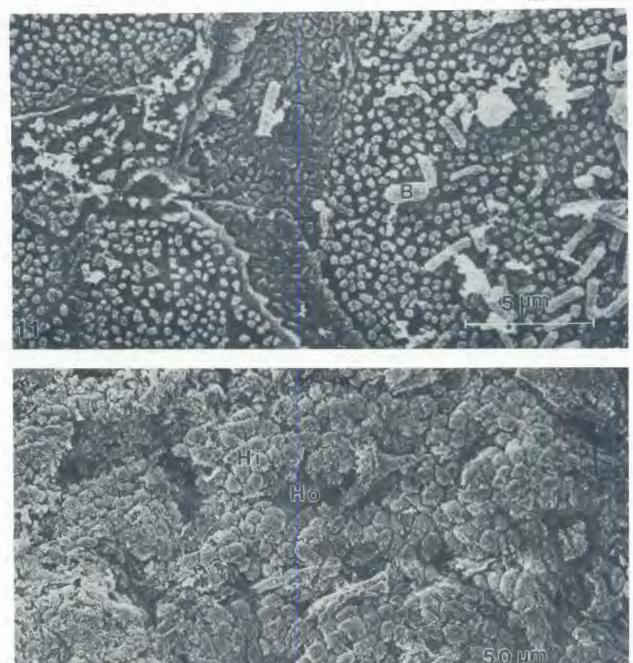


FIG. 11 Surface epithelial cells of the rumen of a grey lamb that had suckled. Note the bacteria (B). 6 000 \times FIG. 12 Abomasal mucosal surface of a black lamb. Hillocks (Hi) and intervening hollows (Ho) are visible. 600 \times

sloughing of the surface epithelial cells of the rumen, reticulum and omasum was demonstrated by Tamate et al. (1971), Scott & Gardner (1973) and Amasaki & Daigo (1988). In the lambs the epithelial cells are tightly packed and seem to overlie one another. In adult animals the cytoplasmic projections on the surface of the epithelial cells appear granular or globular in the rumen and reticulum, and flap-like with a contorted appearance in the omasum (Tamate et al., 1971; Scott & Gardner, 1973). In

newborn black lambs the cytoplasmic projections are similar to that of adult sheep, but they tend to be higher with a less weathered appearance. It would therefore appear that the sloughing of surface epithelial cells and the weathered appearance of the cytoplasmic projections in older animals is due to the presence of ingesta in the fore-stomachs.

Homozygous grey and white Karakul lambs have a paucity of myenteric ganglia and neurons in the walls of the rumen, reticulum and abomasum which

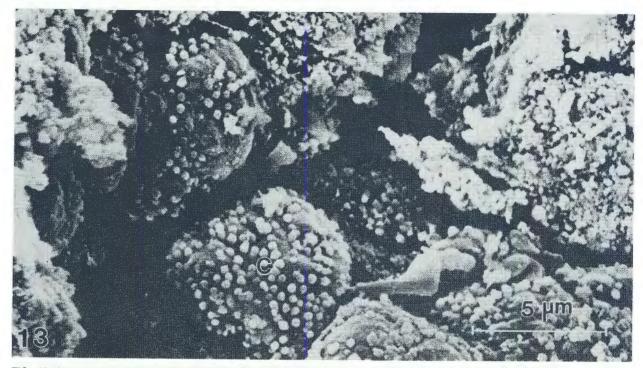


FIG. 13 An abomasal surface epithelial cell (C) of a black lamb showing globular cytoplasmic projections. 6 000 ×

causes disfunction of the oesophageal groove and consequent accumulation of milk in the forestomachs (Groenewald & Booth, 1990). In the present study the shape and size of the papillae in the forestomachs of newborn grey and white lambs that suckled corresponded to those of the newborn black lambs with the exception that sloughing of the surface epithelial cells occurred and the cytoplasmic projections had a weathered appearance.

The appearance of the surface structure of the abomasum of newborn grey, white and black Karakul lambs corresponds to the findings of Asari *et al.* (1981) on the developing bovine abomasum. No differences could be found in the structure of the surface epithelial cells between the different groups of animals in this study.

It is therefore concluded that the sloughing of surface epithelial cells and the weathered appearance of the cytoplasmic projections in the forestomachs of homozygous grey and white lambs is ascribed to the presence of milk. Further support is provided by the observation that the morphology of the surface cells of the forestomachs of grey lambs that were not allowed to suckle and black lambs were similar.

REFERENCES

- AMASAKI, H. & DAIGO, M., 1988. Morphogenesis of the epithelium and lamina propria. *Anatomia Histologia Embryologia*, 17, 1–6.
- ARIAS, J. L., CABRERA, R. & VALENCIA, A., 1978. Observations on the histological development of the bovine rumen papillae. *Anatomia Histologia Embryologia*, 7, 140–151.
- ASARI, M., FUKAYA, K., YAMAMOTO, M., EGUCHI, Y. & KANO, Y., 1981. Developmental changes in the inner surface structure of the bovine abomasum. *Japanese Journal of Veterinary Science*, 43, 211–219.

- CONTESCU, & EPUREANU, 1939. Beitrag der grauen farbe bei den Zurkanaschafen. Zeitschrift fur Tierzucht und Zuchtungsbiologie, 44, 211–218.
- CONTESCU, & LEAGU, 1941. Neue beitrage zu vererbung der grauen farbe bei Zurkanaschafen. Zeitschrift fur Tierzucht und Zuchtungsbiologie, 50, 172–176.
- GARDNER, I. C. & SCOTT, A., 1971. The surface of the ovine omasum as seen with the scanning electron microscope. *Zeitschrift* fur Morphology der Tiere, 70, 229–237.
- GROENEWALD, H. B. & BOOTH, K.K., 1989. An anatomical comparison of the fore-stomachs and abomasum of grey, white and black Karakul lambs. *South African Journal of Science*, 85, 466. (Abstract).
- GROENEWALD, H. B. & BOOTH, K. K., 1990. A histological comparison of the fore-stomachs and abomasum of grey, white and black Karakul lambs. *South African Journal of Science*, 86, 162. (Abstract).
- HABEL, R. E., 1956. A study of the innervation of the ruminant stomach. *Cornell Veterinarian*, 46, 555–633.
- KAMEL, G., ALI, A. M. A., SABER, A. S. & HASSAN, A. H. S., 1984. Scanning electron microscopy and histomorphological studies on the ruminal papillae in the goat. Assiut Veterinary Medical Journal, 13, 287–303.
- McGAVIN, M. D. & MORRILL, J. L., 1976. Scanning electron microscopy of ruminal papillae in calves fed various amounts and forms of roughage. *American Journal of Veterinary Research*, 37, 497–508.
- NEL, J. A., 1965. The effects of the sub-lethal factor on the internal organs of the grey Karakul. Proceedings of the South African Society of Animal Production, 1965, 282–285.
- NEL, J. A. & LOUW, D. J., 1953. The lethal factor in grey Karakul lambs. Farming in South Africa, 5, 169–172.
- SCOTT, A. & GARDNER, I. C., 1973. Papillar form in the fore-stomach of the sheep *Journal of Anatomy*, 116, 225–267.
- SWENSON, M. J., 1984. Dukes' physiology of domestic animals, 10th edn. Ithaca & London: Cornell University Press.
- TAMATE, H., KIKUCHI, T., ONODERA, A. & NAGATANI, T., 1971. Scanning electron microscopic observation on the surface structure of the bovine rumen mucosa. *Archives Histology Japanese*, 33, 273–282.