SCANNING ELECTRON MICROSCOPY OF THE LOWER RESPIRATORY TRACT OF THE ADULT GOAT

KAHWA, C. K. B., PURTON[2] M. and PIRIE[2], M. E. S, [1]Department of Veterinary Anatomy, Sokoine University of Agriculture, P.O. Box 3016, Morogoro, TANZANIA, [2]Department of Veterinary Anatomy, Glasgow Veterinary School, Bearsden Road, G61, Glasgow, UNITED KINGDOM.

SUMMARY

Surface characteristics of the lower respiratory tract of seventeen clinically normal, adult Cashmere goats of both sexes were studied with the scanning electron microscope. Samples were taken from cranial trachea, caudal trachea, extrapulmonary bronchus, caudal lobar bronchus, small bronchi, and lung parenchyma, the latter including bronchiole, terminal bronchiole, respiratory bronchioles, alveolar ducts and alveoli. The trachea and bronchi were characterized by a lining epithelium composed of ciliated, nonciliated microvillous and mucus-producing cells. The degree of ciliation was observed to decrease with decreasing airway diameter, whilst the number of nonciliated microvilli, were in the majority, with ciliated cells presenting poorly developed cilia. Mucus-producing cells were not identified at this level with SEM. Respiratory bronchioles were seen to be present and well developed and their lining epithelium characterized by the presence of both Clara cell and ciliated cells. Alveoli were lined primarily by Type I pneumocytes amongst which occasional Type II pneumocytes were distributed Alveolar pores and alveolar macrophages were both rarely observed.

INTRODUCTION

The development of a rudimentary type of scanning electron microscope (SEM) in 1937 (Wang and Thurlbeck, 1970) and the subsequent dramatic advances of SEM technology, were instrumental in allowing detailed ultrastructural studies of the surface features of mammalian respiratory tract to be undertaken. SEM allows very large areas of tissue to be prepared and examined with ease (Kimoto and Russ, 1969), thus helping to eliminate difficulties in interpretation that might be caused by sampling errors.

Although there is a dearth of information of the surface morphology of the respiratory tract in various domestic animals, such information on the normal appearance in the adult goat appear to be lacking. The purpose of this study was to provide apparently for the first time a systematic account of the luminal surface morphology of the lower respiratory tract airway epithelium of the adult goat by the use of the scanning electron microscope.

MATERIALS AND METHODS

Seventeen clinically normal, adult Cashmere goats of both sexes were used in the preset study. The animals were killed by an overdose of pentobarbital sodium. The tongue, larynx, trachea and lungs were removed from the thoracic cavity and the lungs were infiltrated with Karnovsky's fixative. Samples measuring about 5mm x 5mm and 0.5-2mm thick were taken from dorsal cranial trachea, ventral cranial

trachea, dorsal caudal trachea, ventral caudal trachea, extrapulmonary bronchus, caudal lobar bronchus and lung parenchyma including large bronchiole, terminal bronchiole, respiratory bronchiole alveolar ducts and alveoli. Samples were then left in Karnovsky's fixative overnight, then washed in cacodylate buffer and thereafter cold dehydrated in a series of graded acetone. Samples were critically-point dried using carbon dioxide. The specimens were orientated such that the mucosal surface was uppermost, placed in an oven for 37°C for half an hour and then coated with a gold-palladium. Samples were examined using a 50IB SEM (Phillips, Holland) and viewed at an accelerating voltage of 15 KV using spot sizes between 200 - 1000.

RESULTS

The present study has established that the tracheal mucosa is thrown into longitudinal orientated folds and intervening gutters and lined by a heavily ciliated epithelium with occasional patches of nonciliated microvillous cells. Submucosal gland openings were frequently encountered (Fig. 1) Three cell type were distinguished on the basis of their luminal surface characteristics; These are the ciliated cell, nonciliated microvillous cell and mucus producing cell.

Ciliated cell were easily identified as they carried individually separated, tall slender cilia often appearing matted on the tip (Fig. 2). Nonciliated microvillous cell carried an even distribution of short microvilli and were intermingled in between ciliated cells, occasionally they occurred in patches.

The folded nature of the tracheal mucosa was continued into the bronchi. The bronchial mucosa was ciliated with the gutters being relatively less ciliated than the folds. As the diameter of bronchi decreased there was an increase in the number of nonciliated microvillous cell at the expense of ciliated cells.

Mucus-producing cells appeared in two morphological forms. Those in the gutters presented a depressed apical surface and mucus could be seen be seen being produced in sheet form (Fig. 3). Those seen on the folds, usually carried a protruding apical surface with sparse, uneven surface microvilli. Granules of mucous could sometimes be seen through the plasmalemma. Cells which were identified as regenerating ciliated cells were frequently encountered especially in areas devoid of ciliated cells.

The epithelium of the bronchioles was composed of ciliated cells, mucus-producing cells and nonciliated bronchiolar epithelial cells. Ciliated cells bore relatively shorter cilia compared to those in the upper respiratory tract, and, although cilia formed matted clumps in some individual, these cilia frequently appeared relatively straight and slender. Respiratory bronchioles, characterized by the presence of shallow alveoli were observed to be well developed (Fig. 4).

Nonciliated bronchiolar epithelial cells presented different shapes and different apical cell characteristics, but were always seen to carry a characteristic surface population of many thick, stubby microvilli (Fig. 5). Clara cells also presented different apical cell surface characteristics. Some had a flattened surface (Fig. 5), other a low bulge centrally, with a flattened periphery. Still other Clara cell had their whole apical surface raised into a dome projecting beyond the cilial tips; these were especially numerous in the terminal and respiratory bronchioles (Fig. 6). The number of such dome-shaped Clara cells increased as the diameter of the bronchioles decreased. In most cases this dome-like protrusion appeared full and bulging.

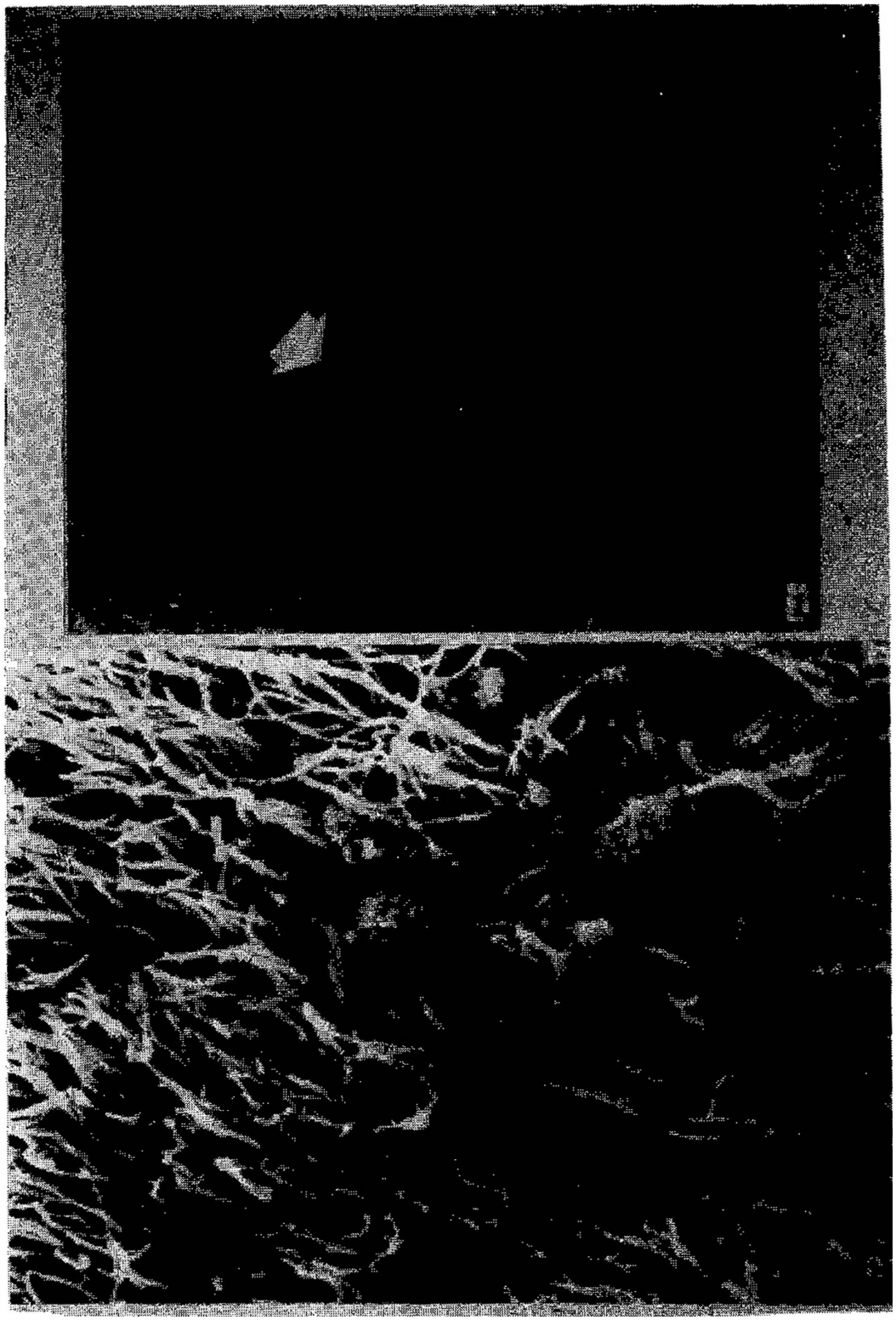

Fig. 1: Trachea, (Cranial dorsal surface). The mucosa is thrown into longitudinal folds and gutters. The exposed part of the gutter (arrow) has relatively fewer ciliated cells. Submucosal gland discharging mucus at the orifice (*). SEM X 360. **Fig. 2**: Trachea, Note the complete ciliation of the lining epithelium. Some cilia appear matted at the cilial tips. SEM X 5600.

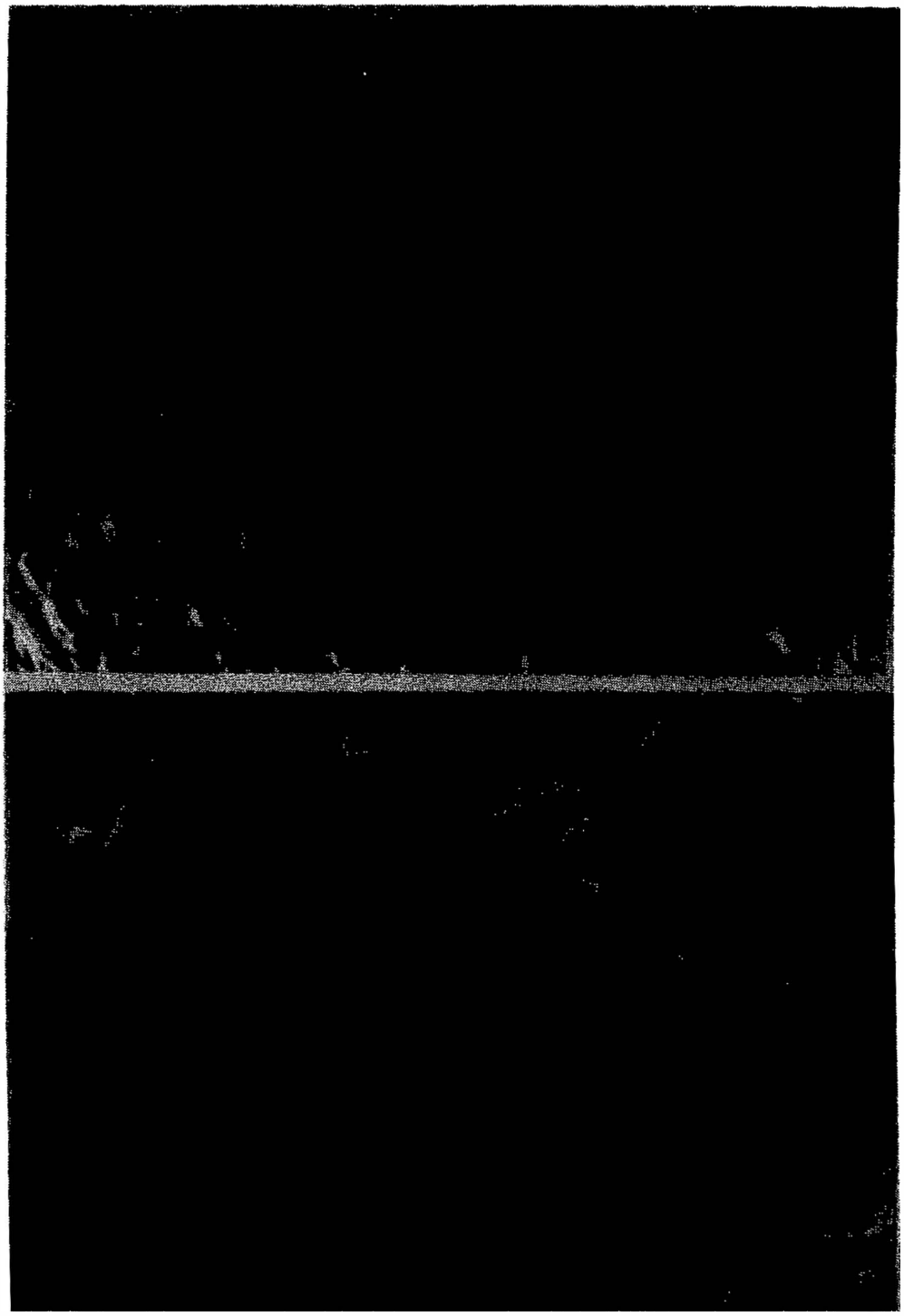

Fig. 3: Extrapulmonary bronchus. Mucus - producing cells with a depressed apical surface. Note a film of mucus on the apical surface (arrow). SEM X 2,800. **Fig. 4: Respiratory** bronchioles. Two respiratory bronchioles (R) terminal bronchiole (T). The former are characterized by the presence of shallow alveoli (*) in their walls. SEM X 360.

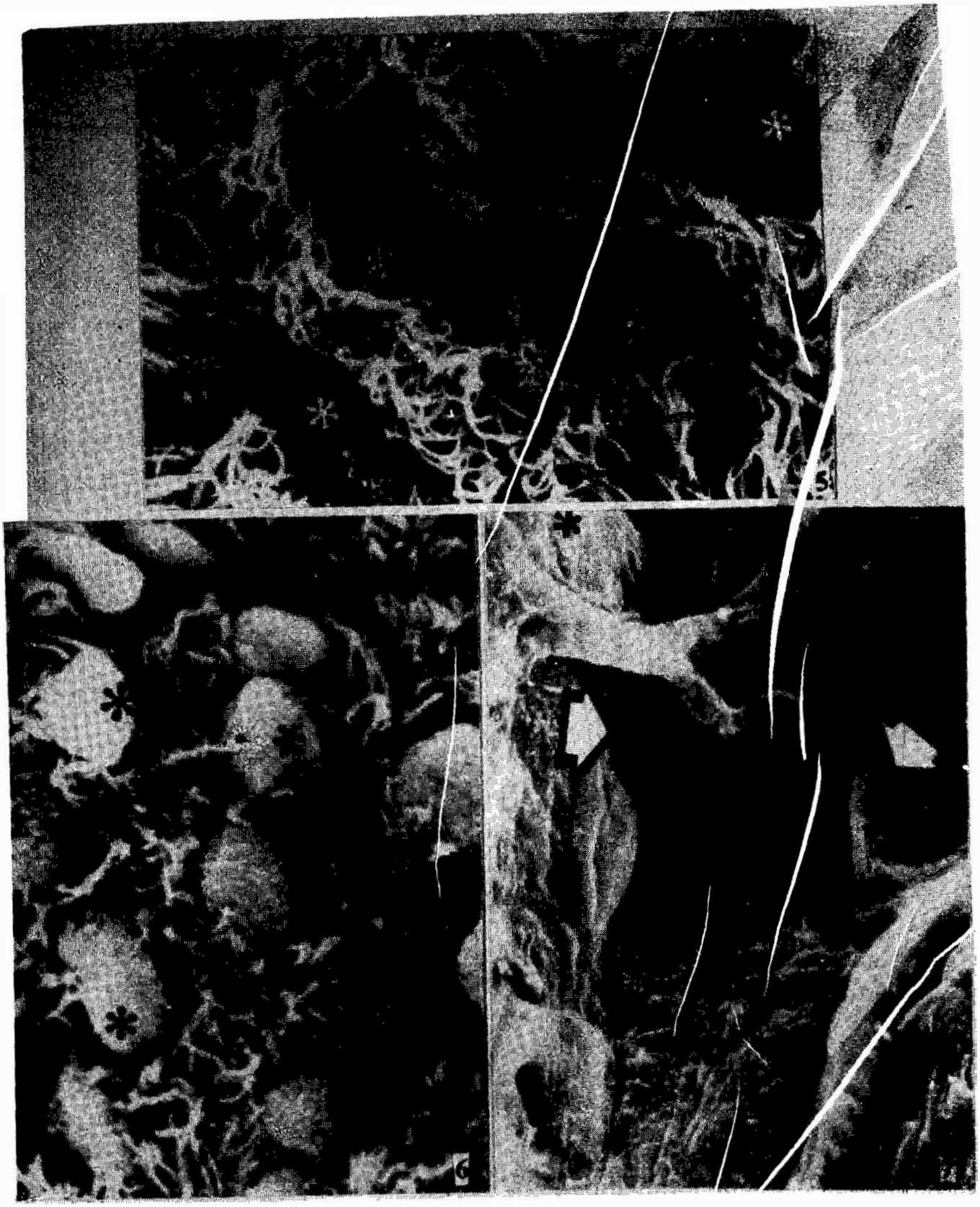

Fig. 5: Bronchiole Clara cells with flattened apical surface bearing short stubby microvilli (*). SEM X 5,600. **Fig. 6**: Respiratory bronchiole. Note the presence of numerous clara cells (*), their apical surface raised in a dome. SEM X 5,600. **Fig. 7:** Alveolar membrane. Note alveolar pore of Kohn (Closed arrow). SEM X 11,250.

The alveoli were lined by alveolar Type I cells (Fig 7) characterized by extensive thin cytoplasmic sheets spreading over the alveolar surface. Alveolar Type II cells were round or oval in outline (Fig. 7), and slightly raised from the epithelial surface. The apical surface. The apical surface bore characteristic, densely packed surface projections in the form of stubby or sometimes thin microvilli. Interalveolar pores were observed as perforations in the alveolar walls (Fig. 7). The number of pores per alveolus was highly variable, with the diameters of the pores also varying widely. With SEM, alveolar macrophages were seldom observe.

DISCUSSION

The folded nature of the tracheal mucosa seen in the goat has been reported in several species including the cat (Tandler *et al.*, 1983[a,b]). The gutters were deeper on the membranous portion of the trachea whereas on the cartilaginous portion the gutters appeared to be shallow, this is also in agreement with observation made in other species (Kahwa, 1992). More ciliated cells were observed on the folds than in the gutters, in the latter, nonciliated microvillous and mucus-producing cells were frequently encountered. This is in agreement with other mammalian species such as the ferret (Hyde et al., 1979) the cat (Tandler et al., 1983[a,b]) the dog (Majid, 1986) and the horse (Pirie, 1990).

The pattern of distribution of the nonciliated microvillous cells, when considered alongside the light microscopic (LM) observations on the numbers and distribution of alcian blue/periodic acid Schiff (PAS) positive cells within the tracheal epithelium, suggests that the majority of these cells identified at SEM level were indeed mucus-producing cells.

The fact that those nonciliated cells on the fold and those in the gutters presented different surface features, thus indicating that mucus-producing cells exhibit different morphological characteristics is supported by other LM and transmission electron microscopic studies (TEM) (Mariassy and Plopper, 1983; (Mariassy and Plopper, 1984).

Some of the nonciliate cells in the goat could definitely be identified as mucus in the form of discrete granules, those in the gutters produced mucus in sheet form. The patches of nonciliated microvillous cells were occasionally found to punctuate the cilial carpet. These were considered to be follicle associated epithelium (FAE), as they appeared to be similar to those reported in the nasopharynx of the sheep by Chen *et al.* (1991).

In the bronchiolar epithelium, with SEM, ciliated cells, mucus-producing cells and nonciliated bronchiolar epithelial (Clara) cells were identified. Clara cells presented different apical surface morphological appearances. These different appearances were regarded as the same cell type seen at different levels of activity; the "withering" type suggests that the secretory granules may have been discharged, while the flat type may cells at an early stage of maturity. Some workers (Lauweryns et al., 1969) have proposed that these three Clara cell types in the mouse are based on the stages of the cell's life cycle describing young (flattened), adult (bulging) and involutionary (withering) forms corresponding to the populations observed by Smith *et al.*, (1979) and to the observations made in the goat in the present study.

In the present study, the identification of prominent, well developed respiratory bronchioles in the goat lung contrasts markedly with observations in the available documented literature suggesting that in ruminants in general (Hare, 1975), and in ox in particular (Iovannitti *et al.*, 1985), respiratory bronchioles are usually

absent, and, if present are poorly developed. It would thus appear inappropriate to suggest that the lack of respiratory bronchioles is a typical feature of the ruminant lung.

A simple cuboidal epithelium composed of ciliated and Clara cells observed in the goat differs from the one observed in the dog where only Clara cells are present and no ciliated cells (Majid, 1986). In rat, human and Rhesus monkey a pseudostratified ciliated epithelium is found accompanying the pulmonary artery (Ten Have-Opbroek et al., 1991). Otherwise, the rest of the epithelium is similar to that of the dog

In the present study the alveolar membrane was lined by alveolar Type I and alveolar Type II cells, similar to those described in other mammalian species (Nowell and Tyler, 1971; Greenwood and Holland, 1972; Mariassy et al., 1975; Andrews, 1979; lovannitti *et al.*, 1985; Majid, 1986; Pirie, 1960). The slightly raised boundaries of the dog (Majid, 1986). The alveolar Type III cell, also known as the alveolar brush cell, which has been reported in the rat (Hijiya, 1978) was not identified in the present study, and indeed does not appear to have been observed in any other species studied to date.

Occasional alveolar macrophages, characterized by a ruffled cell membrane with rounded pseudopodia, were seen adhering to the alveolar membrane, although a considerably large area of lung parenchyma had to be examined before any were located. With other SEM studies in other species , alveolar macrophages were observed with difficulties in routinely prepared SEM specimens, however using material obtained from alveolar lavage, and further TEM work, they were able to confirm that alveolar macrophages were a normal component of the alveolar cell population.

In the goat, interalveolar pores (of Kohn) were found to be few, in contrast to observations made in the ox (lovannitti *et al.*, 1985), rat (Andrews, 1979). Methods of fixation of lung tissue have been seen to produce different results in relation to the abundance of interalveolar pores. The paucity of alveolar pores is considered a normal feature. Such findings are in agreement with similar observations in young adult cattle (Mariassy *et al.*, 1975) where it was reported that interalveolar pores are extremely rare. It has been reported that the numbers of alveolar pores tends to increase with age (Shimura *et al.*, 1986); in the present study this could not be established as there was no significant differences in age among the animals used.

Conclusions

Cellular population has been characterized and ciliated, mucus - producing, nonciliated microvillous, clara cells and Type I and Type II pneumocytes have been identified. Two cell population of mucus producing cells have been distinguished on morphological bases. Different luminal surface appearances of Clara cells represent stages of the cell cycle. Respiratory bronchioles have been seen to be prominent and well developed. Pores of Kohn (interalveolar pores) and alveolar macrophages were rare. Thus, the study has provided the normal appearance of the goats epithelium of the lower respiratory tract, a baseline against which clinical and pathological changes can be assessed.

REFERENCES

Andrews, P.M. (1979). The respiratory system "Biomedical Research Applications of Electron Microscopy." Edited by G.M. Hodges and R.C. Hallowes. Academic Press: New York and London. pp. 177-199.

Chen, W., Alley, M.R., Manktelow, B.W., Hopcroft, M. and Bennet, R. (1991). The potential role of the ovine pharyngeal tonsil in the respiratory tract immunity: A scanning and transmission electron microscopy study of its epithelium. J. Comp. Pathol. 104:47-56.

Greenwood, M.F. and Holland, P. 91972). The mammalian respiratory tract surface. A scanning electron microscopy study. Lab. Invest. 27:296-303.

Hare, W.C.D. (1975). Respiratory system. In "Sisson and Grossman's The Anatomy of the Domestic Animals". 5th Edition. Edited by C.E. Rosenbaum and Goshal, N.G. W.B. Saunders Co. Philadelphia and London. 113-144, 916-936.

Hijiya, A.C. (1978). Electron microscope study of the alveolar brush cell. J. Elect. Micros. 27:223-227.

Hyde, D.M., Samuelson, D.A. Blakeney, W.H. and Kosch, P.C. (1979). A correlative light microscopy, transmission and scanning electron microscopy of the ferret lung scanning Electron Microscopy, III. SEM Inc., AMF: O'Hare, Illinois, USA. pp 891-898.

Iovannitti, B.I.E., Pirie, H.M. and Wright, N.G. (1985). A scanning electron microscopic study of the lower respiratory tract in calves and adult cattle. Res. Vet. Sci. 38:80-87.

Kahwa, C.K.B., (1992). An Ultrastructural and Histological Study Goat. PhD Thesis, University of Glasgow.

Kimoto, S. and Russ, J. (1969). The characteristics and applications of scanning electron microscope. Amer. Sci. 57:112.

Lauweryns, J.M., Cokelaere, M. and Boussauw, L. (1969). L'ultrastructure de L'epithelium bronchique en bronchiolaire de la souris. Bulletin de L'Association des Abatomistes, 146:548-560.

Majid, A., (1986). A combined Histological, Histochemical and Scanning Electron Microscopical Study of the Canine Respiratory Tract. PhD Thesis, University of Glasgow.

Mariassy, A.T. and Plopper, C.G. (1983). Tracheobronchial epithelium of the sheep. I. Quantitative light microscopic study of the epithelial cell abundance and distribution. Anat. Rec. 205:263-275.

Mariassy, A.T. and Plopper, C.G. (1984). Tracheobronchial epithelium of the sheep. II Ultrastructural and morphometric analysis of the epithelial secretory cells types. Anat. Rec. 209:523-534.

Mariassy, A.T., plopper, C.G. and Dungworth, D.L. (1975). Characteristics of the bovine lung as observed by the scanning electron microscope. Anat. Rec. 183: 13-26.

Nowell, J.A. and Tyler, W.S. (1971). Scanning electron microscopy of the surface morphology of the mammalian lungs. Amer. Rev. Resp. Dis. 103: 313-328.

Pirie, M.E.S. (1990). An Ultrastructural and Histological study of the Equine Respiratory Tract in Health and Disease. PhD. Thesis, University of Glasgow.

Shimura, S, Boatman, E.S. and Martin, C.J. (1986). Effects of ageing on the alveolar pores of Kohn and on the cytoplasmic components of alveolar Type II cell in the monkey lung. J. Pathol. 148:1-11.

Smith, M.M., Greenberg, S.D. and Spjut, H.J. (1979). The clara cell: A comparative ultrastructural study in mammals. Am. J. Anat.155:15-29.

Tandler, B., Sherman, J.M., Boat, T.F.

and Wood, E.E. (1983[a]). Surface architecture of the mucosal epithelium of the cat trachea: I cartilaginous portion. Amer. J. Anat. 168:119-131.

Tandler, B, Sherman, J.M., Boat, T.F. and Wood, R.E. (1983[b]). Surface architecture of the mucosal epithelium of the cat trachea: II structure and dynamics of the membranous portion. Amer. J. Anat. 168:133-144.

Ten have-Opbroek, A.A.W., Otto-Verberne, c.T.M. and Dubbeldam, J.A. and Dykman, J.H. (1991). Proximal border of the human respiratory unit, as shown by scanning and transmission electron microscopy and light microscopical cytochemistry. Anat. Rec. 229:339-354.

Wang, N. and Thurlbeck, W.M. (1970). Scanning electron microscopy of lung. Human Pathol. 1:227-231.