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Ultrastructure of the epithelium of the rumen, reticulum and omasum of grey, white and black Karakul lambs

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ABSTRACT

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Mortalities due to digestive disturbances occur in homozygous grey and white lambs after they have reached weaning age. Milk-filled, distended rumens, due to malfunctioning of the oesophageal groove, are found 24 h after birth. Scanning electron microscopical studies revealed that milk caused sloughing of the luminal cells in the forestomachs of the affected lambs, while no sloughing of cells was apparent in control black lambs. The purpose of this study was to compare the ultrastructure of the forestomach mucosa of grey, white and black Karakul lambs; to determine whether the sloughing of luminal cells was evident in sections; and, if possible, to find a reason for the desquamation of the cells. Samples of the forestomach of grey, white and black Karakul lambs were prepared routinely for electron microscopy and studied with a Phillips electron microscope. In all the lambs the mucosa of the forestomach was a stratified squamous epithelium consisting of a stratum basale, stratum spinosum and stratum corneum. In the grey and white lambs the luminal cells of the stratum corneum were electron dense, non-nucleated and vacuolated. Sloughing of luminal cells was observed. In the black lambs no sloughing of cells was evident and the luminal cells were moderately electron-dense, nucleated elements. Desquamation of the luminal cells in the affected lambs revealed the underlying layer with its exposed desmosomal attachment sites. This explained the differences in the appearance of the luminal cells in the three groups of lambs as revealed by the scanning electron microscope.

INTRODUCTION

The Karakul industry has long been adversely affected by mortalities of homozygous grey Karakul lambs. Mortalities in grey lambs of various breeds have been described by a number of authors. Contescu & Epureanu (1939) reported a mortality rate of 30,3 % in grey Zurkana lambs under the age of 10 months. At 5–6 months of age most of the grey Zurkana lambs died owing to digestive disturbances (Contescu & Leagu 1941). Impaction of the abomasum was found in grey Karakul lambs by Hartwigk (1950). He suggested that the condition was due to a reduced ability of the abomasum to contract.

Homozygous grey Karakul lambs are born apparently normal, but at weaning age they develop pot-bellies, become weak and emaciated, and die (Nel & Louw 1953). On post mortem examination Nel & Louw (1953) found large, thin-walled rumens and impacted abomasa in affected lambs. Nel (1965) described enlarged forestomachs in newly born grey

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Karakul lambs and came to the conclusion that digestive tract abnormalities are already present at birth. Studies by Langlet (1949) and Nel (1965) proved this lethal factor to be a genetic disorder. In contrast, Ivanenko (1949) postulated that mortalities attributed to the lethal gene in grey Karakul lambs were, in fact, due to a delicate constitution and that affected lambs could be saved by careful rearing.

Only lambs homozygous for the grey colour are affected. These animals can be identified at birth by the lack of pigmentation of the tongue, palate and ears (Nel & Louw 1953). Theoretically, should there be offspring from the homozygous lambs, all of them would be grey. Heterozygous grey lambs are unaffected and are used for breeding. The ratio of the offspring of the heterozygous lambs is one homozygous grey, two heterozygous grey, and one black lamb. Twenty-five percent of the lambs are therefore affected by the lethal factor and die before reaching breeding age, and 25 % are black. Only 50 % of the progeny can therefore be used for the production of grey pelts, resulting in a relative scarcity of this commodity.

Homozygous white Karakul lambs are born with the same lethal factor. However, they do not become emaciated and develop pot-bellies so soon, and they survive for a longer period. They are typical "poor doers" and, although they usually reach sexual maturity, they eventually die. Black Karakul lambs are unaffected and were used as controls.

The outermost component of the ruminant forestomach mucosa consists of a stratified squamous epithelium (Hyden & Sperber 1965; Prins 1967; Lavker, Chalupa & Dickey 1969; Henrikson 1970a; Henrikson 1970b; Lyford 1988; Schnorr & Hild 1974; Tamate & Kikuchi 1978; Ramkrishna & Tiwari 1979; Fath El-Bab, Schwartz & Ali 1983; Liebich & Scharrer 1984; Hofman & Schnorr 1982; Amasaki & Daigo 1988). There is general agreement that this epithelium consists of a stratum basale resting on a basal lamina, a stratum spinosum, a stratum granulosum and a stratum corneum (Hyden & Sperber 1965; Prins 1967; Lavker *et al*. 1969; Henrikson 1970a; Henrikson 1970b; Lyford 1988; Schnorr & Hild 1974; Tamate & Kikuchi 1978; Ramkrishna & Tiwari 1979; Fath El-Bab *et al*. 1983; Liebich & Scharrer 1984; Hofman & Schnorr 1982; Amasaki & Daigo 1988).

Scanning electron microscopy revealed differences in the appearance of the luminal surface of the rumen, reticulum and omasum in affected grey and white lambs as compared to black lambs (Groenewald & Booth 1992). The luminal surface of the grey and white lambs had a weathered appearance due to sloughing of the surface cells. The appearance of the cytoplasmic processes on the cell surface also differed in the affected and control lambs (Groenewald & Booth 1992). The purpose of this study was to compare the ultrastructure of the mucosa of the rumen, reticulum and omasum in grey, white and black Karakul lambs. Therefore it was possible to determine whether there were any differences between the three groups with respect to the structure and composition of the various cell layers; whether the sloughing of the luminal cells described by Groenewald & Booth (1992) was evident in sections; and whether there was a reason for the desquamation of the cells.

MATERIALS AND METHODS

Five 24-h-old grey, white and black Karakul lambs were slaughtered and samples were taken from corresponding areas of the rumen, reticulum and omasum. Two newborn grey lambs were slaughtered before they had suckled and matching samples of the forestomach were taken. Grey and white lambs with unpigmented tongues, palates and ears were specifically selected and black lambs were randomly selected.

The samples were rinsed in phosphate-buffered saline (PBS) with a pH of 7,4. Small blocks of tissue were immersion-fixed in 4 % glutaraldehyde in Millonig's phosphate buffer for at least 24 h at 4 °C. The blocks were subsequently rinsed in Millonig's phosphate buffer, post-fixed for 1 h at room temperature in similarly buffered 1 % osmium tetroxide and given two final buffer washes. The samples were dehydrated through a graded ethanol series (25 %, 50 %, 75 %, 96 % and 100 % × 2–10 min per step), cleared in propylene oxide and embedded in Polarbed 812 epoxy resin. Semi-thin sections (0,5 μm) were cut from each sample to determine suitable areas for ultra-thin sectioning. Thin sections (0,1 μm) were cut with a diamond knife on a Reichert OmU4 ultramicrotome, stained with uranyl acetate (Watson 1958) (30 min) and lead citrate (Reynolds 1963) (4 min), and examined with a Philips 301 or CM10 transmission electron microscope operated at 80 kV.

RESULTS

The epithelial component of the mucosa of the rumen, reticulum and omasum of the three groups of lambs was a stratified squamous epithelium (Fig. 1). The epithelium consisted of a stratum basale, stratum spinosum and stratum corneum (Fig. 1). A discontinous stratum granulosum was observed.

The cuboidal basal cells displayed a large, round nucleus and were characterized by the presence of numerous mitochondria (Fig. 2). Polyribosomes, a rough endoplasmic reticulum, a Golgi apparatus and fine filaments were also present (Fig. 3). The apical and lateral cell membranes were wavy with cytoplasmic processes and intermittently arranged

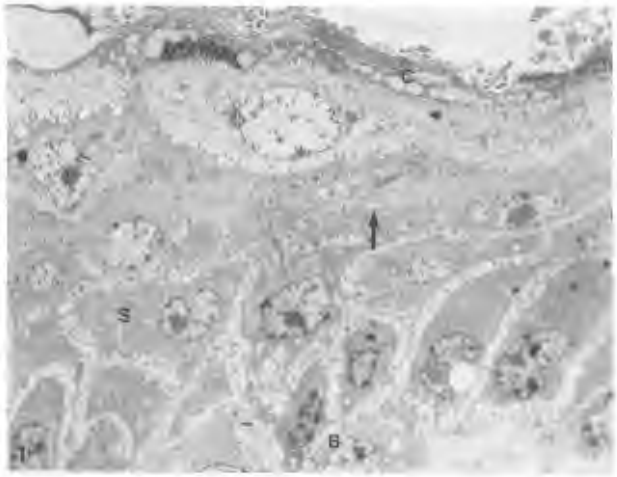

FIG. 1 The stratified squamous epithelium of the reticulum in a grey lamb. The stratum basale (B), stratum spinosum (S) and stratum corneum (C) are visible. Note the reduced intercellular space (arrow) in the more distal level of the stratum spinosum (1300 x)

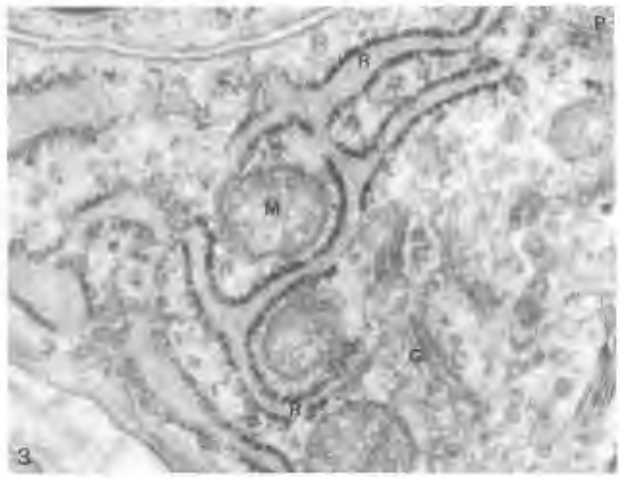

FIG. 3 A basal cell in the rumen of a black lamb. Polyribosomes (P), a rough, endoplasmic reticulum (R), a Golgi apparatus (G) and mitochondria (M) are evident (39000 x)

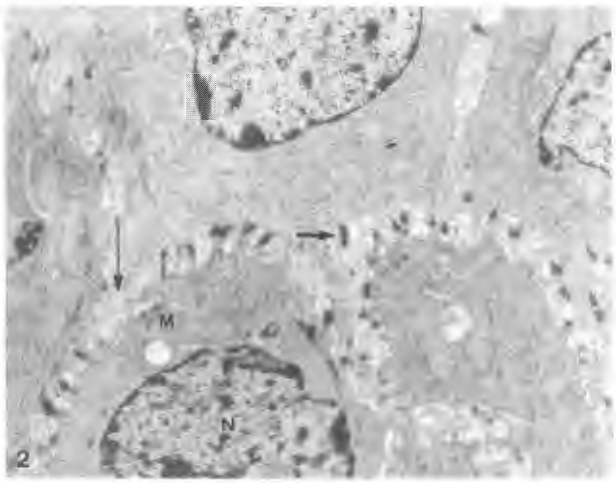

FIG. 2 Basal cells in the omasum of a grey lamb. The nuclei are round (N), and numerous mitochondria (M) and fine filaments (F) are evident. The intercellular space is narrow (long arrow) and numerous desmosomes (short arrow) are apparent (5200 x)

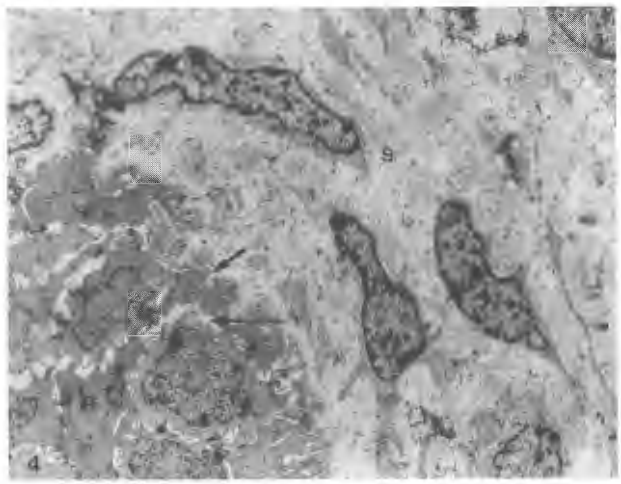

FIG. 4 The slightly folded basal surface (long arrow) of the cells is visible. The basal lamina (short arrow) is situated between the basal cells (B) and submucosa (S) (2950 x)

desmosomes linked neighbouring cells (Fig. 2). The intercellular space was narrow (125 nm) (Fig. 2). The basal surface of the cells was slightly folded (Fig. 4). The epithelium was separated from the connective tissue by a typical basal lamina (Fig. 5) which appeared as an electron-dense structure separated from the basal cell membrane by a less electron-dense space (Fig. 5). The basal lamina was slightly folded and hemi-desmosomes were observed along the basal cell membrane facing the basal lamina (Fig. 5). Micropinocytotic vesicles were observed along the basal cell membrane (Fig. 5). No occluding junctions were observed. Dendritic cells were present between the keratinocytes and, while no Langerhans granules could be found, these cells were most probably Langerhans cells (Fig. 7).

The cells of the stratum spinosum contained more filamentous material than those of the stratum basale (Fig. 6). The proximal cells bordering the stratum basale were polygonal to spherical in shape (Fig. 7) and many finger-like processes projected from their surfaces (Fig. 6). The processes seemed shorter than those of the basal cells and desmosomes were evident where the processes of adjacent cells made contact (Fig. 6). At this level the intercellular

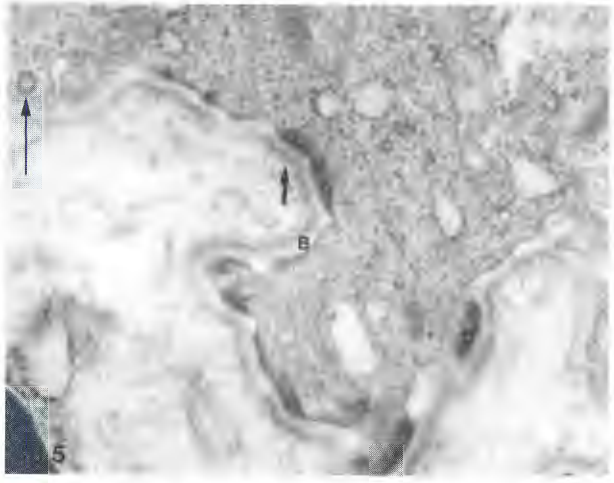

FIG. 5 The basal lamina (short arrow) in the omasum of a black lamb.Hemidesmosomes (D) and micropinocytotic vesicles (long arrow) are evident. Note the electron-dense appearance of the basal lamina and the space (B) between the basal lamina and the cell (28500 x)

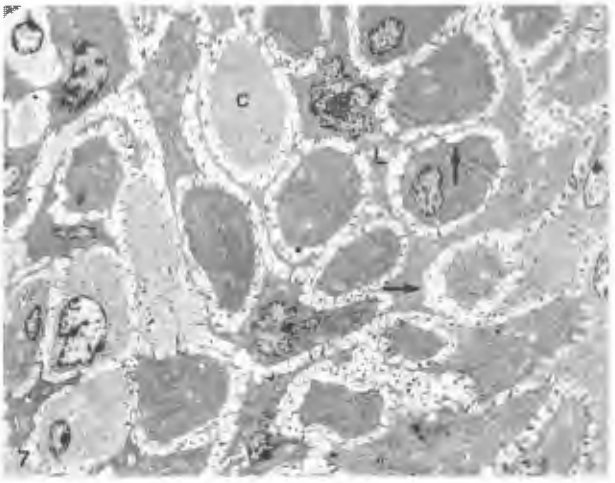

FIG. 7 The stratum spinosum in the reticulum of a white lamb. Note the spherical cells (C) and prominent intercellular spaces (arrow). Dendritic Langerhans cells (L) are evident (1650 x)

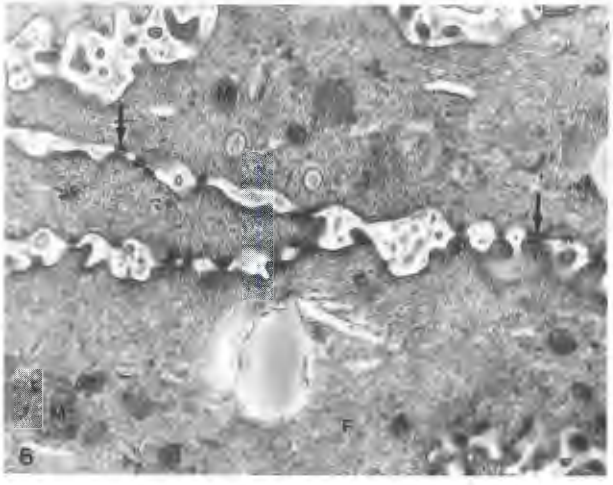

FIG. 6 Cells of the stratum spinosum in the omasum of a grey lamb. Note the abundant filamentous material (F) and mitochondria (M). Cytoplasmic processes (P) and desmosomes (arrows) are conspicuous (11500 x)

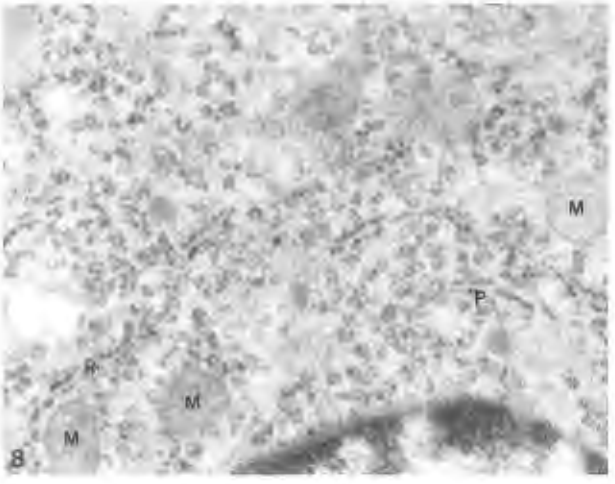

FIG. 8 The cytoplasm of a cell in the stratum spinosum in the reticulum of a white lamb. Mitochondria (M), polyribosomes (P) and a rough endoplasmic reticulum (R) are evident (28500 x)

spaces were prominent (1000 mm) (Fig. 7). Mitochondria were abundant (Fig. 8) and the cells also contained polyribosomes, a rough endoplasmic reticulum and a Golgi apparatus (Fig. 8). At the more distal level of the stratum spinosum, the cells became flattened, they were more compactly arranged, and the intercellular space was reduced (400 nm) (Fig. 1). There was an increase in the amount of filamentous material in the cells. Fewer mitochondria were observed, but free ribosomes were still present in large numbers. The cells contained relatively little rough endoplasmic reticulum. Keratohyalin granules were observed in the stratum granulosum (Fig. 12). Several layers of electron-dense non-keratinized cells (Fig. 9) was characteristic of the stratum corneum in all the lambs. The cells contained abundant tonofibrils (Fig. 10), while no organelles could be positively identified. A trilaminar cell membrane surrounded the cells (Fig. 11). The luminal surface of these cells showed finger-like cytoplasmic projections (Fig. 12) which were covered by an amorphous, fuzzy coating (Fig. 11). The intercellular spaces were narrow (95 nm) (Fig. 13) and numerous desmosomes were evident (Fig. 13). The stratum corneum of the omasum consisted of more cell layers than that of the rumen and reticulum (Fig. 13).

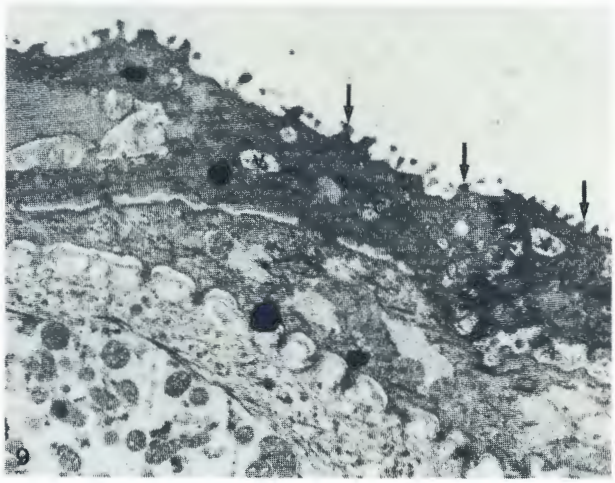

FIG. 9 The stratum corneum in the reticulum of a white lamb. Note the non-nucleated, electron-dense luminal cells (C) with vacuoles (V) in the cytoplasm. The cytoplasmic processes on the cell surface (arrows) are stubby (6610 x)

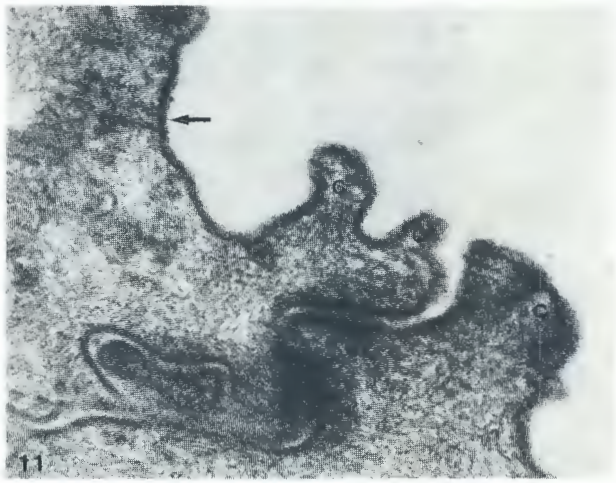

FIG. 11 The luminal surface with cytoplasmic projections (C) of a cell in the stratum corneum in the rumen of a grey lamb. Note the trilaminar cell membrane and the amorphous, fuzzy coating (arrow) (39000 x)

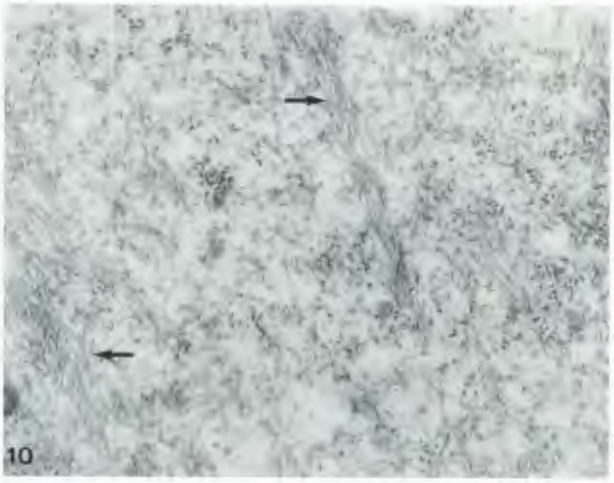

FIG. 10 The cytoplasm of a cell in the stratum corneum in the rumen of a black lamb. The absence of organelles and abundant tonofibrils (arrows) are apparent (39000 x)

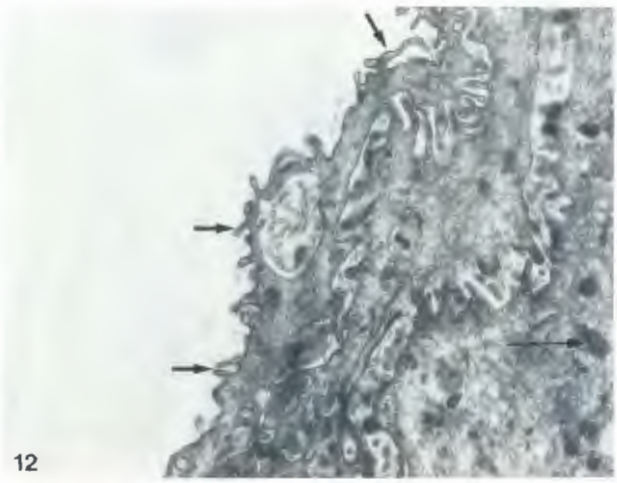

FIG. 12 The luminal surface of a cell in the stratum corneum in the reticulum of a black lamb, with numerous long cytoplasmic projections (arrows). Keratohyaline granules are evident (8900 x)

The appearance of the luminal cells of the stratum corneum in the grey and white lambs differed from that in the black control lambs. In the black lambs these cells were moderately electron-dense, nucleated elements (Fig. 14), and no sloughing of the cells was evident. In the grey and white lambs the luminal cells were electron dense, non-nucleated and vacuolated (Fig. 9). Sloughing of the surface cells was observed (Fig. 15).

The cytoplasmic processes on the surface of the luminal cells were fewer in number and stubbier in the grey and white lambs (Fig. 9) than the same processes in the black lambs (Fig. 12).

The structure of the luminal cells of the stratum corneum in the grey lambs that did not suckle, corresponded to that of the cells in the black lambs.

DISCUSSION

The basic structure of the stratified squamous epithelium of the rumen, reticulum and omasum in the grey, white and black Karakul lambs differed very little from that described by various authors in ruminants (Hyden & Sperber 1965; Prins 1967; Lavker *et al.* 1969; Henrikson 1970a; Henrikson 1970b; Lyford 1988; Schnorr & Hild 1974; Tamate & Kikuchi

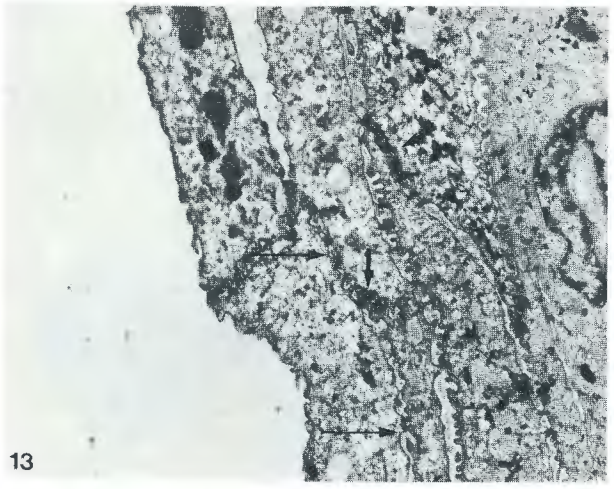

FIG. 13 The stratum corneum in the omasum of a grey lamb. Narrow intercellular spaces (long arrows) and numerous desmosomes (short arrows) are evident (5200 x)

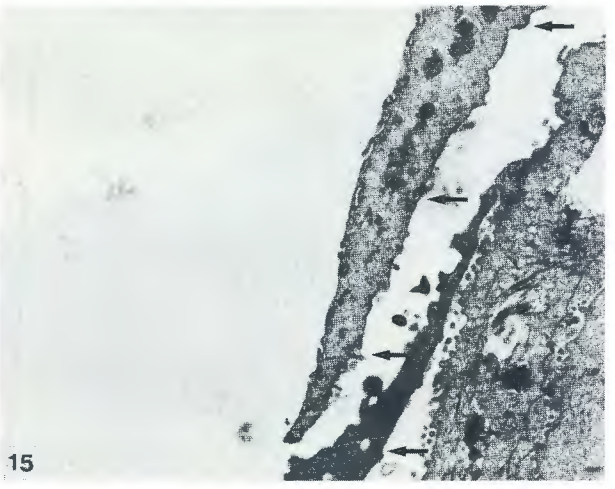

FIG. 15 The stratum corneum in the omasum of a white lamb. Sloughing of the luminal cells (arrows) is evident (5200 x)

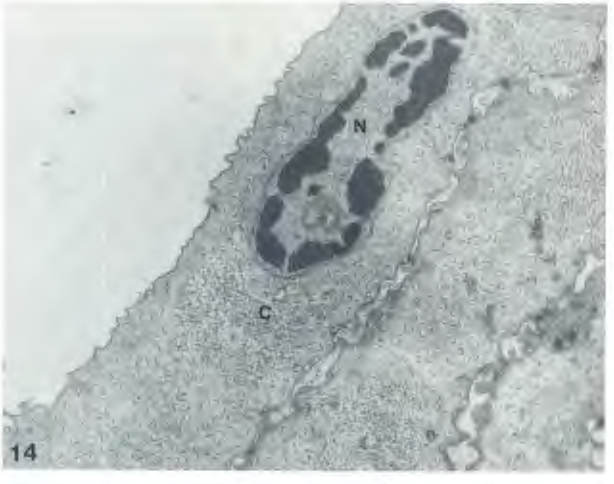

FIG. 14 A nucleated (N) luminal cell with a less electron-dense cytoplasm (C) in the stratum corneum in the rumen of a black lamb (6610 x)

1978; Ramkrishna & Tiwari 1979; Fath El-Bab *et al.* 1983; Liebich & Scharrer 1984; Hofman & Schnorr 1982; Amasaki & Daigo 1988). In the developing rumen the basal lamina originates as a smooth structure, becomes undulated after 5 months of foetal age and is folded in adult sheep (Arias, Fernandes, Oreste, Baeza & Pavez 1991). The basal lamina of the rumen in the 24-h-old lambs used in this study was slightly folded and conformed to the contours of the base of the overlying cells.

In adult sheep the proximal surface of the basal cells resting on the basal lamina, displays a complex array of cellular processes (Hyden & Sperber 1965; Lavker *et al.* 1969; Henrikson 1970a; Henrikson 1970b; Tamate & Kikuchi 1978). In this study the proximal surface was slightly folded and free of interdigitations—as described by Henrikson (1970b)—in lambs a few days old. In adult sheep the width of the space between the proximal surface of the basal cells and the basal lamina varies considerably (Henrikson 1970b). The narrow space between these structures, demonstrated by Henrikson (1970b) in lambs a few days old, was also corroborated in this study. The organelles of the basal cells and the structure of the cell membrane with its associated desmosomes did not differ from that described by other authors (Hyden & Sperber 1965; Lavker *et al.* 1969; Lyford 1988; Henrikson 1970a; Henrikson 1970b; Scott, Gardner, Fulton & McInroy 1972; Tamate & Kikuchi 1978). Langerhans cells as described by Marshall & Steven (1969), Gemmell (1973) and Gerneke (1980), were observed in ruminants, but no Langerhans granules could be demonstrated. No large, membrane-bound vesicles (Lavker *et al.* 1969; Lyford 1988) could be demonstrated in the cells of the stratum basale in any of the lambs studied. The presence of occluding junctions in the stratum basale, noted by Scott *et al.* (1972), could not be confirmed in this study.

The cells of the stratum spinosum with their associated organelles, filamentous material, large intercellular spaces and desmosomes, and the decrease in the number of organelles and increase in the amount of filamentous material within the more distal levels of this layer, confirmed the findings of Hyden & Sperber (1965), Lavker *et al.* (1969), Lyford (1988), Henrikson (1970a), Henrikson (1970b) and Tamate & Kikuchi (1978). The mucous granules reported in sheep by Lavker (1969) were not observed in the stratum spinosum in any of the lambs.

Keratohyaline granules in the stratum granulosum and stratum corneum of the rumen have been reported by several authors (Prins 1967; Lavker *et al.*

1969; Henrikson 1970b; Lyford 1988; Tamate & Kikuchi 1978; Gerneke 1981; Hofman & Schnorr 1982). A discontinous stratum granulosum and keratohyaline granules were observed in the lambs studied. Amasaki & Daigo (1988) declare that in the bovine, ruminal surface cells develop keratohyaline granules from the fifth month of gestation onwards. Henrikson (1970b) describes a non-keratinized epithelium in foetal, 12-h-old and 3-d-old lambs, and states that by the seventh day the epithelium resembles the keratinizing epithelium of adult sheep. Ramkrishna & Tiwari (1979) also describe a non-keratinized epithelium in the foetus of the goat.

The cells of the stratum corneum in the lambs studied contained abundant tonofilament bundles and remnants of nuclear material similar to that described in ruminants (Hyden & Sperber 1965; Lavker *et al.* 1969; Lyford 1988; Henrikson 1970a; Henrikson 1970b; Tamate & Kikuchi 1978).

The superficial layer of the stratum corneum in adult sheep had flattened, non-nucleated, electron-dense, vacuolated surface cells (Hyden & Sperber 1965; Prins 1967; Lavker *et al.* 1969; Henrikson 1970a; Lyford 1988; Tamate & Kikuchi 1978; Hofman & Schnorr 1982). In contrast, the luminal layer of the stratum corneum of the developing rumen displayed larger, less electron-dense, nucleated cells (Arias, Soledad Fernandes & Cabrera 1979; Liebich & Scharrer 1984). The luminal cells of the stratum corneum of the grey and white lambs did not differ in appearance from those of older animals (Hyden & Sperber 1965; Lavker *et al.* 1969; Lyford 1988; Henrikson 1970a; Tamate & Kikuchi 1978), and showed evidence of sloughing. However, in the grey lambs that were not allowed to suckle, and in the black lambs, the luminal cells were larger, less electron dense and nucleated. They contained abundant tonofilament bundles although no other organelles were observed. No sloughing of the cells was evident. Arias *et al.* (1979) described these cells in bovine foetuses as "clear" cells. These clear, luminal cells were also described by Liebich & Scharrer (1984) in 1-week-old lambs.

The surface of the luminal cells in the stratum corneum possesses cytoplasmic projections which give the cells a granular appearance when seen by scanning electron microscopy (Scott & Gardner 1973; Groenewald & Booth 1992). The cells are connected to adjacent cells by means of desmosomes. Scott & Gardner (1973) described the desmosomes in the surface epithelial cells of the stratum corneum of adult sheep while Arias *et al.* (1979) reported on the distribution and nature of desmosomes in the developing ruminal epithelium in the bovine foetus. According to Scott & Gardner (1973) the desmosomes eventually break down to allow the surface layer to slough off, leaving the former attachment sites—in the form of nipple-like cytoplasmic processes—on the surface of the underlying cells. This observation was confirmed in the present study and may explain the differences in the cytoplasmic processes observed between the grey, white and black lambs described by SEM (Groenewald & Booth 1992). The sloughing of the cells in the grey and white lambs is probably due to the presence of milk in the rumens of these lambs (Groenewald & Booth 1992). The underlying cells with the former desmosomal attachment sites on their surface are then revealed.

It is concluded that the different cell type seen on the luminal surface in black lambs (swollen, clear cells), as compared to that on the luminal surface in grey and white lambs (flattened, electron-dense cells), simply reflects the loss of surface cells by the latter due to the presence of milk in the forestomach. The desquamation of the surface cells revealed the underlying cell layer with its exposed desmosomal attachment sites. This also explains the differences in the appearance of the luminal cells in the three groups when studied with the scanning electron microscope.

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