# AN ULTRASTRUCTURAL AND HISTOLOGICAL STUDY OF THE EPITHELIUM OF THE RESPIRATORY TRACT IN THE NEONATAL AND ADULT GOAT.

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## DEDICATION

This work is dedicated to my uncle, the late Tinkasimire Joseph. May the Almighty rest his soul in eternal peace

## DECLARATION

**THIS IS TO DECLARE THAT ALL THE WORK DESCRIBED IN THIS THESIS WAS CARRIED OUT BY ME PERSONALLY. WHERE ASSISTANCE WAS SOUGHT, IT HAS BEEN ACKNOWLEDGED ACCORDINGLY**

## SUMMARY

The objectives of undertaking this study were firstly to characterise the normal surface features of the epithelial lining of the entire respiratory tract of the adult goat by the use of the scanning electron microscope (SEM). Secondly, to further characterise, by the transmission electron microscope (TEM), the epithelial cell population of the distal airways (terminal bronchioles, respiratory bronchioles) and alveolar membrane. Thirdly, to investigate, also by the use of the scanning electron microscope, the development of the respiratory tract epithelium in the neonatal kid. Fourthly, to provide a histological and histochemical picture of the epithelial lining of the respiratory tract. And finally to assess the use SEM in the study of clinical material, with the view of using it as another tool in diagnostic procedures.

It was apparent that information on the gross anatomy of the respiratory tract of the goat was scarce and scattered in many different sources. Thus Chapter 1 was introduced to provide a brief account of the gross anatomy of the caprine respiratory system.

Chapter 2 details the materials, general procedures and methods used in the the whole study. Details of the preparation of different buffers and fixatives are also provided.

In Chapter 3, the histology of the epithelial lining of the respiratory tract was defined, together with the histochemistry of the respiratory tract mucosubstances. 17 clinically normal animals were used in the study. Samples were taken from 18 preselected sites along the entire lining epithelium. Histological sections were stained with H&E and AB/ PAS, the latter used for the histochemical study of the mucosubstances. A stratified squamous type of epithelium lined the nasal vestibule, rostral region of alar and basal folds, caudal region of the nasopharynx, laryngeal surface of the epiglottis, cranial surface of the vocal fold and infraglottic cavity. An intermediate type of epithelium, itself grading from stratified cuboidal to low columnar,

was seen to occupy the transitional zone between the nonciliated and ciliated regions of the rostral nasal mucosa, nasopharynx, vocal fold and infraglottic cavity. A typical respiratory epithelium ( a pseudostratified ciliated columnar epithelium) lined the nasal concha and most of the conducting airways. The bronchioles, proximal to the terminal bronchioles were lined by a respiratory epithelium. Distal to the terminal bronchioles were lined by a simple columnar epithelium which changed into a simple cuboidal in the respiratory bronchioles. It was established that the majority of the individual surface mucus-producing cells were acidic in character. The submucosal glands produced mucosubstances of varying nature and differing proportions of acidic, neutral and mixed glycoproteins. In the bronchioles, surface mucus-producing cells were only seen proximal to the terminal bronchioles, where they produces almost equal amounts of both acidic and mixed mucosubstances.

Chapter 4 detailed the surface characteristics of the lining epithelium of the respiratory tract of the adult goat. Surface characteristics of squamous, nonciliated microvillous, mucus-producing, Clara, alveolar Type I and alveolar Type II cells were described. Two types of mucus-producing cells were distinguished on the basis of their luminal surface characteristics. It was established that the nasal vestibule, and rostral regions of the alar and basal folds were lined by squamous cells which gradually gave way to an intermediate epithelium, characterised by nonciliated microvillous cells with bulging luminal surfaces presenting a "cobblestone" appearance. This type of epithelium gradually changed into a ciliated epithelium in the caudal regions of the alar and basal folds. The latter type of epithelium was also seen to line the ventral, middle and nasal conchae. The intermediate epithelium described in the rostral regions of the nasal cavity was also observed on the nasopharynx, vocal fold and infraglottic cavity, situated between a ciliated epithelium and a nonciliated squamous epithelium. The trachea, bronchi and bronchioles were lined by a ciliated epithelium. The degree of ciliation was observed to decrease with decreasing airway diameter, whilst the numbers of nonciliated microvillous cells increased. At the level of the terminal bronchioles, Clara

cells, characterised by their apical protuberances and the presence of short, stubby surface 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. Alveolar pores and alveolar macrophages were both rarely observed.

Chapter 5 was undertaken to further characterise, by means of TEM, the cell population of the distal airways and alveolar membrane. Five cell types were identified, namely, ciliated, Clara, alveolar Type I and Type II and mucus-producing cells, the latter being only occasionally observed. Essentially all cell types observed presented cytological characteristics similar to those observed in other mammalian species.

Having established the normal surface morphology of the epithelial lining of the respiratory tract of the adult goat, Chapter 6 involved an investigation, by the use of SEM, of the development of this lining epithelium in the neonatal respiratory tract. Twenty kids, aged between 3hrs and 21 days, were used in the study. It was established that at birth the kid presented a relatively well developed epithelium similar to that observed in the adult goat. Some differences between the kid and adult were observed, however, and these included: 1. The cilia were more densely packed and more extensively distributed within the rostral region of the nasal cavity of the kids than they were in adult goats. The large patches of nonciliated microvillous cells seen in adult goats were not a feature of the kid, in which only smaller patches were seen. 2. The epithelium covering the nasal septum was heavily ciliated in new-born to 3-day-old kids, from which time the numbers of nonciliated microvillous cells increased at the expense of ciliated cells. 3. Bronchioles were poorly ciliated in kids compared with the situation in the adult. 4. A cell type, characterised by a large, wrinkled apical surface with short surface microvilli, was frequently observed in the larynx and trachea of the kid, while such cells were not seen in the adults.

5. Lung parenchyma in the kid frequently presented evidence of alveolar formation in the form of low ridges dividing pre-existing alveoli. 6. In the first week of life,

respiratory bronchioles were rarely encountered. 7. Alveolar pores were less numerous in the lung of the kid than in the adult.

The availability of a limited number of clinical cases made it possible to assess the use of SEM in the observation of pathological changes as a result of disease. The details of this study are presented in Chapter 7. Four cases were investigated , three of them suspected to be cases of pneumonic pasteurellosis. It was shown that SEM provided a useful means of assessing changes difficult to assess by any other means. These changes included cilial loss, desquamations and epithelial cell erosion, excessive mucus production, changes of individual cell type surface characteristics. It was concluded that SEM can be successfully used to complement other diagnostic tools available for the study of disease processes and their pathological effects.

Chapter 8 provides a summary of all the studies undertaken in this work and provides conclusions and recommendations where it is appropriate.

In conclusion, the present work has demonstrated the usefulness of SEM in the study, for the first time, of normal caprine respiratory tract surfaces. In addition, it has shown the value of combining LM, SEM and TEM studies for a more complete characterisation of cell types populating the entire airway epithelia. Like all such studies, however, many questions remain unanswered, and more detailed studies of such topics as the developmental relationship between, and functions of, a number of the cell types populating the respiratory airway epithelium await future investigations.

The objectives and aims set in this study have been accomplished, and this work for the first time, provides a morphological account of the lining epithelium of the caprine respiratory tract., a baseline against which future work can be assessed.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| BNF: | Buffered Neutral Formalin |
| H&E: | Haematoxylin and Eosin stain |
| AB /PAS: | Alcian Blue /Periodic Acid Schiff stain. |
| HID: | High Iron Diamine |
| $AB^+$: | Alcian Blue positive |
| $AB^-$: | Alcian Blue negative |
| $PAS^+$: | Periodic Acid Schiff positive |
| $PAS^-$: | Periodic Acid Schiff negative |
| $HID^+$: | High Iron Diamine positive |
| $HID^-$: | High Iron Diamine Negative |
| LM: | Light Microscope (or Microscopy or Microscopical) |
| SEM: | Scanning Electron Microscope (or Microscopy or Microscopical) |
| TEM: | Transmission Electron Microscope (or Microscopy or Microscopical) |
| CAE: | Caprine Arthritis Encephalitis. |
| SER: | Smooth Endoplasmic Reticulum. |
| RER: | Rough Endoplasmic Reticulum. |
| TER: | Tubular Endoplasmic Reticulum. |
| LCDs: | Least Developed Countries. |

## GENERAL INTRODUCTION:

To meet the huge increase in demand for food products from a rapidly rising human population, seen especially in the developing countries, new improved methods of animal husbandry based upon sound scientific research and management techniques are essential.

In many third world countries, the goat has always been of great significance, with over 90% of the world's goat population being located in the tropical and subtropical regions (Wilkinson and Stark, 1987). Its value in terms of meat, milk and hide production is enhanced by virtue of the fact that it can survive and prosper on a relatively poor quality fodder; it also has a high tolerance to tick-borne diseases and trypanosomiasis factors, which tend to limit cattle production in subtropical and tropical regions. Even in developed temperate countries the importance of the goat as a milk producer has increased and their popularity is reflected in the fact that some veterinary practices are presented with more goat than sheep cases (Mews, 1980).

Improved methods of goat husbandry would inevitably entail intensified management systems involving the confinement of large numbers of animals in a limited space. Such conditions usually favour the rapid growth of disease causing organisms.

Respiratory diseases have already been shown to pose a major clinical problem in such intensive goat production systems (Ndamukong *et al.*, 1989) Indeed a number of studies have shown that respiratory problems are one, if not the major cause, of deaths in goats. A mortality rate of 33% due to pneumonia and gastroenteritis and 19.2% by pneumonia alone, was reported by Chawla *et al.* (1982), alsoKumar and Prasad (1986), after examining 4360 goats at post mortem over a period of ten years found a mortality rate of 25.6% due to pneumonia. while Vihara *et al.* (1986) working on Jamunapari goats, even noted a much higher figure of 42%.

Major disease conditions observed in the respiratory system include fibrinous pneumonia, purulent and nonpurulent bronchopneumonia, and pulmonary oedema and

congestion (Jubb *et al.,* 1985); the causative agents of such respiratory diseases may be extremely wide spread. There are however a variety of micro-organisms that have been consistently associated with pneumonia and their aetiological role can not be disputed (Almeida *et al.,* 1986). For example, *Mycoplasma m. mycoides* , the causative agent of contagious caprine pleuropneumonia, has been reported in 31 countries (McMartin *et al.,* 1980) including Nigeria ( Ojo, 1976; Okoh and Kaldas, 1980; Opasina, 1985; Ndamukong *et al.,* 1989), India (Kapur *et al.,* 1974; Sharma *et al.,* 1978; Upadhyaya *et al.,* 1983), Kenya (Rurangirwa *et al.,* 1981), Sweden (Bolske *et. al.,* 1982) Mauritania (Jan *et al.,* 1987), Senegal (Faugere *et al.,* 1987), Brazil (Almeida *et al.,* 1986), Malaysia (Zamri-Saadi *et al.,* 1987) and Portugal (Gonçalves, 1983), whilst *Pasteurella multocida* and *Pasteurella haemolytica* have frequently been isolated from many of the fibrinous pneumonias (Ojo, 1976).

Helminthiasis also plays a role in respiratory conditions of the goat. Lungworms *Dictyocaulus filaria* and *Mullerius capillaris* have been associated with bronchitis. Viral agents, such as the causative agent of stomatitis pneumoenteritis complex, which is immunologically and morphologically identical with *peste des petits ruminants* have also been shown to cause serious clinical problems, especially in temperate regions (Hamdy *et al.,* 1975; Robinson and Ellis, 1984).

Such diseases cause very significant economic losses, and effects include mortalities, delay in reaching slaughter weight, poor carcass quality and increased veterinary expenses. Indeed Akerejola *et al.* (1979) working in Nigeria, estimated a loss of between 30-60 million Naira annually due to contagious caprine pleuropneumonia alone.

It can be appreciated, therefore, that if economic return from intensively or semi-intensively managed goats are to be maintained or even increased, respiratory diseases (amongst others) must be successfully controlled or eradicated where possible. The development of such a successful and necessary therapy depends, in part, on a detailed knowledge of the pathological changes that affect the lining of the respiratory tract as a

result of respiratory infection. Such changes can only be assessed if the normal baseline parameter of the topographical appearance of the lining epithelium (first line of defence) as well as its histochemical nature are available.

It is the purpose of this study therefore to provide for the first time a systematic account of the morphology of the entire airway epithelium of the goat, from the nasal vestibule to the alveoli.

# CHAPTER 1.

# AN OUTLINE OF THE GROSS ANATOMY OF THE RESPIRATORY TRACT OF THE GOAT.

# INTRODUCTION.

Many of the standard textbooks on veterinary anatomy provide little specific information on the gross anatomy of the goat and the reader is usually left to assume that anatomical descriptions provided for the sheep are also to be applied to the goat. What specific information that is available on the caprine respiratory system is scattered piecemeal amongst many different sources and it was therefore thought worthwhile to draw all this information together into a brief description of the gross anatomy of the respiratory system of the goat before embarking on detailed studies at the histological and ultrastructural level. This review thus summarises information derived from the following sources: Hare, 1975; Nickel *et al.*, 1979; Dyce *et al.*, 1987; Garret, 1988; Habel, 1989, and also from observations of dissections of gross specimens in the present study. The respiratory system consists of a conducting portion and a respiratory portion. The former comprises the external nares, nasal cavity, nasopharynx, the larynx, the trachea and, within the lungs, the bronchi and bronchioles as far as the respiratory bronchiole; this portion serves not only to conduct air down into the respiratory regions of the lung, but also to warm, humidify and trap particulate matter, thus improving the quality of the inspired air. The latter, respiratory portion comprises the respiratory bronchioles, the alveolar ducts, alveolar sacs and the pulmonary alveoli, and serves primarily to provide for the exchange of gases between the inspired air and the blood.

# NOSE.

The nose of the goat is embodied in the skeleton of the face and extends from the transverse level of the eyes to the rostral extremity of the head, which carries the two nostrils. The nostrils in the apex lead into the nasal vestibule, and then on into the nasal cavity, to which are connected , directly or indirectly, several paranasal sinuses. The

nasal septum forms a partition between the nostrils and divides the cavity into the right and the left halves. The rostral part of the nasal septum widens along its dorsal and ventral margins to form the dorsal and ventral lateral nasal cartilages. Attached to the dorsal lateral cartilages are the lateral accessory cartilages which provide the ventral and lateral support of the nostrils.

The mobile nostrils (external nares) of the goat appear as narrow slits in a narrow area of modified skin (planum nasale) (Fig. 1.1), which is devoid of hairs and particularly prominent on the dorsal aspect. This area is kept moist by glands which secrete through pores (foveolae) grouped into small polygonal fields.

## NASAL VESTIBULE.

The paired nasal vestibules correspond closely in extent and contour to the cartilaginous portion of the nasal wall, and each forms an entrance chamber to the corresponding half of the nasal cavity. The skin covering the nostril of the goat is reflected to line the vestibule. For a short distance within the vestibule the skin retains its characteristic keratinized nature and carries numerous hairs, after which it changes into a nonkeratinized epithelial lining without hairs.

## NASAL CAVITY (Fig. 1.2).

The roof of the nasal cavity is provided by the dorsal lateral cartilages, the nasal bones and part of the frontal bones, while the floor is formed by the ventral lateral cartilages and the parts of the incisive, maxillary and palatine bones. The lateral walls are irregular and are formed by the lateral parts of the dorsal and ventral lateral cartilages and by parts of the incisive, maxillary, palatine, ethmoid and lacrimal bones. The caudal boundary is formed by the cribriform plate of the ethmoid bone. The cavity is divided

into two halves by a median septum, the nasal septum, and the term "nasal cavity" may refer to the entire cavity or to one of the halves.

Most of the space in the nasal cavity is taken up by the nasal conchae (Fig. 1.2). These are scrolls of bones attached to the lateral walls by a basal lamella; the recesses formed by these scrolls are in wide communication with the nasal cavity. The nasal conchae project mesiad almost to the nasal septum. The dorsal nasal concha is a thick shelf supported by the basal lamella which is made up of compact bone and extends from the level of the supraorbital foramen to the junction of the rostral and middle thirds of the nasal bones. Rostrally, the dorsal nasal concha extends into the nasal vestibular region as the unsupported straight fold. In the caudal two thirds of the concha a spiral lamella is present. It coils first ventrally, then laterally, and then dorsally, and it encloses the dorsal conchal sinus.

The ventral nasal concha originates from the conchal crest and medial plate of the maxilla. The basal lamella passes ventromedially for a short distance where it bifurcates giving rise to two lamellae. The ventral lamella coils ventrally, laterally, dorsally, then medially and ventrally in a small circle; whereas the bigger dorsal lamella coils dorsally, laterally, ventrally, then medially to form one and a half turns. In the goat, the free borders of the spiral lamellae form subdivided bullae which communicate through small openings with their respective recesses. Rostrally, the ventral nasal concha continues to form the alar fold dorsally and the basal fold ventrally.

In the caudal part of the nasal cavity lie the ethmoturbinate bones, of which the longest is referred to as the middle nasal concha, which in the goat has the shape of an arrow-head projecting primarily rostrally and lies just ventral to the dorsal nasal concha and dorsally to the ventral nasal concha. The middle nasal concha consists of a basal lamella and ventral and dorsal spiral lamellae; in the goat, the dorsal spiral lamella encloses a sinus, while the the ventral lamella encloses a second sinus rostrally and a recess caudally.

The dorsal and ventral conchae divide the nasal cavity into three meatuses. The

dorsal nasal meatus is a narrow passage between the roof of the nasal cavity and the dorsal nasal concha, and leads into the caudal part of the cavity. The middle nasal meatus is located between the dorsal nasal concha and the ventral nasal concha and caudally it is split into two channels by the middle nasal concha. An aperture which provides communication between the meatuses and the paranasal sinuses (the nasomaxillary opening) is found in this area. The ventral nasal meatus (which is the largest of these channels) is situated between the ventral nasal concha and the floor of the nasal cavity, and leads into the nasopharynx. The common nasal meatus extends from the roof of the nasal cavity to the floor and is bounded medially by the nasal septum; laterally it is continuous with the other meatuses. Caudally and ventrally the nasal cavity communicates with the nasopharynx through the choanae.

The mucosa covering the walls of the nasal cavity, the nasal septum and the nasal conchae is reddish in colour in fresh specimens, indicating its very vascular nature. In the goat there is a lateral nasal gland producing serous secretions located in the mucous membrane of the nasomaxillary opening, and its duct opens into the nasal cavity close to the nostril in the region of the straight fold.

## NASOPHARYNX (Fig. 1.3).

The nasal part of the pharynx lies caudodorsal to the soft palate and extends from the choanae to the intrapharyngeal ostium. The choanae are separated dorsally by the crest of the vomer, which is covered by a mucosa overlying a thick submucosal venous plexus. In the goat there is an incomplete septum arising from the rostral roof of the nasopharynx and projecting into the narrow fornix.The pharyngeal tonsils are located at the caudal end of this septum, attached to the caudodorsal wall of the pharynx (Habel, 1989). The sides of the tonsil are marked by long ridges and grooves. Each auditory tube opens onto the wall of the nasopharynx just lateral to the tonsil. The opening, which is a mere slit, lies in the transverse plane passing just rostral to the

temporomandibular joint at the level of ears (Habel, 1989).

## PARANASAL SINUSES

These sinuses, which in the goat include maxillary, palatine, lacrimal, and frontal sinuses, surround the nasal cavity almost completely. The maxillary, palatine and lacrimal sinuses communicate with the middle nasal meatus through the nasomaxillary aperture, while the frontal, and most of the conchal, sinuses open separately into the ethmoidal meatuses in the caudal part of the nasal cavity (Nickel *et. al.*, 1979).

Amongst the various functions attributed to the paranasal sinuses is that of olfaction, with the frontal sinus in particular being lined by extensions of the olfactory epithelium.

## LARYNX (Fig. 1.3).

The larynx provides a connection between the caudal region of the pharynx and the trachea. In addition to acting as a valve to prevent foreign material entering the trachea, it controls the entry of air by regulating the size of the glottis, regulates intrathoracic pressure and is also used as a mechanism of phonation. The laryngeal cavity is lined by a mucous membrane and is kept patent by a number of paired and unpaired cartilages including an unpaired cricoid cartilage caudally, an unpaired thyroid cartilage ventrally and laterally, paired arytenoid cartilages dorsally and an unpaired epiglottic cartilage rostrally.

The cavity of the larynx connects the laryngopharynx with the trachea. At the entrance to the larynx the aryepiglottic folds pass from the lateral margins of the epiglottis to the dorsal wall of the larynx bypassing the arytenoid cartilages laterally. That part of the cavity between the entrance to the larynx (aditus) and the level of the

vocal folds is called the vestibule. Some domestic animals have vestibular folds and lateral laryngeal ventricles located in this region, but these are absent in the goat. Because of the absence of the ventricles, the nearly vertical vocal folds are covered with mucosa only medially and rostrally, and consequently appear more like heavy ridges than true folds.

That part of the laryngeal cavity bounded ventrally by the vocal processes and dorsally by the adjacent areas of the medial surfaces of the arytenoid cartilages is known as the rima glottidis and is the narrowest part of the laryngeal cavity. The caudal infraglottic compartment of the cavity is bounded by the cricoid cartilage.

The goat has paraepiglottic tonsils which extend from the free edge of the aryepiglottic folds to the floor of the vestibule. Solitary nodules are present on the epiglottis and the vocal folds.

## TRACHEA.

The trachea is a noncollapsible tube supported by cartilage rings (Fig. 1.3). It extends from the larynx to the tracheal bifurcation. In the neck region the trachea lies ventral to the oesophagus and longus colli and longus capitis muscles which cover the ventral surfaces of the vertebral column. Dorsolaterally, the trachea is accompanied by the common carotid arteries, the vagosympathetic trunks and the tracheal lymphatic trunks.

In the thoracic region the trachea lies dorsal to the cranial vena cava, whilst the oesophagus, which has assumed a lateral position in the caudal cervical region, returns to a position dorsal to the trachea. At the level of the fourth to sixth intercostal spaces, the trachea divides, at the tracheal bifurcation, into the two principal bronchi, and just before it branches, it gives off a tracheal bronchus which supplies the cranial lobe of the right lung. The number of tracheal cartilages is not constant for any species, and varies even between individuals of the same species. In the goat the average is 42. Between

the ends of each tracheal ring there is a considerable gap which is filled by connective tissue and the tracheal muscle. The muscle is made up of smooth muscle fibres arranged in a circular fashion.In the goat the left ends of the cartilage rings overlap the right to form a characteristic dorsal crest.

The walls of the trachea consist of four layers, a mucosa, a submucosa, a musculocartilaginous layer and an adventitia. Seromucous tracheal glands are numerous in the deeper layers of the propria and submucosa. These tracheal glands share essential features with the glands of the larynx and the pulmonary bronchi. The submucosa is thin but well developed dorsally where the cartilages are incomplete. Numerous elastic fibres present in the mucosa help the trachea to return to its normal length after it has been stretched by the extension of neck.

## LUNGS.

Each lung is contained within a pleural sac formed by the parietal and mediastinal pleura. Both sacs occupy the thoracic cavity, although the right pleural sac is larger than the left, in order to accommodate the larger right lung which is subdivided into a greater number of lobes.

The lobes are separated from each other to varying degrees among different animal species but are confluent medially in the vicinity of the hilus of the lung. Hare (1955) defines a lobe as " a large area of pulmonary tissue which is ventilated by a large bronchus arising from a main bronchus or from the trachea; being separated from neighbouring lobes by interlobular fissures which may be continued by connective tissue planes." Using this definition, the right lung of the goat is composed of four lobes: cranial (or apical), a middle (cardiac), a caudal (or diaphragmatic) and an accessory lobe. The left lung is composed of two lobes, a cranial and a caudal lobe.

For the purpose of description, each lung is described as presenting a cranial apex, a caudal (diaphragmatic) base, two surfaces(costal and medial) and three

borders(dorsal, ventral and basal). It is thus cone-shaped with a narrow apex directed into the cupula pleura at the thoracic inlet. The base is wide and concave following the contour of the diaphragm to which it is applied. The large costal surface is convex and is in contact with the ribs and intercostal muscles. The less extensive medial surface is irregular and is divided into a small vertebral part related to the bodies of the thoracic vertebrae and a larger mediastinal part related to the mediastinum and the structures contained therein. Cranially the mediastinal part bears a well marked concave area, the cardiac impression, for the heart.

The ventral border is acute and irregular. In the right lung the ventral border is indented at the level of the heart to form the cardiac notch. The dorsal border is thick and rounded. It forms the dorsal boundary between the costal surface and the vertebral part of the medial surface. The costal and diaphragmatic surfaces meet at the basal border which is sharp and runs in a caudodorsal to cranioventral direction.

## BRONCHIAL TREE.

At the level of the fourth to sixth intercostal spaces, the trachea branches into two thick, but short, principal bronchi.Upon entering the lung, these principal bronchi divide into separate lobar bronchi, each of which ventilates one lobe of the lung. The walls of the principal bronchi outside the lungs resemble that of the trachea. Within the lungs the supporting cartilages of the bronchial walls form irregular plates instead of incomplete rings and the smooth muscle is in the form of a double spiral.

The left cranial bronchus, arising from the left principal bronchus caudal to the hilus of the lung, immediately divides into cranial and caudal segmental bronchi which serve the two parts of the cranial lobe. The right cranial lobe is served by branches of the tracheal bronchus.

The middle lobar bronchus arises from the right principal bronchus a short distance caudal to the hilus and serves the middle lobe. The accessory lobar bronchus

which serves the accessory lobe arises at almost the same level as the middle lobar bronchus and is directed ventromedially.

The continuation of the principal bronchus into the caudal lobe of the lung is termed the caudal lobar bronchus.

The lobar bronchi give rise to a large number of segmental bronchi, each of which enters and ventilates a bronchopulmonary segment. Within this segmental bronchi further branch to give rise eventually to the bronchioles. Each bronchiole ventilates a lung lobule.

The bronchioles are the smallest branches of the conducting tree, and are characterised by a diameter of usually less than 1mm and the absence of cartilage plates in their walls. The bronchioles themselves branch repeatedly, before terminating as terminal bronchioles. Each terminal bronchiole divides into two daughter branches called respiratory bronchioles. They resemble the terminal bronchioles in all aspects save for the fact that their walls are interrupted by saccular outpocketings the alveoli. The respiratory bronchioles terminate in the alveolar ducts, from which the alveolar sacs and alveoli arise.

# CHAPTER 2.
# GENERAL MATERIALS AND METHODS.

## SOURCES OF ANIMALS.

Seventeen normal adult goats used in the study of the normal histology and ultrastructure of the respiratory epithelium. These animals were of various breeds, although the majority were of Cashmere breed, and were aged between eight months and one year. Those animals used in the study of the postnatal development of the respiratory epithelium were also of Cashmere breed and were aged between 3 hours and twenty one days. All animals appeared free from clinical respiratory disease on presentation, and showed no abnormalities of their respiratory systems at post-mortem and histological examination.

Clinical materials were obtained from two small East African goats from Tanzania, aged about one year, and one and half years, respectively, and also from a locally purchased Cashmere goat aged about 10 months. All three individuals presented obvious clinical signs of respiratory distress; *Pasteurella haemolytica* was isolated following microbiological examination in these three cases. A fourth individual, obtained as a normal adult was found to have gross pneumonic lesions at post-mortem, and was therefore included in this clinical section.

## POST-MORTEM TECHNIQUES.

Animals were killed by an overdose of pentobarbital sodium (Euthatal: May and Baker, Dagenham) administered intravenously through the cephalic vein. In each individual an incision was made from the submandibular space down to the thoracic inlet. Access to the thoracic cavity was achieved by cutting through the sternochondral joints and the sternum removed. The tongue, larynx, trachea, heart and lungs were removed intact. The trachea was sectioned at the bifurcation, and one lung was perfused with Karnovsky's fixative and tied off, the other lung being perfused with buffered neutral formalin (BNF). The head was cut off at the atlanto-occipital joint and sagittaly

sectioned using a band saw, so as to gain access to the nasal cavity and nasopharynx. After removing the nasal septum, the surface of one half was washed with Karnovsky's fixative and the other half with BNF. The halves were then used for SEM and LM samples respectively.

Tissue samples were taken from preselected sites as follows (Figs 2.1, 2.2, 2.3)

1. Nasal vestibule.
2. Alar fold.
3. Basal fold.
4. Ventral concha: At the level of 2nd cheek tooth.
5. Dorsal concha: At the level of 2nd cheek tooth.
6. Middle nasal concha: From the rostral tip.
7. Nasal septum: At the level of the 2nd cheek tooth.
8. Nasopharynx: A few millimetres rostral to the opening of the auditory tube.
9. Epiglottis: Laryngeal surface, caudal to the apex.
10. Vocal fold.
11. Infraglottic cavity.
12. Dorsal cranial trachea.
13. Ventral cranial trachea.
14. Dorsal caudal trachea.
15 Ventral caudal trachea.
16. Extrapulmonary principal bronchus.
17. Caudal lobar bronchus.
19. Lung parenchyma to include the following:
    a) Large bronchiole.
    b) Terminal bronchiole
    c) Respiratory bronchiole.
    d) Alveolar duct and alveoli.

## HISTOLOGICAL AND STAINING METHODS.

a) Fixation, embedding and sectioning.

Tissues for examination with the light microscope were fixed in buffered neutral formalin for seven days, then trimmed and post-fixed for two days in mercuric chloride formol. These fixatives were prepared as follows:

Buffered Neutral Formalin

| | |
|---|---|
| Formaldehyde (40%) | 200 ml |
| Sodium chloride | 10 g |
| Sodium sulphate | 30 g |
| Distilled water | 1800 ml |

Mercuric Chloride Formol

| | |
|---|---|
| Saturated aqueous mercuric chloride | 900 ml |
| Formalin | 100 ml |

After fixation, tissues were dehydrated, cleared and impregnated with paraffin wax. Paraffin embedded sections were cut at 3μm with a Leitz Rotary Microtome and mounted on glass slides

b) Staining:

Mounted sections were routinely stained with standard Haematoxylin and Eosin (H&E) and by the Alcian Blue /Periodic Acid Schiff (AB /PAS) (pH 2.5) method for acidic and neutral mucosubstances according to a modification of the method of Mowry and Winkler (1956) as detailed below:

Solutions:

1. 1% Alcian Blue in 3% acetic acid (pH 2.5).

2. 1% Periodic Acid.

3. Schiff's reagent.

Procedure:

1. Hydrate sections.
2. Solution (1) for 4 minutes.
3. Wash in distilled water.
4. Solution (2) for 2 minutes.
5. Wash in distilled water.
6. Solution (3) for 8 minutes.
7. Wash in running water for 10 minutes.
8. Mayer's haematoxylin for 4 minutes.
9. Wash in running water.
10. Differentiate in acid alcohol for 10 seconds.
11. Wash in running water.
12. Blue nuclei in Scotts tap water substitute.
13. Wash in running water.
14. Dehydrate, clean and mount.

Results:

| | |
|---|---|
| Acidic mucosubstances stain | blue |
| Neutral mucosubstances stain | red |
| Mixed mucosubstances stain | purple |

## SCANNING ELECTRON MICROSCOPIC METHODS.

Samples ranging between 0.5mm-2mm thick were left in Karnovsky's fixative overnight, then washed in 0.2M cacodylate buffer for 4hrs and thereafter cold dehydrated in a series of graded acetones as follows:

70% acetone for 4 hours.

90% acetone for 2 hours.

100% acetone for 2 hours.

100% acetone overnight.

The samples were then critically-point dried using liquid carbon dioxide in a critical-point-drier (Polaron: Watford, U.K.).

The specimens were orientated such that the mucosal surface was uppermost, and stuck on aluminium stubs using silver paint and placed in an oven at 37$^{o}$C for half an hour. The specimens were then coated with a gold-palladium mixture in a sputtering system.

The fixatives and buffers were made up as follows.

| 0.2M Cacodylate buffer | (500 ml) |
|---|---|
| 0.4M sodium cacodylate | 250 ml |
| 0.2M hydrochloric acid | 40 ml |
| Distilled water | 210 ml |

Karnovsky's Fixative (500 ml)

10g paraformaldehyde dissolved in 100 ml of distilled water at 60$^{o}$C together with 10 drops of NaOH, and the mixture added to the stock solution.

Stock solution:

| | |
|---|---|
| 0.2M cacodylate buffer | 250 ml |
| 25% glutaraldehyde | 50 ml |
| Distilled water | 100 ml |

## TRANSMISSION ELECTRON MICROSCOPIC METHODS.

Small portions of mucosa from preselected sample site numbered 1 to 18 were removed, minced in a petri dish to sizes of approximately 0.5 $mm^3$, and then placed in chilled Karnovsky's fixative for at least 24 hours.

The fixative was then drained off and 0.2M cacodylate buffer was added. After one hour, the specimens were post-fixed with 1% osmium tetroxide for a further hour. Then the specimens were washed three times using distilled water before being dehydrated in a graded series of acetones. After dehydration the specimens were put through two changes of propylene oxide for 20 minutes each, before being placed in a 1:1 Emscope Emix resin (Emscope, Ashford- Kent) / propylene mixture for an hour. At the end of this time, the mixture was replaced by pure emix resin, in which the specimens were left for 3 hours before being placed in plastic mounting moulds and left to polymerise in an oven at 60$^{o}$C.

Thick sections (at 1μM thickness) were cut from blocks of embedded tissues on an 9LKB microtome, (Croydon, Surrey). These sections were stained with toluidine blue and examined with a Leitz Laborlux II microscope to enable selection of suitable areas for thin sectioning and mounting.

Each block was then subsequently trimmed in preparation for further sectioning. Ultrathin sections in the silver or gold-pale range (60 - 90 nm thick) were cut on an LKB Mk III ultramicrotome, flattened using xylene vapour, and picked up on Polaron 300 mesh grids. Grid specimens were stained with uranyl acetate and lead citrate (see below) and examined with an Hitachi HS8 transmission electron microscope.

Toluidine Blue:

| | |
|---|---|
| 1% Borax (sodium tetraborate) | 1 g |
| 1% Toluidine blue | 1 g |
| Distilled water | 100 ml |
| Staining time | 15 seconds |

Uranyl acetate:

0.2g of uranyl acetate was dissolved in 10 ml of distilled water, providing a saturated solution.

Staining time 5 minutes

Lead citrate:

1.33g of lead citrate and 1.76g of sodium citrate were dissolved in 30ml of distilled water and shaken for 30 minutes.

8 ml of 0.1M NaOH were added, followed by distilled water, to give a final volume of 50 ml.

Staining time 5 minutes

## PHOTOGRAPHIC METHODS

a) Light microscopy:

A Leitz Laborlux 12 microscope equipped with a Wild MPS45 Photoautomat Unit was used. For black and white photography, Agfa PAN 35mm film (12 ASA) was employed. For colour transparencies Kodachrome 25 (25 ASA) film was used. For black and white prints Agfa-Gevaert Rapitome Photographic paper P1-P4 using an Agfa-Gevaert Rapidoprint PD 3700 automatic processor was employed.

b) Scanning electron microscopy:

All SEM samples were examined using a 501B SEM (Philips, Holland) and viewed at an accelerating voltage of 15KV using spot sizes between 200 and 1000. An attached automatic Rolliflex camera fitted with Ilford FP4 120 (125 ASA) film was used in taking pictures. Black and white prints were prepared as for light microscopy.

c) Transmission electron microscopy:

Electron micrographs were taken using Ilford Technical EM plates ($3^1/_4$" x $4^3/_4$"), developed in PQ Universal and fixed in Ilford Ilfospeed fixer. Black and white prints were prepared as above.

# CHAPTER 3.

# HISTOLOGICAL AND HISTOCHEMICAL STUDY OF THE EPITHELIAL LINING OF THE ADULT GOAT.

# INTRODUCTION.

The objective of this study was to provide for the first time a concise account of the basic histology of the epithelial lining of the goat's respiratory tract from the nasal vestibule to the alveolus, as well as a preliminary account of the basic histochemistry of the respiratory tract mucosubstances.

It was hoped that this would provide a baseline against which future histopathological observations could be interpreted, and, in addition, also assist in the interpretation of SEM observations, which, although possessing many advantages in revealing surface details of large areas of tissue at a wide range of magnifications, are limited where examination of subsurface structures are involved.

# LITERATURE REVIEW.

## HISTOLOGICAL STUDIES.

The earliest documented finding that the trachea and bronchi possess a membranous lining was provided by Laurentius in 1602. Later in 1712 Morgagni described precisely the nature of the tracheal glands and their ducts. In 1834, Purkinje and Valentine described the presence of ciliated cells in the epithelium lining the mammalian respiratory tract, observations later supported and augmented with the description of the goblet cell (Sharpey, 1836; Henle, 1837; Bowman, 1847; Schulze, 1872). These descriptions dealt with single cell types however, and it was not until 1880 that a complete account of the basic histology of the lining epithelium of the mammalian respiratory system was first proposed by Aeby. Since then many research workers (Waller and Bjorkman, 1892; Ebner, 1902; Kopsch, 1926; Jarvi, 1935; Huber, 1945; Engstrom, 1951; Bloom and Engstrom, 1953; Moe, 1955; Rhodin and Dalhamn, 1956; Ali, 1965; Bloom and Fawcett, 1976; Mariassy and Plopper, 1983;

Sorokin, 1988; Adams, 1990; Pirie *et al.*, 1991[a,b]) have contributed towards an understanding of the subject in a variety of mammalian species.However the goat, amongst domestic animals, has received little attention as far as studies of the respiratory system are concerned, the available literature only apparently concerning itself with the PAS-positive inclusions in the alveolar Type II cells (Atwal *et al.*, 1979).

Light microscopical studies have shown that the rostral part of the vestibule is lined by a keratinized stratified squamous epithelium continuous at the external nares with the outer skin covering. In the respiratory part of the nasal cavity, this epithelium is replaced by a pseudostratified ciliated columnar (typical respiratory) epithelium (Bloom and Fawcett, 1976; Dellman and Brown, 1976; Sorokin, 1988) via a narrow zone of transitional epithelium (Andrews, 1979; Adams, 1990). The nature and nomenclature of the epithelium lining this transitional zone has been the subject of controversy, although it is now generally accepted that it is primarily composed of stratified cuboidal cells (Andrews, 1979; Sorokin, 1988; Adams, 1990). The respiratory epithelium is typically made up of ciliated, mucus-producing, and basal cells, and covers most of the nasal conchae and nasal septum (Greenwood and Holland, 1972; Andrews, 1979; Boysen, 1982; Adams and Hotchkiss, 1983; Popp and Martin, 1984; Majid, 1986; Menco and Farbman, 1987; Pirie, 1990).

The lining epithelium of the nasopharynx changes from a pseudostratified ciliated columnar epithelium rostrally (similar to that seen in the nasal cavities), through an 'intermediate' epithelial type to a caudally distributed stratified squamous epithelium (Bryant, 1916; Ali, 1965; Ham, 1969; Greenwood and Holland, 1972; Bloom and Fawcett, 1976; Nakano, 1986), continuous at the intrapharyngeal ostium with that lining the oropharynx and laryngopharynx.

While most of the laryngeal cavity is lined by a pseudostratified columnar epithelium, the epiglottis is covered by a stratified squamous epithelium; the vocal cords may be lined by either a pseudostratified ciliated columnar (Dellman and Brown, 1976), a stratified squamous (Bloom and Fawcett, 1976) or an 'intermediate' (Andrews, 1979)

epithelium.

Histologically the lower respiratory tract, from the trachea down to the level of the smaller bronchi, is lined by a typical pseudostratified ciliated epithelium (Bloom and Fawcett, 1976; Dellman and Brown, 1976; Plopper *et al.*, 1983[a]; Sorokin, 1988) in which ciliated, mucus-producing and basal cells are the only cell types discernible. Smaller bronchi and bronchioles are lined by a simple columnar or cuboidal ciliated epithelium (Dellman and Brown, 1976); as the diameters of the bronchioles decrease, the number of mucus-producing cells and ciliated cells decreases, whereas there is a concomitant increase in the number of nonciliated bronchiolar epithelial (Clara) cells and nonciliated microvillous cells (Dellman and Brown, 1976; Sorokin, 1988). At the level of the respiratory bronchioles, characterised by the presence of scattered alveoli opening into their lumina, the epithelial lining is composed primarily of cuboidal Clara cells, some of which have apical protrusions. Previous studies have demonstrated that respiratory bronchioles are present and well developed in rat, mouse, monkey (Castleman *et al.*, 1975), dog (Majid, 1986) and man (Sorokin, 1988), but poorly developed in the ruminants (Getty, 1975) and absent in the horse (Pirie *et al.*, 1991[b]). The alveolus itself, the site of respiratory gaseous exchange, is lined primarily by the alveolar Type I cells, a cell type also known as the squamous alveolar epithelial cell, interspersed between which are the less numerous alveolar Type II cells, also known as cuboidal alveolar epithelial cells (Atwal and Sweeny, 1971; Sorokin, 1988).

SECRETORY CELL TYPES.

It is generally accepted that the epithelial lining of the conducting airways of the mammalian respiratory system contains at least three cells types which are considered to be secretory (Jones and Reid, 1978; Nadel *et al.*, 1979; Dixon, 1992). These are the mucus-producing, serous and nonciliated bronchiolar epithelial (Clara) cells. They have been distinguished from each other by both cellular morphology and anatomical

distribution (Breeze and Wheeldon, 1977; Reid and Jones, 1979). In addition, histological criteria have also been used to further divide the mucus-producing cell type into four categories (Mariassy and Plopper, 1983), although these categories have since been reduced to three on the basis of later ultrastructural studies (Mariassy and Plopper, 1984).

The mucus-producing and serous cells of the submucosal glands and of the surface epithelial lining constitute the secretory apparatus of upper and lower respiratory airways down to the level of the terminal bronchioles (Jones and Reid, 1978; Breeze and Turk, 1984), whereas Clara cells are the secretory cells in the bronchiolar system (Pack *et al.*, 1981; Mariassy and Plopper, 1983), although in some species such as the rabbit they are distributed in the tracheobronchial airways as well (Plopper *et al.*, 1983[b]). By light microscopy Clara cells can be distinguished from mucous cells by the presence of a characteristic apical protuberance, by their location, and also by their negative staining reaction to PAS (Cutz and Conen, 1971; Mariassy *et al.*, 1988).

The submucosal glands are present in the upper respiratory tract as well as in the tracheobronchial tree of the lower respiratory tract. In the latter region, glands lie in the submucosa either between the cartilage and the epithelial surface, or between, and occasionally external to, the plates of cartilage; they are also present in the membranous wall of the trachea (Bozarth and Strafuss, 1974; Mariassy and Plopper, 1983; Iovannitti *et al.*, 1985; Majid, 1986; Pirie, 1990). Studies on the bronchial submucosal gland show that it is composed of tubules, each formed by mucous or by serous cells, connected by a duct system to the airway surface (Meyrick *et al.*, 1969; Spicer *et al.*, 1983). The surface epithelium is seen to dip into the mouth of the gland to form a ciliated duct. This region of the duct system is considered to regulate the balance of electrolytes and water in the bronchial gland secretion (Meyrick *et al.*, 1969; Jones and Reid, 1978). From the collecting ducts arise the secretory tubules, with serous tubules always distal to mucous ones, being usually arranged in clusters at the distal end of the mucous tubule. Thus serous cell secretion flows over the surface of mucous cells before

the total secretion passes into the duct system and onto the airway surface (Jones and Reid, 1978; Spicer *et al.*, 1983).

MUCUS BLANKET.

The earliest study on mucus is that provided by Bostock in 1805, who pointed out the ambiguity which accompanies the use of the term mucus. To date, two centuries later, the situation has not improved and the term is still being defined differently in various disciplines, as discussed by Jones and Reid (1978). Stedman's Medical Dictionary (1966) defines mucus as "a clear, viscid secretion of mucous membranes, consisting of mucin, epithelial cells, leucocytes, and various inorganic salts suspended in water", a similar definition also being provided in Bailliere's Veterinary Dictionary (Blood and Studdert, 1988). The major component of this mucus is water, amounting to 95%, while the other 5% is composed of a fractionated mixture of carbohydrates, proteins, lipids and inorganic materials (Jeffery, 1978; Dixon, 1992). The carbohydrates and proteins are usually found in the form of a number of different glycoproteins, each differing in the ratio of its protein to carbohydrate moieties and its degree of acidification. Mucin itself is the term used to describe this glycoprotein component of the mucus, and is also known to contain 40% carbohydrate in the form of numerous side chains (Hafez, 1977).

The secretions of the mucous cells (a term covering those mucus-producing cells distributed within the lining epithelium of the respiratory surface, and of the submucosal mucous glands) are regarded as glycoproteinaceous in nature, belonging to that group generally referred to as epithelial glycoproteins (Gibbons and Mattner, 1966). The mucus of domestic animals is known to have two discernible layers, the outer viscous gel, whose structure depends primarily on long glycoprotein chains (Cohen and Gold, 1975), and the inner sol which has little if any elastic or gel character (Veit and Farrell, 1978). Submucosal gland secretions appear to contribute significantly to the sol layer,

while surface mucous cells contribute to the gel layer (Dulfano, 1973).The mucus produced from these two sources (Reid, 1954; McCarthy and Reid, 1964; Chakrin and Saunders, 1974; Jones *et al.*, 1975) forms a blanket in which inhaled particles and gaseous pollutants are trapped or dissolved, and this blanket, together with the cilia, forms the mucociliary escalator system which propels trapped material towards the pharynx, where it is then swallowed (Wright *et al.*, 1983; Dixon, 1992).

## THE HISTOCHEMISTRY OF MUCUS

Histochemistry is a biological approach which permits chemical characterisation of cell and tissue components in relation to *in situ* structural organization. Thus it combines histology and analytical chemistry under controlled conditions to identify and localise chemical substances on a cytological scale (Weiss, 1988).

The histochemistry of the carbohydrate moiety of the mucus has been characterised by the use of the Periodic Acid Schiff (PAS) staining reaction (McManus, 1946), a procedure used routinely in most histology and pathology laboratories (Mowry and Winkler, 1956; Wheeldon *et al.*, 1976; Reid and Clamp, 1978; Spicer *et al.*, 1983). It permits the localisation of carbohydrate-rich macromolecules such as glycogen and glycoconjugates (glycoproteins and proteoglycans), the technique thus being used to characterise these glycoconjugates within the mucus blanket and within secretory cells of the respiratory tract. Glycoconjugate itself is a term used to describe polymeric substances consisting of carbohydrates covalently linked to a non-carbohydrate moiety, usually lipid or protein (nucleic acids are excluded).

Two types of glycoconjugates, the proteoglycans and glycoproteins, in which carbohydrate is linked to protein, exist within the mucus. The chief difference between the two is that while the former is made up of long, unbranched carbohydrate chains, most of which have a repeated disaccharide structure, the latter is composed of relatively small carbohydrate units commonly referred to as oligosaccharides (Phelps, 1978; Reid

and Clamp, 1978). Also the two substances tend to occur in different tissues with the proteoglycans occurring in skeletal and supporting tissues, and glycoproteins in body fluids such as blood and in seromucous secretions (Clamp *et al.*, 1978).

Glycoproteins comprise a wide range of materials with differing properties and functions. Mucus glycoprotein, when freshly produced, has a molecular weight of several million and about 50% of this is made up of carbohydrates (Reid and Clamp, 1978). The carbohydrate units project out from the central core of polypeptide. Different acid groups (sialic and sulphate) are terminally attached to some of the oligosaccharide units. Oligosaccharide units do not completely surround the central core of polypeptide but do leave some free stretches ("naked" peptide) to which neighbouring chains can be joined by disulphide cystine bonds.

There are four major groups of glycoproteins which have been identified by histochemical methods, namely neutral glycoproteins, sialylated glycoproteins in which sialic acid is sensitive to the enzyme neuraminidase, sialylated glycoproteins in which sialic acid is resistant to neuraminidase and sulfated glycoproteins.

The Periodic Acid Schiff method employed to identify various carbohydrate moieties recognises the presence of characteristic vicinal hydroxyl groups attached to carbohydrate moieties. A wide range of other stains, including Alcian Blue (AB), are routinely employed to identify the various acid groups, allowing further characterization of the carbohydrate-rich molecules by assessing their degree of basophilia. The presence of sulphate groups or sialic acid in glycosaminoglycan confers a distinct basophilia in appropriately fixed material. Glycans with polyanionic groups can also be identified by staining with Alcian Blue. A combined Alcian Blue/ Periodic Acid Schiff (AB/PAS) stain thus offers a basis for identification of a number of types of glycoprotein (Mowry and Winkler, 1956), all active mucous-producing cells staining with either PAS or AB, or with both. Stained cells can be assigned to one of four main colour groups for qualitative descriptive purposes:

1.Red. Magenta of PAS with no AB; indicative of neutral mucosubstances

2.Red-Blue. Magenta of PAS with some AB; indicative of mainly neutral with some acidic mucosubstances.

3.Blue-Red. Magenta of PAS with AB, the AB predominating, indicative of mainly acidic and some neutral mucosubstances.

4.Blue, strong AB masking the PAS indicative of acidic mucosubstances.

Changes in the glycoproteins of the respiratory mucus have long been known to be associated with the pathogenesis of obstructive lung diseases, usually these changes being brought about by the abnormal activity of the enzyme glycosyltransferase, which is responsible for the synthesis of the mucus. Knowledge of the normal histochemistry of the respiratory tract mucosubstances can therefore be of value in interpreting the pathological changes which occur in different disease conditions, as shown by Wheeldon *et al.* (1976), in cases of chronic bronchitis in the dog, where there was a qualitative shift resulting in increased amounts of acidic mucosubstances being produced.

This study of the mucosubstances in the goat's respiratory tract provides a semiquantitative, and a qualitative assessment of the mucus-producing apparatus based on simple histochemical procedures.

## STUDIES OF MUCOSUBSTANCES IN MAMMALIAN SPECIES.

### *DOG*:

Reports of the histochemical composition of canine respiratory mucosubstances employing the AB/PAS staining technique have been provided by several workers (Goco *et al.*, 1963; Spicer *et al.*, 1971; Wheeldon *et al.*, 1976). The earliest report by Goco *et al.* (1963) using PAS stain only was of use solely for estimating the number of mucosecretory units. Later studies by Spicer *et al.* (1971) were more informative as they employed a variety of histochemical methods and were able to demonstrate that acidic mucosubstances predominated in the canine tracheobronchial tree. This was later

confirmed by Wheeldon *et al.* (1976) in their study of tracheobronchial mucosubstances in normal dogs and in dogs suffering from chronic bronchitis; they found that in both groups, the majority of surface and glandular mucus-producing cells contained predominantly acidic mucosubstances when stained by the AB/PAS method.

Studies on the dog by Majid (1986) incorporated the upper respiratory tract, in addition to the tracheobronchial tree. Using the AB/PAS method, he clearly established that throughout the respiratory airways of the normal dog, mucus-producing cells at the surface and in the submucosal glands contained predominantly acidic or mixed mucosubstances.

*PIG* :

The glycoproteins in the respiratory epithelium of the lobar bronchi of the normal pig have been identified by AB/PAS (Jones *et al.*, 1975), with the use of sialidase digestion and AB staining either at pH 2.6 or pH 1.0. Qualitative analysis of mucus-producing cells shows that in the normal glands most of the glycoproteins are neutral and that the small amount of acidic glycoprotein is sialidase-resistant sialomucin. Other areas of the respiratory airway of the pig do not appear to have been studied.

*COW* :

A histochemical study of the mucosubstances in the bovine respiratory tract has been provided by Allan *et al.* (1977). Samples from three clinically normal 6-month old calves were taken from segmental bronchi. Further characterization of acidic mucosubstances was achieved by neuraminidase digestion followed by AB/PAS at pH 2.6; this enabled the localisation of neuraminidase-sensitive sialomucins. Sulfomucins were identified by acid hydrolysis followed by AB/PAS staining at pH 2.6. Results showed that the bronchial surface mucus-producing cells contained almost exclusively sulfated acidic mucosubstances, but a few sialylated acidic mucosubstances were also encountered.

In the submucosal glands, there was a wide variation in the quantities of the differing mucosubstances, although, overall, approximately equal amounts of neutral mucins, and acidic sialomucins and sulfomucins were produced by the glandular cells.

*RHESUS MONKEY:*

The mucosubstances in the Rhesus monkey have been investigated by St. George *et al.* (1984, 1986), and they established that the surface mucus-producing cells within the trachea are predominantly acidic in nature whilst within the submucosal gland, mucus-producing cells were mainly neutral in character. Later studies (Plopper *et al.*, 1989 included samples from the bronchial airways. The results showed that the majority of all granule-containing cells within the epithelial lining were acidic in nature (i.e. $AB^+$), with sulfomucins predominating (High iron diamine positive, i.e.$HID^+$; a test used to distinguish between sialylated and sulfated glycoproteins). Only a few of the cells were $PAS^+$, these cells being $AB^-$ and $HID^-$. In the glands the staining pattern was reversed, the majority of the secretory products being neutral in nature. Regional variations were apparent however; whilst acidic mucosubstances predominated in the tracheobronchial surface epithelium, in the more distal airways the mucosubstances were mixed in character, with only a few being acidic and $HID^-$. In addition, the amount of sulfated material in both surface epithelial mucous cells and submucosal glands ($HID^+$) decreased in these distal airways.

## PATHOLOGICAL ASPECTS OF MAMMALIAN RESPIRATORY MUCOSUBSTANCES.

Many respiratory diseases are seen to be associated with the impairment of the mucociliary escalator system (Wheeldon *et al.*, 1976; Allan *et al.*, 1977; Jeffery, 1978; Nicholls, 1978; Dixon, 1992). This may be due to deficiencies in the ciliary component

or in the mucous component of the system. The contribution of each component in the dysfunction of the system does not yet appear to have been quantified in any species (Dixon, 1992).

In disease conditions, changes in glycoprotein secretions do occur (Wheeldon *et al.,* 1976; Nicholls, 1978), although these changes are seen to occur in line with the proportions of the various types of glycoproteins normally found. The types of granules are the same, but the proportion of various granules within a cell changes, as does that of the various cell types, so that the mucus produced may be very different (Jones and Reid, 1978). These changes are also reflected in the amount of mucus produced as well as in the viscosity of the mucus, the latter being dependent on the biochemistry of the glycoproteins (Dixon, 1992).

In most disease conditions, e.g. chronic bronchitis, mucus-producing cells of the airway surface epithelium increase in number and extend into the bronchiolar airways (where they are normally absent) down to the level of the respiratory bronchioles. In the bronchial submucosal glands, mucus-producing cell populations increase in number (Reid, 1954, 1960 Ellefsen and Tos, 1972). In these cases, the degree of sulphation of the glycoprotein in mucus-producing cells is increased (Lev and Spicer, 1965; Lamb and Reid, 1969). This increase may be due to a change in the degree of sulphation of molecules, or an increase in the concentration of molecules with sulphate radicals. In addition, in chronic bronchitis or cystic fibrosis associated with lung infection, there is also usually an increase in the proportion of mucous cells in the glands containing neuraminidase-resistant sialylated glycoprotein. The same findings have been reported by Wheeldon *et al.* (1976) while investigating mucosubstances in naturally occurring cases of canine chronic bronchitis.

Experiments in the rat have also shown that irritation of the airway epithelium results in a change in the proportion of cells producing acidic and neutral glycoproteins, and thus in alteration to the regional distribution of acidic, neutral and mixed mucosubstances along the respiratory airway (Jones and Reid, 1978).

Changes in the predominant type of acid group have been noted in mucous cells in hypertrophied bronchial submucosal glands in pigs with enzootic pneumonia induced by intranasal inoculation of *Mycoplasma hyorhinis* (Jones *et al.*, 1975). Increase in gland size was accompanied by a proportional increase in the number of cells containing acidic glycoprotein, along with a relative increase in the amount of neuraminidase-sensitive sialylated and sulfated glycoprotein and a decrease in the neuraminidase-resistant type.

Similar changes have been observed in the nasal epithelial lining of primates exposed for a short period to high ambient levels of ozone, resulting in a significant initial increase in both acidic and neutral glycoconjugates stored in transitional zone and respiratory epithelium of the nasopharynx. However, over a longer period, the nasopharyngeal epithelium was minimally affected (Mellick *et al.*, 1977).

Allan *et al.* (1977), employed a variety of histochemical staining techniques to determine the nature of the mucosubstances in calves with cuffing pneumonia, a proliferative pneumonia characterised histologically by the accumulation of cuffs of lymphocytes around the airway. They found that pneumonic calves, along with an increase in the number of surface epithelial mucus-producing cells, also produced larger amounts of neutral mucosubstances and sulfomucins compared to the normal calves.

## MATERIALS AND METHODS.

Seventeen clinically normal, adult Cashmere goats of both sexes were used in the present study. The method of destruction, post-mortem procedures and sample sites were described in Chapter 2

a) Fixation, embedding and sectioning.

Tissues for examination with the light microscope were fixed in neutral buffered formalin for seven days then trimmed and post-fixed for two days in mercuric chloride formol. After fixation, tissues were dehydrated, cleared and impregnated with paraffin wax. Paraffin embedded sections were cut at 3μm with a Leitz Rotary Microtome and mounted on glass slides

b) Staining:

Mounted sections were routinely stained with standard haematoxylin and eosin (H&E) and by the Alcian Blue /Periodic Acid Schiff (AB /PAS) (pH 2.5) method for acidic and neutral mucosubstances according to a modification of the method of Mowry (1956) as detailed below:

Solutions:

1) 1% Alcian Blue in 3% acetic acid (pH 2.5).

2) 1% Periodic Acid.

3) Schiff's reagent.

Procedure:

1) Hydrate sections.
2) Solution (1) for 4 minutes.
3) Wash in distilled water
4) Solution (2) for 2 minutes.
5) Wash in distilled water.
6) Solution (3) for 8 minutes.
7) Wash in running water for 10 minutes.
8) Mayer's haematoxylin for 4 minutes.
9) Wash in running water.
10) Differentiate in acid alcohol for 10 seconds.
11) Wash in running water.

12) Blue nuclei in Scotts tap water substitute.

13) Wash in running water.

14) Dehydrate, clean and mount.

Results:

| | |
|---|---|
| Acidic mucosubstances | blue |
| Neutral mucosubstances | red |
| Mixed mucosubstances | purple |

## RESULTS.

### NASAL VESTIBULE.

The epithelium lining the rostral portion of the nasal vestibule was of a keratinized stratified squamous type (Fig. 3.1) with a few hairs projecting out from the surface. Within the submucosa, in the rostral region of the vestibule, simple, tubular, sweat glands were abundant.

The caudal part of the vestibule was lined by an epithelium similar to that in the rostral part, except that in this region it was non-keratinized and no hairs were seen.

The basal surface of the epithelium was seen to undulate, forming epidermal papillae which dipped into the submucosa (Fig. 3.1). No mucus-producing cells were observed in the surface epithelium, and glands in the submucosa stained negative with AB/PAS.

### ALAR FOLD.

The rostral mucosa of the alar fold was found to consist of a thick, non-keratinized, stratified squamous epithelium containing a few hair follicles. On moving caudally there was a gradual change from a stratified squamous to a stratified cuboidal

type of epithelium ("intermediate"), the cells of which exhibited a slight apical bulge. Occasional mucus-producing cells, cuboidal in shape, were observed within the epithelium lining the caudal region of the alar fold (Fig. 3.2); these stained blue with AB/PAS, thus indicating the presence of acidic mucosubstances. Many glands were also found in the submucosa; although a few stained purple with AB/PAS, indicative of the presence of mixed mucosubstances, the majority did not stain. Unlike the glands, the gland ducts were lined by cells producing acidic mucosubstances.

BASAL FOLD.

The histology of this region showed that the epithelium was also stratified in nature, with the rostral region being stratified squamous and caudally changing into a stratified cuboidal type. Within the epithelium, mucus-producing cells were observed either singly or aggregated together in a row. With AB/PAS,such mucus-producing cells stained blue, indicating the presence of acidic mucosubstances.

Submucosal glands were abundant. Although most of them did not pick up the AB /PAS stain, those few that did show a positive reaction were found to be mainly mixed in character, with only a small minority being acidic or neutral (Fig. 3.3).

NASAL SEPTUM.

The nasal septum, at the level of the third upper cheek tooth ($PM^3$), was lined by a typical respiratory epithelium, i.e. a pseudostratified ciliated columnar epithelium. However, the majority of the cells within the epithelium were nonciliated with only a few cells bearing short cilia. These nonciliated microvillous cells were observed to be numerous in H&E stained specimens. However, with AB/PAS staining, most of the cells were observed to be mucus-producing cells while the rest stained negatively. The epithelium was quite thick relative to other areas of the nasal cavity examined. Mucus-

producing cells were very numerous throughout the epithelium; these demonstrated exclusively acidic mucosubstances when stained with AB/PAS.

The majority of cells within the submucosal glands demonstrated the presence of mixed mucosubstances, although a few cells were seen to contain neutral or, in very few cases, acidic mucosubstances.

NASAL CONCHAE

The mucosa of the nasal conchae was found to be lined by a pseudostratified ciliated columnar epithelium, in which numerous surface mucus-producing cells were seen (Fig. 3.4). The occasional gutters identified on the surface were lined by both ciliated and mucus-producing cells. Although no quantitative assessment of the mucus-producing cells was carried out, a subjective assessment suggested that they were more numerous in the epithelial lining of the middle concha than that of other conchae. The histochemistry of the nasal conchal epithelial lining indicated that the surface mucus-producing cells contained predominantly acidic mucosubstances (Fig. 3.4). A thin layer of acidic mucosubstances located at base of the cilia was frequently observed in the epithelium covering the concha.

Submucosal glands were abundant on the nasal conchae. On the ventral concha AB/PAS staining indicated the presence of equal amounts of neutral and mixed mucosubstances with very little acidic mucosubstances. The dorsal nasal concha had equal amounts of the three types of mucosubstances, whereas on the middle nasal concha, although neutral, mixed and acidic mucosubstances were also found, the amounts of each decreased in that order.

NASOPHARYNX.

The thickness of the epithelium, as well as the type of epithelium, varied within the nasopharynx. Although the rostral region was lined by a pseudostratified ciliated columnar epithelium, this changed into a transitional "intermediate" epithelial zone, and then into a stratified squamous epithelium caudally. It was within this transitional zone that there was a gradual changing and merging of the typical pseudostratified ciliated epithelium into a stratified cuboidal epithelium, which itself then changed and merged into the caudal stratified squamous epithelium The mucosal lining was frequently highly folded in both the transitional zone and the caudal region of the nasopharynx. Beneath these folds, aggregates of lymphoid tissue were seen (Fig. 3.5). The epithelium covering these lymphoid areas was seen to be attenuated in thickness, with only occasional mucus-producing cells being encountered. In the gutters between the folds, the epithelium was relatively thick and mucus-producing cells more numerous.

Generally surface mucus-producing cells, which were virtually all acidic in nature, decreased in number on moving from rostral to caudal regions. Submucosal glands were numerous, being abundant in the middle and caudal regions of the nasopharynx. These glands were seen deep in the submucosa, usually below the lymphoid tissue in the transitional zone. The glands in the nasopharynx exhibited predominantly acidic mucosubstances with occasional neutral mucosubstances (Fig. 3.5).

EPIGLOTTIS.

The laryngeal surface of the epiglottis was found to be lined by a non-keratinized stratified squamous epithelium. The occasional taste buds observed embedded within the epithelium presented a shape similar to an onion bulb, with the long axis of the constituent cells lying perpendicular to the luminal surface (Fig. 3.6).

Predominantly acidic submucosal glands were seen beneath this lining epithelium, being connected to the luminal surface by ducts lined by low columnar to cuboidal epithelial cells. No mucus-producing cells were seen in the surface epithelium.

VOCAL FOLD.

The rostral surface of the vocal fold was also lined by a typical non-keratinized stratified squamous epithelium (Fig. 3.7). This continued caudally over the fold, changing gradually into a stratified squamous epithelium characterised by a sharp demarcation between a one or two cell thick layer of squamous surface cells resting on an inner mass of underlying cuboidal cells. This stratified squamous epithelium showed an abrupt change to the pseudostratified ciliated epithelium found lining most of the caudal surface of the vocal fold. In this latter region however, ciliated cells, as well as mucus-producing cells, were very few, the majority of cells being nonciliated columnar cells.

The submucosa was rich in glands. The glands demonstrated almost equal amounts of acidic and neutral mucosubstances with AB/PAS staining (Fig. 3.7). Surface mucus-producing cells were exclusively acidic in character.

INFRAGLOTTIC CAVITY.

Three types of epithelia were identified lining the infraglottic cavity. Rostrally, the cavity was lined by a non-keratinized stratified squamous epithelium. This gradually gave way to an "intermediate" type of epithelium. This intermediate epithelium, being similar to that observed in the rostral region of the nasal cavity and also in the nasopharynx, gradually changed from a stratified cuboidal type of epithelium, through a pseudostratified low columnar, into a caudally located typical pseudostratified ciliated columnar epithelium.

Within the epithelium mucus-producing cells were rarely seen, but where present exhibited the presence of acidic mucosubstances with AB/PAS staining. Submucosal glands were very well developed and equal amounts of acidic, neutral and mixed mucosubstances were demonstrated by the use of AB/PAS staining.

TRACHEA.

Except for a slight increase in the number of mucus-producing cells on moving caudally, no striking differences in the histological appearance of the epithelium lining the cranial and caudal portions of the trachea were observed.

Differences were observed between dorsal and ventral tracheal epithelial linings however. The mucosa of the dorsal trachea was highly folded forming alternating relatively tall folds and deep gutters; the former were sometimes approximated leaving the gutter as a narrow cleft. The epithelium was of a pseudostratified ciliated columnar type, punctuated with mucus-producing cells. Ducts leading from submucosal gland orifices were seen opening into the base of the gutters (Fig. 3.8).

In contrast, the mucosal folds of the epithelium lining the ventral trachea were lower and wider than those of the dorsal trachea, resulting in the formation of relatively shallow, widely spaced gutters in this region.

AB /PAS staining indicated that the surface mucus-producing cells, which were few in number, were all acidic in character. A slight increase in the number of mucus-producing cells was noted on moving caudally, although the histochemical nature of the mucosubstances was not altered. Mucosubstances within the submucosal glands were predominantly acidic, with only a few producing a mixed reaction; neutral mucosubstances were only occasionally observed.

BRONCHI.

Although the height of the epithelium decreased as the airway decreased in diameter, a pseudostratified ciliated columnar epithelium still lined the bronchial tree at every level.

The mucus-producing cells encountered within the epithelium increased in number on moving down into the smaller bronchi; in contrast, submucosal glands became less numerous. There was a gradual increase in the amount of mixed mucosubstances in the surface mucus-producing cells with the decrease in airway diameter, such that in the smallest bronchi AB/PAS staining demonstrated almost equal proportions of both acidic and mixed mucosubstances. In the submucosal glands of the larger bronchi, there was a greater proportion of acidic mucosubstances, with only a few neutral being neutral, whereas down the smaller bronchi neutral mucosubstances predominated, with only a few acidic and mixed glands being found (Fig. 3.9).

BRONCHIOLES.

These were identified by the absence of cartilage in their walls. At the level of the terminal bronchioles, the pseudostratified ciliated columnar epithelium that lined the proximal generations of the bronchioles changed into a poorly ciliated, simple columnar epithelium. The lining epithelium of the bronchioles proximal to the terminal bronchioles was composed of ciliated, mucus-producing, and nonciliated bronchiolar epithelial (Clara) cells. The later cell type was identified histologically by its negative reaction to AB/PAS, and also the presence of a characteristic apical protuberance. Further distally into the respiratory bronchioles, characterised by the presence of alveoli along their walls (Fig. 3.10), the lining epithelium was of a simple cuboidal type, composed of a few ciliated cells and numerous Clara cells.

A few mucus-producing cells, producing both acidic and mixed mucosubstances

were observed in the bronchiolar epithelium proximal to the terminal bronchiole (Fig. 3.11). In the terminal bronchioles as well as the respiratory bronchioles, mucus-producing cells were not observed. Submucosal glands were not observed within the bronchiolar tree.

ALVEOLAR MEMBRANE.

The cells lining the alveoli were seen to be attenuated, such that most of the cells consisted of long thin,cytoplasmic processes. Occasional gaps were observed in the alveolar walls.

Two cell types could be identified. One, the alveolar Type II cell, which was almost cuboidal in shape and usually seen to be located in a recess of the alveolar lumen. The second, the alveolar Type I cell, which had a bulging ovoid mass projecting into the lumen of the alveolus and long thin, flat cytoplasmic processes. AB/PAS staining did not indicate the presence of any mucosubstances in the lining epithelium.

## DISCUSSION.

The purpose of undertaking this study was to provide a histological description of the lining epithelium of the entire respiratory tract of the goat, and to establish the histochemical nature of the mucosubstances found in the mucus-producing cells of the epithelial lining and submucosal glands.

The present study established that the nasal vestibule was lined by a thick keratinized stratified squamous epithelium, containing a few hairs, hair follicles and sebaceous glands, together with numerous AB/PAS-negative serous submucosal glands. Such findings reported for the goat are in agreement with observations of the nasal vestibule in the mouse (Greenwood and Holland, 1972), rat (Andrews, 1974) and

dog (Adams and Hotchkiss, 1983; Majid, 1986). As this region of the respiratory tract is exposed to significant mechanical insult, a thick keratinized lining epithelium is necessary to protect the underlying cells and tissues against both wear and tear and fluid evaporation.

Caudal to the nasal vestibule, in the nasal cavity, an "intermediate" epithelium, forming a nonciliated transitional zone between the stratified squamous epithelium of the nasal vestibule and the ciliated epithelium of the nasal concha, was seen to line part of the alar and basal folds. Although the standard histological textbooks appear to describe only a stratified squamous and a typical respiratory epithelium as lining this region of the nasal cavity (Banks, 1981; Weiss, 1988). A similar type of what, in the present study, has been termed "intermediate" epithelium has been described previously, although variously, as transitional in the dog (Adams and Hotchkiss, 1983; Majid, 1986) or stratified cuboidal in the calf and pig (Adams 1986; 1990). This transitional zone of "intermediate" epithelium in the caprine nasal cavity was seen to be lined by a stratified cuboidal epithelium rostrally; further caudally within this zone, the uppermost cells became low columnar in morphology, with a few ciliated cells beginning to appear. A few individual cuboidal (surface) mucus-producing cells were also observed in the rostral region of the zone, the number of these cells increasing caudally. These individual mucus-producing cells stained blue with AB/PAS, indicative of the presence of acidic mucosubstances, contrasting with reports in the rat (Katz and Merzel, 1977) and horse (Pirie, 1990) where the individual mucous cells exhibited a mixed or neutral reaction with AB/PAS.

Within this transitional zone in the goat, submucosal glands were found to be numerous. The majority of such glands exhibited no reaction to AB/PAS staining, a result indicative of their serous nature. The observation of numerous serous glands in this region of the nasal cavity supports similar observations in the rat (Katz and Merzel, 1977) and horse (Pirie, 1990). These glands, as well as those observed in the nasal vestibule, are associated with the copious watery secretions provided by the nasal

epithelium in the goat, such secretions being a familiar feature of the nasal cavity in many mammalian species including rat (Katz and Merzel, 1977), dog (Majid, 1986), man (Thaete *et al.,* 1981) and horse (Pirie, 1990). Such serous secretions are essential to maintain the required humidity of the inhaled air and prevent the dessication of the underlying epithelium. In addition this layer of serous glands, found only in this region, probably provides the source for much of the abundant watery secretion released in allergic or inflammatory states (Phipps, 1981). Long excretory ducts from these AB/PAS negative, compound acinous glands are reported to be lined by mucous cells in the horse (Pirie, 1990) and mouse (Thaete *et al.,* 1981), these were also seen in the goat in the present study.

Caudal to the transitional zone the nasal cavity was lined by a typical pseudostratified columnar (respiratory) epithelium, composed of ciliated and mucus-producing cells. Nonciliated microvillous cells were not always discernible with the light microscope. Individual mucus-producing cells, which produced an acidic reaction with AB/PAS, were seen to increase in number on moving caudally within the cavity. This observation in the goat supports previous observations in man (Tos, 1982) where a rostrocaudal increase in the number of mucus-producing cells was reported on the ventral and middle nasal conchae. The present findings are also in agreement with findings in the Bonnet monkey (Harkema *et al.,* 1987), where it was established that there was a general rostrocaudal increase in the quantity of total epithelial mucosubstances produced along the septal and lateral walls of the nasal cavity, and that there were more acidic than neutral mucosubstances in the caudal nasal airway than in the rostral region.

Whereas acidic mucosubstances predominated in the individual mucus-producing cells in all areas of the nasal conchae, a variation of the types of mucosubstances present in the mucus-producing cells of the submucosal glands of the ventral, dorsal and middle conchal epithelium was noted in the goat. In the dorsal concha, submucosal glands were seen to have equal amounts of mixed, neutral and

acidic mucosubstances, whilst in the ventral and middle conchae, submucosal glands produced primarily neutral and mixed mucosubstances and very little of the acidic type. These observations differ from those made in the dog, where acidic mucosubstances were seen to predominate within the submucosal glands of the middle nasal conchae. (Majid, 1986). Indeed, Bang and Bang (1977), investigating a number of small mammals and over 100 species of birds, noted that the submucosal glands of the nasal cavity showed significantly different staining properties in regard to acidic, neutral or mixed moieties according to the species studied.

In the nasopharynx the lining epithelium differed from the rostral to the caudal regions. Rostrally, the nasopharynx was lined by a relatively thick pseudostratified ciliated columnar epithelium, similar to that which lined the nasal conchae. An intermediate epithelium, similar to that observed and discussed in the rostral region of the nasal cavity, itself grading from a low columnar to a stratified cuboidal epithelium, was then seen to extend further caudally. A stratified squamous epithelium was observed to line the most caudal region of the nasopharynx. Beneath the intermediate and stratified squamous epithelia, aggregates of lymphoid tissue were encountered; the epithelium overlying these structures was seen to be attenuated and devoid of mucus-producing cells.

Such observations in the goat nasopharynx as made in the present study are similar to those made previously in a number of mammalian species including man (Bryant, 1916; Schumacher, 1927; Copenhaver, 1964; Ali, 1965, 1967; Greep, 1966; Ham, 1969; Takahashi, 1973; Bloom and Fawcett, 1976), non-human primates (Leela and Kanagasuntheram, 1973), dog (Majid, 1986) and mouse (Nakano, 1986). In all these studies, however, a number of different terms have been employed to described the lining epithelium of the transitional zone; these terms have included the intermediate epithelium (Bryant, 1916; Ali, 1965, 1967), the transitional epithelium (Ali, 1965, 1967; Leela and Kanagasuntheram, 1973; Majid, 1986) and the stratified columnar epithelium (Schumacher, 1927; Copenhaver, 1964; Greep, 1966; Ham, 1969;

Takahashi, 1973; Bloom and Fawcett, 1976). In the present study, the term intermediate epithelium, as previously defined in the rostral region of the nasal cavity as well as the nasopharynx, has been used to refer to this lining epithelium between the rostral pseudostratified ciliated columnar epithelium and the caudally-directed stratified squamous epithelium. Lymphocytes were often seen infiltrating the nasopharyngeal epithelium in the goat, an observation also noted in previous studies in non-human primates (Leela and Kanagasuntheram, 1973) and the horse (Mair *et al.*, 1987), although, in these latter cases, the observed infiltration of lymphocytes was more noticeable. Mair *et al.* (1987) also observed free lymphocytes in the nasopharyngeal lumen of the horse, although no such observation was made in the goat in the present study. The present observations of follicle-associated epithelium (FAE), commonly referred to as a "lymphoepithelium", overlying the lymphoid aggregates in the nasopharynx in the goat has also been noted in several mammalian species including the horse (Mair *et al.*, 1987; Pirie, 1990), the dog (Majid, 1986), and the sheep (Chen *et al.*, 1991). Such lymphoepithelium was observed in the goat to be attenuated and devoid of mucus-producing cells, features also observed in the horse (Mair *et al.*, 1987; Pirie, 1990) and sheep (Chen *et al.*, 1991). It is believed that the FAE consists of specialised cells which are concerned with the uptake and transport of exogenous proteins (Richardson *et al.*, 1976; Chen *et al.*, 1991; Schuh and Oliphant, 1992).

Individual mucus-producing cells were frequently observed in the rostrally situated respiratory epithelium lining the nasopharynx, their numbers decreasing considerably in the intermediate epithelium, and disappearing completely in the stratified squamous epithelial lining of the caudal region. Such individual mucus-producing cells were found to produce exclusively acidic mucosubstances in the goat, as were those of the well developed submucosal glands usually observed deep below the aggregates of lymphoid tissue.

Very few studies of the histochemistry of the mucosubstances produced by mucus-producing cells in the nasopharyngeal region in mammals appear to have been

carried out. It is therefore all the more interesting that a comparison of the present results with those of other studies available in this area (Pirie, 1990) show that in both the goat and horse individual mucus-producing cells within the epithelium produce acidic mucosubstances, while the mucus-producing cells of the submucosal glands show a marked species difference in the mucosubstances produced, those in the goat being acidic in nature while those in the horse produce both acidic and mixed reactions with AB/PAS.

The entire laryngeal surface of the epiglottis of the goat was lined by a thick, non-keratinized stratified squamous epithelium which functions to provide protection against the wear and tear to which the laryngeal surface is exposed. Whereas in the present study only one type of epithelium was seen to line the laryngeal surface of the epiglottis, in the mouse three types of epithelia (keratinized squamous, transitional (the intermediate epithelium of the present study) and ciliated) have been observed to line the rostral, middle and caudal regions of the laryngeal surface of the epiglottis respectively (Nakano and Muto, 1987). The transitional epithelium reported in the mouse took the form of a stratified cuboidal epithelium. Such an epithelial type has also been observed in the epiglottis of the horse (Pirie, 1990) and the dog (Majid, 1986). A ciliated epithelium located towards the base of the epiglottis has also been reported in man (Copenhaver, 1964, Ham, 1969; Bloom and Fawcett, 1976).

It was also noted in the present study that taste buds are a feature of the epiglottic epithelial lining. These presented an appearance similar to an onionbulb. Such extra-oral taste buds have been previously reported in the lining epithelium of the pharynx and larynx of several mammalian species including human (Lalonde and Eglitis, 1961), ox (Palmieri *et al.*, 1983), goat (Palmieri *et al.*, 1983), sheep (Bradley *et al.*, 1980), cat (Stedman *et al.*, 1983) and mouse (Nakano and Muto, 1987). Some workers have gone further to provide a numerical assessment of these extra-oral taste buds in a number species including the sheep (Bradley *et al.*, 1980) and the cat (Stedman *et al.*, 1983). In some species these taste buds account for a considerable

percentage of the total taste bud population; for example in the hamster they account for about 11.1% (Miller and Smith, 1984). It is believed that these extra-oral taste buds probably play little or no role in food selection, but serve to protect the airway (Stedman *et al.*, 1983) although the mechanisms through which this is achieved are not well established (Travers and Nicklas, 1990).

The absence of individual mucus-producing cells and the presence of numerous submucosal glands in the epithelium lining the epiglottis in the goat are features which have also been noted in many mammalian species including dog (Majid, 1986), rat (Andrews, 1974), horse (Pirie, 1990) and mouse (Nakano and Muto, 1987). Such glands in the goat demonstrated the presence of both acidic and neutral mucosubstances produced in abundancy, whilst mixed mucosubstances, were only occasionally observed. The present findings in the goat are similar to findings in the dog (Majid, 1986) and horse (Pirie, 1990), where both mixed and acidic mucosubstances were encountered. The histochemistry of this region in other mammalian species does not appears to have been widely investigated.

In the present study, three types of epithelia (non-keratinized stratified squamous, intermediate and pseudostratified ciliated columnar) were observed in different locations within the infraglottic cavity. Individual variations in the distribution of these various types of epithelia were noted, with some individuals presenting predominantly a stratified squamous lining epithelium, while other individuals presented mainly an intermediate type of epithelium. The typical pseudostratified ciliated columnar epithelium was only observed in the caudal regions of the infraglottic cavity, and its extension within the cavity was also observed to vary from individual to individual This is in agreement with findings in the dog (Majid, 1986) where, out of 18 animals examined, five had a complete ciliation of the ventral larynx, six had a stratified squamous epithelial lining in this region, and the remaining seven animals, had a larynx lined by both stratified squamous and pseudostratified ciliated epithelia inbetween which a "cobblestone" type of epithelium (intermediate) was observed.

The surface epithelium of the vocal fold and infraglottic cavity had relatively few mucus-producing cells, all producing acidic mucosubstances. The paucity of these cells in the laryngeal mucosa has also been reported in the dog (Majid, 1986). This paucity seems to be compensated for by the well developed submucosal glands in these regions, such glands being seen to be abundant in the vocal folds and infraglottic cavity in the goat, supporting similar observations in the mouse (Pack *et al.*, 1981) and the dog (Majid, 1986). Whereas in the present study acidic, mixed and neutral mucosubstances were found in almost equal amounts in the laryngeal submucosal glands, in the dog only a few neutral mucosubstances were encountered in the glands of the ventral larynx, most mucosubstances being acidic in character (Majid, 1986).

The tracheobronchial system of the goat presented a similar basic histological morphology to that of other mammalian species, being lined by a pseudostratified ciliated columnar epithelium which decreased in thickness with decreasing airway diameter.

In the present study, individual mucus-producing cells were relatively few in the trachea in comparison to those seen in the upper respiratory tract, although the numbers of individual mucus-producing cells gradually increased from the cranial to caudal tracheal regions. Within the bronchial system, however, individual mucus-producing cells became much more numerous. The observation of numerous submucosal glands in the tracheobronchial epithelium in the goat supports similar observations in the sheep (Goco *et al.*, 1963; Mariassy and Plopper, 1983), man (Thurlbeck *et al.*, 1961) and cat (Florey *et al.*, 1932; Gallagher *et al.*, 1975), but contrasts with observations in the rabbit and guinea pig (Nadel *et al.*, 1979) and the mouse (Hansell and Moretti, 1969; Pack *et al.*, 1981), where tracheal submucosal glands are infrequent or even absent. The equine trachea is markedly different from that of the goat in that surface mucus-producing cells are numerous while submucosal glands are few (Pirie, 1990).

As in the larynx, surface mucus-producing cells within the caprine trachea were exclusively acidic in character. Within the bronchial tree, however, a shift in the

histochemistry of individual mucus-producing cells was observed, such that both acidic and mixed mucosubstances were encountered in equal proportions. Submucosal glands exhibited an inverse relationship to surface mucus-producing cells in relation to their abundance;. cranially, the glands were numerous and well developed, but as the airway diameter decreased, fewer glands were encountered. Within the tracheal submucosal glands the mucosubstances were predominantly acidic, with a few being mixed; neutral mucosubstances were only occasionally observed.

The present findings in the goat are in agreement with previous observations made in the dog where it has been established that acidic mucosubstances predominate both in the surface mucus-producing cells and in the submucosal glands (Spicer *et al.*, 1971; Wheeldon *et al.*, 1976; Majid, 1986). In addition, the histochemistry of the surface mucus-producing cells observed in the present study appears to be similar to that observed in a number of mammalian species including the ox (Allan *et al.*, 1977), sheep (Mariassy *et al.*, 1988), Rhesus monkey (St. George *et al.*, 1984) and man (Spicer *et al.*, 1983) and even in species where surface mucus-producing cells are few such as the rat (Mochizuki *et al.*, 1982) and rabbit (Plopper *et al.*, 1984). Species differences in the histochemical nature of the surface mucosubstances has been noted in the hamster, where most of the surface mucus-producing cells in the trachea secrete neutral mucosubstances; in addition, a regional shift in the nature of the secreted mucosubstances, as noticed on moving from the trachea into the bronchial tree in the goat, has also been observed in the hamster where, at the bronchial level, the mucosubstances are primarily mixed (Becci *et al.*, 1978).

The histochemistry of the tracheobronchial submucosal gland mucosubstances of the goat observed in the present study differs markedly from that observed in the sheep (Mariassy *et al.*, 1988), pig (Jones *et al.*, 1975), ox (Allan *et al.*, 1977), man (Spicer *et al.*, 1983), Rhesus monkey (St. George *et al.*, 1986; Plopper *et al.*, 1989), mouse (Pack *et al.*, 1980 1981) and rabbit (Plopper *et al.*, 1984), where neutral mucosubstances have been observed to predominate with only a few acidic

mucosubstances being present.

Within the bronchiolar tree the lining epithelium was seen to differ at different levels. The proximal bronchiolar generations arising from the smaller bronchi were lined by a pseudostratified ciliated columnar epithelium which gradually changed to simple columnar and then low columnar at the level of the terminal bronchioles, the cell population in the bronchioles proximal to the terminal bronchiole being composed of ciliated, nonciliated (AB/PAS negative) and mucus-producing cells. The histology of the bronchioles proximal to the terminal bronchiole in the goat appears therefore to be similar to that seen in most mammalian species, including the pig (Baskerville, 1970[a]), monkey (Castleman *et al.*, 1975), horse (Mair *et al.*, 1987; Pirie, 1990), coyote (Morrison *et al.*, 1983), dog (Majid, 1986) and man (Ten Have-Opbroek *et al.*, 1991).

The histology of the distal conducting airways (terminal bronchioles, respiratory bronchioles and alveolar ducts) has been extensively covered in various mammalian species including pig (Baskerville, 1970[a]), monkey (Castleman *et al.*, 1975; Tyler *et al.*, 1988Plopper *et al.*, 1989;), ferret (Hyde *et al.*, 1979), guinea pig (Lechner and Banchero, 1982), rat (Massaro *et al.*, 1984), coyote (Morrison *et al.*, 1983), rabbit (Hyde *et al.*, 1983; Plopper *et al.*, 1983[b]), dog (Majid, 1986), horse (Pirie, 1990) and man (Ten Have-Opbroek *et al.*, 1991). The present study established that in the goat respiratory bronchioles, characterised by a simple cuboidal epithelial lining and the presence of alveoli within their walls, are prominent and well developed. This contrasts with previous observations that respiratory bronchioles are poorly developed or even absent in ruminants (Getty, 1975), including the ox (Iovannitti *et al.*, 1985). Although respiratory bronchioles are present in most of the mammalian species so far examined, including dog (Majid, 1986), ferret (Hyde *et al.*, 1979), coyote (Morrison *et al.*, 1983), pig (Baskerville, 1970[a]) and guinea pig (Lechner and Banchero, 1982), they have been shown to be rudimentary in the rat (Massaro *et al.*, 1984) and rabbit (Hyde *et al.*, 1983; Plopper *et al.*, 1983[b]), and absent in the horse (Pirie, 1990). In the present study, ciliated cells were seen to be present in the epithelium lining the respiratory bronchiole,

supporting previous observations made in the ferret (Hyde *et al.*, 1979), coyote (Morrison *et al.*, 1983), pig (Baskerville, 1970[a]) and guinea pig (Lechner and Banchero, 1982). However, observation made in the present study of the presence of ciliated cells in the respiratory bronchioles contrasts with findings made in the dog (Majid, 1986), where no ciliated cells were observed in the respiratory bronchioles.

Similar to other mammalian species (Plopper *et al.*, 1989), the present study noted that submucosal glands were absent in the bronchiolar tree of the goat, and thus mucosubstances were produced solely by the individual mucus-producing cells in the lining epithelium. Such mucus-producing cells, relatively fewer in number than those in the bronchi, produced both acidic and mixed reactions to AB/PAS. The paucity of mucus-producing cells in the bronchiolar epithelium was compensated for by the presence of numerous nonciliated bronchiolar epithelial (Clara) cells, which are known to be secretory (Breeze and Wheeldon, 1977; Reid and Jones, 1979; Pack *et al.*, 1981). No mucus-producing cells were observed from the terminal bronchioles distally. The absence of mucus-producing cells in the terminal and respiratory bronchioles noted in the present study, although in agreement with previous observations in numerous mammalian species including the ox (Iovannitti *et al.*, 1985), ferret (Hyde *et al.*, 1979), guinea pig (Davis *et al.*, 1984), dog (Majid, 1986) and horse (Pirie, 1990), differs from observations in the Rhesus monkey (Plopper *et al.*, 1989) and in humans (Ten Have-Opbroek *et al.*, 1991) where individual mucus-producing cells producing acidic to neutral mucosubstances have been observed within these distal airways.

The architecture of the caprine alveolus was observed to be similar to other mammalian species, and indeed at the light microscope level no species differences were observed, although at the ultrastructural level species differences are apparent. The ultrastructure of this region, being part of the distal airways, is discussed in detail in Chapter 5.

In the present study of the alveolar membrane of the goat, AB/PAS staining did not indicate the presence of any mucosubstances in the lining epithelium. Although such

an observation is in agreement with findings in several mammalian species including sheep and buffalo (Atwal *et al.*, 1979), it contrasts with a previous study in the goat (Atwal *et al.*, 1979), where PAS- positive inclusions were observed in alveolar Type II cells.

# CHAPTER 4.

# EPITHELIUM OF THE NORMAL RESPIRATORY TRACT OF THE ADULT GOAT: A SCANNING ELECTRON MICROSCOPICAL STUDY.

## INTRODUCTION.

The development of a rudimentary type of scanning electron microscope in 1937 (Wang and Thurlbeck, 1970), and the subsequent dramatic advances in SEM technology, were instrumental in allowing detailed ultrastructural studies of the surface features of the mammalian respiratory tract to be undertaken. One of the major advantages of the SEM in such studies is that large areas of tissue can be surveyed with ease, either at low or high magnifications, with a high degree of resolution compared with the light microscope or any other electrical optical system (Kimoto and Russ, 1969). In addition, specimen preparation is relatively straight forward. The many SEM studies carried out to date have involved examining different sites within the respiratory tract of various mammalian species (Table 4.1).

## LITERATURE REVIEW.

Information on the presence, distribution and surface characteristics of those cell types previously identified by the LM, the details of which were discussed in the previous chapter, has been considerably augmented by such SEM studies, and a brief review of the major points of these features, as revealed by SEM, is given below.

### UPPER RESPIRATORY TRACT.

SEM studies of the stratified squamous epithelium which lines the nasal vestibule of most mammalian species have demonstrated polygonal, flattened cells on the surface, and such cells are often grouped into patches separated by grooves (Andrews, 1979; Nakano, 1986). Such features are also observed where the stratified squamous epithelium extends onto the rostral part of the alar fold and straight fold (Adams and Hotchkiss, 1983; Adams, 1990).

Caudal to this vestibular region, the cuboidal surface cells of the epithelial lining of the transitional zone appear spherical or irregularly polygonal in outline and are characterised by a bulging apical surface carrying short surface microvilli (Andrews, 1979).

Mucus-producing cells throughout the epithelial lining of the respiratory tract are seen to have variable surface characteristics (Popp and Martin, 1984). Typically, the apical surface has a prominent central protuberance beneath which mucous granule outlines are often seen (Andrews, 1979; Davis and Smallman, 1988). Other cells demonstrate a flat, sometimes depressed apical surface, with an aggregation of microvilli around the periphery (Popp and Martin, 1984).

SEM studies have shown that the ciliated cells of the pseudostratified epithelium covering the nasal conchae, walls and septum of the nasal cavity bear microvilli intermingled between straight or wavy cilia exhibiting smooth, rounded or curled tips. At the ultrastructural level regenerating ciliated cells can be seen to have many more microvilli and fewer cilia per cell than those seen on ciliated cells (Menco and Farbman, 1987). Polygonal or rounded nonciliated microvillous cells, characterised by a convex apical cell surface carrying a dense population of microvilli, are another cell type revealed at the ultrastructural level in the nasal cavity epithelial lining (Boysen, 1982).

The detailed SEM studies carried out by Nakano (1986) on the mouse, as well as confirming histological observations that the nasopharynx was lined rostrally by a typical respiratory epithelium and caudally by a stratified squamous epithelium, also demonstrated the presence of an intermediate type of epithelium organised into zones composed of numerous different cell types. Andrews (1979), Majid (1986) and Pirie *et al.*, (1991[a]) made similar observations on the presence of a transitional zone, lying between the rostral ciliated and the caudal squamous epithelia, in the mouse, dog and horse respectively

SEM studies of the rat (Andrews, 1974), dog (Majid, 1986) and mouse (Nakano, 1986) have shown that the dorsal surface of the epiglottis is lined by a

typically flattened stratified squamous epithelium, the surface cells of which are characteristically similar to those seen in the nasal vestibule. Squames are frequently seen detaching from the underlying surface.

Similar studies have shown that the vocal folds are lined primarily by a stratified squamous epithelium, the cells of which have a tendency to bulge out from the surface. In some individuals, however, an intermediate type of epithelium, composed of nonciliated microvillous cells with surface microprojections, appears to line the caudal region of the folds. A few progenitor ciliated cells are found intermingled inbetween the nonciliated cells (Andrews, 1979). Most of the infraglottic region is seen, under SEM, to have a lining epithelium organised into longitudinal folds and is clothed by a heavy carpet of cilia (Majid, 1986; Pirie *et al.*, 1991[a]).

LOWER RESPIRATORY TRACT.

At low magnification, the lining respiratory epithelium of the trachea and bronchi of most mammalian species studied (Table 4.1) is seen to be organised into longitudinal folds and gutters, with submucosal gland orifices being located mainly in the latter. This typical respiratory epithelium is composed of ciliated, mucus-producing and nonciliated microvillous cells (Greenwood and Holland, 1972; Alexander *et al.*, 1975; Andrews, 1979; Wilson *et al.*, 1984; Pirie *et al.*, 1991[b]). SEM studies have shown that the distribution and proportion of these cell types along the tracheobronchial tree differ from species to species, and sometimes, within a species, from individual to individual (Hyde *et al.*, 1979Tandler *et al.*, 1983[a,b]).

At the bronchiolar level, there is the appearance of the nonciliated bronchiolar epithelial (Clara) cell, which, under SEM, may exhibit different morphologies. Typically, the round, oval or polygonally shaped Clara cells are characterised by dome-shaped protuberances at the apical surface (Mariassy *et al.*, 1975); these protrusions do not usually carry surface microvilli (Plopper *et al.*,1983[a,b]) and instead have a rough,

knobby surface (Andrews, 1979). None of the mucus-producing cells in the bronchioles appear to exhibit the typical central secretory protuberances characteristic of many of these cells elsewhere in the respiratory tract. Instead they have a flattened or low concave apical surface with a sparse distribution of microvilli, similar to mucus-producing cells sometimes seen on the nasal septum. Ciliated cells in the bronchioles are similar to those seen elsewhere, although their cilia seem to be relatively shorter and fewer per cell (Mariassy *et al.*, 1975).

Where present, low magnification SEM observations of the respiratory bronchioles, which are characterised by the presence of small numbers of alveoli opening off the bronchiolar lumen, show the epithelial lining to have a "roughened" appearance due to the presence of many Clara cells exhibiting typical dome-shaped apical protuberances. In some species, such as the dog (Majid, 1986), the epithelium appears devoid of ciliated cells, although in other species such as the rat and mouse, ciliated cells, although few in number, remain a component of the epithelial lining of the respiratory bronchioles (Wang and Thurlbeck, 1970Mariassy *et al.*, 1975).

Much of the work on the mammalian respiratory tract with SEM has dealt primarily with the ultrastructural features of the lung parenchyma itself (Table 4.1) The alveoli, which constitute the bulk of the lung parenchyma, are separated by thin interalveolar septa. SEM studies have provided detailed observations of the surface features of the alveolar Type I and Type II cells which line the alveolar walls.

The alveolar Type I cell is characterised by a bulging central region, and extensive cytoplasmic sheets ending in slightly raised cell borders, this cell type has relatively few surface microprojections. In contrast, the alveolar Type II cell, which is raised above the general surface, has a characteristically rounded outline and a dense population of surface microprojections (Nowell and Tyler, 1971; Mariassy *et al.*, 1975Greenwood and Holland, 1972; Andrews, 1979). A third epithelial cell (alveolar brush cell or Type III cell) has been reported in the rat (Meyrick and Reid, 1968; Hijiya, 1978[a,b]).

Alveolar macrophages have been observed, appearing to have variable shapes (Majid, 1986), and are occasionally seen migrating through alveolar pores. The latter appear as perforations in the alveolar walls and are rounded or oval in outline with variable diameters. Their abundancy differs from species to species (Mariassy *et al.*, 1975; Iovannitti *et al.*, 1985; Pirie, 1990).

TABLE 4.1

SCANNING ELECTRON MICROSCOPY OF THE RESPIRATORY TRACT OF VARIOUS MAMMALIAN SPECIES.

| SPECIEs | SAMPLE SITE /CELL TYPE | REFERENCE |
|---|---|---|
| Rat | Alveolus | Kuhn and Finke (1972) |
| | Trachea and Bronchi | Alexander *et al.*,(1975) |
| | Bronchioles | Ebert and Terracio (1975[b]) |
| | Alveolar brush cell | Hijiya, (1975[a,b]) |
| | Larynx and Trachea | Smolich et al.(1977) |
| | Trachea and Bronchi | Luchtel (1978) |
| | Terminal Bronchiole | Lum *et al.*, (1978) |
| | Clara cell | Smith *et al.*, (1979) |
| | Pulmonary Macrophage | Warheit and Hartsky(1988) |
| | Alveolus | Scheuermann *et al.*, (1988) |
| | Nasal cavity | Spit et al.(1989) |
| Mouse | Clara cell | Okada (1969) |
| | Lung: bronchiole | Wang and Thurlbeck (1970) |
| | Nasal Cavity | Adams, (1972) |
| | Respiratory Tract Surface | Greenwood and Holland, (1972) |
| | Lung: Alveolus | Kuhn and Finke (1972) |
| | Alveolus | Amy *et al.*, (1977) |
| | Lung: Bronchiole and Alveolus | Zitnik et al. (1978) |
| | Lung: Neuroepithelial bodies | Hung *et al.*, (1979) |
| | Clara cell | Smith *et al.*, (1979) |
| | Nasopharynx | Nakano (1986) |
| Hamster | Lung: Alveolus | Kuhn and Finke (1972) |
| | Tracheobronchial Epithelium | Becci *et al.*, (1978) |
| | Lung | Krause and Leeson (1973) |
| | Trachea | Althoff *et al.*, (1981) |
| Guinea Pig | Clara cell | Okada (1969) |
| | Trachea | Dahlgren *et al.*, (1972) |
| | Distal airway | Davis *et al.*, (1984) |
| Rabbit | Lung: Alveolus | Holma (1969) |
| | Bronchus | Sturgess (1977) |
| | Lung: Neuroepithelial Body | Cutz *et al.*, (1978) |
| | Clara cell | Smith *et al.*, (1979) |

| | | |
|---|---|---|
| Coyote | Entire Respiratory Tract | Morrison *et al.*(1983) |
| Ferret | Lower Respiratory Tract | Hyde *et al.*(1979) |
| Monkey | Trachea, bronchi and alveolus | Greenwood and Holland, (1973) |
| | Intrapulmonary airway | Castleman *et al.*, (1975) |
| | Lung: Respiratory Bronchiole | Mellick *et al.*, (1977) |
| | Tracheobronchial epithelium | Wilson *et al.*, (1984) |
| | Nasal epithelium | Harkema *et al.*, (1987) |
| | Lung: Bronchus | Maina (1988) |
| | Lung: Bronchiole | Tyler *et al.*, (1988) |
| Dog | Lung: Parenchyma | Groniowski *et al.*, (1972) |
| | Lung: Bronchiole and Alveolus | Kondo *et al.*, (1973) |
| | Trachea, Gland orifice | Nadel (1977) |
| | Lung: Bronchiole, Alveolus | Hyde *et al.*, (1978) |
| | Interalveolar Pores | Parra *et al.*, (1978) |
| | Nasal Cavity | Edwards *et al.*, (1983) |
| | Trachea and Lung | Wright *et al.*, (1983) |
| Cat | Trachea | Tandler *et al.*, (1983[a,b]) |
| | Lung: Bronchiole | Plopper *et al.*, (1983[a]) |
| Ox | Bronchus,Bronchiole & Alveolus | Mariassy *et al.*, (1975) |
| | Clara Cell | Smith *et al.*, (1979) |
| | Lower Respiratory Tract | Iovannitti *et al.*, (1985) |
| Sheep | Lung: Bronchiole,Alveolus | Tyler *et al.*, (1971) |
| | Pharynx | Chen *et al.*, (1991) |
| Pig | Lung: Bronchiole | Wang and Thurlbeck (1970) |
| | Trachea and Bronchi | Mebus and Underdahl (1977) |
| | Trachea and Lung | Williams and Gallagher (1978) |
| | Lung: Bronchiole and Alveolus | Winkler and Cheville (1984) |
| | Nasal Mucosa | Adams (1990) |
| Man | Lung: Bronchiole | Wang and Thurlbeck (1970) |
| | Larynx | Biondi and Biondi-Zappala, (1974) |
| | Lung | Gonzales-Crussi and Boston, (1974) |
| | Bronchus | Ebert and Terracio (1975[a]) |
| | Trachea and Lung | Greenwood and Holland, (1975) |
| | Clara cell | Smith *et al.*, (1979) |
| | Nasal Mucosa | Boysen (1982) |
| | Nasal Mucosa | Winther *et al.*, (1984) |
| | Nasal Cavity | Moor-Gillon (1985) |
| | Proximal border of respiratory unit | Ten Have-Opbroek *et al.*(1991) |

| | | |
|---|---|---|
| Horse | Lung: Bronchiole and Alveolus | Tyler *et al.*, (1971) |
| | Lung: Bronchus and Alveolus | Nowell and Tyler (1971) |
| | Respiratory Bronchiole | Tyler *et al.*, (1988) |
| | Upper Respiratory Tract | Pirie *et al.*, (1991[a]) |
| | Lower Respiratory Tract | Pirie *et al.*, (1991[b]) |

## MATERIALS AND METHODS.

Seventeen clinically normal, adult Cashmere goats of both sexes were used in the present study. The method of destruction, post-mortem procedures and sample sites were described in Chapter 2.

For SEM, samples measuring about 5mm x 5mm and 0.5mm-2mm thick were left in Karnovsky's fixative overnight, then washed in cacodylate buffer and thereafter cold dehydrated in a series of graded acetones.

The samples were then critically-point dried using liquid carbon dioxide (Polaron; Watford, U.K.) in a critical-point drier (Polaron; Watford, U.K.).

The specimens were orientated such that the mucosal surface was uppermost, stuck on aluminium stubs using silver paint, and placed in an oven at 37$^{o}$C for half an hour. The specimens were then coated with a gold-palladium mixture in a sputtering system. All SEM samples were examined using a 501B SEM (Philips, Holland) and viewed at an accelerating voltage of 15KV using spot sizes between 200 and 1000. An attached automatic Rolliflex camera fitted with Ilford FP4 120 (125 ASA) film was used in taking pictures.

LM samples were fixed in neutral buffered formalin for seven days then trimmed and post-fixed for two days in mercuric chloride formol.After fixation, tissue were dehydrated, cleared and impregnated with paraffin wax. Paraffin embedded sections were cut at 3μm with a Leitz Rotary Microtome, mounted on glass slides and routinely stained with standard Haematoxylin and Eosin (H&E) and by the Alcian Blue /periodic acid Schiff (AB /PAS) (pH 2.5) method Mowry (1956).

# RESULTS.

## NASAL VESTIBULE.

The epithelial surface in the rostral part of the vestibule was composed of flattened, polygonally shaped squamous cells (Fig. 4.1),characterised by the presence of numerous microplicae on the apical surface and frequently seen detaching, either singly or in groups, from the underlying layers. A few caudally directed hair shafts were seen projecting from the hair follicles (Fig. 4.1).

Further caudally in the vestibule, hair follicles disappeared although the squamous surface cells of the now highly folded epithelium retained their typical surface characteristics. Occasional circular arrangements of squamous cells surrounding a central pore (Fig. 4.2) were seen on the surface; these were assumed to be the openings of submucosal serous glands identified histologically as being present in this region.

Further caudally towards the alar fold, the squamous nature of the cells changed to present a more rounded outline (Fig. 4.3) and microplicae or microvilli, or a mixture of the two, on their apical surfaces (Fig. 4.4). Individual cell boundaries were clearly seen. In this region the presence of mucus-producing cells was revealed by the presence of mucous strands being extruded at the apical surface (Fig. 4.5). Occasional cells presenting a wrinkled apical surface were observed; these were presumed to be dying cells (Fig. 4.3).

## ALAR FOLD.

A narrow strip of rostral epithelium lining the alar fold had squamous surface cells characteristically similar to those seen in the middle of the nasal vestibule.

On moving away from this rostral zone, surface cells of the epithelium were cuboidal cells. The histology of this region showed a stratified cuboidal type of

epithelium (Fig. 4.6). With the SEM these polygonal cuboidal cells, sometimes exhibiting rounded cell boundaries, were seen to have bulging apical surfaces, giving the epithelium a "cobblestone" type of appearance (Fig. 4.7). At high magnification, a dense population of microvilli was observed on the apical surface (Fig. 4.8). Some of the apical cells had their surfaces thrown into folds, thus giving them a wrinkled appearance, and some could be seen detaching from the cells beneath.

In the caudal region of the alar fold, another cell type, smaller than the cuboidal cells and characterised by a flattened or slightly depressed apical surface carrying a sparse population of microvilli, was observed (Fig. 4.9). The concentration of surface microvilli around the periphery of this cell made the cell borders much more prominent; such cells were considered to be mucus-producing cells (Fig. 4.10). Submucosal gland orifices were occasionally found opening onto the epithelial surface (Fig. 4.11).

BASAL FOLD.

Light microscopy of the rostral region of the basal fold showed the epithelium to be stratified and composed of cuboidal cells. With SEM, the "cobblestone" appearance of the epithelium, similar to that of the alar fold, was again obvious (Fig. 4.12). SEM studies also showed that, although most of these cells carried microprojections in the form of short microvilli, these were not easily discernible on some of the cells. Intermingled between the cuboidal cells were mucus-producing cells characterised by slightly depressed apical surfaces and a peripheral aggregation of microvilli. Most of the mucus-producing cells in this region were seen actively secreting coalescing mucous granules (Fig. 4.13).

Although in some individuals there was a gradual transition, in a few cases even an abrupt transition (Fig. 4.14), from this more rostral nonciliated cuboidal epithelium to a caudally located ciliated epithelium, in most individuals these two epithelia were separated by a transitional zone composed of a mixture of ciliated and nonciliated cells,

the latter often in different stages of ciliogenesis (Fig. 4.15). Where present, this ciliated epithelium was marked by short, longitudinal grooves (Fig. 4.16) of variable depth. Mucus-producing cells were also present within this ciliated epithelium, being concentrated in and around the gutters and typically presenting a slightly shallow apical surface carrying a sparse distribution of microvilli. Outwith the gutters, mucus-producing cells were usually encountered singly or in groups of three or four. These frequently exhibited an irregular apical protuberance projecting above the level of the cilial tips (Fig. 4.17), such actively secreting cells being apparently absent within the gutters.

VENTRAL NASAL CONCHA.

The ventral concha was covered by a ciliated epithelium within which nonciliated cells were distributed either individually or in groups (Figs. 4.18, 4.19). Most ciliated cells exhibited a high density of individually separated, tall, slender cilia, often appearing matted at their tips (Fig. 4.19). In a few areas, the ciliated cells carried short, matted cilia, of uneven lengths and of low surface density (Fig. 4.20); these cells were considered to be regenerating ciliated cells.

Small patches composed primarily of two types of nonciliated microvillous cell were observed within the ciliated epithelium. One cell type was characterised by a polygonal shape presenting distinct cell borders and a primarily convex apical surface with a small radius of curvature, carrying an even distribution of short microvilli (Fig. 4.20). The other cell type, characterised by a peripheral aggregation of microvilli, could be identified as a mucus-producing cell either by a depressed apical surface frequently covered by a thin layer of mucus (Fig. 4.21) or a central apical protuberance resulting from mucus granules accumulating beneath the plasmalemma (Fig. 4.22).The distribution and abundancy of ciliated and nonciliated cells varied from one area to another in a given individual, as well as from individual to individual; however in

totality the ciliated cells appeared to be the dominant cell type (Fig. 4.21).

Occasional bigger patches consisting of regenerating ciliated cells were also seen (Fig. 4.23); these cells were characterised primarily by the presence of cilia of unequal lengths and sparse surface distribution. Both mature and regenerating cells carried surface microvilli, relatively longer than those seen on the cuboidal cells of the basal fold.

Although under light microscopy numerous submucosal gland orifices were frequently observed, with SEM these were only occasionally encountered.In three individuals, no orifices could be visualised as the surface was completely covered by sheets of mucus.

DORSAL NASAL CONCHA.

At low magnification the heavily ciliated epithelium, containing numerous individually scattered microvillous cells, was normally seen to be organised into longitudinal folds. Patches of nonciliated microvillous cells, many of which appeared to be mucus-producing cells and some to be regenerating ciliated cells, were observed (Fig. 4.24). Occasional submucosal gland openings, usually surrounded by a rim of nonciliated microvillous cells, were noted (Fig. 4.25).

In some of the specimens examined the surfaces were extensively covered by mucous sheets. In the few occasional areas devoid of mucus, the underlying epithelium was seen to be ciliated, although the cilia were matted and disorganised (Fig. 4.26).

MIDDLE NASAL CONCHA.

The epithelial lining of the middle nasal concha presented a characteristic longitudinally orientated pattern of folds and gutters of variable depths, the former being seen to run for only a short distance compared to those on the ventral and dorsal nasal

conchae (Fig. 4.27).

Individual mucus-producing cells were frequently observed protruding above the surface of a heavily ciliated epithelium (Fig. 4.28), with mucous granules frequently being seen through the plasmalemma. In some individuals, the scattered nonciliated cells showed a relatively flat apical surface covered by a thin layer of mucus (Fig. 4.29). Patches of nonciliated microvillous cells were very infrequently distributed throughout the ciliated epithelium.

The ciliated cells were similar to those on both the dorsal and ventral nasal concha, bearing long, slender and free-standing cilia, in most cases with a wave in their middle (Fig. 4.30). Occasionally cilia would appear clumped and matted (Fig. 4.31). Islands of regenerating ciliated cells were not uncommon.

NASAL SEPTUM.

The nasal septum was normally covered by a ciliated epithelial lining differing from that of the middle nasal concha in containing many small, scattered, irregularly shaped patches of nonciliated microvillous cells, giving the epithelium as a whole a "moth-eaten" appearance. A few individuals had a complete carpet of cilia lining the nasal septum. The cilial carpet was occasionally interrupted by single mucus-producing cells protruding from the surface in the characteristic manner seen in the nasal conchae (Fig. 4.19). Again, as in the ventral nasal conchae, developing ciliated cells seen in different stages of ciliogenesis had fewer cilia than the normal mature cell, these cilia appearing disorganised, and of unequal lengths (Fig. 4.32).

Two apparently separate types of nonciliated microvillous cell could be identified. One was polygonal in outline with pentagonal or hexagonal borders predominating, with a slightly convex apical surface and a high density of apical microvilli. The other had a more or less circular cell boundary, with a slightly depressed apical surface carrying fewer although taller microvilli (Fig. 4.32). Some of the latter

type of nonciliated microvillous cells could be seen discharging mucus in the form of granules.

NASOPHARYNX.

The epithelium of the rostral part of the nasopharynx was seen to be similar to that lining the nasal conchae. It was again organised into longitudinally orientated gutters, and composed of ciliated cells amongst which nonciliated microvillous cells were scattered either individually or in groups of two or three (Fig. 4.33). In some areas the carpet of cilia was interrupted by islands of nonciliated microvillous cells, some of which were identifiable as mucus-producing cells, characterised by a typical apical protuberance of mucus (Fig. 4.34).

In the middle, transitional zone, region of the nasopharynx the epithelial lining was seen to exhibit a gradual change-over from a heavily ciliated rostral region, through a less ciliated but more secretory epithelium, to a final squamous epithelial lining typical of the caudal region of the nasopharynx. In this middle region the epithelium was initially seen to be thrown into deeper and more irregular corrugations (Fig. 4.35), and the number of ciliated cells, themselves carrying fewer and shorter cilia per cell, were seen to decrease at the expense of microvillous cells (Figs. 4.36). The nonciliated microvillous cells in this transitional zone were polygonal, or occasionally rounded, with distinct cell borders and slightly bulging apical surfaces (Figs. 4.37, 4.38). Small numbers of cells were commonly seen extruding thick columns of mucus in this region (Figs. 4.39, 4.40). Further caudally, but still in the transitional zone, the epithelial lining was characterised by a lack of ciliated cells and the presence of exclusively microvillous cells arranged in a "paving-stone" fashion(Fig. 4.41); the latter were hexagonal in outline, with evenly distributed surface microprojections, the lengths of which varied from cell to cell (Figs. 4.42). Occasionally cells would be seen lifting off from the epithelial surface (Fig. 4.43).

Further caudally, towards the oropharynx, the epithelium was lined by squamous surface cells. Flattened squames could be seen detaching from the underlying surface. Only occasional submucosal gland orifices were encountered.

In the transitional and caudal regions of the nasopharynx, the epithelium was seen to be punctuated by low domes (Fig. 4 44). In the latter region these domes were characterised by covering squamous surface cells which were arranged in a circular fashion and had relatively larger apical surfaces than the cells surrounding the dome, which appeared wrinkled (Fig. 4 44). Within the dome, intercellular pores or fissures were sometimes found to contain what appeared to be cellular aggregates. In the transitional zone, where the lining epithelium was made up of both ciliated and nonciliated microvillous cells, exaggerated fissures between adjacent cells covering the domes were a common feature (Fig. 4.45).

## EPIGLOTTIS.

The epithelial surface of the laryngeal aspect of the epiglottis was composed of squamous surface cells (Fig. 4.46), and was of a similar nature to that observed in the nasal vestibule. The flattened squamous cells, bearing apical surface microplicae (Fig. 4.47), were arranged in an irregular paving-stone fashion; a few desquamating cells could be seen at the surface. Towards the base of the epiglottis there was a tendency for the mucosa to form transverse folds. Here the squamous cells had a "spongy" appearance, with the majority of cells exhibiting a wrinkled apical surface carrying microplicae or microvilli.

The rounded or oval orifices of submucosal glands were seen to be sparsely distributed across the epiglottal surface (Fig. 4.46).

Taste buds were also often seen. They were characterised by a central pore from which sensory hairs projected out, and occasional droplets were seen being discharged (Fig. 4.47).

VOCAL FOLD:

In most of the individuals examined (13/17), the epithelium lining the vocal fold was found to be of a squamous type. In these individuals the epithelium was similar to that seen on the laryngeal surface of the epiglottis, except that the squamous cells were relatively thicker and most of them carried surface microvilli instead of microplicae. Occasionally squamous cells with wrinkled apical surfaces were seen to be lifting off from the underlying surface; these were thought to be dying cells. This part of the larynx differed also from the epiglottis in that no taste buds were encountered, in spite of an extensive search of the area. However, submucosal gland orifices were not uncommon (Fig. 4.48).

In three individuals, flattened polygonal microvillous cells and mature and regenerating ciliated cells covered the surface (Fig. 4.49).

The change-over from the nonciliated to a ciliated epithelium was quite abrupt (Fig. 4.50). In the ciliated portion of the epithelium, a few regenerating ciliated cells could be seen, characterised by the presence of surface microvilli interspersed between short cilia of uneven lengths (Fig. 4.51). The ciliated portion of the epithelium had many ciliated cells carrying dense aggregations of cilia of approximately equal lengths; these cilia were, however, of relatively short length as compared to those in the nasal cavity.

INFRAGLOTTIC CAVITY.

At low magnification the mucosa of the infraglottic cavity in all individuals was seen to form irregular longitudinal ridges separated by deep (Fig. 4.52) or occasionally shallow gutters (Fig. 4.53). The nature of the epithelial lining of the infraglottic cavity, however, exhibited a great deal of individual variation. In two of the individuals

examined, the cranial half of the cavity was covered by a squamous type of epithelium (Fig. 4.54), while the caudal half was lined by an intermediate epithelium composed of nonciliated microvillous and regenerating ciliated cells. In three individuals, the cranial half of the infraglottic cavity was lined by this intermediate epithelium and the caudal half by a ciliated epithelium with occasional patches of nonciliated microvillous cells. In four individuals the infraglottic cavity was lined by a ciliated epithelium except for a narrow cranial zone which was lined by the intermediate epithelium. The majority of animals examined, (8/17) had an infraglottic cavity lined entirely by a ciliated epithelium composed primarily of cells bearing tall, slender cilia similar to those seen in the caudal regions of the nasal conchae. Readily identifiable mucus-producing cells, characterised by apical protuberances, were infrequently observed.

Submucosal gland orifices of relatively bigger diameters than those seen in the nasal conchae were frequently encountered in the gutters; in areas where the epithelium was ciliated, the orifices were surrounded by nonciliated microvillous cells.

TRACHEA.

Cranial dorsal trachea.

Under low magnification the dorsal surface was seen to be thrown into high folds and deep intervening gutters (Fig. 4.55). In most of the individuals examined, the epithelium covering the folds was extensively ciliated (Fig. 4.56), with only occasional patches of nonciliated microvillous cells apparent. Nonciliated microvillous cells found on the folds had slightly raised apical surfaces carrying a dense population of microvilli of uniform lengths; some cells had short microvilli while others in the vicinity had relatively taller microvilli (Fig. 4.57). Regenerating ciliated cells, characterised by few cilia of uneven lengths, were occasionally found inbetween the nonciliated microvillous cells (Fig. 4.57). Although it was not always possible to visualise the floors of the gutters due to the highly folded nature of the epithelium, in areas where the floors were

exposed, there were relatively few ciliated cells, the majority of the cell population being of the nonciliated microvillous type (Fig. 4.58). These latter cell types, however, were different from those found on the surface of the folds in that their apical surfaces were flat or shallow (Fig. 4.59), bearing relatively taller and more sparsely distributed microvilli; corresponding histological study showed this cell type to be, in all probability, a mucus-producing cell. Microvillous cells were also observed around submucosal gland orifices, the latter being infrequently observed, and located in the exposed portions of the gutters.

A few functional mucus-producing cells characterised by the presence of typical apical protuberances were observed, primarily on the folds, scattered singly or in pairs amongst the ciliated cells.

Cranial ventral trachea.

The mucosa lining the ventral surface of the cranial trachea was heavily ciliated with many irregular depressions giving the epithelium a characteristic surface pattern (Figs. 4.60, 4.61). In contrast to the dorsal cranial trachea, submucosal gland orifices were frequently observed opening onto the floors of the gutters. Regenerating ciliated cells, characterised by numerous microvilli intermingled with few cilia of uneven lengths, were not uncommon, and were observed amongst patches of nonciliated microvillous cells.

Caudal trachea.

In general, the lining epithelium of the caudal region of the trachea resembled that of the cranial trachea. The only noticeable difference under SEM was that, caudally, fewer patches of nonciliated microvillous cells were seen, especially on the ventral surface. The dorsal surface, which was still heavily ciliated, remained virtually unchanged along the entire length of the trachea.

## EXTRAPULMONARY BRONCHUS.

The extrapulmonary bronchial epithelium was similar to that of the trachea in being ciliated and thrown into folds. Submucosal gland orifices were significantly fewer in the bronchial epithelium than in the cranial ventral trachea, and were confined to the gutters, where the density of ciliated cells was much less compared with that on the fold.

A marked increase in the number of nonciliated microvillous cells distributed amongst the ciliated cells was noted on moving from the trachea into the extrapulmonary bronchus. These nonciliated cells were characterised by a relatively flat apical surface carrying sparsely distributed microvilli. A cell type, identified as a mucus-producing cell, was characterised by a circular and depressed apical surface covered by a thin film of mucus (Fig. 4.62). More typical mucus-producing cells with apical protrusions were occasionally observed, mostly on the fold.

## CAUDAL LOBAR BRONCHUS.

The folded ciliated epithelium of the extrapulmonary bronchus continued into the caudal lobar bronchus. At low magnification the epithelium was again seen to be thrown into alternating folds and gutters. The depths of gutters were variable; in some areas they were quite wide and very shallow, whereas in other areas they were relatively deep. Within the gutters a number of oval or rounded submucosal gland openings were observed, these orifices being surrounded mainly by nonciliated microvillous cells, some of which could be seen discharging mucous droplets (Fig. 4.63). The lining epithelium carried a thick carpet of cilia, being more densely organised on the folds rather than on the floor of the gutters; the cilia were straight and slender, and had rounded tips which curved to form small hooks (Fig. 4.64). Scattered, nonciliated microvillous cells and identifiable mucus-producing cells could be seen between the

ciliated cells.

In the smaller bronchi, there was a marked decrease in the ratio of ciliated to nonciliated microvillous cells. In addition, the number of submucosal gland openings decreased.

In the smallest bronchi, ciliated cells, the cilia of which appeared curved and entangled with each other, especially at the tips, were organised into small patches separated by mucus-producing cells (Fig. 4.65).

BRONCHIOLE.

Bronchioles were not readily distinguishable from the smallest bronchi by their epithelial surfaces; only those bronchioles confirmed as such in fractured specimens by the absence of cartilage plates in their walls were therefore examined.

Immediately after the bronchioles branched off from small parent bronchi, the epithelium was found to be composed of a mixture of four readily identifiable cell types, the ciliated, nonciliated microvillous, mucus-producing and nonciliated bronchiolar epithelial (Clara) cells. In the bronchioles proximal to terminal bronchioles, the relative numbers of these cells differed from individual to individual, although in each case ciliated cells were numerically dominant. Nonciliated microvillous cells were less numerous, while Clara cells were few in number, and identifiable mucus-producing cells infrequently observed.

Ciliated cells bore relatively shorter cilia compared to those in the upper respiratory tract, and, although cilia formed matted clumps in some individuals, these cilia frequently appeared relatively straight and slender (Fig. 4.66). Mucus-producing cells had a rounded, slightly depressed surface with relatively few tall apical microvilli; their identification was confirmed by the presence of mucus granules, observed in fractured specimens. Nonciliated microvillous cells had flattened apical surfaces carrying a dense aggregation of relatively thin microvilli.

Clara cells presented different shapes and different apical surface characteristics, but were always seen to carry a characteristic surface population of many thick, stubby microvilli (Fig. 4.67). Some had a polygonal cell surface outline with prominent raised pentagonal boundaries predominating (Fig. 4.67). The circular cell boundaries of other cells were not prominent. Clara cells also presented different apical cell surface characteristics. Some had a flattened surface (Figs. 4.66, 4.68), others a low bulge centrally, with a flattened periphery (Fig. 4.67). Still other Clara cells had their whole apical surface raised into a dome projecting beyond the cilial tips; these were especially obvious in the terminal and respiratory bronchioles (Fig. 4.70). Such dome-shaped Clara cells became more numerous as the diameter of the bronchiole decreased. In most cases this dome-like protrusion appeared full and bulging, and carried few short surface microvilli. Occasionally it was seen to be "withered" and collapsing (Fig. 4.71).

TERMINAL BRONCHIOLE.

From the level of the terminal bronchioles distally, there was a marked shift in ratio of cell types, with an increase in the number of Clara cells, a significant decrease in the number of ciliated cells, and the disappearance of mucus-producing cells. In these smaller bronchioles, ciliated cells appeared to carry fewer cilia per cell, these cilia appearing relatively short and wavy compared to those in the larger bronchioles. In these regions, Clara cells presenting a dome-shaped apical surface predominated over those which had a flat, oval apical surface

RESPIRATORY BRONCHIOLE.

The presence of respiratory bronchioles arising at the termination of terminal bronchioles in the goat lung was established at low magnification with SEM. These were identified as short tubes whose walls were perforated by a number of alveoli (Fig.

4.69). The epithelium was essentially composed of two cell types, ciliated cells and nonciliated bronchiolar epithelial (Clara) cells. The latter were the more numerous of the two cell types, ciliated cells being fewer in number and appearing to be squeezed between adjacent Clara cells (Fig. 4.70). The cilia of these ciliated cells were short, ruffled and fewer in number per cell in comparison to ciliated cells of the upper respiratory tract or trachea, and frequently clumped at their tips (Fig. 4.71). Many microvilli of variable lengths were intermingled between cilia.

Although Clara cells of variable surface morphologies, such as those in the proximal bronchiolar generations, were frequently encountered,Clara cells exhibiting an apical dome-like protrusion were much more dominant in the respiratory bronchioles (Fig. 4.70). Occasionally, Clara cells presenting a "withered" apical protuberance were also observed (Fig. 4.71).

At high magnification the junction between the respiratory bronchiole and the alveolus was seen to be an abrupt one, such that ciliated cells and Clara cells had a common boundary with the alveolar Type I cellof the alveolar wall (Fig. 4.72).

ALVEOLUS.

Alveoli were first observed at low magnification in the respiratory bronchiole as shallow outpocketings (Fig. 4.69; 4.72) lined predominantly by alveolar Type I cells characterised by extensive thin cytoplasmic sheets spreading over the alveolar surface, away from the raised central nuclear region. Thin, slightly raised borders sometimes defined junctions between two adjacent alveolar Type I cells.

Alveolar Type II cells were round or oval in outline, and slightly raised from the epithelial surface. The apical surface bore characteristic, densely packed surface projections in the form of stubby or sometimes thin microvilli (Fig. 4.73). Although the lengths of the microvilli on the same cell were fairly uniform, they were seen to vary between cells. Sometimes a few droplets could be seen on the surface of these cells.

Capillaries were seen as elevated ridges running across alveolar walls, and sometimes characteristic outlines of erythrocytes could be seen within them. Interalveolar pores (of Kohn), defined by smooth rounded or oval margins, were observed as perforations in the alveolar walls. The number of pores per alveolus was highly variable, with the diameters of the pores also varying widely (Fig. 4.73).

With SEM, alveolar macrophages were seldom observed. They were encountered infrequently in the alveoli and were even more infrequent in the respiratory bronchioles (Fig. 4.74), a considerable number of specimens having to be examined in order to locate one. When seen, they were of variable shapes, their cell surfaces being either smooth or ruffled and exhibiting pseudopodia-like extensions of the cytoplasm.

## DISCUSSION.

### UPPER RESPIRATORY TRACT.

The upper respiratory tract is under the constant influence of inspired air, which may contain microorganisms, potential allergens and obnoxious gases, and more often than not is at unsuitable temperature and humidity levels. Thus the upper respiratory tract, and nasal cavity in particular, plays an important role in improving the quality of inspired air by warming, humidifying and trapping particulate matter (Mygind *et al.*, 1982). In spite of these important functions performed by this region of the respiratory tract, the upper respiratory tract of the goat has not been widely investigated. The purpose of this study, therefore, was to provide a detailed account of the surface morphology of the epithelial lining of the caprine upper respiratory tract by the use of the scanning electron microscope.

This study has established that the upper respiratory tract of the goat is lined basically by three different types of epithelia. The nasal vestibule and the rostral portion

of the alar and basal folds are lined by a squamous epithelium which is continued caudally by a narrow transitional zone of an intermediate type of epithelium. The major part of the nasal cavity is lined by a ciliated epithelium. All three types of epithelia were encountered in the nasopharynx as well as in the larynx

In the present study, it was established that the rostral region of the nasal vestibule of the goat was lined by keratinized squamous surface cells. Occasional hairs were seen projecting from the surface in this region. The present observations on the squamous nature of the epithelium lining the nasal vestibule are in agreement with similar findings in the rat (Andrews, 1974, 1979; Katz and Merzel, 1977), mouse (Greenwood and Holland, 1972), dog (Adams and Hotchkiss, 1983), horse (Pirie, 1990), monkey (Harkema *et al.*, 1987) and, in less detail, in several mammalian species (Graziadei, 1970) and the pig (Larochelle and Martineau-Doize, 1990). Although these squamous cells did not appear to present any obvious surface characteristics at low power magnifications, appearing simply smooth-surfaced and cornified, a feature also noted in the rostral region of the nasal vestibule of the rat (Andrews, 1979), at higher power magnifications the cell surfaces presented a pitted appearance. The same high power surface characteristics have been reported by Cleaton-Jones (1976) in the keratinized squamous cells of the soft palate of rats, and seem to be a feature of this cell type in general.

In the caudal region of the vestibule, hairs were no longer present, and the squamous epithelial surface cells in this region presented prominent surface microvilli or microplicae, and sometimes both. Such microplicae are a characteristic feature of squamous cells and have been reported to be present on cells in the epiglottis (Nakano and Muto, 1987), nasopharynx (Leela and Kanagasuntheram, 1973; Nakano, 1986), alimentary tract, cornea and conjunctiva (Andrews, 1976). Different terms have been used to describe these structures, such as microridges (Nakano, 1986) and microfolds (Fawcett, 1981). Andrews (1976) carried out a detailed study of microplicae and contended that they arise from plasmalemmal folds which once provided for intercellular

interdigitations and desmosome adhesions between adjacent cells. He further argued that although microplicae may merely represent the remnants of intercellular interdigitations, they may also have another specific function. He speculated that the interplical grooves may function to hold a layer of lubricating and cushioning mucin which would protect the underlying plasmalemma from abrasive abuse. The same view was also held by Fawcett (1981).

It was also established in the present study that further caudally in the nasal vestibule, apical microplicae of the surface cells were replaced completely by microvilli which became much longer in protected areas of the nasal cavity. The same observations have been noted in the rat (Andrews, 1974, 1979) , mouse (Greenwood and Holland, 1972) and pig (Adams, 1986). Whereas Andrews, (1979) reported an abrupt transition from the cornified squamous epithelium of the rostral region of the nasal vestibule in the rat to the caudal region, where the cells are covered by either microvilli or microplicae, in the present study this transition was observed to be gradual. In the horse the transition was also seen to be abrupt (Pirie, 1990).

The alar and basal folds were seen to be lined rostrally by a narrow zone of squamous epithelium. However, most of the alar and basal folds were lined by an intermediate type of epithelium primarily composed of nonciliated cuboidal cells exhibiting a typical "cobblestone" appearance probably due to the bulging nature of the surface cells, also previously described in the dog (Majid, 1986), horse (Pirie, 1990) and pig (Adams, 1990). The stratified nature of both these epithelial types was determined on histological sections. Such observations on this intermediate epithelium in the goat are in agreement with descriptions previously reported in several mammalian species (Adams *et al.*, 1970; Adams, 1972; Lenz, 1973; Andrews, 1979; Mygind *et al.*, 1982; Adams and Hotchkiss, 1983; Monteiro-Riviere and Popp, 1984; Popp and Martin, 1984; Majid, 1986; Harkema *et al.*, 1987; Adams, 1990; Larochelle and Martineau-Doize, 1990; Pirie, 1990), although this nonciliated cuboidal lining has only been recognised as a separate entity in dog (Adams and Hotchkiss 1983 Majid, 1986),

bonnet monkey (Harkema *et al.*, 1987), pig (Larochelle and Martineau-Doize, 1990) and horse (Pirie, 1990). Although the cuboidal epithelium has been referred to as a transitional epithelium (Majid, 1986), Pirie (1990) argues that it is not appropriate to refer to it as transitional since histological and SEM observations in the horse, and indeed in the dog (Majid, 1986) and goat in the present study, do not conform to the classical description of transitional epithelium (now renamed "uroepithelium") as found in the urinary tract.. A similar type of epithelium has been described in the nasopharynx of the mouse by Nakano (1986), who refered to it as an intermediate epithelium, a term which has been employed in the present study in the goat. Boysen (1982) has argued that such an intermediate epithelium observed in the human nasal cavity is a result of metaplasia, based on the fact that the area of distribution of this particular epithelial type was seen to increase with age. Larochelle and Martineau-Doize, (1990) also observed that this particular type of epithelium increased its area of distribution with age in pigs, and suggested that the increase was probably associated with additional protection against bacterial colonisation and infection, with the cuboidal cells probably involved in the non-specific antigen interaction. However, the present observations have been interpreted as being in agreement with recent findings in the monkey (Harkema *et al.*, 1987), dog (Majid, 1986) and horse (Pirie, 1990), where this nonciliated stratified cuboidal epithelium is regarded as normal and not a result of deciliation and metaplasia induced by any specific toxicant.

Two cell types were identified in the intermediate epithelial covering of the alar and basal folds. The first presented a dense aggregation of microvilli on its apical surface and tended to bulge slightly outwards, while the other cell type was identified as a mucus-producing cell.The latter cell type had sparse microvilli and the apical surface was either depressed or bulged with mucous granules which were visible through the plasmalemma. Histological sections stained with AB/PAS confirmed the presence of mucus-producing cells in this region. Adams (1990) also reported the presence of goblet cells, although numerically they formed a minor constituent of the epithelium

lining the folds. The use of the term "goblet cell" by Adams (1990) seems to be inappropriate, as these cells do not show a classical "goblet" shape when viewed with the light microscope.

Mucus-producing cells have been reported to present different morphological appearances with SEM depending on the stage of the cells' life cycle (Boysen, 1982; Monteiro-Riviere and Popp, 1984; Harkema *et al.*, 1987; Pirie, 1990). Immature mucus-producing cells have been described with a very low apical bulge with relatively dense aggregations of surface microvilli. As the cells become mature, the apical surface develops into a dome with mucous granules sometimes seen through the plasmalemma, and eventually surface microvilli disappear. Discharged cells usually present a collapsed apical surface with a pore or two through which mucus has been discharged.

Submucosal gland orifices observed in the alar and basal folds of the nasal mucosa of the goat are not unique to this species, as similar structures have also been reported in the pig (Adams, 1990), horse (Pirie, 1990), dog (Adams and Hotchkiss, 1983) and Bonnet monkey (Harkema *et al.*, 1987).

Caudal to the stratified cuboidal epithelium in the basal fold, there was a gradual change to a ciliated epithelium and in a few cases this change was seen to be abrupt. Where the change was gradual, a narrow zone composed of nonciliated microvillous, mucus-producing and ciliated cells was observed, the latter bearing short cilia of unequal lengths. These cells with imperfectly developed cilia were considered to be regenerating ciliated cells and have been described before by other workers (Bryant, 1916; Andrews, 1974, 1979; McDowell *et al.*, 1978; Iovannitti *et al.*, 1985; Pirie, 1990). Caudal to this transitional zone, the mucosa was thrown into alternating short folds and gutters which were longitudinally orientated and covered by a thick carpet of cilia.

The ciliated epithelium covering the caudal region of the basal fold was similar to that observed on the ventral, middle and dorsal nasal conchae. As observed in other mammalian species (Andrews, 1974; Adams and Hotchkiss, 1983; Popp and Martin,

1984; Majid, 1986; Harkema *et al.*, 1987), there was a gradual increase in the number of ciliated cells from rostral to caudal regions of the nasal cavity. Mygind and Bretlau (1973) also observed that in the anterior part of the human nasal cavity the cilia seldom formed a thick carpet as is the case distally in the respiratory tract.

This cilial carpet provides an essential component of the mucocilliary apparatus which moves trapped foreign material towards the pharynx from where it is eventually swallowed (Wright *et al.*, 1983; Dixon, 1992). The mucous blanket originates from two sources, from mucus-producing cells which are abundant in the gutters as well as being distributed on the folds, and from submucosal glands which are numerous in the gutters (Reid, 1954; McCarthy and Reid, 1964; Chakrin and Saunders, 1974; Jones *et al.*, 1975). Whereas the latter contribute to the sol layer of the mucous blanket, the former contribute significantly to the gel layer (Dulfano, 1973).

Studies by Larochelle and Martineau-Doize (1990) show that, although there are variations in the areas of distribution of the lining (squamous, transitional, respiratory and olfactory) epithelia in the nasal cavity of different age groups of piglets, the respiratory epithelium, similar to that described in the present study, lines more than 50% of the nasal mucosa from the level of the canine tooth up to the third premolar. Adams and Hotchkiss (1983) have estimated that between 40-50% of the total nasal cavity is lined by a typical ciliated respiratory epithelium, and although Majid (1986) does not give the percentage of the nasal mucosa covered by this type of epithelium, his findings appear similar to those reported by Adams and Hotchkiss. Studies by Harkema *et al.* (1987) in the Bonnet monkey also indicate that respiratory epithelium lines more than 60% of the lateral wall of the nasal cavity. In the present study, taking into consideration that the first ciliated cells started to appear at the level of the basal fold, and were the dominant cell type at the level of 2nd cheek-tooth, it would appear that ciliated respiratory epithelium covers more than half of the total area of the nasal cavity in the goat.

Patches of nonciliated microvillous cells were frequently observed on the ventral

and dorsal nasal conchae, with a few regenerating ciliated cells and mucus-producing cells amongst them. On the more caudally located middle nasal concha, such patches were infrequent in the goat, supporting earlier observations that the density of ciliation increased on moving caudally within the nasal cavity (Menco and Farbman, 1987). Harkema *et al.* (1987) have reported that of all the epithelial cell types to reach the lumen on the nasal conchae, 35% are nonciliated microvillous cells, although, they do not mention whether these are distributed individually amongst ciliated cells or occur in patches. Pirie (1990) reported the presence of large patches of nonciliated microvillous cells in the conchofrontal sinus of only one out of 23 horses examined; occasional nonciliated microvillous cells were encountered on the dorsal nasal conchae of the others.

The epithelium lining the middle regions of the nasal septum of the goat was seen to be ciliated, although the cilial carpet was disrupted by numerous nonciliated microvillous cells, most of which were identified by SEM as mucus-producing cells. This gave the epithelium a characteristic 'moth-eaten' appearance. Such an observation contrasts with findings reported by Pirie (1990) in the horse, where the nasal septum was nonciliated in these middle regions, being only completely ciliated in its most caudal part.. Ciliated cells on the nasal septum of the goat had short, poorly formed cilia.

Such observations in the goat contrast with findings in the rat (Popp and Martin, 1984), where, although different regions of the septum had a relatively constant mixture of cell types, being covered with ciliated cells and numerous interspersed mucus-producing cells, patches of nonciliated microvillous cells were not observed.

These patches of nonciliated microvillous cells observed on the nasal septum appeared to be composed primarily of two cell types. One exhibited a rounded or occasionally polygonal outline and carried a sparse population of surface microvilli, and was identified as a mature mucus-producing cell. The second cell type was characterised primarily by a dense population of surface microvilli and was thought to represent an

immature stage of a mucus-producing cell, a suggestion proposed for similar cell types found in man (Boysen, 1982), rat (Monteiro-Riviere and Popp, 1984), Bonnet monkey (Harkema *et al.*, 1987) and horse (Pirie, 1990). This suggests, therefore, that in the goat the number of mucus-producing cells in the epithelium lining the nasal septum is substantial. This is in agreement with observations in the rat (Popp and Martin, 1984; Katz and Merzel, 1977).

Although the nasopharynx of the goat occupies an extensive area, and the sample site used in this study was limited to an area a few millimetres rostral to the opening of the auditory tube, this opening was used as a landmark to ensure constancy in sampling. From observations in this study, it appears that this area represents a transitional zone, as up to three types of epithelia were encountered on a single sample. These were pseudostratified ciliated, stratified cuboidal and stratified squamous, the stratified nature of the epithelia being confirmed on histological sections.

Rostrally, the mucosa was thrown into longitudinally orientated alternating rows of folds and gutters; this region was narrow and resembled the epithelium lining the nasal conchae. Caudal to this zone there was a middle, much wider transitional zone, characterised by a progressive shift in the ratio of ciliated to nonciliated microvillous cells, until the most caudal region of this zone was entirely composed of the latter cell type. Caudal to this middle zone, a non-keratinized stratified squamous epithelium, similar to that lining the caudal region of the nasal vestibule, was observed. Such observations in the goat are in general agreement with similar observations made in the mouse (Nakano, 1986), where the rostral part of the nasopharynx is lined by ciliated epithelium, and the caudal part by an intermediate epithelium, merging finally into a stratified squamous epithelium. The presence of nonciliated microvillous cells as the predominant cell type in this transitional zone of intermediate epithelium, as observed in the goat, was also noted in the horse (Pirie, 1990) and rat (Andrews, 1974), but not in the dog, where the nasopharynx was reported to be covered rostrally by pseudostratified ciliated columnar epithelium containing numerous goblet cells and

caudally, towards the free border of the soft palate, by a non-keratinized squamous epithelium (Majid 1986). This lack of any mention of the presence of an intermediate epithelium in the dog may be due to the fact that the transitional zone between ciliated and stratified squamous epithelium was narrow and was therefore missed in the sampling procedures or was absent altogether, as is the case in the gibbon (Leela and Kanagasuntheram, 1973) .

Studies in other nonhuman primates (Leela and Kanagasuntheram, 1973) have revealed that the transition from a pseudostratified ciliated columnar to a stratified squamous type of epithelium is a graded one with different types of epithelia mixing and changing over gradually. This contrasts with a rapid appearance of a single intermediate type of epithelium forming this transitional zone as described by Bryant (1916) in the human. Nakano (1986) provided a detailed account of the nasopharyngeal epithelium in the mouse and was able to distinguish, by SEM and TEM, three types of epithelia (pseudostratified ciliated low columnar, stratified cuboidal and stratified squamous) and demonstrated that there was a gradual change from one type to the other. Although no TEM studies were done in the present study, SEM findings are in agreement with those made in the mouse.

The patches of nonciliated microvillous cells, arranged in a circular manner and raised into a low dome, which were encountered in the present study, may represent follicle-associated epithelium (FAE) similar to that which has been described in the nasopharynx of sheep (Chen *et al.*, 1991). Histological examination of these areas showed the overlying epithelium to be attenuated, composed of low columnar nonciliated cells and devoid of mucus-producing cells. This modified lymphoepithelium has been described histologically by Pirie (1990) and related to the prominent bulging areas observed by SEM in the nasopharynx of the horse. Chen *et al.* (1991) describe the FAE in the nasopharynx of the sheep as composed of either tall or flattened nonciliated cells presenting characteristic surface microvilli or microfolds, often intermingled with non-keratinized squamous cells. They have suggested that such cells represent cells

equivalent to the M cells described in other mucosal-associated lymphoid tissue (MALT) in animals and man (Wolf and Bye, 1984; Bienenstock, 1985). The M cells in the pharyngeal region were not distinguished from other microvillous cells with SEM but were identified by TEM in the rabbit (Bienenstock and Johnston, 1976), ox (Anderson *et al.*, 1986) and rat (Spit *et al.*, 1989).

The laryngeal mucosa of the goat in the present study was examined in three sites, namely the epiglottis, vocal fold and infraglottic cavity. The epiglottis was lined entirely by non-keratinized squamous cells similar to those described in the caudal regions of the nasal vestibule or nasopharynx. Such observations are in agreement with similar observations in the rat (Andrews, 1974), dog (Majid, 1986), mouse (Nakano and Muto, 1987) and mammals in general (Nickel *et al.*, 1979).

Pirie (1990) describes the epithelium of the dorsal surface of epiglottis of the horse to be mixed in character, with the stratified squamous merging into a stratified cuboidal epithelium, the latter containing a few mucus-producing cells. This contrasts with our observation in the goat, as no stratified cuboidal epithelium nor mucus-producing cells were observed. But this difference may be because in the present study the base of the epiglottis was not examined; the thickening of squamous cells towards the base that was observed suggests that, in the goat, the base may be lined by a stratified cuboidal epithelium similar to that in the horse. The number of submucosal gland duct orifices were few and did not correlate with the abundant submucosal glands observed on histological sections. The abundancy of these submucosal glands has been noted by LM in the dog (Majid, 1986) and horse (Pirie, 1990), although no mention of submucosal gland duct orifices at SEM level is made.

The laryngeal surface of the epiglottis of the goat was studded with taste buds. These appeared in the form of pores from which gustatory hairs were observed protruding. Extra-oral taste buds have been found to be present in the mucosa of the soft palate, pharynx and larynx of several mammalian species (Travers and Nicklas, 1990). The relative importance of these structure and the mechanisms under which they

are stimulated are not well understood. It is however assumed, because of their location, that they serve to protect the airway and play no role in food selection (Travers and Nicklas, 1990).

Individual variation was noted in the epithelium lining the vocal cords. In most of the goats the vocal cords were lined by squamous cells similar to those of the epiglottis. A few individuals had vocal cords lined by an intermediate epithelium and /or a ciliated epithelium. Such individual variation was much more pronounced in the infraglottic cavity of the larynx, with animals presenting a stratified and intermediate, or intermediate and ciliated, or completely ciliated, epithelial lining. The results of this study compare with similar observations made on the laryngeal mucosa of the dog, where individual variations from animal to animal with regard to the presence or absence of respiratory epithelium showed that in only four of the 18 animals examined was the infraglottic cavity lined by ciliated respiratory epithelium (Majid, 1986); in the present study, in only 3 out of the 17 animals examined was the infraglottic cavity completely ciliated. Such observations in the goat and dog, however, contrast with those made in the horse (Pirie, 1990), where the laryngeal epithelium caudal to the vocal fold was reported to be completely ciliated in all individuals examined. In the bovine, the cranial laryngeal mucosa was found to be lined by a stratified squamous epithelium whereas the caudal larynx was lined by a pseudostratified ciliated epithelium (Veit and Farrell, 1978). However these workers did not indicate the extent of either type of epithelium because precise anatomical landmarks were not described.

LOWER RESPIRATORY TRACT.

The present study has established that the tracheal mucosa of the goat is thrown into longitudinally orientated folds and intervening gutters, and lined by a heavily ciliated epithelium within which are found occasional patches of nonciliated microvillous cells. Submucosal gland duct openings are frequently encountered. Three

cell types are distinguished on the basis of their luminal surface characteristics, the ciliated, nonciliated microvillous and mucus-producing cells.

The present detailed SEM observations of the tracheal lining of the goat in consisting primarily of a ciliated epithelium composed of ciliated, mucus-producing and nonciliated microvillous cells in varying proportions, correspond to those previously described for the dog (Majid, 1986), horse (Pirie, 1990), ox (Iovannitti *et al..*, 1985), cat (Tandler *et al.*, 1983[a,b]), mouse (Pack *et al.*, 1980), ferret (Hyde *et al.*, 1979) and in lesser details for the mouse (Greenwood and Holland, 1972), human (Wang and Thurlbeck, 1970), monkey (Greenwood and Holland, 1973Wilson *et al.*, 1984), and rabbit (Plopper *et al.*, 1983[b]) In the present study, the tracheal mucosa was heavily populated with ciliated cells. These cells presented similar characteristics to those reported in other species and were more numerous on the folds, whereas in the gutters nonciliated microvillous cells became more frequent. The same distribution of ciliated cells has been reported in the ferret (Hyde *et al.*, 1979), cat (Tandler *et al.*, 1983[a,b]), dog (Majid, 1986) and horse (Pirie, 1990). In the present study, dorsal and ventral surfaces were compared and no significant differences in cilial lengths were noted. In SEM studied on nonhuman primates, however, Wilson *et al.* (1984) established that cilia were shorter on the anterior (ventral) compared to the posterior (dorsal) tracheal surface.

Cells which were identified as regenerating ciliated cells were frequently encountered, especially in areas devoid of ciliated cells. Such cells were characterised by numerous microvilli and very few cilia of unequal lengths, such features being similar to those described for regenerating ciliated cells in the rat by Andrews (1979). Menco and Farbman (1987) have provided a detailed study of thegenesis of cilia and microvilli in rat nasal epithelium, and, along with studies of developing respiratory tract cilia in the rabbit by Kanda and Hilding (1968), these observations generally support the description of regenerating ciliated cells in the present study. The presence of these cells is a result of normal sloughing and the subsequent regeneration of new ciliated

cells (Rhodin, 1966).

Within the tracheal epithelial lining of the goat, nonciliated microvillous cells were frequently encountered in the gutters and occasionally found on the folds. On the folds, they were present either singly or in groups of two or three cells, each cell exhibiting a low luminal surface bulge carrying densely organised microvilli of uniform length. The same cell type has been described in a number of mammalian species including the cat (Tandler *et al.*, 1983[a,b]), the hamster (Becci *et al.*, 1978), the ferret (Hyde *et al.*, 1979), the rat (Alexander *et al.*, 1975; Andrews, 1979), the dog (Wright *et al.*, 1983; Majid, 1986), and nonhuman primates (Wilson *et al.*, 1984),. The cells which populated the gutters had different surface characteristics in that their luminal surface was slightly depressed and carried relatively fewer surface microvilli. This latter type has been observed in cats to be abundant in gutters and very rare on the folds (Tandler *et al.*,1983[a,b]).

The pattern of distribution of these nonciliated microvillous cells, when considered alongside the light microscopical observations on numbers and distribution of AB/ $PAS^{+}$ cells within the tracheal epithelium, suggests that the majority of these cells identified at the SEM level were mucus-producing. 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 in part by the studies of Mariassy and Plopper (1983) on the tracheobronchial epithelium in sheep. With the light microscope they identified four categories of mucus-producing cells (M1, M2, M3 and M4), the difference between them being the relative sizes of the cells and the secretory granules, and the nature of the secretory granules produced. Subsequent morphometric studies of these cells at transmission electron microscopic level (Mariassy and Plopper, 1984) showed very little difference between M1 and M2, suggesting that these two categories may be mere variations of one and the same cell type.

Some of the non-ciliated microvillous cells in the goat could definitely be

identified as mucus-producing cells of the one or the other type, as they appeared to present observable differences in the form of mucus discharged. Those usually found on the fold presented a characteristic apical protuberance in which, on some occasions, discrete mucous granules could be seen accumulating beneath the plasmalemma prior to release. Those exclusively confined to the gutters usually produced a sheet of mucus which tended to mat the adjacent cilia and often form a covering film of mucus on the luminal surface of the cells. The viscosity of the mucus produced by these two populations of cells also seemed to differ, taking into account the way the mucus was released from these cells. The fact that the viscosity of mucus is dependent on biochemical (especially disulphide bonds) and physical properties (Dixon, 1992) suggests that these two cell types may therefore be distinct both morphologically and histochemically.

SEM studies of the trachea of the rat (Alexander *et al.*, 1975; Andrews, 1979) and the ferret (Hyde *et al.*, 1979) have identified a fourth cell type characterised by a pentagonal luminal surface outline carrying a dense population of long, thick microvilli, and referred to as a brush cell. Such a cell type was not identified in the present study despite careful examination of the tracheal samples from 17 different individual animals. Other SEM studies in the cat (Tandler *et al.*, 1983[a,b]), ox (Iovannitti *et al.*, 1985), dog (Frasca *et al.*,1968; Majid, 1986) and horse (Pirie, 1990) have also failed to identify the presence of this particular cell type in the trachea. However, TEM studies in the cow (Allan, 1978), pig (Baskerville, 1970[a,b]) and mouse (Pack *et al.*, 1981) have indicated its presence, but only in constituting up to 1% of the total tracheal cell population. The failure to observe these cells in the caprine trachea in the present study may possibly be explained partly by the fact that these cells are relatively rare and partly by the fact that they may be obscured by the cilial carpet. The function of this particular cell type is not clear (Breeze and Wheeldon, 1977), although both chemoreceptive and absorptive functions have been attributed to it (Breeze and Wheeldon, 1977; Allan, 1978).

The patches of nonciliated microvillous cells occasionally found distributed in

the tracheal mucosa of the goat in the present study have also been reported to punctuate the tracheal cilial carpet of the dog (Majid, 1986), horse (Pirie, 1990) and ox (Iovannitti *et al.*, 1985). Pirie (1990) encountered these patches in only a few horses and attributed these to subclinical infection. However in the present study, these patches were found in every individual examined and it is thought likely that the patches are a normal feature of the tracheal epithelium of the goat. It is possible that such patches represent a covering epithelium associated with respiratory-tract-associated Lymphoid Tissues (RTALT). Such tissues have been studied in sheep (Chen *et al.*, 1991) and in cattle (Anderson *et al.*, 1986) by the use of SEM and a characteristic type of epithelium has been identified overlying these aggregates of lymphoid tissue. This epithelium is characterised by nonciliated microvillous cells, is devoid of mucus-producing cells and appears similar to the patches observed in the present study.

Submucosal gland duct orifices surrounded primarily by nonciliated microvillous cells were seen in the gutters. This is in agreement with observations made in the rat (Alexander *et al.*, 1975), cat (Tandler *et al.*, 1983[a]), ox (Iovannitti *et al.*, 1985), dog (Wright *et al.*, 1983; Majid, 1986), monkey (Wilson *et al.*, 1984) and horse (Pirie, 1990). Although Tandler *et al.* (1983[a]) were able to categorise these ostia into three types in the cat, the present study was unable to confirm their findings in the goat.

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 the bronchi decreased there was an increase in the number of nonciliated microvillous cells at the expense of ciliated cells. This gradual decrease of ciliated cells towards the distal conducting airways has been reported in most mammalian species including the rat (Alexander *et al.*, 1975), guinea pig (Davis *et al.*, 1984), hamster (Becci *et al.*, 1978), dog (Wright *et al.*, 1983; Majid, 1986), pig (Winkler and Cheville, 1984), ox (Mariassy *et al.*, 1975Iovannitti *et al.*, 1985), horse (Nowell and Tyler, 1971; Pirie 1990), monkey (Greenwood and Holland, 1973; Castleman *et al.*, 1975 Wilson *et al.*, 1984;) and man (Wang and Thurlbeck, 1970;

Ebert and Terracio, 1975[a]; Greenwood and Holland, 1975).

In the bronchial mucosa of the goat, submucosal gland duct orifices also became progressively fewer with decreasing airway diameter.It is known that in mammalian species, mucus is produced from two sources namely, surface mucus-producing cells and submucosal glands (Reid, 1954; McCarthy and Reid, 1964; Chakrin and Saunders, 1974; Jones *et al.*, 1975), it implies therefore, that in the bronchial mucosa, the former became more prominent as a source of tracheal mucus. Such observations are in agreement with studies in the dog (Majid, 1986), horse (Nowell and Tyler, 1971; Pirie, 1990), ox (Mariassy *et al.*, 1975; Iovannitti *et al.*, 1985), rat (Alexander *et al.*, 1975), mouse (Wang and Thurlbeck, 1970; Greenwood and Holland, 1972) and ferret (Hyde *et al.*, 1979). Examination of the extrapulmonary bronchial epithelium revealed that the cellular population and distribution more closely resembled that of the trachea than the lobar bronchi.

A number of similarities and differences in the bronchiolar epithelium were noted between the goat and other mammalian species. Proximal to the terminal bronchiole, epithelial morphology was essentially the same as that which has been described in man (Wang and Thurlbeck, 1970; Ebert and Terracio, 1975[a]; Greenwood and Holland, 1975), rat (Andrews, 1974, 1979; Alexander *et al.*, 1975), mouse (Wang and Thurlbeck, 1970; Greenwood and Holland, 1972), hamster (Becci *et al.*, 1978), monkey (Greenwood and Holland, 1973; Castleman *et al.*, 1975; Wilson *et al.*, 1984; Tyler and Plopper, 1985), pig (Winkler and Cheville, 1984), horse (Nowell and Tyler, 1971), ox (Iovannitti *et al.*, 1985; Mariassy *et al.*, 1975) and dog (Wright *et al.*, 1983; Majid, 1986). Histological studies showed that the epithelium lining the bronchioles was of a pseudostratified ciliated columnar type, whilst with SEM, ciliated cells, mucus-producing cells and nonciliated bronchiolar epithelial (Clara) cells were identified. The same epithelial type and cellular population has been reported in the Rhesus monkey (Tyler and Plopper, 1985), dog (Majid, 1986), ferret (Hyde *et al.*, 1979) and rat (Andrews, 1979), although such findings contrast with studies in the

horse which show that the type of epithelium lining the bronchiole is simple columnar to cuboidal in type (Pirie, 1990). However, the bronchiole - terminal bronchiole junction was seen to be rather abrupt in terms of epithelial height and cell types in the goat, with the pseudostratified columnar epithelium of ciliated and nonciliated microvillous cells lining the bronchioles changing into a simple columnar or cuboidal epithelium, composed primarily of Clara cells, lining the terminal bronchioles. Such an arrangement has also been reported in the ferret (Hyde *et al.*, 1979), rodents (Plopper *et al.*,1980[a]; Plopper *et al.*, 1983[b]) and stumptail and macaca monkeys (Castleman *et al.*, 1975).

Mariassy *et al.* (1988) described the bronchiolar mucosa in the sheep as composed of approximately equal numbers of ciliated and nonciliated cells. In the goat this was only true in the very distal generations of the bronchioles; in the proximal generations, ciliated cells were always found to be in the majority. These workers further described ciliated cells in the bronchiolar region as having slightly shorter cilia than those in the bronchi. The same observation was noted in the present study and by other workers in the rat (Andrews, 1979) and the ox (Iovannitti *et al.*, 1985). The folds and gutters observed in the trachea and bronchi were reduced to shallow surface folds in the larger bronchioles, such folds being reduced to shallow undulations in smaller bronchioles. The same observation has been reported in the horse (Pirie, 1990).

In the goat, as observed in the present study, the surface of the Clara cells usually presented a characteristic, dome-shaped apical protuberance in line with previous descriptions at SEM level in a variety of mammalian species including mouse (Okada, 1969), rabbit (Plopper *et al.*, 1983[b]), ox (Iovannitti *et al.*, 1985), horse (Pirie, 1990), rat (Andrews, 1979) and man (Wang and Thurlbeck, 1970). Such protuberances occasionally appeared to be "withering" instead of full. Sometimes the Clara cells presented a flattened surface appearance, occasionally with a very low central bulge, a form also observed in the rabbit (Plopper *et al.*, 1983[b]). 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 be cells at an early stage of maturity. Smith *et al.*, (1979) have grouped these different forms of the Clara cell into separate populations and have pointed out that these may co- exist in the same animal. In addition, 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 described by Smith *et al.* (1979) and to the observations made in the goat in the present study.

In the goat some of these nonciliated bronchiolar cells, especially those with apical protuberances, appeared to resemble, in some respect, the mucus-producing cells of the tracheobronchial epithelium. The two cell types could be distinguished, however by the use of histological sections stained with AB/PAS. This produced a negative reaction in the Clara cells of the terminal bronchiolar epithelium, as also observed by Azzopardi and Thurlbeck (1969). It could be argued that maybe these nonstaining cells were immature mucus-producing cells, as only mature mucus-producing cells with stored glycoproteins could be expected to take the stain. However the present study showed that many of these nonciliated bronchiolar epithelial cells appeared to be mature or adult cells similar to those described by Lauwyerns *et al.* (1969) which, if mucus-producing, would have stained positively with AB/PAS; this did not occur, confirming that in all probability these were Clara cells.

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 (Getty, 1975), and in the ox in particular (Iovannitti *et al.*, 1975), respiratory bronchioles are usually absent, and, if present, are poorly developed. It would therefore appear inappropriate to suggest that the lack of respiratory bronchioles is a typical feature of the ruminant lung. Respiratory bronchioles have been reported to be present in the dog (Majid, 1986), ferret (Hyde *et al.*, 1979), coyote (Morrison *et al.*, 1983), pig (Baskerville, 1970[a]; Winkler and Cheville, 1984), human (Ten Have-Opbroek *et al.*, 1991) and monkey (Tyler *et al.*,

1983; Tyler and Plopper, 1985) but were absent in the horse (Pirie, 1990).

A simple cuboidal epithelium composed of ciliated and Clara cells was observed in the present study to line the respiratory bronchioles of the goat lung. This epithelial organization differs considerably from that of other mammalian species in which respiratory bronchioles are present. In the dog, although the epithelium is simple cuboidal, the cellular population is quite different, with the Clara cell being the only cell type found in this region (Wright *et al.*, 1983; Majid, 1986). In the rat (Andrews, 1979), Rhesus monkey (Tyler and Plopper, 1985) and humans (Ten Have-Opbroek *et al.*, 1991), although the lining epithelium of the respiratory bronchioles is similar to that seen in the goat, an additional type of pseudostratified ciliated epithelium is also found overlying accompanying branches of the pulmonary artery.

SEM studies of bronchioles in the lungs of laboratory animals (Cutz *et al.*, 1978; Hung *et al.*, 1979) have indicated the presence of bronchiolar neuroepithelial bodies which appear as isolated organoids along the entire length of the bronchiolar tree, usually at branching points. In the present study, such organised morphological entities could not be identified. Failure to observe neuroepithelial bodies is not surprising as the previously reports confirming their presence have been confined to fetuses and neonates. SEM studies by Wright *et al.* (1983) also failed to reveal their presence in the lungs of the puppy.

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; Kuhn and Finke, 1972; Mariassy *et al.*, 1975; Andrews, 1979; Wright *et al.*, 1983; Iovannitti *et al.*, 1985; Majid, 1986; Pirie, 1990). The slightly raised boundaries of the Type I cell were not easily identifiable, supporting previous observations in 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[a,b]), 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, characterised 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. Majid (1986),in his study in the dog, also encountered difficulties in observing alveolar macrophages with routinely prepared SEM tissues; however, using material obtained from alveolar lavage, and further TEM work, he was able to confirm that alveolar macrophages were a normal component of the canine alveolar cell population. Numbers of alveolar macrophages appear to vary from species to species, being apparently numerous and easily identified in the ferret (Hyde *et al.*, 1979), fewer in number in the horse (Pirie, 1990), where they present long filamentous pseudopodia, and rare in monkeys (Greenwood and Holland, 1972). In the ox such cells were easily identified by Iovannitti *et al.* (1985), but Mariassy *et al.* (1975) found them to be rare. Alveolar macrophages form an important defence mechanism against bacteria and other foreign bodies. Thus in pathological conditions the number of alveolar macrophages has been seen to increase considerably. (Greenwood and Holland, 1972; Majid, 1986).

In the goat, interalveolar pores (of Kohn) were found to be few, in contrast to observations made in ox (Iovannitti *et al.*, 1985), rat (Andrews, 1979), dog (Wright *et al.*, 1983; Majid, 1986), ferret (Hyde *et al.*, 1979) and man (Burri, 1985). Methods of fixation of lung tissue have been seen to produce different results in relation to the abundancy of interalveolar pores. Intravascular fixation of the lung parenchyma tended to preserve the alveolar surfactant, thus covering the pores, whilst airway instillation of fixative had the effect of removing the surfactant and revealing pores (Parra *et al.*, 1978; Shimura *et al.*, 1986). As the latter method was used in the present study, 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 number of alveolar pores tends to increase with age (Shimura *et al.*, 1986Pirie, 1990); in the present study this could not be established as there were no significant differences in

age among the animals used.

# CHAPTER 5.

# TRANSMISSION ELECTRON MICROSCOPY OF THE DISTAL AIRWAYS AND ALVEOLAR MEMBRANE IN THE LUNG OF THE ADULT GOAT.

# INTRODUCTION.

The purpose of this study was to use the transmission electron microscope to further characterise and identify, cytologically, those cell types previously observed by light and scanning electron microscopy to populate the distal airways, and to define the ultrastructural characteristics of the alveolar membrane.

The use of different terminologies in the study of pulmonary morphology has led to some confusion in trying to identify structures described at different levels of the respiratory tract by different workers (Iovannitti, 1984). In the present study, the term "distal airway" is applied to"the region of the respiratory system including the terminal bronchioles through alveoli"as defined by Davis *et al.* (1984) and used previously in an undefined context (Baskerville, 1970[a]; Hyde *et al.*, 1979; Tyler and Plopper, 1985).

TEM studies were concentrated on the distal airways because of their physiological and clinical importance in the gaseous exchange mechanism between tissues and the environment, and the fact that it is these airways that frequently show the primary effects of pulmonary pathology caused by such factors as infectious agents, genetic disorders and inhaled toxic agents (Denny *et al.*, 1977; Castleman *et al.*, 1980; Boucher *et al.*, 1983; Pirie, 1990). It is understandable, therefore, that many recent studies on the ultrastructure of the lung have concentrated on the distal airways (refer Table 4.1) with the aim of defining the epithelial cell populations lining these regions. Ultrastructural studies of the distal airways in farm animals have been carried out in the pig (Baskerville,1970[a];Epling,1964[a,b]), the ox (Mariassy *et al.*, 1975; Iovannitti *et al.*, 1985) and the horse (Gillespie and Tyler, 1967; Nowell and Tyler, 1971; Pirie *et al.*, 1991[b]). However, in the goat, such reports appear to be limited to the ultrastructure of the alveolar epithelial cells and to descriptions of the transmission electron microscopic anatomy of the pulmonary blood-air barrier (Atwal, 1988; Atwal and Sweeny, 1971; Atwal et al., 1979; Atwal and Saldanha, 1985).

In Chapters 3 and 4 the entire epithelial lining of the respiratory airway of the

goat, from the nasal vestibule to the alveoli, was investigated by the use of the light and scanning electron microscope with the aim of identifying both the surface characteristics of the different cell types and their distribution within the airways. In the lower respiratory tract, bronchioles were seen to be characterised by different cell types at different levels: Proximal to the terminal bronchioles they were lined by a pseudostratified ciliated epithelium composed of ciliated, mucus-producing and nonciliated microvillous cells, whilst at the level of the terminal bronchiole the epithelium changed to a simple low columnar to cuboidal lining composed of ciliated and nonciliated bronchiolar epithelial (Clara) cells. At this level, mucus-producing cells could not be identified. Respiratory bronchioles, characterised by the presence of alveoli within their walls, were lined by a simple cuboidal epithelium in the non-alveolar region, and a simple epithelium composed of alveolar Type I and alveolar Type II cells in the alveolar region. The latter two cell types typically characterised the alveolar lining.

The SEM characteristics of the cells lining the distal airways of the goat lung, as described in Chapter 4, are similar to those described in other mammalian species. Previous studies have shown however that, whereas SEM characteristics are fairly uniform, TEM ultrastructural characteristics appear to differ from species to species, especially those of the nonciliated bronchiolar epithelial (Clara) cell, which has been extensively investigated (Cutz and Conen, 1971; Smith and Moosavi, 1974; Smith et al., 1979; Plopper *et al.*, 1980[a-c]; Plopper *et al.*, 1983). Not only do the ultrastructural characteristics of the cell types differ between species, but also their distribution.

## LITERATURE REVIEW.

At least eleven different cell types have been identified in the epithelial population lining the airways of the lower respiratory tract in mammals. These cells have been characterised and differentiated from each other on the basis of both cellular

morphology and distribution (Breeze and Wheeldon, 1977; Mariassy and Plopper, 1983). The cell types so far identified are mucus-producing, ciliated, nonciliated bronchiolar epithelial (Clara), brush, basal, intermediate, special, serous, pulmonary neuroendocrine, alveolar Type I and alveolar Type II cells. Three of these cell types are thought to be secretory, the mucus-producing (goblet), the serous and the nonciliated bronchiolar epithelial (Clara) cells (Breeze and Wheeldon, 1977; Reid and Jones, 1979). A brief review of the cellular characteristics of each individual cell type is given below.

MUCUS-PRODUCING CELLS.

Mucous cells are found throughout almost the entire lining epithelium of the respiratory tract (Breeze and Wheeldon, 1977), where they usually occur in groups of three or four and account for about a quarter of the total cell population of the columnar layer (Frasca *et al.*, 1968). However their presence in terminal bronchioles and respiratory bronchioles has not been ascertained in many mammalian species.

Mucus-producing cells have a narrow, columnar shape, with a base usually tapering off towards the basement membrane. Each individual mucus-producing cell is surrounded by an irregular space that is closed at the luminal surface by tight junctional complexes between adjacent cells (Bozarth and Strafuss, 1974). The nucleus is usually located towards the base of the cell and is round to slightly oval in shape (Rhodin, 1966).

The cytoplasm is relatively dense and electron-opaque compared to that of ciliated or basal cells, due in part to the presence of many mucous granules and ribosomes which are found in their apical cytoplasm. The granules are of variable size, the density of their contents varying from a high density fibrillar to a low density homogeneous nature (Frasca *et al.*, 1968).

In the supranuclear position, a well developed Golgi apparatus is usually found

(Breeze and Wheeldon, 1977). It is generally accepted that the mucous granules develop as pre-mucous granules from Golgi membranes and vacuoles (Rhodin, 1966).

One of the characteristics of a mature mucus-producing cell is the dome-shaped apical surface, which usually protrudes between the adjacent cells into the lumen of the respiratory tract (Baskerville, 1970[a]). It is in this apical protuberance that mucous granules are concentrated; fusion of the granules may occur as the limiting membranes become less distinct (Rhodin, 1966). The mucous granules are discharged from the cell, often still within an intact limiting membrane (Breeze and Wheeldon, 1977); such secretion is thus of the apocrine type (Freeman, 1966).

Cells which are presumed to be immature mucus-producing cells are also encountered in the epithelium, and have been extensively investigated by Frasca *et al.* (1968). In size and shape they resemble ciliated cells except that, instead of cilia, many cytoplasmic processes (microvilli) project from their apical surfaces. Surface microvilli are often found around the apical cell margin and, although found on mature cells, are more numerous on immature and discharged cells, where they may be found all over the cell surface (Baskerville, 1970[a,b]; Greenwood and Holland, 1972; Andrews,1974).

CILIATED CELLS.

Ciliated cells are found in airway epithelium from the nasal cavity down to the small bronchi (Sturgess and Czegledy-Nagy, 1978), and are the commonest cell type in the conducting portion of the mammalian respiratory tract, averaging, for example, five ciliated cells to every mucus-producing cell in the human trachea (Rhodin, 1966).

Within the epithelium the ciliated cell extends from the basement membrane to the luminal surface, being narrow and roughly columnar in shape, with a tendency of tapering basally. The lower lateral surfaces form complex interdigitations and desmosomal attachments with adjacent cells (Frasca *et al.*, 1968; Hansell and Moretti, 1969; Baskerville, 1970[b]); cell contacts towards the luminal surface usually tend to be

straight.

The cytoplasm is usually more electron-lucent compared to the mucus-producing and other nonciliated epithelial cells, due to the relative lack of secretory products or granules and the sparse distribution of ribosomes (Rhodin, 1966; Breeze and Wheeldon, 1977). Lysosomes are numerous, and intracytoplasmic tonofilaments are found within the cytoplasm although they are not as abundant as those found in the basal cells (Rhodin, 1966).

Towards the tapered end of the cell, an irregularly spherical to ovoid nucleus is found, the shape of which corresponds to the shape of the cell (Rhodin, 1966; Tesik, 1984). In most mammalian species, the Golgi apparatus is well developed and is found in the immediate supranuclear position (Frasca *et al.*, 1968; Breeze and Wheeldon, 1977). Large aggregates of glycogen rosettes have been found in many ciliated cells; although these are usually in close association with the basal aspect of the nucleus, they are occasionally found at the apical aspect and, on rare occasions, surround the nucleus (Frasca *et al.*, 1968).

One of the chief characteristics of ciliated cells, which has been clearly elucidated by the use of SEM and TEM, is the presence of numerous cilia at the luminal surface of these cells. The cilia are anchored within the cytoplasm by means of basal bodies from which rootlets extend into the apical part of the cell (Friedmann and Bird, 1971). The cilia have an average length of about 6μm, although the length and density of the cilia have been seen to differ considerably depending on the stage of development of the cell (Hilding, 1965; Hilding and Hilding, 1966). The structure of the cilia is basically the same for all ciliated cells in animals, each cilium containing nine paired peripheral microtubules surrounding a central pair. The microtubules are longitudinally orientated and surrounded by an extension of the cell membrane (Frasca *et al.*, 1968; Baskerville, 1970[a]). The two central tubules fuse at the tip of the cilium and do not extend to the basal bodies as the others do.

Filiform projections, referred to as microvilli, which are short and have no

internal structure, are also characteristic of ciliated cells. The plasma membrane covering the microvilli has a "fuzzy" appearance due to the presence of a 'naplike' coating of fine filaments which may interweave with those from the neighbouring microvilli (Okano and Sugawa, 1965; Adams, 1986).

Ciliated cells, through the action of cilial propulsive force, transport trapped material within the mucus blanket to the pharyngeal region, where it is eventually swallowed. Thus ciliated cells form an important component of the mucociliary escalator system (Kilburn, 1968; Kaltreider, 1976). In vitro studies have suggested that the ciliated cell may also have a secretory function, as it has been observed to play a role in the transport of ions and water (Nadel *et al.*, 1985) and the release of macromolecules across its luminal surface (Varsano *et al.*, 1987). However, details of in vivo release and the significance of these macromolecules are uncertain at this time (Basbaum and Finkbeiner, 1989)

NONCILIATED BRONCHIOLAR EPITHELIAL(CLARA) CELLS.

A description of the nonciliated bronchiolar epithelial (Clara) cell was first provided by Kolliker in 1881, its presence being later confirmed by Clara (1937) after whom it was named.

Clara cells are primarily distributed within the bronchioles. They are most numerous in the terminal bronchioles (Smith et al., 1979; Plopper, 1983), with 66% of terminal bronchiolar epithelium in the rabbit, 73% in the guinea pig, 55% in the hamster and 67% in the mouse being composed of nonciliated bronchiolar epithelial cells (Plopper *et al.*, 1980[a]); in the horse they account for 55-75% of the terminal bronchiolar epithelial population (Plopper, 1983). They have been encountered as far proximally as the trachea in the mouse (Hansell and Moretti, 1969; Pack *et al.*, 1981) and rabbit ( Plopper, 1983), and distally as far down as the respiratory bronchioles in the dog (Majid, 1986).

The Clara cell rests on the basement membrane, the lateral plasma membranes forming complex interdigitations, including desmosomes, with adjacent epithelial cells (Cutz and Conen, 1971).

With the light microscope, nonciliated bronchiolar epithelial cells appear columnar and sometimes cuboidal, have a deeply invaginated central nucleus, and usually have a club-shaped protoplasmic process projecting into the lumen beyond the ciliated cells (Breeze and Wheeldon, 1977). With SEM, the luminal surface is seen to be bulging in shape, and carry short and stubby microvilli. Some species differences have been noted as regards the surface microvilli - in rabbits, for example, Clara cells have a relatively smooth luminal surface with relatively few, small microvilli (Cutz and Conen, 1971), whereas in other species, such as the rat, the guinea pig and the mouse, the apical surface is studded with numerous short stubby microvilli (Plopper *et al.*, 1980[a-c]).

There is a considerable variation between species in the TEM ultrastructure of the nonciliated bronchiolar epithelial cells (Smith *et al.*, 1979; Plopper *et al.*, 1980[a-c]; Widdicombe and Pack, 1982). The latter compared the ultrastructure of Clara cells in fifteen mammalian species, including man, and described three morphologically distinct categories based on the presence and abundance of secretory granules, rough endoplasmic reticulum (RER) and smooth endoplasmic reticulum (SER). Secretory granules and SER are major cell components in the horse and sheep, but not in the ox; in dogs and cats, however, the cells are characterised by an abundancy of cytoplasmic glycogen granules. SER is not a prominent feature of Clara cells in man.

Although there is a controversy as regards the function of Clara cells (Majid, 1986), it is generally accepted that the cells are secretory in nature (Breeze and Wheeldon, 1977; Massaro, 1989) as indicated by the histochemical profile, the abundant mitochondria, extensive SER and prominent Golgi zone. Cutz and Conen, (1971) have suggested that Clara cells might be an important source of the hypophase component of the alveolar lining layer. Since they contain abundant cytochrome P-450,

it has also been suggested that they play a role in the metabolism of pulmonary toxins through a mixed-function oxidase system (Plopper *et al.*, 1980[a-c]). These functions will be considered in the discussion.

Several workers have isolated a common antigen in secretory proteins of Clara cells and alveolar Type II cells of both man (Balis *et al.*, 1984; and ratWalker *et al.*, 1986), and their findings suggest that the two cells may share a functional role. Recent studies in man have suggested that the cuboidal Clara cells of the respiratory bronchioles are Type II cells (Ten Have-Opbroek *et al.*, 1991).

## BRUSH CELLS.

The infrequent occurrence of brush cells was first reported by Rhodin and Dalhamn, (1956) in the rat tracheal epithelium. Since then their presence has been reported in the bronchi of pigs (Baskerville, 1970[a]) and calves (Allan, 1978), in the upper and lower airways of the mouse (Hama and Nagata, 1970; Breeze and Wheeldon, 1977), in the trachea of the guinea pig (Inoue and Hogg, 1974) and in the trachea and principal bronchi of the rat (Alexander *et al.*, 1975).

The brush cell is tall and always extends from the basement membrane to the luminal surface. It seldom occurs singly in between ciliated cells, but is usually surrounded by four to six mucus-producing cells (Rhodin and Dalhamn, 1956; Andrews, 1979). The apical surface is commonly pentagonal (Alexander *et al.*, 1975) and carries very regularly arranged, more densely packed, uniformly sized microvilli, which are wider and taller than the microvilli of other nonciliated cells, their intracellular axial filaments being finer and much more uniform (Allan, 1978).

The cytoplasm is more electron-lucent than that of mucus-producing cells (Rhodin and Dalhamn, 1956) and contains free ribosomes as well as glycogen granules. The nucleus is spherical with indentations, so as to appear multilobate, and is located in the lower part of the cell just below the Golgi zone (Adams, 1986), at more or less the

same level as the nuclei of neighbouring ciliated and mucus-producing cells. Desmosomes are infrequently observed (Meyrick and Reid, 1968). Mitochondria are abundant in the supranuclear region of the cell. Vacuoles of variable diameter, bounded by a thick, opaque membrane enclosing a much less opaque centre, are commonly located in the basal region of the cell (Rhodin and Dalhamn, 1956).

Although the ultrastructural details of the brush cell have been studied in depth, their function is still speculative. The presence of pinocytotic vesicles located at the cell apex, as well as the presence of intracytoplasmic vacuoles, strongly suggests that the cell has an absorptive function, and this hypothesis has gained much support (Allan, 1978; Baskerville, 1970[a]; Jeffery and Reid, 1975). Breeze and Wheeldon (1977) have suggested that the nerve endings observed to contact the lateral borders of the brush cell are related to a chemoreceptor role for these cells. Jeffery and Reid (1975) have also suggested that the brush cell may function as a stretch receptor due to the presence of intracytoplasmic filaments, the latter having been noted by Allan, (1978).

BASAL CELLS.

These are the smallest cells of the epithelial cell population. They usually rest on the basement membrane and characteristically do not reach the luminal surface; it is this feature that accounts for the observed pseudostratified nature of the epithelium. Basal cells are most numerous in the trachea and decrease in numbers from trachea to bronchi (Evans and Shami, 1989). In the rat, they are found as far distally as the bronchioles (Jeffery and Reid, 1975). They are characteristically either ovoid in shape, or rather flat and sometimes triangular (Plopper *et al.*, 1983).

The irregular intercellular space surrounding the basal cell is crossed by numerous cytoplasmic projections attaching the cell to adjacent cells, or sometimes to the basement membrane, frequently by means of desmosomes (Rhodin and Dalhamn, 1956; Frasca *et al.*, 1968).

Most of the cell is taken up by the electron-dense nucleus, which is centrally situated and has an irregular outline with shallow indentations (Frasca *et al.*, 1968). The nucleolus is prominent. The Golgi apparatus occupies only a small zone near the nucleus, usually at an indentation. A few short ovoid mitochondria with well formed cristae usually surround the nucleus. The cytoplasm contains many ribosomes and intracytoplasmic filaments, these then accounting for the observed increased cytoplasmic opacity as compared to ciliated cells or mucus-producing cells (Monteiro-Riviere and Popp, 1984).

Basal cells were originally considered to be the precursors of nonciliated and ciliated cells (Blenkinsopp, 1967). However recent studies (McDowell *et al.*, 1984[a,b]; McDowell *et al.*, 1985; Evans *et al.*, 1986; Plopper *et al.*, 1986) have confirmed that the basal cell is not responsible for cell renewal in the columnar epithelium, since it was also observed that basal cells do not increase in number following injury (Keenan *et al.*, 1982[a-c]). It is now generally accepted that the cellular specialization of the basal cell is the formation of desmosomes with adjacent cells, and hemidesmosomes with the basement membrane (Kawanami *et al.*, 1979). Since it has been observed that the columnar epithelial cells themselves do not appear to form hemidesmosomes with the basement membrane, it has been proposed that the function of the basal cell may be to aid in the attachment of the columnar epithelium to the basement membrane (Evans and Shami, 1989).

INTERMEDIATE CELLS.

Intermediate cells have been described in the tracheal epithelium of rats (Rhodin and Dalhamn, 1956) and man (Rhodin, 1966), and in the bronchial epithelium of pigs (Baskerville, 1970[a,b]). They are situated just above the basal cells and they may or may not reach the luminal surface. They are spindle-shaped with a large ovoid nucleus.

Rhodin (1966) suggests that since the intermediate cell is less specialised, a

further differentiation would turn it into either a ciliated cell or a mucus-producing cell; cytoplasmic opacity depends, to a great extent, on the line upon which cell differentiation proceeds, such that variations in cytoplasmic densities may be seen even within species (Jeffery and Reid, 1975). Within the individual cell the cytoplasm contains abundant mitochondria, lysosomes and tonofilaments, the latter however being less numerous than those seen in basal cells (Breeze and Wheeldon, 1977).

In addition to being a stem cell for the ciliated and mucus-producing cells, It is also suggested that the cell may play a role in protein transport (Breeze and Wheeldon, 1977).

SPECIAL CELLS.

The rarely observed special cell was first described in the tracheobronchial tree of the dog by Frasca *et al.* (1968). The wedge-shaped cell, sometimes resembling the basal cell in size and shape, is located in between adjacent columnar or basal cells and rests on a basement membrane. The cell tapers towards its apex, but has not been observed to reach the lumen of the airway (Frasca *et al.*, 1968).

The special cell is characterised by the presence of numerous intracytoplasmic membrane-bound granules which appear as discs, or curved or straight rods. The nucleus is smooth and oval in outline and usually has a visible nucleolus. The Golgi apparatus is found in the supranuclear region of the cell, while mitochondria are elongated and have prominent cristae. Ribosomes are abundant and scattered uniformly throughout the cytoplasm. A little rough endoplasmic reticulum is present and occasionally lysosomes are seen (Frasca *et al.*, 1968). The function of the special cell remains unknown (Majid, 1986).

EPITHELIAL SEROUS CELLS.

Serous cells were first reported in the trachea and extrapulmonary bronchi of rats by Jeffery and Reid (1975). They have since been reported in the trachea and extrapulmonary bronchi of cat, hamster and human foetus as well (Jeffery and Reid, 1977).

The serous cell appears columnar in shape and extends from the basement membrane to the airway lumen, where a few microvilli are present on the apical surface. The electron-dense cytoplasm contains a basally located irregular nucleus, abundant rough endoplasmic reticulum and a variable number of rounded, membrane-bound secretory granules.

The secretory granules, appearing smaller than those in the mucus-producing cells, have been reported to produce a low viscosity secretion contributing to the periciliary liquid layer below the tracheobronchial mucus (Jeffery and Reid, 1977).

PULMONARY NEUROENDOCRINE CELLS.

The endocrine cell has been found at all levels of the tracheobronchial tree (Hage, 1971, 1972; Scheuermann, 1987). The cell is roughly triangular in shape and lies adjacent to the basement membrane. The tapering apical portion of the cell may or may not reach the luminal surface, with contradictory observations of this between species (Moosavi *et al.*, 1973) and even between individuals within a species (Moosavi *et al.*, 1973; Cutz *et al.*, 1974).

Several terminologies, including clear cell (Feyrter, 1954), endocrine-like cell (Hage, 1971), Feyrter cell (Moosavi *et al.*, 1973), neurosecretory-appearing cell and K cell (Breeze and Wheeldon, 1977) have been used to describe this particular cell type. However, it is now referred to principally as a pulmonary neuroendocrine cell (Johnson and Georgieff, 1989; Gosney, 1990).

The cytological characteristics of the endocrine cell have been described in detail

(Breeze and Wheeldon, 1977; Wasano and Yamamoto, 1981). The cytoplasm, appearing electron-lucent, contains a round or oval nucleus, a prominent Golgi apparatus, numerous free ribosomes and abundant smooth endoplasmic reticulum.

Granules, which have clear haloes between the electron-dense cores and the limiting membrane, are usually located in the basal cytoplasm and, together with bundles of microfibrils, form the characteristic features of the cell. The cell has been included in the APUD (amine-precursor uptake and decarboxylation) group of cells (Pearse, 1969; Hage, 1971, 1973[a,b]) encountered elsewhere in the body (Gail and Lenfant, 1983).

Pulmonary neuroendocrine cells are usually distributed singly; where they do occur in groups, however, they are referred to as neuroepithelial bodies. Aggregations of endocrine cells were first reported by Frohlich (1949) and later described in detail in human infants (Lauweryns and Peuskens, 1971). They are found throughout the entire tracheobronchial and bronchiolar airways, even within the alveolar ducts and alveoli, but they appear to be particularly numerous in the bronchioles, at least in rabbits (Lauweryns and Goddeeris, 1975).

A review of the structure, distribution and histochemistry of pulmonary endocrine cells (Sorokin *et al.*, 1983) has suggested that they could respond to changes in the airway gases by releasing, from the dense-cored vesicles, vasoactive substances which regulate airflow (Gail and Lenfant, 1983).

The close association of the neuroepithelial bodies to various morphological types of nerve endings (Lauweryns and Goddeeris, 1975) further suggests that these cells may act as intrapulmonary chemoreceptors. They are also thought to be regulators of airway epithelial differentiation (Johnson and Georgieff, 1989).

ALVEOLAR TYPE I CELLS (Type I pneumocytes).

Alveolar Type I cells cover most of the alveolar surface of the lung with long,

thin cytoplasmic extensions or plates (Winkler and Cheville, 1984). The cells account for about 93% of the alveolar surface in humans and 97% in the dog (Crapo *et al.*, 1983). The nucleus-bearing part of the cell is cuboidal and contains a few cell organelles. In the cytoplasmic extensions, although vesicles are numerous (Winkler and Cheville, 1984), other cellular organelles are scarce, reflecting the highly differentiated nature of this cell type, which has subsequently lost its capacity to divide. In spite of the paucity of cytoplasmic organelles, the cell has a high metabolic activity (Burri, 1985). The characteristically very thin cytoplasmic plates, a feature related to the function of the cells in ensuring an efficient gaseous exchange (Burri, 1985), can extend as far as 50μM from the nuclear region. Sparsely distributed microvilli are observed on the luminal surface of the cytoplasmic extensions of alveolar Type I cells.

ALVEOLAR TYPE II CELLS (Type II pneumocytes).

Alveolar Type II cells are roughly cuboidal in shape, with apical surfaces that either project slightly into the lumen or, in some cases, appear level with the surrounding alveolar surface. The luminal surface is covered by short microvilli except at the centre, where extrusion of lamellated bodies occurs (Nowell and Tyler, 1971).

The cell cytoplasm appears vacuolated and contains many well developed mitochondria and a well developed and widely dispersed Golgi apparatus (Baskerville, 1970[b]; Sorokin, 1988). Lamellated inclusion bodies, said to be rich in phospholipids (Williams, 1977, 1978), are found throughout the cytoplasm and form a characteristic feature of the Type II cell.

The functions of alveolar Type II cells include the synthesis, storage and secretion of pulmonary surface-active material, the re-epithelialisation of the alveolar wall after lung injury, and transepithelial solute transport to limit the volume of alveolar fluid and perhaps to regulate its composition (Voelker and Mason, 1989).

ALVEOLAR TYPE III CELLS (Alveolar brush cells).

In 1968, Meyrick and Reid described an alveolar brush cell, also known as the alveolar Type III cell, in the lungs of the rat. This cell is characterised by a pyramidal shape and rests on the basal lamina, its lateral surfaces being covered by cytoplasmic extensions of alveolar Type I cells, which then form attachments around the free luminal surface of the Type III cell. This free apical surface is densely packed with large, blunt microvilli (Burri, 1985).

Investigations using SEM and TEM strongly suggest that alveolar Type III cells are derived from alveolar Type II cells (Hijiya, 1978[a,b]). Speculations have been made regarding their function, and the close relationship of these cells to nerve fibres has led to the hypothesis that they may function as receptor cells (Hijiya, 1978[a,b]; Burri, 1985).

## MATERIALS AND METHODS

### ANIMALS:

Four adults cashmere goats, aged between 10 and 18 months were used in this study. Post-mortem procedures were carried out as described in the general materials and methods section provided in Chapter 2. Samples were taken from the lung parenchyma to include bronchioles, alveolar ducts and alveoli.

### TRANSMISSION ELECTRON MICROSCOPY:

Small portions of mucosa preselected sample site numbered 1 to 18 were removed, minced in a petri dish to sizes of approximately 0.5 $mm^3$, and then placed in chilled karnovsky's fixative for at least 24 hours, washed with buffer, dehydrated through a graded series of acetones and embedded in Emix. Thick sections were cut and stained with uranyl acetate and lead citrate, and examined with a Hitachi HS 8 transmission

electron microscope.

## RESULTS.

TEM studies confirmed the previous LM observations on the morphology of the lining epithelium, in that the epithelium lining the terminal bronchiole was observed to be of a simple columnar to a simple cuboidal type (Figs. 5.1, 5.2). Two cell types, the ciliated and the nonciliated bronchiolar epithelial (Clara) cell, formed the major components of the cell population, with the mucus-producing cell being observed only occasionally. The cells rested on a prominent basal lamina, and the whole epithelium was thrown into folds.

The epithelium lining the respiratory bronchioles was predominantly of a simple cuboidal type, being occasionally interrupted by areas of simple squamous epithelium. Nonciliated bronchiolar epithelial cells and a few ciliated cells bearing a small number of cilia, together with alveolar Type I and Type II cells, formed the cell population of the respiratory bronchiolar epithelium. No mucus-producing cells were encountered at this level.

The alveolar membrane of the goat was comprised of a continuous, simple squamous epithelial lining and a centrally situated capillary which was surrounded by connective tissue, the amount of which differed from region to region. Two cell types were observed to constitute the epithelial lining, namely alveolar Type I and Type II cells (Fig. 5.8).

### CILIATED CELLS.

Ciliated cells were observed in the terminal bronchioles and as far distally as the respiratory bronchioles, and were seen to vary both in number and height. Whereas those in the terminal bronchioles were columnar, ciliated cells in the respiratory

bronchioles were fewer in number and were exclusively cuboidal in shape. However, the cytological characteristics were the same. The cells were large and extended from the basal lamina to the airway lumen. The luminal surfaces were seen to bear cilia and numerous microvilli (Figs. 5.2, 5.3). The cilia were seen to arise from the apical cytoplasm and were anchored onto the basal bodies. Microvilli were seen to be much thinner than cilia, and had no obvious internal structure (Fig. 5.3).

The cytoplasm of ciliated cells was much more electron-lucent than that of the neighbouring nonciliated microvillous cells (Fig. 5.2), and a nucleus, oval in shape, was usually observed occupying the basal portion of the cell. A well-developed Golgi apparatus was usually seen situated above the nucleus, and a number of mitochondria with relatively few cristae were seen occupying a region immediately below the basal bodies (Fig. 5.3). A few intracytoplasmic membrane-bound inclusion bodies containing homogeneous electron-dense material were observed (Fig. 5.1, 5.4). Profiles of smooth endoplasmic reticulum were observed in the apical region of the cell.

A narrow intercellular space was seen surrounding the lateral cell surface, except at the luminal surface where tight junctions with adjacent cells were present (Fig. 5.3).

Occasionally, cells which were presumed to be developing ciliated cells were encountered in the terminal bronchiole. Such cells, which were columnar in shape, arose from the basal lamina, projected up to the airway lumen, and were characterised by the presence of numerous microvilli and the presence of a number of basal bodies (Fig. 5.4). The cytoplasm was of a medium electron-density and a few intracytoplasmic multivesicular bodies were observed. A prominent Golgi body and a basally situated nucleus were characteristic features of this cell type.

## NONCILIATED BRONCHIOLAR EPITHELIAL (CLARA) CELL.

Nonciliated bronchiolar epithelial (Clara) cells were observed in the terminal bronchioles and all the way into the respiratory bronchioles, where they were in the

majority. Clara cells were observed with TEM to be columnar to cuboidal in shape (Figs 5.1, 5.2) and often presented characteristic apical protuberances (Fig. 5.5). The apical surfaces were usually studded with numerous short, thick stumpy microvilli. The cells were attached apically to each other or to adjacent cells by tight junctions; in the basal region, numerous interdigitations with neighbouring cells were always observed (Fig. 5.5).

Individual cell cytoplasm was usually seen to be electron-dense compared to adjacent ciliated cells (Fig. 5.2). The relatively large nucleus was frequently observed in the central region of the cell, although occasionally located in the apical protuberance. Patches of dense heterochromatin were distributed in the periphery of the nucleus and a nucleolus was often present.

Accumulation of smooth endoplasmic reticulum was observed especially in the apical region (Fig. 5.6); a few inclusion bodies were also encountered.

A few discrete, electron-dense granules, devoid of internal structures and lacking a clear limiting membrane, were observed, mainly in the apical region. Moderate numbers of elliptical mitochondria with an electron-dense matrix were observed (Figs. 5.5, 5.6).

MUCUS-PRODUCING CELLS.

These were very occasionally observed in the terminal bronchioles only. The cell was cuboidal in shape and was attached to adjacent cells by tight junctional complexes (Fig. 5.7). The luminal surface carried a few short microvilli concentrated around the periphery of the cell. A large nucleus with a prominent nucleolus was basally situated. The cytoplasm was much more electron-lucent compared to that of the Clara cell, although, of a higher electron-density than that of the ciliated cell. Numerous heterogeneous granules were distributed in the supranuclear region of the cell. Abundant rough endoplasmic reticulum and a small amount of smooth endoplasmic

reticulum were encountered, particularly in the apical half of the cell (Fig. 5.7).

BASAL CELLS.

These were not observed in the present study of the distal airways of the goat.

ALVEOLAR TYPE I CELL.

This was observed to contain an oval nucleus surrounded by a limited perikaryon from which very long cytoplasmic extensions, covering most of the alveolar surface, arose. The cytoplasm appeared to be devoid of cellular organelles except for occasional pinocytotic vesicles. Occasionally short microvillous-like protrusions were observed projecting from the luminal surface of the cell (Fig. 5.8).

ALVEOLAR TYPE II CELL.

The large cuboidal-shaped cells, occupying positions in the alveolar recesses (Fig. 5.9) and sometimes bulging out into the alveolar lumen (Fig. 5.8), were identified as alveolar Type II cells. These cells were also characterised by the presence of numerous thin microvilli on their free surfaces, and were seen to form junctional complexes with neighbouring alveolar Type I cells.

The cytoplasm was electron-dense and contained characteristic lamellated inclusion bodies. The lamellated bodies, consisting of spirals of osmiophilic material, appeared to vary in size and number from one individual cell to another (Fig. 5.9 inset). Numerous, relatively large and well formed mitochondria with an electron-dense matrix were observed distributed in the cytoplasm, along with granular endoplasmic reticulum and numerous ribosomes (Fig. 5.9). A Golgi apparatus was occasionally observed. Lipid vacuoles of variable sizes were observed distributed in the cytoplasm; whereas

some appeared empty (lipid material having been lost during processing of specimens), many of them still contained electron-lucent material (Figs. 5.9, 5.11). The large centrally-placed nucleus was often observed to contain a prominent nucleolus; sometimes two nucleoli were seen.

On rare occasions, large Type II cells dramatically protruded into the alveolar lumen near to the basal lamina; the lateral surfaces of such cells were seen to be attached to the cytoplasmic extensions of the alveolar Type I cells by desmosomes and tight junctional complexes. The protruding surface was studded with microvilli (Fig. 5.10). Occasional cross section through the apical dome of such cells gave an impression of these cells being free in the alveolar lumen (Fig. 5.11).

ALVEOLAR SEPTUM.

Capillary endothelial cells in the alveolar septa were attenuated except for the nuclear region which bulged into the lumen. The cytoplasmic extensions presented numerous deep invaginations both in the luminal and basal surfaces of the cells (Fig. 5.12). Within the capillary lumen, white blood cells and red blood cells were observed

The amount of connective tissue within the alveolar septa varied from region to region and was located between the basal lamina of the epithelial cell and that of the endothelium. Aggregates of collagen fibres were observed (Fig. 5.12), and fibroblasts and occasional mast cells were also encountered. In some areas, where the septal connective tissue was absent, the basal laminae of endothelial and epithelial linings fused to form a basal lamina common to both.

ALVEOLAR MACROPHAGES.

Free cells, unattached to a basal lamina, were occasionally observed in the alveolar spaces and were identified as alveolar macrophages (Fig. 5.13). The cell

surface was to a large extent smooth and, at intervals, long pseudopodia-like extensions were observed. The cytoplasm contained smooth endoplasmic reticulum, a patchy distribution of rough endoplasmic reticulum, many small ellipsoid-shaped mitochondria, and a large irregularly-shaped nucleus (Fig. 5.14).

Large vacuoles and numerous membrane-bound vesicles of varying sizes and containing a dense matrix were observed in the cytoplasm. Spherical bodies containing osmiophilic material or lamellated lattice-like structures were also observed distributed within the cell (Figs. 5.13, 5.14).

## DISCUSSION.

The present study confirmed LM observations (Chapter 3) that, in the goat, the terminal bronchioles are lined by a simple columnar epithelium, the height of the cells decreasing with the decrease in airway diameter to form a simple cuboidal epithelium in the respiratory bronchioles.

These observations on the types of epithelia lining the distal airways in the goat are in agreement with those made in several mammalian species including the dog (Majid, 1986), pig (Baskerville, 1970[a]), cat (Al-Tikriti *et al,* 1991), horse (Pirie, 1990), ox (Iovannitti *et al.*, 1985) and rat (Andrews, 1979), although they contrast with observations made at LM and TEM level in humans (Ten Have-Opbroek *et al.,* 1991) and in nonhuman primates (Tyler and Plopper, 1985; Plopper *et al.*, 1986), where two types of epithelia have been described lining the respiratory bronchioles. In the primates, in addition to the simple cuboidal epithelial lining of the respiratory bronchiole, a strip of pseudostratified columnar epithelium has been described located on the bronchiolar wall apposed to the associated pulmonary arterial branch. Such an arrangement was not observed in the goat in the present study.

This lining epithelium of the distal airways, as observed in the goat, was seen to

be populated by five different cell types, identified and characterised by the use of the transmission electron microscope (TEM). These were the ciliated, nonciliated bronchiolar epithelial (Clara) and mucus-producing cell types, and the alveolar Type I and alveolar Type II cell types. Alveolar macrophages were also observed.

## CILIATED CELLS.

Ciliated cells were observed both at the level of the terminal bronchiole and the respiratory bronchiole. The cells were easily identified by the characteristic presence of cilia on their apical surfaces and the less opaque nature of the cytoplasm in comparison with that of the adjacent cells (Okano and Sugawa, 1965). Although ciliated cells are frequently refered to as being columnar in shape (Rhodin and Dalhamn, 1956; Rhodin, 1966; Gail and Lenfant, 1983), the present study has observed that the height of the cell may vary while still retaining the same cytological characteristics; indeed, the ciliated cells observed in the respiratory bronchioles were cuboidal in shape.

Cilia presented a typical cytoskeletal arrangement of 9 peripheral tubules arranged in a regular circle about a pair of central tubules, a description which basically still remains the same as it was when described by Rhodin and Dalhamn (1956) almost half a century ago. This time-tested description of the structure of the cilium appears to be similar in all mammalian species so far examined (Breeze and Wheeldon, 1977; Gail and Lenfant, 1983).

Other characteristic features observed in the present study included a prominent Golgi body, basal bodies to which the cilia were anchored, numerous mitochondria , tight junctions on the luminal surface and an electron-lucent cytoplasm. These features are in agreement with the general cytological features as provided by Breeze and Wheeldon (1977). However, the aggregations of glycogen rosettes observed in the ciliated cells of the tracheobronchial epithelium of the dog (Frasca *et al.*, 1968) were not observed in the present study, neither were they reported in the horse (Pirie, 1990), or

in later studies in the dog (Majid, 1986). Also the intracytoplasmic tonofilaments reported in the ciliated cell of the rat (Rhodin, 1966) were not observed in the goat.

The Golgi complex of the ciliated cell is said to be mainly concerned with protein synthesis within the cell itself (Varsano *et al.*, 1987), while the mitochondria, in addition to providing energy for this activity, play a more important role in providing energy for the ciliary beat, which may explain their supranuclear position just below the basal bodies.

The membrane-bound inclusion bodies observed in the ciliated cell in the present study have also been observed in other mammalian species (Tyler and Plopper, 1985); they are clear on photomicrographs provided for the horse (Pirie, 1990) and have also been observed in the domestic fowl (Mohammed, 1989). However, their exact nature and function remains unclear; whether these inclusions are related to the secretion of macromolecular glycoconjugates by ciliated cells, which has been established in vitro (Varsano *et al.*, 1987), can only be speculative.

Only a few short cilia were observed on the apical surface of ciliated cells in the distal airways. This is in agreement with observations by Breeze and Turk (1984), who reported that cilia decrease in length progressively with succeeding airway generations in the peripheral lung.

The cilia carpet forms an intergral part of the mucociliary apparatus responsible for clearing the airways by propelling trapped foreign bodies, including micro-organisms, through the ciliary beat, towards the pharynx were they are eventually swallowed. The important role played by the cilia in the surface defence mechanisms of the respiratory airways is thus well recognised (Kilburn, 1968; Kaltreider, 1976), and the paucity of cilia in the distal airways observed in the present study may account for the vulnerability of this region to diseases, as noted in the review of literature. The association of deciliation with pathological conditions is well documented and is discussed later in Chapter 7.

Microvilli were observed intermingled in between cilia, and have been reported

to be present on ciliated cells of all mammalian species studied (Sturgess, 1989). Friedmann and Bird (1971) reported that microvilli have bundles of filaments forming a central core. Such details were not observed in the present study, the microvilli appearing to have no internal structure. Indeed, the presence of filaments within microvilli does not appear to have been observed in many of the ultrastructural studies.

The function of these microvilli on the ciliated cells of the respiratory airways remains elusive. As it is generally accepted that microvilli increase the surface area and there are reports that ciliated tracheal epithelial cells synthesise and transport sulfated macromolecular glycoconjugates which are eventually released from the cell surface, it may be that microvilli play a role in such processes (Varsano *et al.*, 1987).

Cells which were presumed to be developing ciliated cells were encountered in the present study. Although no cilia were observed on the apical surfaces of such cells, the presence of numerous basal bodies suggested that these cells were destined to become ciliated. It was established that, at the level of the terminal bronchioles in the goat, basal cells (which have been presumed to be stem cells of columnar cells in a pseudostratified epithelium) are not represented in the cell types composing the epithelial lining; this begs the question as to which cell type is involved in cell renewal in the distal airways?

It has been proposed (McDowell *et al.*, 1984[a,b]) and confirmed experimentally (Evans *et al.*, 1986), that the nonciliated bronchiolar epithelial (Clara) cell is capable of both cell division and cell differentiation to form new secretory and ciliated cells. Thus it is now accepted that the Clara cell is the primary progenitor cell in the distal airways.

NONCILIATED BRONCHIOLAR EPITHELIAL (CLARA) CELLS.

The clara cells found throughout the terminal and respiratory bronchioles in the present study were usually seen to carry apical protuberances, but were also occasionally observed to present a flattened apical surface.

The TEM appearance of the Clara cell in the mammalian respiratory tract lining

epithelium shows considerable variation in its observed ultrastructure. For example, Plopper *et al.*, (1980[c]) have shown the presence of considerable qualitative and quantitative interspecies variations in Clara cell morphology based on the presence and abundance of secretory granules, and on whether smooth, rough or both types of endoplasmic reticulum are present.

Clara cells observed in the present study contained numerous profiles of smooth endoplasmic reticulum; this supports similar observations made in the Clara cells of the horse and sheep, where SER is a major cell constituent, but differs from observations made in the ox, dog, cat and man (Plopper *et al.*, 1980[a-c]) and nonhuman primates including Rhesus, Bonnet and stumptail monkeys (Castleman *et al.*, 1975), where SER is not a prominent intracytoplasmic feature. In addition, the presence of secretory granules in the Clara cell of the goat is in agreement with observations made in a number of other mammalian species, where they have been consistently observed, although such granules were reported to be absent in the Clara cell of the Rhesus monkey and the cat (Plopper *et al.*, 1980[a-c]). Secretory granules in the goat were observed to be rather spherical than ovoid, as has been reported in other species.

Glycogen granules were not observed in the present study in the goat. Although this is in agreement with observations made in the guinea pig, rat, hamster and mouse (Plopper *et al.*, 1980[a]) and in the horse (Plopper *et al.*, 1980[b]; Pirie, 1990), this present observation contrasts with reports in the ox, cat, and ferret (Plopper *et al.*, 1980[b]) and in the dog (Plopper *et al.*, 1980[b]; Majid, 1986) where glycogen granules were abundant. While the present observations are in agreement with the general morphological descriptions provided for these cells (Breeze and Wheeldon, 1977), it is apparent that Clara cells exhibit great interspecies diversity in many of their ultrastructural characteristics. Indeed it has been noted (Plopper *et al.*, 1980[a-c]) that , aside from the nucleus which appears to be common to all 15 species studied, the cytoplasmic extensions from the lateral cell surfaces, also observed in the present study, may be the only other common feature.

Although the function of the Clara cell is uncertain and controversial, it is generally accepted that the cell is secretory in nature (Cutz and Conen, 1971; Becci *et al.*, 1978; Smith *et al.*, 1979; Plopper *et al.*, 1980[a-c]; Tyler and Plopper, 1985; Majid, 1986). The proposed function of the cell has been based on the presence of two of the cellular characteristics which have also been commonly used to distinguish nonciliated bronchiolar epithelial cells, namely numerous membrane-bound granules and abundant SER. The granules are said to contain either proteins or phospholipids and have been associated with the production of serous secretions (Breeze and Turk, 1984). SER, in addition to being associated with such secretions (Kuhn *et al.*, 1974), is also associated with the metabolism of pulmonary toxins. Cytochrome P-450-dependent mono-oxygenases provide a major pathway for the oxidative metabolism of xenobiotics present in the environment. These enzymes have been isolated in the Clara cell (Boyd, 1977; Serabjit-Singh *et al.*, 1980), and thus there is much evidence to suggest that the Clara cell is a primary site for detoxification. Indeed studies in the rabbit have further suggested that the Clara cell is involved in the metabolism of noxious compounds (Boyd, 1977; Serabjit-Singh *et al.*, 1980), a proposal supported by Plopper *et al.*, (1983).

Studies in the rat exposed to nitrogen dioxide, oxygen and ozone (Evans *et al.*, 1973; 1978; Lum *et al.*, 1978) have also suggested that Clara cells function as progenitor cells for the bronchiolar epithelium. Since no basal cells ( which are considered to provide a renewal of cells in the proximal airways) were observed in the distal airways in the present study, this further supports the view that Clara cells are responsible for cell renewal at this level.

A recent study in humans appears to provide a different opinion as regards the nature of the nonciliated bronchiolar epithelial cell. By means of immunocytochemical techniques, also supported by histochemistry and TEM observations, Ten Have-Opbroek *et al.* (1991) have demonstrated the presence of glycoproteins in the columnar types of Clara cells. At the same time they have suggested that the cuboidal type of

Clara cells are Type II precursor cells, based on ultrastructural criteria for embryonic Type II cells ((Ten Have-Opbroek *et al.*, 1990), including a cuboidal shape, a large and roundish nucleus, presence of both SER and RER, osmiophillic multivesicular bodies, and dense bodies. In addition, the cuboidal cells showed a cytoplasmic staining pattern for surfactant protein-A , identified immunocytochemically. These workers have therefore suggested that Clara cells in man instead of being a separate cell type, may instead be varieties of mucus-producing or alveolar Type II cells. These studies were based on previous histochemical observations of the respiratory bronchioles in primates which gave some indication that mucus-producing cells were present at this level (Tyler and Plopper, 1985; Plopper *et al.*, 1989).

Histochemical observations made in the goat in Chapter 3, however, provide no evidence of mucus-producing cells in the distal airways. In addition, the present TEM studies did not show the presence of osmiophillic multivesicular bodies characteristic of these cells in man (Ten Have-Opbroek *et al.*, 1991). Whether this represents a species difference between the goat and man can not be judged on the present results alone. The observation by Widdicombe and Pack (1982), that the Clara cells are still the "mystery cells of the lung", is therefore still valid and further work would be justified to be able to elucidate the nature and function of these cells.

MUCUS-PRODUCING CELLS.

In the present study an occasional mucus-producing cell was encountered in only one individual animal. This was differentiated from the nonciliated bronchiolar epithelial (Clara) cell by the heterogeneous nature of the secretory granules, and the presence of both smooth and rough endoplasmic reticulum along with the basally situated nucleus and the electron-lucent nature of the cytoplasm.

This TEM observation of mucus-producing cells in the present study contrasts with the earlier LM and SEM studies (chapter 3 and 4), and also contrasts with

observations in other mammalian species including the dog (Majid, 1986), ox (Mariassy *et al.*, 1975; Iovannitti *et al.*, 1985) and horse (Pirie, 1990). It is worth noting, however, that mucus-producing cells constitute the major secretory cell type in the distal airways of humans (Ten Have-Opbroek *et al.*, 1991) and the Rhesus monkey (Tyler and Plopper, 1985).

Observations of the bronchiolar epithelium in man, demonstrating that smoking results in a reduction in the number of Clara cells and an increase in the number of mucus-producing cells (Ebert and Terracio, 1975[a]), have suggested that the Clara cell, which is known to be a progenitor cell (Evans *et al.*, 1973, 1978; Lum *et al.*, 1978), may differentiate into a mucus-producing cell following irritation of the epithelial lining. It could be speculated that the individual animal showing the presence of these mucus-producing cells in the present study was, although free from detectable respiratory distress, exhibiting an epithelial response to a mild, but clinically undetectable, irritation of the respiratory mucosa.

ALVEOLAR TYPE I CELLS.

This cell type, identified by its attenuated cytoplasm joined with the alveolar Type II cell by tight junctions and desmosomal attachments, was seen to line most of the alveolar surfaces. Except for the frequently observed mitochondria and endoplasmic reticulum in the perinuclear region, the ultrastructural characteristics observed in the present study are similar to those described in earlier studies in the goat (Atwal and Sweeny, 1971). Similar ultrastructural features have been described in other mammalian species including the horse (Pirie, 1990), ox (Epling, 1964[a]; Rybicka *et al.*, 1974[a]), pig (Baskerville, 1970[b]) and coyote (Morrison *et al.*, 1983).

The alveolar Type I cells are estimated to cover 95% of the alveolar surface in the rat (Meyrick and Reid, 1970), and because of their thin squamous nature they facilitate gaseous exchange. Atwal (1988) reported for the first time a significant amount

of tubular endoplasmic reticulum (TER), which is a modified SER and pinocytotic vesicles in the Type I cells of the goat's lung, and linked these structures to the transport of fluid and electrolytes across the cell membrane of the pneumocyte. Pinocytotic vesicles were observed in the cytoplasmic extensions in the present study, although the TER reported by Atwal (1988) was not identified. This could have been due to the differences in the methods of fixation employed; whereas Atwal, (1988) used vascular perfusion, in the present study, airway perfusion was employed in fixing the material.

ALVEOLAR TYPE II CELLS.

The alveolar Type II cell was easily identified by the use of TEM and was distinguished from the alveolar Type I cell by its cuboidal shape, the presence of numerous apical surface microvilli, and the characteristic presence of intracytoplasmic osmiophilic inclusion bodies, the latter being the cellular form of surfactant (Kuhn, 1976; King, 1979)

The Type II ultrastructural features observed in the present study are similar to earlier descriptions provided for this cell type in the goat (Atwal and Sweeny, 1971). Large membrane-bound inclusions containing electron-lucent material, and presumed to be be lipid vacuoles, were observed in the goat in the present study, confirming previous observations in the goat by Atwal and Sweeny (1971). Such vacuoles do not appear to have been reported in the normal lung of other mammalian species. However, similar vacuoles have been reported in guinea pigs exposed to hypoxia in low-pressure chambers, leading to the suggestion that perhaps the development of such hypoxic features in the alveolar Type II cells is a significant factor in high altitude pulmonary conditions, this vacuolar response being involved, in some way, with changes in the surfactant-producing mechanism (Valdivia *et al.*, 1966). Alternatively, it has been suggested that the presence of lipid vacuoles may be a result of the high respiratory quotient of the goat (Homer 1977), which favours the formation of fat, and the

mammalian lung is known to participate in *de novo* synthesis of fatty acids (Mason, 1976). This has led to the suggestion that metabolic pathways in the goat lung are mostly orientated towards the formation of lipids, and this leads to lipid accumulation in the metabolically active alveolar Type II cells (Atwal and Sweeny, 1971).

It is generally accepted that alveolar Type II cells actively synthesise and secrete the surface-active phospholipids (surfactant) of the alveolar lining (Kikkawa *et al.*, 1965; Kuhn, 1976; King, 1979; Morrison *et al.*, 1983; Kikkawa and Smith, 1983; Breeze and Turk, 1984), and the osmiophilic lamellated inclusions appear to be associated with this function. In addition to the production and storage of pulmonary surfactant, the alveolar Type II cell is also known to be a stem cell of the alveolar epithelium, involved in regenerating the epithelium following injury (Kauffman, 1980). Other postulated functions of alveolar Type II cells have included the defence of the lung against oxidant injury (Freeman *et al.*, 1981) and the metabolism of xenobiotic substances (Devereux *et al.*, 1981; Devereux and Fouts, 1981).

Occasional large alveolar Type II cells dramatically protruding into the alveolar lumen, as observed in the present study, have been observed in other species (Krause *et al.*, 1976) and appear to be a feature of the neonatal animal. Observation of such cells in the present study could imply that alveolar formation, characterised by Type II cell proliferation, may also continue into adult life in the goat.

## ALVEOLAR MACROPHAGES.

Although alveolar macrophages were rarely observed in previous investigations with SEM (Chapter 4), in the present study alveolar macrophages were frequently observed with the use of TEM. Majid (1986) had previously reported difficulties in observing the alveolar macrophages in the dog using SEM, and attributed his failure to detect them in spite of studious searches to the intrabronchial instillation of fixative. However, change in technique to intravascular perfusion also failed to detect any

macrophages with SEM although they were frequently observed with TEM. To quoteMajid (1986) 'it appears inconceivable that, despite the greater surface area available for inspection with SEM compared to the relatively small specimens used for TEM studies, alveolar macrophages could only be identified with certainty in the latter'.

The present study can only speculate that maybe airway instillation of fixative causes the macrophages to stick together, as they were often observed in pairs (Refer Fig. 5.14); as a result they were confused with debris on SEM studies. However, this does not provide an answer as to why they could not be detected following vascular perfusion.

The ultrastructural characteristics of the alveolar macrophage of the goat as described in the present study are in agreement with a previous study in the goat (Atwal and Sweeny, 1971). They are also similar to those of other mammalian species including the horse (Pirie, 1990), ox (Epling, 1964[a]; Rybicka *et al.*, 1974[a]) and dog (Majid, 1986).

In the present study lamellar material, similar in appearance to the lamellated material observed in the alveolar Type II cells, was observed in the alveolar macrophages. This material, characteristic of surfactant, has also been observed in the alveolar macrophages in the ox (Epling, 1964[b]; Rybicka *et al.*, 1974[a]) and has led to the suggestion that the macrophage cells participate in controlling alveolar surfactant levels (Veit and Farrell, 1978). The latter suggestion has been supported by recent work which has localized by immunocytochemical methods, the major surfactant apoprotein in the alveolar Type II cells, Clara cells and alveolar macrophages of rat lung (Walker *et al.*, 1986)

Alveolar macrophages are known to phagocytose an assortment of inhaled debris, including infectious agents and inorganic minerals (Green and Kass, 1964; Heppleston and Young, 1973; Gilka *et al.*, 1974), and thus their major function is defence.

The present study, in cytologically characterising the cell populations that line

the distal airway in the goat lung, has thus provided additional information which, when combined with that obtained from previous LM and SEM studies, provides a basic understanding of the morphological features of the cell population of the epithelium lining the goat's respiratory tract.

# INTRODUCTION.

The respiratory epithelium, through its mechanical, cellular and humoral actions, plays an important part in defending the respiratory tract against obnoxious substances or disease-causing organisms, and thus its stage of development could be expected to effect its efficiency. The degree of development of this system at birth has an added degree of significance when it is realised that respiratory related problems have been shown to account for more than 50% of all mortalities in kids, with the majority of such deaths usually occurring within the first three weeks of life (Osuagwuh and Akpokodje, 1981).

Investigations of the lungs of various mammals have since brought growing evidence for the general concept that the development of the mammalian lung is not yet completed at birth and that new alveoli are formed postnatally. This has been demonstrated for the lung of mice (Engel,1953), rats (Engel, 1953; Neuhauser, 1962; Neuhauser and Dingler, 1962; Weibel, 1967), rabbits (Dingler, 1958), dogs (Boyden and Tompsett, 1961) and also man (Emery and Mithal, 1960; Mithal and Emery, 1961; Dunnill, 1962; Boyden, 1965, 1967; Boyden and Tompsett, 1965; Emery and Wilcock, 1966; Reid, 1967; Zeltner and Burri, 1987).

The degree of development of the lung at birth varies widely, and Engel's studies (1953) on various mammalian species culminated in a postulate that the degree of lung maturity at birth reflects the stage of general body development. Whereas extremely altricious species (such as the opossum) have markedly underdeveloped lung structures at birth, precocious neonatal animals exhibit relatively well developed lungs (De Lorimier *et al.*, 1969; Alcorn *et al.*, 1981; Lechner and Banchero, 1982; Winkler and Cheville, 1984).

Although there is plenty of information on the foetal development of the mammalian respiratory system, there is still a dearth of information as far as systematic investigations of postnatal development of lungs in domestic animals is concerned

(Winkler and Cheville, 1984). A few investigators have attempted to provide some information on the subject, although these studies are limited in scope as they either deal with only selected sections of the respiratory tract (mainly the lung tissue) or with only a few animals, thus making it difficult to reach a satisfactory conclusion.

Despite the economic significance of respiratory diseases in kids, very little attention has been paid to the study of the postnatal development of the respiratory system of the goat, with information on the postnatal development of the epithelium of the respiratory of the goat in particular being apparently unavailable. It is the purpose of this study to provide, apparently for the first time, an account of the postnatal development of the lining epithelium of the entire respiratory tract in the goat.

## LITERATURE REVIEW.

Lung development appears to follow the same basic pattern in all mammalian species, the difference amongst them being based primarily on the timing of events. The more active or independent the species at birth, the more developed the lung is in the new-born. It has been observed that the neonatal young of the opossum, which are solely dependent on their mother at birth as they are born blind and naked, have a very rudimentary, saccular lung with no alveoli. In contrast, new-born lambs, which are seen to be on their feet within hours of their birth, possess a relatively well developed lung with many alveoli.

### FOETAL LUNG DEVELOPMENT.

Thurlbeck (1975) has provided a comprehensive review of foetal lung development and the following brief review incorporates his findings along with studies by Burri (1974, Alcorn *et al.* (1981), Langston *et al.* (1984), ), Zeltner and Burri (1987) and Latshaw (1987). Foetal lung development is divided into the an early

embryonic period (or phase), followed by a pseudoglandular period, a canalicular period, and finally a terminal sac period. The latter phase may, depending on the species, be followed by a possible alveolar period.

The embryonic period includes the earliest phase of lung development, which involves the evagination of the pharynx to form the laryngotracheal groove and the separation of the oesophagus from the trachea by formation of a tracheoesophageal septum. The trachea then divides dichotomously to form two buds. These paired lung buds elongate to form lobar buds which continue to divide dichotomously, the divisions of which essentially represent the developing bronchial tree.

At this stage the lung enters the pseudoglandular period with airways being lined by columnar epithelium and separated from each other by poorly differentiated mesenchyme.

This stage is followed by a canalicular period characterised by the proliferation of the mesenchyme and the development of a rich blood supply within it, together with flattening of the epithelium that lines the airways. At this stage the epithelium is usually irregularly thinned and continuity between epithelial cells is sometimes seen to be lost at the cell margins, being maintained only at their bases. During this canalicular period, but especially towards the end, areas of thin blood-air barrier resembling that of the adult begin to appear and the various types of alveolar lining epithelial cells (alveolar Type I and Type II cells) can be identified, together with osmiophilic bodies in alveolar Type II cells.

The terminal sac period is characterised by a progressive thinning of the epithelium and a protrusion of capillaries into the airways resulting in a greater surface area of the blood-air barrier. The terminal generations of the airways are lined only by flattened epithelium. During this phase, although true alveoli are not yet present, respiration can be maintained and most altricious animals, notably the rat and mouse (Burri and Moschopulos, 1992), are born during this period. Terminal sac phase involves alveolar formation in such species as the cat (Al-Tikriti et al., 1991) and dog

(Boyden and Tompsett, 1961) but in other species including the rat and mouse (Burri and Moschopulos, 1992), alveolar formation is a separate phase following the terminal sac phase.

POSTNATAL DEVELOPMENT.

The postnatal growth of the lung has been investigated for many years, especially in laboratory mammals. As early as 1881, Kolliker had concluded that adult structures of the lung (the presence of alveoli, with a single capillary network) were already present in the new-born infant and that growth involved expansion only. More than half a century later his view was still held to be true and was supported by Short (1950). More recent work however has indicated that the lungs of many new-born mammals are qualitatively different from those of adults of the same species in that alveoli are few or absent at birth (Amy *et al.*, 1977). Thus the lungs of many species at birth are usually in their final part of the terminal saccule stage and /or at the beginning of the alveolar period. Those species in which most or all alveoli in the lung are formed after birth include the rat (Weibel, 1967; Burri, 1974), rabbit (Engel, 1953), cat (Engel, 1953; Dingler, 1958) and man (Boyden and Tompsett, 1965; Reid, 1967).

In contrast, ruminants are well into the alveolar stage at birth and thus have relatively more alveoli than do other domestic mammals (Alcorn *et al.*, 1981; Castleman and Lay, 1990). At birth, the horse and the pig have fewer alveoli than ruminants but more than are found in carnivores (Latshaw, 1987), which themselves have more alveoli than new-born infants, but are still in the terminal saccule stage at birth. However, regardless of how advanced or not lung development is at birth, it has been established that all mammals form alveoli postnatally, and the increase in the number of alveoli soon after birth accounts for the major part of the early postnatal growth of the lung (Bartlett, 1972; Latshaw, 1987; Winkler and Cheville, 1984).

The process of alveolar formation itself is controversial and several explanations

have been provided. These include subdivision of peripheral lung units, alveolar 'sprouting', alveolarization of bronchioles, and peripheral airway branching (Amy *et al.*, 1977). It has been established in the rat that subdivision of peripheral lung units (primary saccules) is an active process involving proliferation of interstitial and endothelial cells, and not a mere expansion of the septal tissue mass as had been originally stated by Short (1950). The 'sprouting' theory has been described as a form of alveolar formation by several workers (Broman, 1923; Wilson, 1928; Bremer, 1935, 1936, 1937) and involves the outgrowth of tubular buds at the end of the bronchiolar tree.

Alveolarization of bronchioles was first described as a mode of alveolar formation by Boyden and Tompsett (1961; 1965). They found that bronchioles are converted into respiratory bronchioles by the local transformation of the bronchiolar wall, which involves the outpocketing of circumscribed areas and the formation of the blood-air barrier, resulting in the formation of true alveoli.

Peripheral airway branching was first proposed by Loosli and Potter (1959), and this involves centripetal partitioning of the air spaces starting from the most peripheral.

## POSTNATAL DEVELOPMENT OF THE EPITHELIAL LINING OF THE RESPIRATORY TRACT IN VARIOUS MAMMALIAN SPECIES.

As noted earlier on, the postnatal development of the lining epithelium of the mammalian respiratory tract has received very little attention, with the literature that is available apparently dealing primarily with the lung parenchyma. Very little information appears to be available concerning the postnatal development of other regions of the respiratory tract lining epithelium.

Tracheobronchial tree.

Wright *et al.* (1983) investigated the normal development of the ciliation pattern in the dog, as demonstrated by the distribution of ciliated cells in the lower respiratory tract of 25 puppies aged between 4 hrs and 6 months, using SEM. They observed that in the new-born puppy only the dorsal wall was ciliated, complete ciliation of the trachea not being achieved until day 5 of age. In addition, bronchi of new-born puppies were uniformly poorly ciliated, but at the age of 2 days complete ciliation was attained. Smaller bronchi were poorly ciliated, although there was an increase in number of ciliated cells with age. In addition, Pirie *et al.* (1991[b]) working with horses, found that 2-day-old foals had a complete ciliation of the trachea and bronchi comparable to the adult pattern.

It has also been observed in calves (Iovannitti *et al.*, 1985) that, at one week of age, the distribution of ciliation in the lower respiratory tract is similar to the pattern in adult animals. The first week of life was not investigated.

Bronchial tree and lung parenchyma.

There is a substantial amount of information available on the postnatal development of the lung in rats and mice (Weibel, 1967; Burri, 1974; Amy *et al.*, 1977; Scheuermann *et al.*, 1988), indicating that at least in the rat postnatal development proceeds in three consecutive stages (Burri, 1974). The first stage is characterised by expansion of the primitive gas exchange airspaces (primary saccule), and this usually lasts up to 4 days postnatally. The second stage, which extends from days 4 to 13, basically involves a rapid enhancement of specific lung tissue mass involving an extremely rapid enlargement of the alveolar and capillary surface areas as well as formation of alveoli, the latter being formed by septation of the primary saccule. The third stage, which lasts from the 13th to 21st day, consists of the restructuring of

interalveolar tissue components, the double capillary network being transformed into a single network and the alveolar septa becoming thinner (Burri, 1974; Caduff *et al.*, 1986).

Recently, extensive studies of postnatal development and growth of the mammalian lung have been carried out by Zeltner and Burri (1987) in humans aged between 26 days and 64 months, and also in cats (Al-Tikriti *et al.*, 1991) aged 1, 7, 14 and 21 days postnatally. It was found that in humans at one month of age alveolar formation, which begins in late foetal life, was well under way and secondary septa were seen to subdivide peripheral airspaces into shallow alveoli. The parenchymal septa present during and after alveolar formation were immature in that they still retained a double network of capillaries. The results in humans at this stage were comparable to those seen in rats in the first to second week following birth (Burri, 1974; Scheuermann *et al.*, 1988). This stage was followed by a few months of septal maturation and was characterised by a reduction in the interstitial tissue mass and the restructuring of the capillary network into a single capillary layer. By one and half years of age, most of the parenchymal septa were comparable to those seen in the adult.

The cat was seen to have patches of primitive airspaces and very few alveoli at birth (Al-Tikriti *et al.*, 1991). Primary saccules were seen to be thick-walled and very cellular, with alveolar Type II cells being the major cell type lining the saccule. In 7-day-old kittens a progressive septation of the primary saccule was observed. The septum was lined by both alveolar Type I and alveolar Type II cells. In 21-day-old lungs some areas of the septum were seen to retain a double capillary network, although in general the septa became thinner and increased in length with a concomitant decrease in cellularity.

Winkler and Cheville (1984) have provided some information on the ultrastructural morphology and postnatal development of the terminal airways and alveolar region of the pig. They observed that the porcine lung at birth exhibited a high degree of maturity, as thick-walled primary saccules, described in mice (Engel, 1953;

Amy *et al.*, 1977), rats (Weibel, 1967; Burri *et al.*, 1974), humans (Zeltner and Burri, 1987) and cats (Al-Tikriti *et al.*, 1991), were not observed. Subsequent morphometric studies (Winkler and Cheville, 1985) augmented their previous findings that the porcine lung is relatively well advanced at birth and the morphology of alveolar septa in newborn pigs was seen to resemble the ones in 30-day-olds. However, it was also established that lung growth was far from being complete, and the first two weeks of life involved further subdivision of the airspaces and a decrease in septal thickness as well as the remodelling of capillaries from a double to a single network. In 60-day-old animals the size and shape of alveoli were discernible, appearing smaller than the primary saccule, having more regular walls and with less protrusions of capillaries into alveolar lumina. Alveolar pores were infrequently observed.

There is very little literature available on the subject of postnatal lung development in ruminants. Castleman and Lay (1990) recently conducted a morphometric and ultrastructural study of postnatal lung growth and development in calves with a view to determining whether there were any especially rapid periods of postnatal bronchiolar or alveolar growth comparable to those described in rodents; they also investigated maturation of nonciliated bronchiolar epithelial (Clara) cells. The three basic conclusions drawn from their work were that the basic architecture of the bovine lung is essentially developed at birth, the alveoli are formed with a single capillary network in contrast with a double capillary network found in other mammals (Caduff *et al.*, 1986; Zeltner and Burri, 1987), and that there are age-associated increases in alveolar number and surface area.

As far as small ruminants are concerned, it appears that a systematic postnatal investigation of the normal development of the respiratory epithelium has not been carried out. The only apparently available information on sheep and goat is based on qualitative lung morphology and morphometric studies dealing with lung volume and respiratory surface area in relation to body weight (De Lorimier *et al.*, 1969; Tyler *et al.*, 1971; Bartlett and Areson, 1977). Recent information on pulmonary development in the

lamb is provided by Docimo *et al.* (1991) in the form of a morphometric study of the alveolar surface area, lung volume and interalveolar wall thickness in fetuses, with only an occasional mention of new-born animals.

## MATERIALS AND METHODS.

### ANIMALS.

Twenty kids of Cashmere breed, representing the following age groups : Day 1, Day 2, Day 3, Day 5, Day 7, Day 9, Day 15 and Day 21, were used in this study. Post-mortem procedures and sample sites were similar to those described in the general materials and methods section in Chapter 2.

### SCANNING ELECTRON MICROSCOPY:

For SEM, samples measuring about 5mm x 5mm and 0.5mm-2mm thick were left in Karnovsky's fixative overnight, then washed in 0.2M cacodylate buffer for 4hrs and thereafter cold dehydrated in a series of graded acetones.

The samples were then critically-point dried using liquid carbon dioxide in a critical-point drier (Polaron: Watford, U.K.).

The specimens were orientated such that the mucosal surface was uppermost, stuck on aluminium stubs using silver paint, and placed in an oven at 37$^{o}$C for half an hour. The specimens were then coated with a gold-palladium mixture in a sputtering system. All SEM samples were examined using a 501B SEM (Philips, Holland) and viewed at an accelerating voltage of 15KV using spot sizes between 200 and 1000. An attached automatic Rolliflex camera fitted with Ilford FP4 120 (125 ASA) film was used in taking pictures.

LIGHT MICROSCOPY:

LM samples were fixed in buffered neutral formalin for seven days, then trimmed and post-fixed for two days in mercuric chloride formol.After fixation, tissues were dehydrated, cleared and impregnated with paraffin wax. Paraffin embedded sections were cut at 3μm with a Leitz Rotary Microtome, mounted on a glass slides and routinely stained with standard Haematoxylin and Eosin (H&E) and by the Alcian Blue /Periodic Acid Schiff (AB /PAS) (pH 2.5) method (Mowry and Winkler, 1956).

## RESULTS.

### NASAL VESTIBULE.

The initial part of the respiratory tract was lined by squamous cells which histological examination showed to be the surface cells of a stratified squamous epithelium. Rostrally, the cells were very flattened and could be seen detaching. A few hairs were also seen. Caudal to this region, squamous cells had wrinkled apical surfaces (Fig. 6.1). Numerous gland orifices were seen opening onto the epithelial surface. Individual squamous cells carried surface microvilli and microplicae (Fig. 6.2), especially prominent in newly-born to three-day-old kids. Whorl-like microplicae, similar to those encountered in adults were rarely observed. Occasionally, mucus strands were seen extruding from the cell surfaces in the caudal region of the vestibule (Fig. 6.3).

A feature observed in all ages, from the new-born to the three-week-old kids, was the presence of dome-shaped areas in the caudal regions of the nasal vestibule (Fig. 6.4). These areas were covered by squamous cells having smooth luminal surfaces, in contrast to squamous cells elsewhere, which exhibited the characteristic folded luminal surface. A central pore was usually encountered on the summit of these dome-shaped areas, and two to three spherical cells would often be seen extruding from these pores

(Fig. 6.4).

There were no other major differences in the epithelium or in individual cell morphology between animals of different ages.

ALAR FOLD:

In all the kids examined in this study, from a 3-hr-old to twentyone-day-old, LM observations showed that the alar fold was lined by a narrow zone of stratified squamous epithelium rostrally (Fig. 6.5), with the major part of the fold being lined by a stratified cuboidal epithelium. The latter gave way caudally to a narrow zone of ciliated epithelium.

SEM studies showed that, in the middle region of the alar fold, the surface cells of the stratified cuboidal epithelium had prominent boundaries and luminal surfaces which were frequently dimpled or folded (Fig. 6.6); these features were observed in kids of all ages. Although at low magnification the luminal surfaces appeared to be smooth, at high magnifications surface microplicae could be seen.

Submucosal gland orifices were often encountered in this region, along with numerous nonciliated microvillous cells with sparsely populated microvilli; the latter cells were considered to be mucus-producing cells (Fig. 6.7).

The caudal region of the alar fold was fairly heavily ciliated even in new-born kids, with mucus-producing cells scattered amongst the ciliated cells (Fig. 6.8). The same extent and degree of ciliation was seen in all kids examined in the present study. Dome-shaped circumscribed areas with a central pore, similar to those encountered in the nasal vestibule, were also observed in this region, although the dome was much flatter than in those seen in the nasal vestibule (Fig. 6.9).

BASAL FOLD.

In the rostral region of the basal fold, the mucosal surface was lined by squamous cells, the cells appearing thicker on moving caudally. Most of these surface cells were characterised by depressed and sometimes folded luminal surfaces (Fig. 6.10). Surface microvilli were not easily discernible at low power, although at high power they were seen to be short and closely packed. On moving caudally, a narrow zone composed of nonciliated microvillous cells, regenerating ciliated cells and a few mature ciliated cells was seen (Fig. 6.11). The caudal region was completely ciliated, the degree of ciliation being much more pronounced than on the alar fold, even at the early age of 3 hrs. (Fig. 6.12). Individual mucus-producing cells were observed within the cilial carpet, while patches of nonciliated microvillous cells were not uncommon (Fig. 6.13).

NASAL CONCHAE.

The epithelium lining the ventral concha, which was organised into longitudinal folds with short gutters, was seen to be heavily ciliated in kids of all ages, the cilia frequently being seen to be matted. Submucosal gland orifices were numerous and these were located primarily within the gutters. Mucus-producing cells, identifiable by their characteristic apical protuberances, were numerous and these were seen to increase with age, being fewer in new-born animals and more numerous in three week-old-kids (Fig. 6.14). Patches of nonciliated microvillous cells and a few ciliated and regenerating ciliated cells were also occasionally encountered

The heavy ciliation of the lining epithelium of the ventral concha was also observed to extend onto the middle and dorsal conchae. However, on these conchae, patches of nonciliated microvillous cells were frequently observed, and differences in the degree of ciliation between kids of different ages were noticeable. This was much

more obvious on the middle nasal concha ,where the new-born kids presented a more sparsely ciliated epithelial surface (Fig. 6.15) compared to the three-week-old animals (Fig. 6.16).

NASAL SEPTUM.

In new-born kids (3 hrs to one-day-old) the epithelium lining the nasal septum was found to be heavily ciliated, the cilia frequently being matted (Fig. 6.17). From the age of nine days onwards there was a gradual reduction in the degree of ciliation, such that in the three-week-old kids the nasal septum exhibited extensive patches of nonciliated microvillous cells. These cells, which were sometimes seen to be flattened and appearing to lift off from the underlying surface, presented a large luminal surface frequently seen to be wrinkled and studded with prominent surface microvilli. Mucus-producing cells identified by their characteristic apical protuberances were numerous at all ages. By the age of 3 weeks, the nasal septum presented a "moth-eaten" appearance characterised by large numbers of nonciliated and mucus-producing cells, and a few scattered regenerating ciliated cells, interspersed amongst the ciliated cells (Fig. 6.18); such an appearance was also a characteristic feature of this epithelium in the adult.

NASOPHARYNX:

In all age groups, the mucosa of the rostral portion of the nasopharynx was seen to be organised into longitudinal folds and gutters, and to be lined by a ciliated epithelium. Frequently, ciliated cells were seen to be matted and forming clumps (Fig. 6.20). Interspersed between the ciliated cells were mucus-producing cells presenting globular apical protrusions. A few nonciliated microvillous cells were also observed.

Occasional submucosal gland orifices, often seen extruding mucus, were observed in the gutters. In the new-born animals, the degree of ciliation was observed to be relatively poor compared to older kids (15 and 21-day-old animals), the cilia being short and the epithelium containing relatively more nonciliated microvillous cells (Fig. 6.19). Occasionally patches of densely ciliated epithelium were however also observed in these younger kids (Fig.6.20).

Caudal to the ciliated epithelium, a transitional zone was observed. This was characterised by an epithelium composed of numerous nonciliated microvillous cells, regenerating ciliated cells and a few ciliated cells. In the new-born, 2-day-old and 3-day-old kids, although most of the cells presented a bulging apical surface some of the nonciliated microvillous cells presented apical surfaces which appeared to have folds or dimples (Fig. 6.21). Occasionally circumscribed areas composed exclusively of nonciliated microvillous cells were observed on the epithelial folds; these cells carried very dense aggregations of short surface microvilli ,discernible only at high power (Fig. 6.22).

LM observations demonstrated that a stratified epithelium, starting off as stratified low cuboidal rostrally and continuing into a stratified squamous epithelium caudally, lined the region caudal to the transitional zone. The caudal region presented a similar appearance in animals of all ages. A few submucosal gland orifices were encountered in this region .

EPIGLOTTIS:

The epiglottis was seen to be lined by squamous cells characterised by the presence of microplicae on their luminal surfaces. Submucosal gland orifices and taste buds (Fig. 6.23) were encountered. No differences in the basic appearance were noted amongst kids of different ages .

VOCAL FOLD.

LM observations showed that three types of epithelia lined the vocal folds in all ages, an intermediate type of epithelium being observed to form a narrow transitional zone between a cranially located stratified squamous epithelium and a caudally situated ciliated epithelium.

The stratified squamous epithelium was characterised, at the level of SEM, by flattened surface squames, most of them presenting wrinkled luminal surfaces. In this region, circumscribed, dome-shaped areas were frequently encountered, the squamous cells on the dome appearing flat and smooth (Fig. 6.24). On moving caudally the squamous cells became more "spongy" and their luminal surfaces appeared to be much more wrinkled and pitted (Fig. 6.25).

The transitional zone was lined by an intermediate type of epithelium characterised by the presence of numerous regenerating ciliated and nonciliated microvillous cells amongst which were distributed a few ciliated cells (Fig. 6.26). Some of the nonciliated microvillous cells appeared to have 'pits' on their luminal surfaces from which droplets of mucus could be seen extruding.

The mucosa lining the caudal surface of the vocal folds was organised into folds and short gutters, and was lined by a ciliated epithelium. The cilia appeared relatively shorter than those seen on the nasal conchae and were frequently seen to be matted. Intermingled between the ciliated cells were nonciliated microvillous cells which presented large, wrinkled luminal surfaces. Occasional patches of normal nonciliated microvillous cells were observed (Fig. 6.27).

Age differences were noted in relation to the nonciliated microvillous cells, which were much more wrinkled and numerous in new-born to 15-day-old kids, and less numerous in the three-weeks-old ones.

INFRAGLOTTIC CAVITY

The mucosa lining the cavity was organised into low folds and gutters which were longitudinally orientated. Squamous cells were observed to line a narrow cranial region of the cavity whilst ciliated cells lined the remainder. Ciliation was observed in animals of all ages, although in the new-born to 3-day-old kids it was not as well developed as in 21-day-old animals. Nonciliated microvillous cells were frequently seen, either individually distributed amongst the ciliated cells or organised into patches. Most of the individually distributed cells, and a few of those grouped into patches, presented a very obvious wrinkled luminal surface carrying numerous short microvilli (Fig. 6.28). LM observation of this region demonstrated the presence of mucus-producing cells.

TRACHEA.

The mucosa of the trachea, organised into folds and gutters, was seen to be lined by a relatively heavily ciliated epithelium as early as 3 hrs postnatally. However, the cilia appeared shorter and not so densely organised as those seen in the adult. Patches composed of intermingled nonciliated microvillous and regenerating ciliated cells were often observed (Fig. 6.29). Occasionally, smaller circumscribed areas of nonciliated microvillous cells, without regenerating cells, were encountered along the tracheal mucosa; the cells appeared to present flattened luminal surfaces with a dense population of microvilli.

Characteristic of the new-born to 15-day-old kids were cells presenting large,wrinkled microvillous or microplicate apical surfaces. These were often seen to be distributed singly amongst ciliated cells (Fig. 6.30).

Mucus-producing cells with depressed luminal surfaces were seen distributed in the gutter areas in all ages, along with numerous submucosal gland orifices which

decreased in number on moving caudally down the trachea. LM sections show that there were relatively fewer mucus-producing cells in the dorsal tracheal epithelium than in the ventral epithelium; in both areas such cells were located mainly in the gutter areas. In addition, cilia were frequently seen to be matted and clumped. Mucus-producing cells presenting apical protuberances were very infrequently observed on the folds in the larynx and trachea. It was not until the first week of life that such cells were observed in small numbers on the folds in the trachea. A layer at the base of the cilia, staining blue with AB/PAS, was often observed by LM.

EXTRAPULMONARY and CAUDAL LOBAR BRONCHI.

The bronchial epithelium in all ages was also thrown into alternating folds and gutters, with ciliated cells being more numerous on the folds than in the gutters. In new-born to 5-day-old animals, a number of individually distributed nonciliated microvillous cells were observed on the folds; occasionally these would appear in groups of twos or threes and usually presented a slightly convex luminal surface.

In animals of all ages the gutters were primarily populated by nonciliated microvillous cells with slightly depressed luminal surfaces. Such cells were identified as mucus-producing cells as they were often seen discharging mucus in sheet form. Few ciliated cells were found in the gutters.

As the diameter of the bronchial tree decreased, regardless of age there was a concomitant decrease in the number of submucosal gland openings and ciliated cells, whereas the number of nonciliated microvillous cells and mucus-producing cells increased (Fig. 6.31).

## RESPIRATORY BRONCHIOLES.

Respiratory bronchioles were rarely encountered in the first week of life. It was only at the age of 7 days that structures comparable to the adult respiratory bronchioles were first observed (Fig. 6.32) . These bronchioles were characterised by a few shallow depressions in their walls, the depressions being surrounded by relatively thick ridges at the rims (Fig. 6.33). The epithelium was essentially composed of two cell types: nonciliated bronchiolar epithelial (Clara) cells and ciliated cells (Fig. 6.34). The former usually presented typical apical protuberances and short, stubby surface microvilli; the latter were characterised by very few cilia per cell, the cilia being of unequal lengths and frequently matted, with microvilli distributed amongst them.

At the age of 15 days, respiratory bronchioles had relatively more alveoli within their walls, although they still remained shallow. A gradual increase in the number of ciliated cells relative to nonciliated bronchiolar epithelial cells was noted. As the age of the kids advanced up to 21 days, the numbers and lengths of individual cilia per cell increased.

## ALVEOLI.

Low power micrographs of lung parenchyma from new-born kids were seen to present numerous alveoli, the appearance being similar to that observed in adult animals (Fig. 6.35). However, high power magnification of the same areas indicated that, although the majority of alveoli were lined with flattened alveolar Type I cells, in the new-born to 3-day-old kids, alveolar Type II cells were frequently seen to be clumped together, in some situations appearing to form the whole of the alveolar wall (Fig. 6.36). Such aggregations of Type II cells were also occasionally observed in the other age groups.

Alveolar sacs with low septa separating individual alveoli were much more common in new-born to 3-day-old animals than in the 15 to 21-day-old kids (Fig. 6.37).

Alveolar pores (of Kohn) and alveolar macrophages were rarely encountered in kids of any age.

## DISCUSSION.

A review of the literature indicated that studies of the postnatal development of the respiratory system in domestic mammals are very limited, with information concerning the goat in particular being apparently unavailable.

The review established that the pattern of foetal and postnatal lung development in mammalian species is essentially the same, with species differences being related primarily to the timing of events. For species such as the goat, in which the new-born kid has to be on its feet and active as soon as possible following birth, it is obviously advantageous to have as fully functional and efficient a respiratory system as possible at birth. Such a system would be expected to be structurally very similar to that of the adult animal.

Such an assumption was confirmed in the present study which established that the epithelium lining the respiratory tract of the new-born kid is relatively well developed and essentially resembles that found in the adult goat. However, the differences which did exist in the structural organisation of the epithelial lining in the kid were characteristic, and are discussed below.

UPPER RESPIRATORY TRACT:

The epithelium lining the nasal vestibule and rostral region of the basal fold and alar fold was of a squamous type, observed to be stratified on histological sections. This epithelium presented similar characteristics in kids of all ages from new-born to 21-day-old. Such observations are in agreement with studies in the neonatal pig (Adams, 1990; Larochelle and Martineau-Doize, 1990).

In these regions in the present study, dome-shaped areas covered by smooth squamous cells surrounding a central pore were commonly observed. These areas, previously identified as lymphoepithelium (Chapter 4), were more frequently encountered in the kid than in the adult goat, suggesting that neonatal kids have more nasal-associated lymphoid tissue (NALT) than adult goats. The sentinel position of the NALT suggests that it plays a significant role in host defence (Mair *et al.*, 1987), and since young animals are usually more susceptible to infection, it would therefore appear appropriate that these structures are most frequently encountered in young animals. Although the present observations thus also appear to suggest that NALT may decrease with age in the goat, such mucosal-associated lymphoid tissue (MALT) increases or decreases with age are not well established. Mair *et al.* (1987) found that there was a marked variation between individual horses in the amount of NALT, as well as in the amount of bronchiole-associated lymphoid tissue (BALT), present. This variation did not appear to be age related, and they proposed that it was due to the variation in the degree of exposure to environmental antigen. Such a proposal is supported by the observations of Jericho *et al.* (1971) and Gregson *et al.* (1979) who found that BALT is poorly developed in germ-free and specific pathogen-free animals. In the present study however, the young animals (especially the new-born kids) had had little environmental antigenic stimulation and it would therefore appear that the variation in occurrence and distribution of this NALT tissue in the goat is more probably age related.

In animals whose young are not immediately active, the development of MALT

appears to be be somewhat delayed. In the rat, associated lymphoid tissue in the bronchioles is first found 2 weeks after birth, and continues to develop beyond 8 weeks of age (Emery and Dinsdale, 1973). In man, BALT aggregates are not found at birth but appear at approximately 1 week of age (Emery and Dinsdale, 1973). These observations in the rat and man reflect the immature stage of lung development at birth in these species compared to that of the goat.

It was established in the present study that at birth the neonatal kids also present a ciliated epithelium in those areas of the nasal cavity that are normally ciliated in adult animals. Although the caudal regions of the alar and basal folds of adult animals are ciliated, the present study in kids established that the degree of ciliation was heavier even in the 3-hr-old kids. Further into the nasal cavity, the development of ciliation on the nasal conchae appeared to be somewhat delayed; indeed it was not until three weeks of age that the middle nasal concha presented a heavily ciliated epithelium similar to that observed in the adult animals. Such observations suggest that the postnatal development of cilia in the nasal respiratory epithelium of the kid follows a rostrocaudal axis, beginning in the rostral region of the nasal cavity, and developing later in the caudal region. These observations receive some support from the studies of Kanda and Hilding (1968) in the rabbit, where ciliogenesis occurs along a craniocaudal axis, being first observed in the nasal cavity, followed by the larynx and then the trachea. However, there is a disagreement with findings obtained in the rat (Menco and Farbman, 1987), where cells with both cilia and microvilli first start to appear in the caudal region of the nasal cavity near the olfactory region, and develop later in the rostral region of the nasal cavity.

It was observed in the present study that the epithelium lining the nasal septum was heavily ciliated in new-born kids up to the age of 9 days, from which time onwards the degree of ciliation was markedly reduced to resemble that of the adult animal, as described in Chapter 4. This relative reduction in ciliation in the lining epithelium with age, as seen in the present study, may well be due to the effect of airflow in the nasal

cavity postnatally. Such a view receives some support from observations in new-born babies (Mygind, 1978), where the pseudostratified ciliated epithelium covering the nasal conchae and extending into the caudal region of the nasal vestibule later shows marked deciliation, assuming the characteristic adult pattern of a transitional zone epithelium. In addition,cessation of nasal breathing, as brought about by mouth breathing in rhinitis patients or in cases of tracheotomy, results in the nasal epithelium becoming more heavily ciliated (Ewert, 1965; Jahnke, 1972; Mygind *et al.*, 1974).

In the present study, mucus-producing cells identified at both SEM and LM levels were encountered even in the new-born kid. Although no quantitative assessment of mucus-producing cells was attempted in the present investigation, a qualitative assessment suggested that there was an increase in the number of these cells with age. This observation is in agreement with studies in man, where it has been observed that goblet cells on the nasal conchae tend to increase with age in both prenatal and postnatal phases of development (Tos, 1982; Boysen, 1982).

The rostral regions of the nasopharynx of 1 and 3-day-old kids exhibited a poor cilial carpet, although the degree of ciliation was seen to increase with age. The other regions of the nasopharynx presented a surface morphology similar to that seen in the adult goats. The surface morphology of the epiglottis was also similar in appearance to that of the adult goat, even in the new-born kid.

A nonciliated microvillous cell type, not identified in adult goats, was encountered in all age groups of kids examined in the present study. This cell type was frequent in ages up to 1 week, although thereafter it was seen to decrease numerically. It was characterised by a large, wrinkled apical surface studded with short microvilli and, very occasionally, with microplicae. Such cells, frequently seen singly or in groups of two or three, were first observed at the laryngeal level, and again in the trachea. Such cells were not observed elsewhere.

Smolich *et al.* (1977) investigating the postnatal development of the epithelium of the larynx and trachea in the rat, described a nonciliated microvillous cell type larger

than other epithelial cell types and presenting an irregular polygonal apical surface with a low electron response and sparsely distributed microvilli. Beyond the third postnatal week these cells were seen to have disappeared. Although such cells did not present the typical wrinkled appearance characteristic of similar cells observed in the present study, their distribution and postnatal pattern of development showed some similarities. Such cells, as observed and described in the present study, were not seen in the adult goat and do not appear to have been reported in any other mammalian species. Patches of similar cells have been reported, however, in the trachea of the broiler chicken (Mohammed, 1989), where it was suggested that they might represent sites of ciliated cell metaplasia.

In the present study it was observed that, although the pattern of ciliation in the larynx and trachea was already established at birth, it had not reached the same stage of development as that observed in those regions in the adult goat, nor even that in the nasal cavity of the new-born. This observation supports previous observations in rabbit (Kanda and Hilding, 1968) and dog (Wright *et al.*, 1983) that the pattern of cilia development in the mammalian respiratory tract follows a cranial-caudal axis.

Such observations might suggest that the wrinkled nonciliated microvillous cells observed in the larynx and trachea in the present study may represent an undifferentiated cell type capable of developing into either a ciliated or mucus-producing cell. Such a suggestion receives some support from the proposition, experimentally confirmed (Evans *et al.*, 1986), that the nonciliated microvillous cell is a primary progenitor cell , capable of developing into a ciliated or a mucus-producing cell (McDowell *et al.*, 1984[a]).

Given that typical mucus-producing cells were very infrequent in the larynx and trachea of the neonatal kid but numerous in these regions in the adult, and that the distribution and location of the wrinkled, nonciliated microvillous cell types was similar to the distribution and location of the mucus-producing cell in these locations in the adult goat, it is tempting to suggest that these wrinkled, nonciliated cell types may

represent a developing form of the mucus-producing cell.

However, such speculation begs the question as to why such a developmental form of mucus-producing cell was not observed in other areas of the lining epithelium. Certainly, more studies on the nature of these cells are required, especially with the use of TEM to further characterise these cells at the ultrastructural level.

LOWER RESPIRATORY TRACT.

The tracheobronchial epithelium of the goat was ciliated at birth; indeed, the general morphological appearance of this epithelium in kids of all ages examined in the present study was similar to that of the adult. The only noticeable difference between kids and adults was that the ciliation in kids was relatively less dense than that seen in the adult. Also nonciliated cells presenting a large, wrinkled apical surface, similar to those observed in the larynx of the kid, were again observed in the trachea of kids of all ages in the present study. As the airway diameter decreased, the degree of ciliation was also seen to decrease, an observation in agreement with that made in the sheep, and supporting the suggestion that the development of the conducting airways in the foetal lung proceeds centripetally (Alcorn *et al.*, 1981).

The present observation of the relatively heavy, almost adult pattern of ciliation in the tracheobronchial tree of the new-born kid contrasts markedly with observations in the dog by Wright *et al.* (1983). These workers investigated the pattern of cilia formation in the tracheobronchial tree of 25 animals aged between 4 hrs. and six months, and were able to show that the new-born dog is born deficient of its full complement of cilia, apart from a carpet of cilia running along the dorsal wall of the trachea. They found, however, that the dog quickly developed its full complement of ciliated cells within five to seven days after birth. In the ox, it was observed that calves at one week of age had also attained the adult pattern of ciliation (Iovannitti *et al.*, 1985). However, because this latter study of cilial development was not carried out

during the first week of life, the pattern of cilia distribution at parturition in the calf was not established.

Although observations made in the goat in the present study are in agreement with those made previously in the sheep (Alcorn *et al.*, 1981) and in the bovine (Castleman and Lay, 1990) in that the basic architecture of the lung is well developed at birth in these species, the present study of young goats has established that respiratory bronchioles are not well developed at birth. They are rarely encountered in the first week of life, after which they are encountered with increasing frequency as the animal ages. These observations are in agreement with those of Mariassy *et al.* (1975) who found that in the bovine, where, like in the goat, the degree of lung development is well advanced at birth, alveolization of respiratory bronchioles at birth is poor. Such observations are also similar to those of Castleman and Lay (1990) who were unable to find respiratory bronchioles in the lung of new-born calf and also observed that the volume densities of airways, vessels and alveolar tissue undergo no major changes with age for the first 5 months of life, suggesting that the distal airways are fully developed at birth and undergo no major changes thereafter. Whether or not respiratory bronchioles develop later in the adult bovine lung is not well established, as such structures were not observed in the adult lung by Iovannitti *et al.* (1985), although Mariassy *et al.* (1975) reported their presence. The presence of respiratory bronchioles in the lung of the adult goat has been previously described in the present study.

The present observation of a postnatal development of respiratory bronchioles in the goat is similar to reports in the dog (Boyden and Tompsett, 1961) where it was demonstrated that alveolization of conducting airways to form respiratory bronchioles also occurs during postnatal development; this is not surprising in this species as the general architecture of the lung of young dogs at birth differs considerably from that of the adult. However, present findings contrast with those of the guinea pig (Lechner and Banchero, 1982) where terminal bronchioles were observed to grade into typical respiratory bronchioles by 58 days of gestational age. The alveolization of the

conducting airway has also been shown to begin halfway through gestation in the Rhesus monkey (Tyler *et al.*, 1983); whether respiratory bronchioles continue to develop postnatally was not established in this species.

The present study in the goat has established that at birth the lung has relatively well formed alveoli, comparable to those of the adult. Such observations can also be applied to the sheep (Alcorn *et al.*, 1981) where the process of alveolar formation, which is seen to begin around the $120^{th}$ day of gestation, results from the subdivision of the saccules by alveolar crests; the associated wall attenuation results in the formation of small thin-walled alveoli. SEM of lung parenchyma in the goat revealed numerous alveoli lined by alveolar Type I and alveolar Type II cells. Most of the alveolar septa were relatively thin, similar to those seen in the adult animal. Findings in the goat in the present investigation further support Engel's (1953) postulate that the degree of lung maturity at birth reflects the stage of body development. It has been observed (Bartlett and Areson, 1977) that large species such as the sheep and cow have well developed lungs at birth, with a higher alveolar surface area per unit of oxygen consumption. This pattern reflects the extensive metabolic requirements which must accompany, among other activities, the new-born animal's struggle to stand and walk efficiently as soon possible after birth.

The present study has revealed, however, that the process of alveolar formation in the kid is not entirely complete at the time of birth. This was evidenced by the occasional presence of ridge-like elevations seen subdividing what appeared to be large saccules. In addition, in some of the alveoli the walls were seen to be lined by numerous cuboidal alveolar Type II cells. It is generally accepted that alveolar Type I cells are formed from alveolar Type II cells (Thurlbeck, 1975), such Type II cell proliferation usually being associated with normal alveolar development (Kauffman *et al.*, 1974; Adamson and Bowden, 1975) or alveolar repair following lung damage (Evans *et al.*, 1973; Adamson and Bowden, 1975). It is therefore likely that such aggregations of alveolar Type II cells lining some of the alveolar sacs are precursors of

the alveolar Type I cells later involved in the remodelling of the alveolar wall.

A double capillary network associated with alveolar development in rat and mouse (Burri, 1974), man (Boyden, 1967), and cat (Al-Tikriti *et al.*, 1991) was not observed in the goat lung with either the SEM or LM. The present study suggested that only a single capillary network was associated with the alveoli in the developing lungs, observations which have also been reported in the bovine (Castleman and Lay, 1990) and sheep (Alcorn *et al.*, 1981Docimo *et al.*, 1991). Such a transitory double capillary network, therefore, does not appear to be a feature of alveolar formation in ruminants.

A paucity of alveolar pores (of Kohn), compared to those observed in the adult goats (chapter 4), was noted in the lung of the kid in the present study. This observation is in general agreement with reports in the cat (Al-Tikriti *et al.*, 1991) where, although alveolar pores were not observed in the developing lung, they were seen to be present in that of the adult. Such an increase in the number of pores with age has also been reported in a number of other mammalian species including monkey (Shimura *et al.*, 1986), dog (Gillette *et al.*, 1989), man (Loosli, 1937; Pump, 1976), rat (Mercurio and Rhodin, 1984) and horse (Pirie, 1990). In the latter case it was reported that numbers of alveolar pores were fewer in animals under 6 years of age but increased in older animals. It has been suggested that such increases in the number of alveolar pores are a pathological manifestation associated with senescence of the alveolar membrane (Gillette *et al.*, 1989).

In summary, although the epithelium lining the respiratory tract of the kid is essentially similar to that of the adult goat, characteristic differences, representing developmental stages, between the two were observed. These include:

1. The cilia were more densely packed and more extensively distributed within the rostral region of the nasal cavity of the kids than they were in adult goats. The large patches of nonciliated microvillous cells seen in adult goats were not a feature of the kid, in which only smaller patches were seen.
2. The epithelium covering the nasal septum was heavily ciliated in new-born to 3-day-

old kids, from which time the numbers of nonciliated microvillous cells increased at the expense of ciliated cells.

3. Bronchioles were poorly ciliated in kids compared with the situation in the adult.

4. A cell type, characterised by a large wrinkled apical surface with short surface microvilli ,was frequently observed in the larynx and trachea of the kid, while such cells were not seen in the adult.

5. Lung parenchyma in the kid frequently presented evidence of alveolar formation in the form of low ridges dividing pre-existing alveoli.

6. In the first week of life, respiratory bronchioles were rarely encountered.

7. Alveolar pores were less numerous in the lung of the kid than in the adult.

# CHAPTER 7.

# OBSERVATIONS ON THE USE OF THE SCANNING ELECTRON MICROSCOPE IN THE STUDY OF THE CAPRINE RESPIRATORY TRACT EPITHELIUM IN THE DISEASED SITUATION.

## INTRODUCTION:

Respiratory diseases are a major constraint in goat production systems (Shavulimo *et al.*, 1988; Ndamukong *et al.*, 1989). It was noted in the general introduction (Chapter 1) that the major causative agents of these diseases include such pathogens as viruses, bacteria and helminths. The commonest bacterial agents associated with respiratory conditions include *Mycoplasma m. mycoides* and *Pasteurella haemolytica* (Ojo, 1976; Ngatia *et al.*, 1985; Jasni *et al.*, 1991), the causative agents of contagious caprine pleuropneumonia and pneumonic pasteurellosis respectively. Both organisms have been shown to produce marked changes in the SEM structure and organization of the respiratory lining epithelium in other species (Mebus and Underdahl, 1977; Jones *et al.*, 1985; Ackermann *et al.*, 1991).

The availability of a limited number of goats with clinical respiratory problems provided an opportunity to determine possible changes in the SEM organisation of the epithelial lining and surface characteristics of individual cell types in the caprine respiratory tract, with a view to assessing the use of the SEM as an additional tool in the battery of diagnostic procedures available for studying respiratory diseases.

The presence and extent of such changes could only be appreciated and properly assessed by having a firm knowledge of the normal SEM morphological appearance of the respiratory tract epithelium in the goat. This was previously established and described in Chapter 4.

## LITERATURE REVIEW.

Scanning electron microscopy allows very large areas of tissue to be prepared and examined with relative ease, thus helping to eliminate difficulties in interpretation that might be caused by sampling errors. It has been used effectively to study the numerous morphological surface changes occurring in the lining epithelium of the respiratory tract as a result of the challenge arising from such factors as pathogens, environmental pollutants, chemical agents and mechanical insults.
The types of changes observed are reviewed below:

### LOSS OF CILIATION.

This appears to be the most frequently observed response to pathological processes, and is certainly a feature of all chronic cases. An extensive loss of ciliation was observed with SEM in the trachea and bronchi of gnotobiotic pigs infected with *Mycoplasma hyopneumonia* (Mebus and Underdahl, 1977), and also in porcine tracheal ring and lung explant organ cultures infected with the same organism (Williams and Gallagher, 1978). Cilial loss was also appreciated under SEM in the the trachea of the mouse damaged following aspiration of gastric contents (Wynne *et al.*, 1981). Loss of ciliation has been reported in SEM studies of the respiratory lining epithelium of horses infected with *Streptococcus equi* (Pirie, 1990) and in calves infected with bovine herpesvirus I (Allan and Msolla, 1980).

SEM has also been used to assess the damage caused by low levels of ozone on the respiratory epithelial lining (Hyde *et al.*, 1978; Zitnik *et al.*, 1978), where cilial loss was reported to be a feature of the pathological lesion. Mechanical injuries resulting from tracheal intubation have also been successfully investigated with SEM, being seen to cause cilial loss (Althoff *et al.*, 1981).

CELL DESQUAMATION.

With SEM, sloughing of cells following infection has been reported. Muse *et al.* (1977) used SEM to demonstrate the effect of *Bordetella pertussis* on tracheal tissue cultures, and exfoliation was effectively demonstrated. Similar observations have been observed with SEM in calves infected with bovine herpesvirus I (Allan and Msolla, 1980). SEM investigations in mice following aspiration of gastric contents demonstrated the desquamation of cells in the tracheal epithelial lining (Wynne *et al.*, 1981). The effects of *Pasteurella haemolytica* on the nasal mucosa of the goat were successfully investigated with SEM (Jasni *et al.*, 1991), where erosions and desquamation of epithelial cells were among the changes observed as a result of the damaging effects of the organism; lesions were observed to be severe in animals inoculated with pure cultures of *Pasteurella haemolytica.*

INCREASED MUCUS PRODUCTION.

Extensive sheets of mucus have been reported by the use of SEM to cover the respiratory epithelial lining in disease processes. Both Majid (1986) and Pirie (1990) observed extensive sheets of mucus almost obscuring the details of the epithelial lining in dogs and horses infected with *Bordetella bronchiseptica* and *Streptococcus equi* respectively. Increased mucus production was also appreciated with SEM in the trachea of pigs inoculated with *Mycoplasma hyopneumoniae* (Mebus and Underdahl, 1977).

It is known that mucus is produced from two sources, the superficial mucus-producing cells and the submucosal glands. The former have been confirmed by the use of SEM to contribute to the observed abnormal sheets of mucus as a result of their numerical increase in pathological conditions. This increase in numbers of mucus-producing cells in disease situation as observed by the use of SEM, has also been reported by several investigators in both mammalian species (Mebus and Underdahl,

1977; Majid, 1986; Pirie, 1990; Jasni *et al.*, 1991) and in birds (Mohammed, 1989).

CHANGES IN CELL SURFACE MORPHOLOGICAL CHARACTERISTICS.

SEM has been useful in studying morphological changes in the lining epithelial cells of the respiratory tract due to pathological processes. Carcinoma-like cells of the distal airways in sheep infected with ovine pulmonary adenomatosis virus (Payne and Verwoerd, 1984) could be distinguished on the basis of surface characteristics observed by SEM. Metaplastic changes were also easily appreciated with the use of SEM in the tracheal epithelium of the golden hamster instillated with benzo(a) pyrene-ferric oxide (Becci *et al.*, 1978), where patches of epithelium with protruding neoplastic cells characterised by short, stubby microvilli or microridges were revealed, findings confirmed concurrently by the use of TEM.

SEM changes in the characteristics of surface cilia, observed as bulbous or curved cilial tips, or a sometimes highly disorientated ciliary pattern, have been associated with disease processes such as immotile cilial syndrome in dogs (Edwards *et al.*, 1983) and *Bordetella bronchiseptica* infection in dogs (Majid, 1986) respectively.

Changes in surface characteristics of Clara cells have been observed with SEM in 3-methylindole-induced bronchiolitis (Turk *et al.*, 1983), in chronic obstructive pulmonary disease (COPD) of horses (Pirie, 1990; Pirie *et al.*, 1992) and in the bronchiolar epithelium of the rat exposed to high levels of ozone and oxygen (Lum *et al.*, 1978; Zitnik *et al.*, 1978). Unusual crater-like lesions were observed with SEM in the apical surfaces of alveolar Type II cellsof horses with COPD (Kaup *et al.*, 1990), and were easily distinguished from the normal.

Hyde *et al.* (1978) investigating the long term effect of high ambient levels of air pollutants on the respiratory system of the dog, noted that SEM revealed the apparent loss of alveolar walls more clearly. The persistent nature of bronchiolar cell proliferation was also clearly demonstrated by the use of SEM.

PRESENCE OF CAUSATIVE AGENT.

The use of SEM has made it possible to observe, and in some cases identify with certainty, the actual causative agent of the pathological changes observed in the lining epithelium of the respiratory tract.

Murphy *et al.* (1980) observed filaments characteristic of *Mycoplasma pneumonia* adhering to the cilia of infected hamsters. Also spherules of *Mycoplasma hyopneumoniae* have been observed with SEM in the tracheobronchial epithelial lining of infected pigs (Mebus and Underdahl, 1977), their size and shape being confirmed by SEM observation of the pure culture. Rods of *Bordetella bronchiseptica* were observed under SEM in the trachea and bronchi of dogs, and the numerical increase of the organisms with time post-infection was appreciated by the use of SEM alone (Majid, 1986).

RESPIRATORY DISEASE IN GOATS.

It was noted earlier in the introductory section that pneumonic pasteurellosis is one of the important diseases in goat production systems, especially in the tropical and subtropical regions. Although the disease has been reported worldwide, it does not appear to be of great economic importance in temperate regions, where caprine arthritis encephalitis virus (CAE) is the most frequently encountered disease condition. Although CAE is basically a disease of the nervous system, pneumonia, either as a result of the virus or as a consequence of the recumbency caused by locomotor paralysis, is an eventual development. On returning home to Tanzania for a short visit, animals exhibiting clinical manifestations typical of pneumonic pasteurellosis became available for study, thus the opportunity was seized to examine the respiratory tract using SEM techniques.

Although there is a dearth of information on the gross pathology, histopathology, and clinical manifestations of these conditions, a summary of which is provided below, the effects of the disease processes on the surface characteristics of the epithelial lining of the respiratory tract do not appear to have been investigated by SEM.

Pasteurella infection in goat.

Pneumonic pasteurellosis is one of the most important caprine diseases in the world (Jasni *et al.*, 1991) and causes high mortality rates in goats of all ages (McSporran *et al.*, 1985). *Pasteurella haemolytica* is frequently isolated from normal goats and has been recognised as part of the normal flora of the upper respiratory tract (Ojo, 1976; Ngatia *et al.*, 1985; Hayashidani *et al.*, 1988); however in disease conditions it is the most frequent isolated bacteria. Although *Pasteurella haemolytica* is frequently isolated in pneumonic lungs, the aetiology of this disease remains uncertain.

Stress factors, including poor housing, transportation, inclement weather and the damaging effects of general viral infections, have been associated with outbreaks of pneumonia in goats (Ngatia *et al.*, 1986; Hayashidani *et al.*, 1988; Fodor *et al.*, 1990) *Pasteurella haemolytica* also plays a role in respiratory disease of other ruminants such as cattle and sheep (Jericho and Langford, 1978; Panciera and Corsvet, 1984; Mishra, 1988). In cattle, it has also been noted that *Pasteurella haemolytica* alone does not necessarily cause the disease, the aetiology being multifactorial and involving stress factors usually associated with shipping, primary viral infection (including infectious bovine rhinotracheitis and parainfluenza type 3 virus) or bacterial infection (*Mycoplasma bovis* and *Pasteurella multocida*) (Jericho and Langford, 1978; Panciera and Corsvet, 1984).

Pneumonic pasteurellosis in the goat is clinically characterised by elevated body temperature and respiration rate, coughing and bilateral mucoid nasal discharge and dullness (Ngatia *et al.*, 1986). Microscopically, pathological lesions of consolidated

lungs range from an acute exudative necrotising bronchopneumonia with numerous neutrophils infiltrating the visceral pleura and peribronchiolar and perivascular connective tissue (Buddle *et al.*, 1990[a,b]), to a predominantly proliferative pneumonia. Exudative-proliferative lesions are characterised by relatively less neutrophils than in the exudative type and more macrophages, hyperplasia of lymphoid tissue, and epithelialisation of alveoli. Proliferative-exudative lesions are characterised by pronounced hypertrophy and hyperplasia of bronchiolar epithelium, moderate hyperplasia of lymphoid tissue, and fibrosis of interlobular septa and perivascular regions, together with patchy epithelialisation of alveoli.

Caprine arthritis-encephalitis virus infection.

Caprine arthritis-encephalitis (CAE) is a relatively new disease complex that affects domestic goats of all ages and probably all breeds, and is caused by a non-oncogenic retrovirus, a member of the lentiviruses (Clements *et al.*, 1980; Sundquist, 1981). The virus can cause three different diseases in different age groups of the host. These include are a rapidly progressive leukoencephalitis in newborn and young goats, a chronic arthritis and mastitis in adult goats, or a sporadic slowly progressive pneumonia-encephalitis.

The most frequently encountered of these three disease syndromes is the arthritic disease of goats aged between 2 and 9 years. The arthritis is insidious in onset and slowly progressive over a period of months to years; joints (usually the carpal joints), bursae and tendon sheaths are the targets. Depending on severity affected animals are usually thin to emaciated and have long, coarse hair. Animals show signs varying from lameness and reluctance to walk, to severe restrictions in joint movements, leading to recumbency. Unless there are septic complications, the animals usually appear alert, afebrile and appetite is maintained.

Rapidly progressive neurological disease of young goats is the other major

syndrome associated with this virus infection (Cork *et al.*, 1974). The syndrome is clinically characterised by posterior paresis (which may progress to include the fore-limbs), circling signs and apparent blindness. Kids with neurological disease also often develop a transient subclinical interstitial pneumonia and the lungs fail to collapse completely at autopsy. Histologically, these lesions consist of lymphoid hyperplasia with frequent nodular arrangements, thickening of alveolar septa and pronounced infiltration by macrophages (Narayan and Cork, 1990).

In spite of the fact that respiratory diseases are one of the major constraints in goat production systems, investigations of disease processes in this species with the use of the scanning electron microscope do not appear to have been attempted. The purpose of this study therefore, was to use SEM to provide a three-dimensional view of the diseased respiratory tract epithelium of the goat, and compare it with the normal

## MATERIALS AND METHODS.

### SOURCE OF CLINICAL CASES.

Two animals, TZ1 and TZ2, aged three and two years respectively, were obtained from the abbatoir in Dar-es-salaam, Tanzania. During routine ante-mortem inspection the two animals were seen to be suffering from a respiratory condition. Their history revealed that these goats had been introduced into a small herd only a few days before showing any clinical signs. Upon clinical examination their body temperatures were elevated, with temperatures of $40^{o}$ and $41^{o}C$ respectively (cf. $39.3^{o}C$). Both animals were coughing and had an increased respiratory rate of 30 and 35 per minute respectively (cf. 15 -20 per minute), with goat TZ1 showing a mucoid discharge from the nostrils. They were slightly underweight (18-20 kg) (cf. ~25 Kg.), with poor hair coats.

Animal CC1 was referred to the Anatomy Department, Glasgow University, with a history of poor performance; it was unable to stand upright, walking on its carpal joints, and had exhibited a long term loss of appetite. Clinical examination revealed a loss of co-ordination, especially of the fore-limbs, together with dyspnea and an obviously stunted growth. Secondary bacterial pneumonia following caprine arthritis encephalitis (CAE) virus infection was suspected. No attempts were made to isolate the virus, but bacterial culture of lung tissue later revealed the presence of *Pasteurella haemolytica*.

Animal MC5: This animal was obtained from among the normal goats used in the study of Chapter 4, and on clinical examination presented no signs of respiratory embarrassment. However, post-mortem examination revealed gross pathological lesions in the lungs of this animal and it was decided to include it in this section.

POST-MORTEM PROCEDURES AND TISSUE COLLECTION.

The animals were killed using an overdose of pentobarbital sodium (Euthetal: Mayer and Baker), the head sagittally sectioned, and the lungs and trachea removed and examined for gross pathological lesions. Samples for both histology and scanning electron microscopy were taken from those sites described in Chapter 3. Tissue samples from the lung parenchyma from animal TZ1, TZ2 and CC1 were removed for bacteriology assessment. No attempts were made to isolate the virus from CC1.

LM AND SEM SAMPLES.

Samples for LM and SEM were processed in the same manner as described in Chapter 3.

# RESULTS.

## GROSS PATHOLOGY.

### Animals TZ1 and TZ2.

Within the nasal cavity, the nasal conchae of TZ1 were covered with excessive mucoid material. Examination of the lungs revealed extensive consolidation of the cranial lobes and conspicuous lobulation in both lungs. The consolidated areas were greyish red and firm on palpation. Similar gross pathological lesions of the lungs were observed in TZ2, except that there was no excessive mucus in the nasal cavity.

### Animal CC1.

Excessive mucoid material was observed within the trachea, together with extensive consolidation of the cranial lobes of both lungs and the middle lobe of the right lung. On cutting these consolidated areas, thick plugs of mucoid-like material, cheesy in consistency, were seen to block the small airways.

### Animal MC5.

This animal had extensive pleurisy involving most of the surface of the lungs. Focal areas of consolidation were observed in the cranial lobes of both lungs and the middle lobe of the right lung. The trachea contained excessive mucoid material.

## HISTOPATHOLOGY.

### Animals TZ1 and TZ2.

Upper respiratory tract: In animal TZ2 a mild form of rhinitis was observed, characterised by mononuclear cell infiltration in the lamina propria, as well as in the

epithelium. Mucus was seen lying on the luminal surface. Changes in animal TZ1 were relatively much more pronounced than in animal TZ2. The epithelium and dermis of the nasal vestibule was seen to be infiltrated by inflammatory cells, mainly plasma cells and lymphocytes (Fig. 7.1). A mild form of rhinitis, characterised by polymorphonuclear cell infiltration in the epithelium and mononuclear cell infiltration in the lamina propria, was a feature. Congestion and mild oedema was also observed. Some loss of surface epithelial cells was encountered. Mucus and debris was also seen lying on the luminal surface (Fig. 7.2).

Lower respiratory tract: Animal TZ2 presented mild changes in the lower respiratory tract, although there was some evidence of tracheitis and bronchitis characterised by mononuclear cell infiltration in the lamina propria (Fig. 7.3). Lymphocytic infiltration was observed around some bronchioles. Foci of alveolar collapse was a feature. Again changes in the lower respiratory tract of animal TZ1 were relatively pronounced. Lymphocytic "cuffing-type" lesions were frequently encountered around bronchi, and hyperplastic submucosal follicles were seen. Small bronchi and bronchioles were often seen to contain inflamatory exudate composed of neutrophils and mucoid material (Fig. 7.4). Foci of alveolar collapse were also observed.

Animal CC1:

Upper respiratory tract: Focal necrosis and apical cell erosion was observed in the epithelium lining the nasal vestibule. The epidermis, as well as the dermis, was heavily infiltrated by neutrophils. Acute rhinitis associated with dense infiltration of neutrophils in the epithelium was found in the basal fold

The epithelium covering the nasal conchae presented varying degrees of changes, characterised by an accumulation of mononuclear cells, mainly plasma cells and lymphocytes, in the lamina propria; a few of them infiltrated the epithelium. Debris, composed of inflammatory cells, dead and dying epithelial cells and mucus, was observed lying on the epithelial surface. In some areas most of the epithelial cells were

seen to be exfoliated, exposing the basal lamina (Fig. 7.5).

In the nasopharynx, there was heavy infiltration of the lamina propria by lymphocytes and plasma cells. Lymphatic nodules had distinct germinal centres. The epithelium overlying lymphatic nodules was frequently seen to be disrupted, with lymphocytes seen passing through clefts in the epithelium onto the epithelial surface (Fig. 7.6).

A very mild infiltration of mononuclear cells was seen in the lamina propria of the epiglottis, most of the epithelium showing a normal organisation except for a few necrotic foci in the epithelium. In the vocal fold, a ruptured microabscess was seen in the epithelium; the lamina propria was also infiltrated by a few plasma cells and lymphocytes.

Lower respiratory tract: The trachea and bronchi presented similar changes characterised by infiltrations of lymphocytes and plasma cells within the lamina propria, a mixture of inflammatory cells, debris and mucus lying on the luminal surface, and a mild degree of lymphoid hyperplasia in the cranial dorsal trachea. The bronchioles were blocked by inflammatory exudate (Fig. 7.7) composed of neutrophils, mucus and some exfoliated epithelial cells.

Most of the changes were seen in the lung parenchyma. There was an extensive degree of alveolar collapse, together with thickening of the alveolar membrane; this thickening was due to the heavy infiltration of neutrophils and plasma cells in the interstitial tissue (Fig. 7.8). Occasionally a mild degree of oedema was observed in the interstitium. Some of the alveoli were filled with mucus.

Animal MC5:

Upper respiratory tract: The mucosa of the alar and basal folds presented varying degrees of inflammation, being more pronounced in the latter. The epithelial lining was infiltrated by polymorphonuclear cells while the lamina propria was infiltrated by mononuclear cells, mainly plasma cells and lymphocytes. Some macrophages were also

observed, especially in the basal fold. Debris, consisting of desquamated cells, mucus and some inflammatory cells, was observed on the surface of the epithelial lining.

Changes in the nasal conchae were characterised by mononuclear cell infiltration of the lamina propria and an increase in numbers of surface mucus-producing cells. Mucosal lymphoid hyperplasia was observed in the ventral and middle nasal conchae. Changes in the dorsal nasal concha were of a relatively mild nature, with an increase in numbers of mucus-producing cells being the prominent change.

Except for an occasional infiltration of mononuclear cells seen in the vocal fold, the epiglottis and vocal folds presented no striking changes in the lining epithelium. The infraglottic cavity exhibited prominent changes in the lamina propria, with an infiltration of mononuclear cells, mainly plasma cells and lymphocytes.

Lower respiratory tract: The dorsal cranial trachea appeared normal. Inflammatory changes were prominent in the ventral trachea, however, these changes being characterised by the infiltration of mononuclear cells consisting of lymphocytes, plasma cells and some macrophages, into the lamina propria. Other salient features were mucosal lymphoid hyperplasia and an increase in numbers of surface mucus-producing cells. Debris, consisting of mucus and desquamated cells, was observed on the epithelial surface. Relative to changes observed in the trachea, those in the bronchi were of a mild nature. Mucoid material was observed in the lumina of the terminal and respiratory bronchioles with AB / PAS staining, and numerous numbers of mucus-producing cells were observed (Fig. 7.9).

There was a degree of alveolar collapse, and thickening of the alveolar septa. Some lymphocyte infiltration of the septa was observed. A mild degree of fibrosis was encountered, together with cellular exudate in the alveolar lumina.

## SCANNING ELECTRON MICROSCOPY.

### Animal TZ1, TZ2 and MC5.

It was observed that the lesions in the epithelial lining of the respiratory tracts of the three animals, TZ1, TZ2 and MC5, were similar. Thus to avoid monotony of repetition in describing the observed changes, it was decided to discuss the findings in these three animals together, highlighting only significant individual differences.

Nasal vestibule and alar fold: Debris, appearing to be made up of a mixture of desquamated cells and mucus, was seen to be entangled in between the hairs in the rostral portion of the nasal vestibule. Sheets of desquamating cells were also seen on the luminal surface. In the exposed regions a few micro-organisms, presenting varying shapes ranging from stellate to coccoid to coccobacilli, were seen lying on the luminal surface (Fig. 7.10). Most of the surface squamous cells in this region presented a number of pores on their luminal surface (Figs. 7.10, 7.11); the numbers of such cells were seen to increase towards the alar fold.

Within the alar fold mucus strands, debris and micro-organisms were also seen lying on the luminal surface (Fig. 7.12), and in the caudal regions mucus strands were seen being extruded from the cells beneath (Fig. 7.12).

In the alar fold, there was evidence of the loss of considerable numbers of the surface cuboidal cells. The areas from which the cells had detached were marked by depressions or pits; in these areas, the intercellular spaces between adjacent cells were seen to be wider than normal, leaving gaps between adjacent cells.

Basal fold: The epithelium was confirmed by histology to be stratified cuboidal in nature. Numerous mucous cells could be seen extruding mucus (Fig. 7.13). In a few places, the uppermost layer of cuboidal cells was seen to have been lost, leaving a rugged surface carrying debris mixed with mucus.

Further caudally on the basal fold, there emerged a number of regenerating ciliated cells characterised by few short cilia of uneven lengths; their numbers increased caudally in the nasal cavity. Many micro-organisms, mostly coccoid in shape, were seen adhering to the luminal surface, being primarily attached to the cilia.

Nasal septum: In animal TZ2 the nasal septum was lined by an epithelium composed of a few regenerating ciliated cells interspersed between normal ciliated cells. Other ciliated cells, carrying much longer but fewer cilia per cell, were also encountered. In contrast the nasal septum of goat TZ1 presented an epithelial lining completely devoid of cilia (Fig. 7.14). Here the majority of cells at the luminal surface were of the nonciliated microvillous type, with microvilli length varying between cells, although uniform on individual cells.

In both animals, mucus-producing cells were numerous. Some were seen extruding mucus in the form of apical protuberances, while others carried a shallow crater on their apical surface with an occasional film of mucus on top. Mucous sheets and debris covered most of the luminal surface of the nasal septum. Micro-organisms were only seen in areas which had some ciliated or regenerating ciliated cells.

Ventral nasal concha: The ventral nasal concha was extensively covered by sheets of mucus. In those areas where the surface epithelium was exposed, nonciliated microvillous cells were the major cell type making up the epithelium, although occasional patches of ciliated cells bearing relatively short cilia were also observed (Fig. 7.15). Mucus-producing cells were seen discharging mucus; in some places these were numerous and presented a dome-shaped apical surface. Submucosal gland orifices were also a feature, and could be seen extruding mucus.

Dorsal nasal concha: The dorsal nasal concha showed an extensive loss of cilia, the majority of cells comprising the epithelium presenting a microvillous apical surface

(Fig. 7.16). Occasionally a few patches of ciliated cells, covered by a surface debris composed of desquamated cells, mucus and inflammatory cells, were observed.

The mucosa was thrown into alternating folds and gutters, both of which were devoid of cilia; the few ciliated cells that were seen had short, poorly developed cilia. The lining epithelium contained mucus-producing cells in greater numbers than normal, some of which exhibited protuberances of mucus at the apical surface.

Middle nasal concha: The middle nasal concha was also covered by mucous sheets to some extent. The lining epithelium was composed of nonciliated microvillous cells and a few interposed patches of ciliated cells.

Larynx: Most of the epiglottis appeared normal, the epithelium being composed of surface squamous cells. In some areas there were extensive sheets of mucus covering the epithelium. A few micro-organisms were seen scattered on the luminal surface of the epiglottis (Fig. 7.17). The cranial surface of the vocal fold had a similar epithelial lining to that seen on the epiglottis, whilst the caudal surface had fewer ciliated cells than normal, the majority of the component cell type being the nonciliated microvillous cell.

In these animals the lining epithelium of the infraglottic cavity had a characteristically matted appearance. Sheets of mucus, together with cellular debris and inflammatory cells, were observed covering the epithelial lining.

Trachea and extrapulmonary bronchi: The ciliated epithelium found lining the dorsal regions of the tracheal lumen was folded to form alternating ridges and gutters as normal. However, these gutters were also narrowed, and in some areas the resultant clefts were plugged with mucus (Fig. 7.18). In most areas examined the cilia were characteristically matted by mucus; in some areas the latter formed a heavy blanket covering the luminal surface of the trachea. There was a great variation in density of cilia from one area to another. In some areas the cilial carpet was thick, whilst in several

areas the carpet was thinner, the ciliated cells here presenting fewer cilia per cell, with the cilia usually ruffled and intertwined. Highly active mucus-producing cells were seen extruding columns of mucus (Fig. 7.19). A number of micro-organisms were again seen attached to the cilia; the majority were coccoid in shape.

The ventral tracheal mucosa presented an undulating surface with shallow gutters. A mucus sheet containing cellular debris and dead cells was again seen lying on the luminal surface. Areas denuded of cilia were common, resulting in a larger than normal population of nonciliated microvillous cells bearing short microvilli on their apical surfaces. These would sometimes exhibit a slight apical bulge with a wrinkled cell membrane. In some regions there were patchy, irregularly defined areas where the epithelium had been eroded down to the basal lamina. Occasionally in other regions, where erosion appeared to be in its early stages, ciliated cells could be seen detaching from each other and being extruded from the epithelium.

Intrapulmonary bronchi and bronchioles: Compared to the trachea and extrapulmonary bronchus, the epithelial changes seen in the bronchi and bronchioles were relatively minor. Such changes included an increase in the number of mucus-producing cells, poorly developed ciliated cells and increased surface mucus, especially in the terminal and respiratory bronchioles. Microvilli on Clara cells were not readily discernible, the rough apical surface being raised into a dome (Fig. 7.20).

Alveoli: TZ2 was characterised by greatly thickened alveolar septa. Although alveolar Type II cells were not easily identified, the nuclear outlines of alveolar Type I cells protruding into the alveolar lumina were more apparent than normal. In some areas the luminal surfaces of the alveoli were covered by amorphous sheets of material (Fig. 7.21), making it impossible to see the details of the alveolar surface. Blood capillaries were greatly engorged and thus were more obvious than normal. (Fig. 7.22)

The thickness of alveolar septa in goat TZ1 appeared normal. The only

noticeable abnormality was an accumulation of inflammatory cells in the alveolar lumina. A few macrophages, bearing a rough surface with blunt pseudopodia, were seen. Some flocculent material was occasionally seen lying on the alveolar surfaces.

Animal CC1.

Alar and basal folds: Apical squamous cells lining the rostral regions of the alar and basal folds were frequently seen desquamating singly or in patches. Further into the nasal cavity, the apical cells were mainly cuboidal in shape and these also were seen detaching to leave a depression (Fig. 7.23). Some micro-organisms, coccoid in shape, were observed on the epithelial surface. Mucus was often seen to be produced in the form of long filamentous strands.

Ventral nasal concha: The epithelial lining of the ventral concha was extensively covered by sheets of mucus (Fig. 7.24). Where the epithelial surface was exposed, a dense carpet of matted cilia was observed.

Dorsal nasal concha: In contrast to the ventral nasal concha, extensive deciliation was observed, with the majority of the component cell type being the nonciliated microvillous cell. Numerous regenerating ciliated cells were frequently observed. Mucus, together with cellular debris and some fibrous debris, was observed lying on the epithelial surface (Fig. 7.25). Mucus-producing cells appeared to be more numerous than normal.

Middle nasal concha: The surface was similar to the ventral nasal concha, however sheets of mucus were not as pronounced. Mucus-producing cells, often occurring in patches and exhibiting apical protuberances, were encountered in large numbers.

Larynx: Changes in the vocal fold were characterised by the presence of sheets of mucus and surface debris containing cellular exudate, and frequent patches of nonciliated microvillous cells. Extensive areas of desquamation (Fig.7.26), sometimes leaving a bare basal lamina, were observed in the infraglottic cavity. Cells appearing to be detaching from adjacent cells and lifting off from the surface were often observed.

Trachea: The epithelial lining of the dorsal surface of trachea was extensively deciliated, the few ciliated cells that were present having a small number of cilia on their apical surfaces. Sheets of mucus were frequently observed. In some areas there appeared to be evidence of desquamation, with most of the epithelial cells lost to leave, in some cases, a bare basal lamina.

The epithelial lining of both the cranial and caudal portions of the ventral surface of the trachea showed mild changes in comparison to the dorsal surface. Such changes included an increase in number of the domed patches of nonciliated microvillous cells, increased mucus production with mucous plugs being occasionally observed in submucosal gland orifices and a general decrease in number of the ciliated cells (Fig. 7.27).

Bronchi: Changes were relatively mild in comparison to those observed in the trachea. In addition to a general reduction in the number of ciliated cells, patches of nonciliated microvillous cells were frequently observed such that their numbers appeared to be greatly increased compared to those observed in normal animals.

In one of the specimens a single cell, characterised by a dense aggregation of tall spiky, uniform surface microvilli, was observed surrounded by ciliated cells (Fig. 7.28); this cell was considered to be a brush cell. Mucus-producing cells, actively producing sheets of mucus, were also frequently observed.

Bronchioles: The lumina of the smaller bronchioles, the terminal bronchioles and the

respiratory bronchioles contained accumulations of flocculent material which, in many cases, covered the entire epithelial surface (Fig. 7.29). In the few areas that were exposed, the ciliated cells had markedly disorientated cilia, with some flocculent material being held in between cilia. The majority of the Clara cells presented flattened apical surfaces, while some presented shrivelled ('withered') apical protuberances (Fig. 7.30).

Alveoli: There was evidence of an increase in the number of alveolar Type II cells (Fig. 7.31), most of them presenting a number of pores on a central region sparsely populated by microvilli, in contrast to the densely populated peripheral regions (Fig. 7.32). Flocculent material was often observed covering the alveolar surfaces, and occasionally accumulating in the alveolar lumina.

## BACTERIOLOGY

*Pasteurella haemolytica* was isolated from animals TZ1, TZ2 and CC1. Samples from MC5 were not taken for bacteriology.

# DISCUSSION.

The present study was undertaken to give an indication of the use of SEM as an additional tool for examining disease processes within the caprine respiratory system. It was not intended to provide an examination of any particular disease process. In addition it was intended to highlight how a knowledge of normal SEM morphology could be used in clinical situations.

Animals used in this study had natural infection and, in all the cases except MC5, *Pasteurella haemolytica* was isolated. The gross pathological examinations carried out, however, revealed that the animals used in the present study were suffering from respiratory disease, possibly due to infections by *Pasteurella haemolytica*. This organism is known to be part of the normal flora of the nasal cavity of the goat (Ojo, 1976; Ngatia *et al.*, 1985), but may become associated with disease processes when other factors, often stress-related, come into play.

## UPPER RESPIRATORY TRACT.

The presence of a small number of micro-organisms, possessing coccoid to coccobacillic morphological characteristics similar to those previously described for *Pasteurella haemolytica* by Buxton and Fraser (1977) on the epithelial surface of the rostral regions of the nasal cavity as described by SEM support the indication of a possible role for Pasteurella in the observed clinical conditions. The small numbers of these micro-organisms observed in the present study, however, contrast with previous studies where *Bordetella bronchiseptica, Streptococcus equi* and *Mycoplasma hyopneumonia* were observed in large numbers on the lining epithelium of the nasal cavity of the dog (Majid, 1986), horse (Pirie, 1990) and pig (Mebus and Underdahl, 1977) respectively. The numbers seen in the present study seem to be on the low side given the severity of morphological changes on the lining epithelium. These low

numbers may well be a true reflection of the degree of infection, although numerous factors may alter them.

SEM observations made in the present study demonstrated marked changes from the normal appearance of the lining epithelium of the rostral regions of the nasal cavity, with large numbers of desquamating surface cells forming, together with inflammatory cells, an accumulation of surface debris often seen to entangle in hairs of the nasal vestibule.

Desquamation of cells as reported in the present study is in agreement with a previous report in the goat (Jasni *et al.*, 1991) on the effect of *Pasteurella haemolytica* on the nasal mucosa. Erosions and epithelial cell desquamations were characteristic changes observed with SEM. Such observations have also been reported in the lining epithelium of the respiratory tract of other species. Allan and Msolla (1980) observed by SEM a considerable degree of desquamation in the trachea of calves infected with bovine herpes virus. Sloughing of cells was also observed in tissue cultures infected with *Bordetella pertussis* (Muse *et al.*, 1977). Also, patches where desquamation of cells had occurred, were observed in the nasal cavity of dogs infected with *Bordetella bronchiseptica* (Majid, 1986). It would appear that SEM presents the best means of assessing the extent of damage in the form of cell desquamation, which appears to be a common pathological finding in bacterial and viral infections of the lining epithelium of the respiratory tract (Muse, 1977; Allan and Msolla, 1980).

Present SEM observations also indicated that occasionally the surface lining cells presented pores on the luminal surface. Such an observation may be an indication of the damage to the surface plasmalemma, an assumption consistent with observations reported by Whiteley *et al.* (1991), who investigated alteration in pulmonary morphology and peripheral coagulation profiles caused by intratracheal inoculation of live and ultraviolet light-killed *Pasteurella haemolytica* A1 in calves. They observed that *Pasteurella haemolytica* A1 produced cell necrosis and pore formation. Indeed *Pasteurella haemolytica* has already been shown to secrete a leukotoxin known to form

pores in cell surface membranes (Clinkenbeard, *et al.* 1989[a,b]). In addition, *Pasteurella haemolytica* produces several factors including endotoxins (Kiess *et al.*, 1964; Rimsay *et al.*, 1981) a protein exotoxin (Kaehler *et al.*, 1980; Shewen and Wilkie, 1982Chang *et al.*, 1986), neuraminidase (Tabatabai and Frank, 1981) and a neutral protease (Otulakowski, 1983) that may also be involved in early damage of the plasmalemma of susceptible cells. Other bacteria such as *Streptococcus equi* have also been shown to produce soluble products including toxins and enzymes, which are presumed to be involved in local pathogenic damage (Bazeley, 1943; Taussig, 1984).

In the most caudal regions of the nasal cavity, the extensive deciliation and increased mucus production observed in the present study in the goat is in agreement with previous observations (Jasni *et al.*, 1991), where these workers reported extensive cilial collapse onto the epithelial surface, and the micrograph they provideded reveal extensive deciliation. The present findings are also in agreement with previous observations in the pig nasal epithelium colonised by a toxigenic strain of *Pasteurella haemolytica* Type D. (Ackermann *et al.*, 1991), in dogs infected with *Bordetella bronchiseptica* (Majid, 1986), in horses infected with *Streptococcus equi* (Pirie, 1990) and also in a viral condition associated with perennial rhinitis in man (Mygind and Bretlau, 1973) Such studies have shown the ciliated cells to be extremely sensitive to infections, resulting in the localised loss of cilia followed by regeneration, provided that the injury is not repetitive or lethal (Sturgess, 1989). Such regeneration of ciliated cells was also observed in the present study.

Although there was no quantitative assessment of either the individual mucus-producing cells or submucosal glands, which are both responsible for contributing to the formation of the mucous blanket (Chakrin and Saunders, 1974; Jones *et al.*, 1975), a subjective assessment of the individual surface mucus-producing cells suggested that they were more numerous than usual in the epithelial lining of the nasal cavity in the diseased animals. The observed increase in numbers of mucus-producing cells supports previous observations that irritation of the lining epithelium of the nasal cavity either

mechanically (Reid, 1963), chemically by inhalation of various gases such as sulphur dioxide (Mawdesley-Thomas *et al.*, 1971; Collier, 1980) or due to infection (Jones *et al.*, 1975; Wheeldon *et al.*, 1976; Majid, 1986) results in an increase in numbers of mucus-producing cells. The main role of the mucous blanket is to trap inhaled substances, both particulate (Gail and Lenfant, 1983) and microbial (Newhouse, 1982), and then provide for their elimination by activating the mucociliary clearance mechanism (Kilburn, 1974).

In respiratory diseases there is an increase in the numbers of mucus-producing cells and this is usually coupled with changes in the histochemistry of the mucus produced (Jones *et al.*, 1975; Wheeldon *et al.*, 1976; Mellick *et al.*, 1977).

SEM observations in the present study showed that the surface morphology of the epiglottis was not affected in any of the clinical cases examined. Such findings support previous SEM studies in horses infected with *Streptococcus equi* (Pirie, 1990) and also in dogs infected with *Bordetella bronchiseptica* (Majid, 1986). It would thus appear that this region of the respiratory tract is very resistant to pathological changes.

LOWER RESPIRATORY TRACT.

In the present study, the trachea of all four cases examined showed SEM surface changes in the form of cilial loss, mucosal erosion and excess mucus production. There were regional variations in the degrees of change observed, these being more pronounced in the cranial regions of the trachea than in the caudal regions. In addition, the dorsal tracheal epithelial surface exhibited more changes than the ventral surface. Such variations in the regional severity of observed lesions within the trachea are comparable to SEM findings in the Rhesus monkey (Mellick *et al.*, 1977) exposed to high ozone and oxygen levels, where deciliation was more marked on the dorsal membranous aspect than on the lateral or ventral aspect of the tracheal epithelial lining. The present findings in the goat also support previous SEM studies in the dog (Majid,

1986), ferret (Chevance *et al.*, 1978) and horse (Pirie, 1990) where regional variations in observed pathological responses to challenges by *Bordetella bronchiseptica,* influenza A virus and *Streptococcus equi* respectively was also observed. Although the latter case is considered to be a disease primarily of the upper respiratory tract, SEM revealed that some changes also occurred in the lower respiratory tract where patches of nonciliated microvillous cells were present in the usually well ciliated surfaces of these regions. SEM examination of horses affected with chronic obstructive pulmonary disease (COPD) revealed more extensive patches of nonciliated microvillous cells than normal, with ciliated cells exhibiting fewer and more disorganised cilia in the tracheal epithelial lining (Pirie *et al.*, 1992).

It has been suggested that the regional variation in damage caused to the tracheobronchial epithelium by inspired pathogenic organisms or chemical agents would be primarily influenced by three factors, namely the concentration of the aetiological agent at the site, the variation in the degree of protection provided by the covering mucus, and the regional differences in susceptibility to the aetiological agent residing in the inherent sensitivity of the component cell population of the lining in any given region (Mellick *et al.*, 1977).

In the present study, very few micro-organisms with the morphological characteristics of *Pasteurella haemolytica* were observed at this level of the respiratory tract. In addition to an increased mucus production, such as that observed in the diseased animals in the present study, the nature of the histochemistry of the mucosubstances produced in clinical cases has also been observed to change (Ellefsen and Tos, 1972; Wheeldon *et al.*, 1976; Nicholls, 1978)

Experiments in the rat have shown that irritation of the airway epithelium results in a change in the proportion of cells producing acidic and neutral glycoproteins (Jones and Reid, 1978) and thus in alteration to the regional distribution of acidic, neutral and mixed mucosubstances along the respiratory airway. Allan *et al.* (1977) observed changes in the mucosubstances of calves with cuffing pneumonia, where mucus-

producing cells producing larger amounts of neutral mucosubstances than normal were observed; also the amount of sulphation in such cases was observed to be greatly increased. Wheeldon *et al.*, (1976) also reported an increase in the amount of neuraminidase-resistant sialylated glycoprotein in cases of chronic bronchitis in dogs. In cases of chronic bronchitis in man, the histochemical nature of mucus has also been observed to change, with the mucus produced containing greater proportions of neuraminidase-resistant acid glycoprotein than in normal cases (De Haller and Reid, 1965).

There are two schools of thought in explaining the observed changes in the histochemistry of the mucosubstances produced in clinical situations. One is that the change is due to the disruption of glycoprotein synthesis by the pathogen, whereas the other school of thought suggests that the change in histochemistry is produced in order to neutralise or counter the damaging effects of the pathogen.

It is tempting to think that the regional variation observed in the present study may in part be due to the regional differences in susceptibility to pathogenic organisms in the lining cell population. However observations made in the present study can only allow a speculative comment on this point, and further work in this area would be necessary.

In the present study, mucosal erosion was observed both on the ventral and dorsal tracheal epithelial surfaces. Patches of desquamated epithelial cells were obvious and in some areasthe basal lamina was exposed. Observations made in the present study are in agreement with observations made in calves infected with bovine herpes virus (Allan and Msolla, 1980), where desquamation and sloughing of cells was observed. Cell desquamation has also been noted in hamsters infected with *Bordetella pertussis* (Muse *et al.*, 1977), and in ovine and caprine tracheal cultures infected with *Mycoplasma ovipneumoniae* and *Mycoplasma arginini* (Jones *et al.*, 1985).

In the bronchial epithelium of one individual animal, a single cell, characterised by tall spiky, densely organised uniform microvilli, was revealed. These surface

characteristics of this cell were identical to those described for a brush cell (Alexander *et al.*, 1975; Andrews, 1979) and it was therefore identified as such in the present study. Brush cells, constituting a small percentage of the tracheobronchial cell population, have also been described in SEM studies of the normal epithelial lining of the respiratory tract of several mammalian species including the pig (Baskerville, 1970[a]), horse (Nowell and Tyler, 1971) and rat (Andrews, 1979; Popp and Martin, 1984).

Although in the present study the histopathology of the bronchial and bronchiolar mucosa indicated pronounced changes, characterised by mononuclear infiltrations in the lamina propria, lymphoid hyperplasia, and accumulation of inflammatory exudate in the lumina, SEM observations showed only minor changes, characterised as matted cilia and accumulation of surface debris. Compared to the trachea, mucosal erosion in the bronchi was uncommon.

Although SEM demonstrated that the number of ciliated cells decreased and the cells had few cilia, the major changes noted in the bronchioles in this study involved the collapse of the characteristic apical protuberances of the nonciliated bronchiolar epithelial (Clara) cells. Such observations have also been noted in the distal airways of the rat (Lum *et al.*, 1978) and mouse (Zitnik *et al.*, 1978) exposed to high ambient ozone or oxygen levels, in the bronchiolar epithelium of smokers (Ebert and Terracio, 1975[a]), in cases of equine bronchiolitis induced by 3-methyl-indole (Turk *et al.*, 1983) and in cases equine chronic obstructive pulmonary disease (Kaup *et al.*, 1990; Pirie *et al.*, 1992). Previous observations have also suggested that the collapse of the Clara cell apical protuberances is also associated with a reduction in number of contained secretory granules (Lum *et al.*, 1978; Zitnik *et al.*, 1978; Kaup *et al.*, 1990). The collapse of the Clara cell protuberance made positive identification of the cells difficult at this level with the SEM in the present study, a difficulty also encountered by Pirie *et al.* (1992) in cases of equine COPD, Lum *et al.* (1978) in the rat and Zitnik *et al.* (1978) in the mouse.

It could be argued that the observed flattening of the nonciliated bronchiolar

epithelial cells was a shift in the cell type, with more of them changing into mucus-producing cells.

More recent studies in monkeys (Plopper *et al,* 1989) and in man (Ten Have-Opbroek *et al.,* 1991) have suggested that, at least in these species, the nonciliated bronchiolar epithelial cell is indeed a mucus-producing cell, a proposition which departs from the classical view that, at this level, the secretory cell type is the Clara cell. However, it has also been noted that the Clara cell serves as a stem cell of the small conducting airways (Evans *et al.*, 1976, 1978[a], 1986[a]) and indeed may change into a mucus-producing cell. Thus, the increase in mucus production observed at this level in the goat may be due to an increase in the number of these mucus-producing cells, an assumption that receives some support from histopathological and SEM studies in mice (Zitnik *et al.,* 1978) rats (Lum *et al.*, 1978) and man (Wang and Thurlbeck, 1970; Ebert and Terracio, 1975[a]). The observed increase in mucus production, together with the reduction in the ciliary escalator system, results in the accumulation of mucus, inflammatory exudate and surface debris, which cover large parts of the bronchiolar surface. Such observations are in agreement with those in horses affected with COPD (Kaup *et al.*, 1990; Pirie, 1990) and in mice (Zitnik *et al.*, 1978) and rats (Brummer *et al.*, 1977) exposed to ozone, where accumulation of inflammatory exudate was a feature of affected bronchioles.

At the alveolar level it was observed in the present study that in localised regions, there was an increase in the number of alveolar type II cells. Such an increase is usually associated with alveolar formation in young normal animals (Kauffman *et al.*, 1974; Adamson and Bowden, 1975; Kauffman, 1980), but in diseased conditions, an increase in the number of alveolar Type II cells has been shown to be associated with the repair of the damaged alveolar lining epithelium (Rosin, 1947; Hers, 1955; Freeman *et al.*, 1972; Mellick *et al.*, 1977; Kaup *et al.*, 1990; Pirie, 1990). The fact that this increase in alveolar Type II cell numbers was only occasionally observed suggests that the lesions in the lung parenchyma were localised, an observation supported by LM

findings. The alveolar Type II cells observed in the present study usually exhibited fewer than normal surface microvilli and showed a number of pores on the luminal surfaces. Such features of the alveolar Type II cells have been described previously in horses affected with COPD (Kaup *et al.*, 1986; Pirie, 1990). It has been suggested that these pores are associated with hyperactivity of the alveolar Type II cells, the pores being sites of surfactant release. The ease with which they are seen in certain disease conditions has been considered indicative of an increased rate of surfactant production (Kikkawa and Smith, 1983).

In one animal (TZ2), the alveolar surfaces were extensively covered by sheets of amorphous material. In normal situations the alveolar membrane is lined by surfactant material (King, 1979) which, during normal SEM preparation, is usually washed away. The reasons for the persistence of this lining as observed in this particular individual animal must be speculative, possibly being due to either an increased production of surfactant material and /or a spill-over of bronchiolar secretions. The latter, which appear to be a mixture of Clara and mucus-producing cell secretions, have also been noted in certain disease conditions, such as horses with COPD (Pirie *et al.*, 1992).

Another feature observed by SEM in the present study of the diseased respiratory epithelium of the goat was the accumulation of inflammatory exudate in the alveoli, which appeared to be cellular in character. However the amount of exudate did not correlate with LM observations, where accumulation of this cellular exudate appeared to be abundant, filling alveolar lumina.

Macrophages were observed with considerable ease, an observation which contrasted with their scarcity in the normal animal. The present findings of increased numbers of alveolar macrophages in certain disease conditions supports previous studies in the horse (Kaup *et al.*, 1986, 1990; Pirie, 1990), dog (Majid, 1986) mouse (Zitnik *et al.*, 1978) and monkey (Mellick *et al.*, 1977). Recruitment of extra numbers of alveolar macrophages observed in the present study may have been brought about as a response to the infectious agent, as alveolar macrophages are reported to phagocytose

an assortment of debris, including infectious agents and inorganic materials (Green and Kass, 1964; Heppleston and Young, 1973Gilka *et al.*, 1974). Hyperventilation, which is associated with some respiratory diseases such pneumonic pasteurellosis (Rybicka *et al.*, 1974[b]) and COPD (Pirie, 1990), also results in an increase in surfactant production. It is tempting to suppose that the increase in number of alveolar macrophages may also be involved in controlling the quantity of surfactant. This function of the alveolar macrophages has been proposed because of the observed presence of lamellated material, characteristic of surfactant, within alveolar macrophages (Rybicka *et al.*, 1974[b]). Work by Miles *et al.* (1985) went further, to confirm that alveolar macrophages are capable of rapidly degrading natural surfactant. These workers suggested that it seems likely that alveolar macrophages may play an important role in the handling of surfactant that is not recycled into alveolar Type II cells.

The present study has shown that SEM can reveal significant surface epithelial changes brought about by disease processes within the respiratory tract, to complement conventional histopathological examinations. Thus SEM can be successfully employed as a complimentary diagnostic tool in the diagnosis of respiratory diseases amongst the battery of other diagnostic tools available.

# CHAPTER 8.
# SUMMARY AND CONCLUSIONS.

# SUMMARY AND CONCLUSIONS.

The goat is an important animal in the economics of the Least Developed Countries (LDCs) of the tropical and subtropical regions, where 90% of the world's goat population is found. Although respiratory diseases account for up to 40% of all mortalities observed in the goat, any attempt to understand and assess the nature and progression of the relevant disease process, in order to develop appropriate curative and / or control measures, can only be successfully undertaken if a basic knowledge of the normal morphological features of the respiratory tract is available.

A review of the available literature established that studies on mammalian respiratory tract epithelial morphology have dealt mainly with laboratory animals, including the rat, mouse, hamster and monkey. It is only recently that attention has been focused on the domestic mammals, and extensive studies of the dog, bovine and horse have been undertaken with a view to defining the surface morphology of the lining epithelium within the respiratory tract by the use of SEM. Such studies have also employed TEM techniques to characterise the cell types within the epithelial lining. The lack of available information on epithelial morphology in the respiratory tract of the goat, however, determined that the primary objective of this study was to characterise the normal surface features of the entire respiratory tract of the goat by the use of the scanning electron microscope. In addition to this core objective, the histology and the histochemistry of the respiratory tract epithelial lining were also investigated. TEM was used to further characterise the cell types that populate the distal airways and the alveolar membrane. The development of the respiratory tract epithelium was also investigated in new-born kids up to the age of three weeks.

Having established the normal surface topographical appearance, the last chapter dealt with how the SEM could be used to examine surface changes in the respiratory tract lining epithelium as a result of respiratory diseases.

As most standard anatomical texts do not provide an account of the morphology

of the caprine respiratory system in its own right, assuming structures to be similar to those in the sheep unless otherwise indicated, it was felt that an introductory account of the gross anatomy of the respiratory system in the goat as provided in Chapter 1 would provide a useful basis for embarking on the main objective of the histological and ultrastructural study of the epithelial lining of the tract.

As a prelude to the ultrastructural studies, the histology and the histochemistry of the normal respiratory tract lining epithelium was studied as detailed in Chapter 3. Seventeen clinically normal goats were used in both the light microscopical and ultrastructural studies. Animals were killed, and samples taken and processed, as detailed in the procedures described in Chapter 2.

It was established that the nasal vestibule and rostral regions of the alar and basal folds were lined by a stratified squamous epithelium which graded caudally, through an intermediate zone composed of stratified cuboidal cells, into a pseudostratified ciliated epithelium. The latter type of epithelium was found to line the major part of the nasal cavity. The olfactory region was not investigated in the present study.

Individual surface mucus-producing cells were not observed in the nasal vestibule, and were very occasionally observed in the alar and basal folds. Such cells were more numerous on the nasal conchae and nasal septum, and demonstrated the presence of exclusively acidic mucosubstances. Submucosal gland orifices were numerous in the rostral regions of the nasal cavity and indeed elsewhere on the nasal conchae. It was established that the histochemistry of the submucosal glands differed from region to region. Whereas in the rostral region of the nasal cavity (nasal vestibule, alar fold, basal fold), the secretions were serous in nature, on the nasal conchae the proportions of acidic, neutral and mixed secretions were seen to vary between the ventral nasal concha, where the glands produced little acidic and equal amounts of both neutral and mixed mucosubstances, the dorsal nasal concha, where equal amounts of acidic, neutral and mixed mucosubstances were produced, and the middle nasal concha,

which produced neutral, mixed and acidic mucosubstances, although the amounts of each decreased in that order.

The epithelium lining the nasopharynx was seen to vary, changing from a rostrally situated pseudostratified ciliated epithelium through an intermediate epithelium, itself grading from pseudostratified ciliated low columnar to stratified cuboidal, into a stratified squamous epithelium in the caudal regions of the nasopharynx. Individual mucus-producing cells were numerous in the rostral region, their numbers decreasing on moving into the caudal regions. Lymphoid aggregations were observed below the epithelial lining, the latter being attenuated and devoid of mucus-producing cells. Submucosal glands, producing primarily acidic and but mixed mucosubstances, were abundant.

Regional variations in the type of epithelium lining different regions of the larynx was observed. Whilst a stratified squamous epithelium lined the laryngeal surface of the epiglottis and cranial regions of the vocal fold and infraglottic cavity, the caudal surfaces of the latter two regions were lined by a pseudostratified ciliated epithelium. An intermediate type of epithelium, similar to that observed in the rostral regions of the nasal cavity and in the nasopharynx, was observed between the cranial stratified squamous epithelium and the caudally-directed pseudostratified ciliated epithelium on both the vocal fold and infraglottic cavity. Surface mucus-producing cell were exclusively acidic in character, but the submucosal glands exhibited both mixed and acidic staining reactions.

The trachea was lined by a pseudostratified ciliated columnar epithelium. Individual surface mucus-producing cells were relatively few in number and predominantly acidic character.; there was however, a general cranio-caudal increase in numbers of these cells. Submucosal glands were numerous and produced predominantly acidic mucosubstances with only a few producing a mixed reaction; neutral mucosubstances were rarely observed.

The type of epithelium that lined the bronchial tree was similar to that observed

in the trachea, with only a reduction in the height of the epithelial lining being apparent. In addition, the numbers of individual surface mucus-producing cells were seen to increase with a decrease in airway diameter. In contrast, the numbers of submucosal glands decreased with decreasing airway calibre. Both mixed and acidic mucosubstances were seen to be produced in equal amounts in the bronchial surface mucus-producing cells, whilst neutral mucosubstances predominated in the submucosal glands, acidic and mixed mucosubstances being rarely observed.

The bronchiolar epithelium was seen to vary at different levels of the bronchiolar tree. Proximal to the terminal bronchiole, the epithelial lining was of a pseudostratified ciliated type, with only a few surface mucus-producing cells being present. The terminal bronchiolar epithelium was a simple columnar epithelium, composed of ciliated and nonciliated bronchiolar epithelial (Clara) cells, the latter being identified by their negative staining reaction to AB /PAS. Mucus-producing cells were not observed at this level. In the distal generations of the terminal bronchioles, the lining epithelium was of a simple cuboidal type. The same type of epithelium was observed to line the respiratory bronchioles, although the epithelial lining of the latter was also interrupted by alveoli. Submucosal glands were not observed within the bronchiolar tree.

The alveolar membrane was thin and attenuated. At the level of the LM two cell types could be identified, the cuboidal alveolar Type II cell and the alveolar Type I cell with its long cytoplasmic processes. At this level no mucosubstances were detected by AB /PAS staining procedures.

The LM studies detailed in Chapter 3 provided a basis for the examination of the surface morphology of the lining epithelium of the respiratory tract by means of SEM. Results of this study are detailed in Chapter 4. This appears to be the first time that such an account of the surface morphology of the entire caprine respiratory tract epithelial lining has been provided. Seventeen clinically normal animals were used in this particular study, and a total of eighteen sample sites, covering the lining epithelium from the nasal vestibule to the lung parenchyma, were utilised.

In the upper respiratory tract it was established that squamous cells, carrying microplicae on their luminal surfaces, lined the nasal vestibule and the rostral regions of the alar and basal fold. A large area of intermediate epithelium lined the caudal regions of the alar and basal folds and exhibited a typical "cobblestone" surface appearance. The surface cells of this intermediate epithelium carried numerous microvilli. Mucus-producing cells were identified by their slightly depressed apical surfaces and a sparse distribution of surface microvilli. Occasionally mucous granules were observed within such cells.

The mucosa of the nasal conchae was thrown into folds and was lined by a ciliated epithelium, the cilia forming a dense carpet. Patches of nonciliated microvillous cells were also observed. Mucus-producing cells were distributed individually on the folds and were more numerous in the gutters. The apical surfaces of those observed on the fold usually exhibited a characteristic protuberance within which mucous granules were sometimes observed. Submucosal gland orifices were observed, especially in the gutters. The epithelium lining the nasal septum contained small numbers of ciliated cells. The whole epithelial surface presented a "moth eaten" appearance characterised by numerous nonciliated microvillous cells and mucus-producing cells intermingled in between the few mature and regenerating ciliated cells.

The rostral region of the nasopharynx was seen to be heavily ciliated, with nonciliated microvillous cells intermingled, individually or occasionally in groups, in between the ciliated cells. Individual surface mucus-producing cells, exhibiting the typical apical protuberance, were also observed. In the middle region of the nasopharynx the numbers of ciliated cells were seen to decrease progressively at the expense of nonciliated microvillous cells. In this region, low domes representing follicle-associated epithelium were observed, the cells covering the domes having smooth apical surfaces studded with microvilli; an occasional pore was seen on such domes. The caudal regions of the nasopharynx were lined by squamous epithelium, similar to that observed in the nasal vestibule. This same type of epithelium lined the

laryngeal surface of the epiglottis and cranial regions of the vocal fold and infraglottic cavity. Taste buds, characterised by a pore from which numerous sensory hairs protruded, were observed on the laryngeal surface of the epiglottis. A few submucosal gland orifices were also observed. On the vocal fold, the change-over from the nonciliated intermediate epithelium to a ciliated epithelium was seen to be abrupt.

The infraglottic cavity was lined, cranially by a squamous epithelium, and caudally by a ciliated epithelium; in between an intermediate type of epithelium was observed. There was a great diversity in the extent and distribution of each epithelial type between individual animals.

In the lower respiratory tract the dorsal tracheal mucosa was seen to be thrown into high alternating folds and gutters; although the ventral surface was also organised into alternating folds and gutters, the latter were seen to be much wider and shallower. The cilial carpet was more densely organised on the folds than in the gutters, and punctuated with patches of nonciliated microvillous cells. Mucus-producing cells were seen to present two morphologically distinct surface characteristics. On the folds, they were frequently observed with a typical apical protuberance, whilst in the gutters they presented a shallow apical surface with mucus appearing to be produced in a sheet-like form. Along the bronchial tree, there was a gradual decrease in the density of the cilial carpet, with an increase in the number of nonciliated microvillous cells.

At the bronchiolar level nonciliated bronchiolar epithelial (Clara) cells were observed. They frequently presented a characteristic apical protuberance studded with short stubby microvilli. Various morphological forms of Clara cell were observed, including those with an obvious apical bulge and others presenting a flattened apical surface. They appeared to represent developmental stages of the Clara cell. Respiratory bronchioles characterised by the presence of alveoli within their walls were observed to be prominent and well developed.

At the alveolar level two cell types were identified. The alveolar Type II cell usually presented a protruding luminal surface studded with numerous microvilli. The

alveolar Type I cell was characterised by its long cytoplasmic processes and an occasionally identifiable central bulging nuclear region. Alveolar pores were seen to be rare and alveolar macrophages were infrequently observed.

In Chapter 5, TEM was employed to further characterise the different cell types populating the distal airways and alveolar membrane of the goat lung. Four clinically normal animals aged between 10 and 18 months were used in the study. Five cell types, namely ciliated, nonciliated bronchiolar epithelial (Clara), mucus-producing, alveolar Type I and alveolar Type II cells were identified and characterised ultrastructurally.

The ciliated cells presented ultrastructural characteristics similar to those described for other mammalian species. Some developing ciliated cells, characterised by the presence of a number of basal bodies and a medium electron-dense cytoplasm, were observed.

The nonciliated bronchiolar epithelial (Clara) cell of the goat was seen to present an electron-dense cytoplasm containing numerous profiles of smooth endoplasmic reticulum concentrated in the apical region.The presence of significant amounts of rough endoplasmic reticulum was not established in the present study. A few membrane-bound secretory granules were observed. Short stubby microvilli were observed on a luminal surface which was frequently seen protruding into the lumen. Occasional Clara cells presenting a flattened luminal surface were also observed.

In one of the animals examined, a possible mucus-producing cell was identified at this level. It was characterised by the presence of heterogeneous membrane-bound secretory granules and a cytoplasm with an electron density between that of a ciliated cell and Clara cell. Profiles of rough and smooth endoplasmic were abundant and the nucleus was large.

Alveolar Type I cells presented long cytoplasmic processes containing occasional pinocytotic vesicles but very few other cellular organelles. Alveolar Type II cells were identified by their characteristic cuboidal shape and were frequently observed in alveolar recesses, where the luminal surface, itself studded with numerous microvilli,

was usually seen to be level with the general lining of the alveolus but occasionally protruded into the alveolar lumen. Osmiophilic lamellated bodies were a constant feature of these cells, as were frequent large intracytoplasmic lipid vacuoles.

Alveolar macrophages were observed lying free in the alveolar lumina. They presented numerous pseudopodia, a large nucleus, numerous ellipsoid mitochondria and numerous membrane-bound inclusion bodies of various sizes, possibly representing lysosomes. A feature of these cells was the presence of vacuoles containing lamellated bodies, similar to those seen in the alveolar Type II cells. Smooth endoplasmic reticulum was seen distributed within the cytoplasm, whilst the distribution of rough endoplasmic reticulum was only patchy.

The combined LM, SEM and TEM studies described in Chapters 3, 4 and 5 have provided ,for the first time, a detailed morphological account of the entire lining epithelium of the respiratory tract of the normal adult goat, thus providing a sound morphological basis for future studies in goats with respiratory diseases.

Chapter 6 was undertaken to investigate, by the use of SEM, the postnatal development of the lining epithelium of the respiratory tract of the neonatal kid. Twenty kids aged between 3 hrs and 21 days were used in the study. The age groups examined were 1 day, 2 days, 3 days, 5 days, 7 days, 9 days, 15 days and 21 days old. Two to three animals were examined in each age group.

This study revealed that the general pattern of ciliation throughout the respiratory tract is well established at birth, and that with age there is a change in the degree of ciliation, varying from region to region. Whereas the nasal septum for example was seen to become less ciliated with age, in contrast the degree of ciliation increased in the distal conducting airways.

Patches of nonciliated microvillous cells were frequently observed in the nasal cavity and, less evidently, elsewhere along the respiratory tract. Although no quantitative assessment was carried out, the patches in the nasal cavity of kids were encountered more frequently than in adults (Chapter 4). The present study has linked

these patches to nasal-associated lymphoid tissue.

Individually scattered or aggregated cells presenting wrinkled apical surfaces were frequently observed in the larynx and trachea; with increase in age, they were less frequently observed. The distribution of such cells, as observed in this study, suggested that they may be mucus-producing cells. However, the findings in relation to these cells were only speculative and no reason could be advanced as to why, at the age of seven days, these cells were no longer observed. TEM characterisation of these cells would be necessary in order to confirm their exact nature.

The study also revealed that respiratory bronchioles, although present, are poorly developed and not well established at birth, and that further differentiation and growth of these structures is a post-natal occurrence. Whereas most alveoli are well formed at birth, the study has revealed that some are formed post-natally. Alveolar pores were very rarely observed in young goats, although by the third week of life their numbers had increased.

This study has, for the first time, provided information on the morphology of the lining epithelium in the respiratory tract of the neonatal goat and its subsequent development during the first three weeks of life. It has highlighted the considerable degree of development attained by the caprine respiratory tract at the time of birth and discussed such features in relation not only to studies in other species, but also in relation to the requirement for an efficient respiratory system in the neonatal kid. It is apparent however that relatively little information appears to be available in the literature dealing with developmental aspects of the lining epithelium in the respiratory tract of the mammal.

The availability of a limited number of goats with clinical respiratory problems provided an opportunity to determine possible changes in the SEM organisation of the epithelial lining and in the surface characteristics of individual cell types, with a view to assessing the use of the SEM as an additional tool in the battery of diagnostic procedures available for studying respiratory diseases. The results of this study are

presented in Chapter 7.

Extensive deciliation and desquamation was observed in the nasal cavity, along with an increase in the extent and distribution of the mucous blanket. Inflammatory exudate was observed lying on the luminal surface. Although no quantitative assessment was made, a qualitative assessment suggested that there was an increase in the numbers of surface mucus-producing cells. Similar changes were observed in the nasopharynx. The epiglottis appeared to be unchanged. Within the larynx more changes were observed in the infraglottic cavity than anywhere else and these included extensive deciliation, desquamation, and increased mucus production. Patches of nonciliated microvillous cells were more frequent in the trachea of diseased animals than in the normal animal. Regional variations in the degree of severity of the lesions were also noted, with the dorsal tracheal surface being more affected than the ventral surface. Similar, relatively milder changes were observed in the bronchial tree with SEM than were observed by histopathological examination.

Within the bronchiolar tree ciliated cells were observed to carry poorly developed and matted cilia. Clara cells frequently presented collapsed apical protuberances, and debris, together with cellular exudate, covered most of the bronchiolar surface

Lesions in the lung parenchyma were focal in nature and at the alveolar level they affected an increase in numbers of alveolar Type II cells. Alveolar macrophages were also seen to increase in response to the disease processes. Alveolar lumina usually contained significant cellular inflammatory exudate. In one animal a layer of what appeared to be surfactant material and /or bronchiolar secretion covered most of the alveolar lining membrane.

Although these SEM observations were complimented by histopathological examinations, the extent of deciliation and desquamation was best appreciated by SEM. In addition, a number of cellular changes, such as observation of the collapsing apical protuberances of the Clara cells observed at the bronchiolar level, could not have been

observed as clearly by histopathology examination.

In conclusion, the present work has demonstrated the usefulness of SEM in the study, for the first time, of normal adult and neonatal caprine respiratory tract surfaces. In addition, it has shown the value of combining LM, SEM and TEM studies for a more complete characterisation of cell types populating the entire airway epithelium, and show how the basic morphology of the lung epithelium may be affected by respiratory disease. Like all such studies, however, many questions remain unanswered, and more detailed studies of such topics as the functions of, and the developmental relationship between a number of the cell types populating the respiratory airway epithelium await future investigation.

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# APPENDIX.

## APPENDIX: CHAPTER 3.

Distribution and semi-quantitative assessment of mucosubstances in the respiratory tract of the goat as detected by the Alcian Blue /Periodic Acid Schiff method.

EXPLANATION OF TERMS USED.

| | | |
|---|---|---|
| Acid | = | Cells stain blue. |
| Neutral | = | Cells stain red. |
| Mixed | = | Cells stain purple. |
| 1+ | = | Very few cells. |
| 2+ | = | Few cells. |
| 3+ | = | Many cells. |
| 4+ | = | Very many cells. |
| ND | = | Not done, or epithelium not available for assessment. |
| - | = | Negative. |

| | EPITHELIUM | | | GLANDS | | |
|---|---|---|---|---|---|---|
| ANIMAL | ACID | NEUTRAL | MIXED | ACID | NEUTRAL | MIXED |
| MC1 | 1+ | - | - | - | - | - |
| MC2 | 1+ | - | 1+ | - | - | - |
| MC3 | - | - | - | - | - | - |
| MC4 | 1+ | - | - | - | - | - |
| MC5 | 1+ | - | - | - | - | - |
| MC6 | 1+ | - | - | - | - | 3+ |
| MC7 | 2+ | - | 1+ | 1+ | - | 3+ |
| MC8 | ND | ND | ND | ND | ND | ND |
| MC9 | 1+ | - | 1+ | - | - | 1+ |
| MC10 | 4+ | - | - | - | - | 4+ |
| MC11 | 1+ | - | - | - | - | - |
| MC12 | 1+ | - | - | - | - | - |
| MC13 | 2+ | - | - | - | - | - |
| MC14 | 3+ | - | - | 1+ | - | - |
| MC15 | 1+ | - | - | - | - | - |
| MC16 | 2+ | - | - | - | - | - |
| MC17 | 1+ | - | - | - | - | - |

BASAL FOLD

| | EPITHELIUM | | | | GLANDS | |
|---|---|---|---|---|---|---|
| ANIMAL | ACID | NEUTRAL | MIXED | ACID | NEUTRAL | MIXED |
| MC1 | ND | ND | ND | ND | ND | ND |
| MC2 | 4+ | 3+ | - | - | - | 2+ |
| MC3 | 3+ | - | - | - | - | 3+ |
| MC4 | 4+ | 1+ | - | 1+ | 1+ | 3+ |
| MC5 | 2+ | - | - | - | - | 3+ |
| MC6 | ND | ND | ND | ND | ND | ND |
| MC7 | ND | ND | ND | ND | ND | ND |
| MC8 | 4+ | - | - | - | - | - |
| MC9 | 3+ | 1+ | 1+ | - | 2+ | 3+ |
| MC10 | ND | ND | ND | ND | ND | ND |
| MC11 | 3+ | - | - | 1+ | 1+ | 3+ |
| MC12 | 3+ | - | - | 1+ | 1+ | 3+ |
| MC13 | 3+ | - | - | 1+ | - | 3+ |
| MC14 | 3+ | - | - | 1+ | 1+ | 4+ |
| MC15 | 3+ | - | - | 1+ | 2+ | 4+ |
| MC16 | 3+ | - | - | 1+ | 1+ | 3+ |
| MC17 | ND | ND | ND | ND | ND | ND |

VENTRAL NASAL CONCHA

| | EPITHELIUM | | | GLANDS | | |
|---|---|---|---|---|---|---|
| ANIMAL | ACID | NEUTRAL | MIXED | ACID | NEUTRAL | MIXED |
| MC1 | 3+ | 1+ | 2+ | 1+ | 4+ | 2+ |
| MC2 | 3+ | - | - | 2+ | 4+ | 2+ |
| MC3 | 3+ | - | - | - | 4+ | - |
| MC4 | 3+ | - | - | 1+ | - | 3+ |
| MC5 | ND | ND | ND | ND | ND | ND |
| MC6 | ND | ND | ND | ND | ND | ND |
| MC7 | 3+ | - | - | 1+ | 3+ | 2+ |
| MC8 | 3+ | - | - | - | - | - |
| MC9 | 4+ | - | - | 1+ | 4+ | 2+ |
| MC10 | ND | ND | ND | - | 3+ | 1+ |
| MC11 | 3+ | - | - | 1+ | 4+ | 1+ |
| MC12 | 3+ | - | - | 1+ | - | 3+ |
| MC13 | 2+ | - | - | - | - | 2+ |
| MC14 | ND | ND | ND | 1+ | 3+ | 2+ |
| MC15 | 3+ | - | - | 1+ | 3+ | 2+ |
| MC16 | 3+ | - | - | 2+ | 2+ | 3+ |
| MC17 | 3+ | - | - | 1+ | 2+ | 2+ |

DORSAL NASAL CONCHA

| | EPITHELIUM | | | GLANDS | | |
|---|---|---|---|---|---|---|
| ANIMAL | ACID | NEUTRAL | MIXED | ACID | NEUTRAL | MIXED |
| MC1 | 3+ | - | - | - | 4+ | 2+ |
| MC2 | ND | ND | ND | ND | ND | ND |
| MC3 | 2+ | - | - | - | 2+ | 4+ |
| MC4 | 2+ | - | - | 2+ | 1+ | 2+ |
| MC5 | 4+ | - | - | 3+ | 1+ | 2+ |
| MC6 | ND | ND | ND | ND | ND | ND |
| MC7 | ND | ND | ND | - | 2+ | 4+ |
| MC8 | ND | ND | ND | ND | ND | ND |
| MC9 | 3+ | - | - | 3+ | - | - |
| MC10 | 3+ | - | - | - | 3+ | 3+ |
| MC11 | ND | ND | ND | - | 1+ | 4+ |
| MC12 | 3+ | - | - | - | 2+ | 3+ |
| MC13 | ND | ND | ND | ND | ND | ND |
| MC14 | 3+ | 1+ | - | 1+ | 4+ | 2+ |
| MC15 | 4+ | - | - | 2+ | 2+ | 2+ |
| MC16 | 3+ | - | - | 2+ | 2+ | 3+ |
| MC17 | ND | ND | ND | ND | ND | ND |

MIDDLE NASAL CONCHA

| | EPITHELIUM | | | GLANDS | | |
|---|---|---|---|---|---|---|
| ANIMAL | ACID | NEUTRAL | MIXED | ACID | NEUTRAL | MIXED |
| MC1 | 3+ | - | 1+ | 2+ | 3+ | 2+ |
| MC2 | ND | ND | ND | ND | ND | ND |
| MC3 | 3+ | - | - | 2+ | 4+ | 3+ |
| MC4 | 3+ | - | - | 2+ | 3+ | 3+ |
| MC5 | 4+ | - | - | - | 4+ | 3+ |
| MC6 | 3+ | - | - | 1+ | 3+ | 2+ |
| MC7 | 3+ | 1+ | - | 1+ | 3+ | 2+ |
| MC8 | 4+ | - | - | - | 3+ | 2+ |
| MC9 | 3+ | - | - | - | 3+ | 1+ |
| MC10 | 2+ | - | - | - | 3+ | 1+ |
| MC11 | 4+ | - | - | 2+ | 3+ | 2+ |
| MC12 | 4+ | - | - | 2+ | 3+ | 2+ |
| MC13 | 2+ | - | - | 2+ | 3+ | 2+ |
| MC14 | 4+ | - | - | 1+ | 4+ | 2+ |
| MC15 | 3+ | - | - | 2+ | 4+ | - |
| MC16 | 3+ | - | - | 2+ | 2+ | 3+ |
| MC17 | 2+ | - | - | 1+ | 3+ | 3+ |

NASAL SEPTUM.

| | EPITHELIUM | | | GLANDS | | |
|---|---|---|---|---|---|---|
| ANIMAL | ACID | NEUTRAL | MIXED | ACID | NEUTRAL | MIXED |
| MC1 | 4+ | - | 1+ | 2+ | 1+ | 3+ |
| MC2 | 3+ | - | - | 2+ | 1+ | 4+ |
| MC3 | 3+ | - | - | 3+ | 2+ | 3+ |
| MC4 | 4+ | - | 2+ | 2+ | - | 4+ |
| MC5 | 4+ | 2+ | 3+ | 3+ | 4+ | 2+ |
| MC6 | 3+ | - | - | 2+ | - | 3+ |
| MC7 | 3+ | - | - | 3+ | 1+ | 4+ |
| MC8 | 4+ | - | - | 2+ | - | 3+ |
| MC9 | 3+ | - | 1+ | 2+ | - | 3+ |
| MC10 | ND | ND | ND | ND | ND | ND |
| MC11 | 3+ | - | - | 3+ | 2+ | 3+ |
| MC12 | 3+ | - | - | 3+ | - | 3+ |
| MC13 | ND | ND | ND | ND | ND | ND |
| MC14 | 3+ | - | - | 3+ | - | 3+ |
| MC15 | 3+ | - | 1+ | 3+ | - | 4+ |
| MC16 | 3+ | - | - | 3+ | - | 3+ |
| MC17 | 3+ | - | - | 2+ | - | 4+ |

| | EPITHELIUM | | | GLANDS | | |
|---|---|---|---|---|---|---|
| ANIMAL | ACID | NEUTRAL | MIXED | ACID | NEUTRAL | MIXED |
| MC1 | 1+ | - | - | 4+ | 2+ | - |
| MC2 | 2+ | - | - | 4+ | 1+ | - |
| MC3 | 2+ | - | - | 4+ | 3+ | - |
| MC4 | ND | ND | ND | 4+ | 1+ | - |
| MC5 | 2+ | - | - | 4+ | 1+ | 1+ |
| MC6 | 2+ | - | - | 4+ | - | - |
| MC7 | 2+ | 1+ | - | 4+ | 1+ | - |
| MC8 | 2+ | - | - | 4+ | - | - |
| MC9 | ND | ND | ND | ND | ND | ND |
| MC10 | 2+ | - | - | 4+ | 2+ | - |
| MC11 | 1+ | - | - | 4+ | 2+ | - |
| MC12 | 2+ | - | - | 4+ | - | - |
| MC13 | 2+ | - | - | 4+ | - | - |
| MC14 | 2+ | - | - | 4+ | - | - |
| MC15 | 3+ | - | - | 4+ | - | - |
| MC16 | 2+ | - | - | 3+ | - | - |
| MC17 | 3+ | - | - | 4+ | - | - |

EPIGLOTTIS

| | EPITHELIUM | | | GLANDS | | |
|---|---|---|---|---|---|---|
| ANIMAL | ACID | NEUTRAL | MIXED | ACID | NEUTRAL | MIXED |
| MC1 | - | - | - | 4+ | 2+ | - |
| MC2 | - | - | - | 4+ | 2+ | - |
| MC3 | - | - | - | 4+ | 3+ | - |
| MC4 | - | - | - | 3+ | 4+ | - |
| MC5 | ND | ND | ND | ND | ND | ND |
| MC6 | - | - | - | - | 3+ | 3+ |
| MC7 | - | - | - | 4+ | 3+ | - |
| MC8 | - | - | - | 4+ | 4+ | 1+ |
| MC9 | ND | ND | ND | ND | ND | ND |
| MC10 | - | - | - | 4+ | 4+ | - |
| MC11 | - | - | - | 3+ | 4+ | - |
| MC12 | - | - | - | 3+ | - | - |
| MC13 | - | - | - | 4+ | 1+ | 2+ |
| MC14 | - | - | - | 3+ | 3+ | 1+ |
| MC15 | - | - | - | 4+ | 3+ | 2+ |
| MC16 | - | - | - | 3+ | 3+ | - |
| MC17 | - | - | - | 4+ | 3+ | - |

| | EPITHELIUM | | | GLANDS | | |
|---|---|---|---|---|---|---|
| ANIMAL | ACID | NEUTRAL | MIXED | ACID | NEUTRAL | MIXED |
| MC1 | 1+ | - | - | 3+ | 3+ | - |
| MC2 | ND | ND | ND | ND | ND | ND |
| MC3 | - | - | - | 4+ | 2+ | 2+ |
| MC4 | 1+ | - | 1+ | 3+ | 3+ | 2+ |
| MC5 | ND | ND | ND | ND | ND | ND |
| MC6 | ND | ND | ND | ND | ND | ND |
| MC7 | 1+ | - | 1+ | 3+ | 3+ | - |
| MC8 | 1+ | - | - | 3+ | 4+ | - |
| MC9 | 2+ | - | - | 3+ | 3+ | 1+ |
| MC10 | - | - | - | 3+ | 4+ | 2+ |
| MC11 | 1+ | - | 1+ | 3+ | 4+ | 3+ |
| MC12 | 1+ | - | - | 3+ | 4+ | - |
| MC13 | - | | - | 3+ | 4+ | 1+ |
| MC14 | 1+ | - | - | 4+ | 3+ | - |
| MC15 | - | - | - | 4+ | 3+ | - |
| MC16 | - | - | - | 3+ | 4+ | 1+ |
| MC17 | 1+ | - | - | 4+ | 3+ | - |

| | EPITHELIUM | | | GLANDS | | |
|---|---|---|---|---|---|---|
| ANIMAL | ACID | NEUTRAL | MIXED | ACID | NEUTRAL | MIXED |
| MC1 | ND | ND | ND | 3+ | 3+ | 2+ |
| MC2 | ND | ND | ND | 3+ | 3+ | 2+ |
| MC3 | 1+ | - | - | 2+ | 2+ | 2+ |
| MC4 | - | - | ND | - | 2+ | 3+ |
| MC5 | ND | ND | ND | ND | ND | ND |
| MC6 | ND | ND | ND | ND | ND | ND |
| MC7 | ND | ND | - | ND | ND | ND |
| MC8 | 1+ | - | - | 4+ | 2+ | 3+ |
| MC9 | 1+ | - | ND | 3+ | 2+ | 3+ |
| MC10 | ND | ND | 1+ | 4+ | 2+ | 3+ |
| MC11 | 2+ | - | - | 3+ | 1+ | 3+ |
| MC12 | 2+ | - | - | 3+ | 2+ | 3+ |
| MC13 | - | - | - | 3+ | 2+ | 3+ |
| MC14 | 1+ | - | - | 4+ | 3+ | 2+ |
| MC15 | 1+ | - | - | 3+ | 3+ | 2+ |
| MC16 | 2+ | - | - | 4+ | 2+ | 2+ |
| MC17 | 1+ | - | - | 3+ | 3+ | 3+ |

DORSAL CRANIAL TRACHEA

| | EPITHELIUM | | | GLANDS | | |
|---|---|---|---|---|---|---|
| ANIMAL | ACID | NEUTRAL | MIXED | ACID | NEUTRAL | MIXED |
| MC1 | ND | ND | ND | ND | ND | ND |
| MC2 | 2+ | - | 2+ | 2+ | 3+ | 3+ |
| MC3 | 1+ | - | 1+ | 2+ | - | 2+ |
| MC4 | ND | ND | ND | 4+ | 4+ | 1+ |
| MC5 | 2+ | - | 1+ | 2+ | - | 3+ |
| MC6 | ND | ND | ND | ND | ND | ND |
| MC7 | ND | ND | ND | 2+ | - | 2+ |
| MC8 | 2+ | - | - | 2+ | - | 1+ |
| MC9 | ND | ND | ND | ND | ND | ND |
| MC10 | ND | ND | ND | ND | ND | ND |
| MC11 | ND | ND | ND | ND | ND | ND |
| MC12 | 3+ | - | - | 3+ | - | - |
| MC13 | 1+ | - | - | 2+ | - | - |
| MC14 | 3+ | - | - | 3+ | - | 2+ |
| MC15 | 2+ | - | - | 3+ | - | - |
| MC16 | ND | ND | ND | ND | ND | ND |
| MC17 | 3+ | - | - | 3+ | - | 1+ |

VENTRAL CRANIAL TRACHEA

| | EPITHELIUM | | | GLANDS | | |
|---|---|---|---|---|---|---|
| ANIMAL | ACID | NEUTRAL | MIXED | ACID | NEUTRAL | MIXED |
| MC1 | ND | ND | ND | ND | ND | ND |
| MC2 | 1+ | - | - | 3+ | - | 3+ |
| MC3 | ND | ND | ND | ND | ND | ND |
| MC4 | ND | ND | ND | 3+ | - | - |
| MC5 | 2+ | - | - | 2+ | - | 4+ |
| MC6 | ND | ND | ND | ND | ND | ND |
| MC7 | ND | ND | ND | ND | ND | ND |
| MC8 | ND | ND | ND | ND | ND | ND |
| MC9 | 2+ | 1+ | 2+ | 4+ | 1+ | 2+ |
| MC10 | ND | ND | ND | 4+ | - | 2+ |
| MC11 | ND | ND | ND | 4+ | - | 2+ |
| MC12 | 2+ | - | 1+ | 3+ | 2+ | 2+ |
| MC13 | 1+ | - | - | 2+ | - | - |
| MC14 | 3+ | - | - | 3+ | 3+ | 2+ |
| MC15 | 2+ | - | - | 2+ | 1+ | 2+ |
| MC16 | ND | ND | - | 2+ | 1+ | 2+ |
| MC17 | 2+ | - | - | 3+ | 2+ | 2+ |

DORSAL CAUDAL TRACHEA

| | EPITHELIUM | | | GLANDS | | |
|---|---|---|---|---|---|---|
| ANIMAL | ACID | NEUTRAL | MIXED | ACID | NEUTRAL | MIXED |
| MC1 | 2+ | - | 1+ | 3+ | - | 2+ |
| MC2 | ND | ND | ND | ND | ND | ND |
| MC3 | 1+ | - | - | 3+ | 2+ | 1+ |
| MC4 | ND | ND | ND | 4+ | 4+ | - |
| MC5 | 1+ | - | - | 2+ | 1+ | 2+ |
| MC6 | ?? | 1+ | 1+ | 3+ | 3+ | 3+ |
| MC7 | ND | ND | ND | 2+ | 2+ | 2+ |
| MC8 | ND | ND | ND | ND | ND | ND |
| MC9 | 1+ | 1+ | 1+ | 2+ | 2+ | 2+ |
| MC10 | ND | ND | ND | ND | ND | ND |
| MC11 | 2+ | - | 1+ | 2+ | - | 2+ |
| MC12 | 2+ | - | - | 3+ | - | 2+ |
| MC13 | 2+ | - | - | 3+ | - | - |
| MC14 | 3+ | - | - | 2+ | - | - |
| MC15 | 3+ | - | - | 3+ | - | 2+ |
| MC16 | 2+ | - | - | 2+ | - | 2+ |
| MC17 | 1+ | - | 1+ | 3+ | - | 1+ |

VENTRAL CAUDAL TRACHEA

| | EPITHELIUM | | | GLANDS | | |
|---|---|---|---|---|---|---|
| ANIMAL | ACID | NEUTRAL | MIXED | ACID | NEUTRAL | MIXED |
| MC1 | 3+ | - | - | 4+ | 1+ | 2+ |
| MC2 | 2+ | - | - | 3+ | 3+ | 2+ |
| MC3 | 1+ | - | - | 4+ | - | 2+ |
| MC4 | 2+ | - | - | 3+ | 1+ | 4+ |
| MC5 | 1+ | - | - | 3+ | 3+ | 3+ |
| MC6 | 2+ | ND | ND | 3+ | 2+ | 3+ |
| MC7 | 2+ | - | 1+ | 3+ | 2+ | 2+ |
| MC8 | ND | ND | ND | ND | ND | ND |
| MC9 | 2+ | - | 1+ | 4+ | 2+ | 3+ |
| MC10 | ND | - | 1+ | ND | ND | ND |
| MC11 | 2+ | ND | ND | 3+ | 2+ | 3+ |
| MC12 | 2+ | - | - | 4+ | 2+ | 2+ |
| MC13 | ND | - | - | 3+ | - | ?? |
| MC14 | 2+ | - | - | 3+ | - | 2+ |
| MC15 | 2+ | - | - | 3+ | 1+ | 2+ |
| MC16 | 2+ | - | - | 4+ | - | 2+ |
| MC17 | 3+ | - | - | 3+ | 1+ | 2+ |

EXTRA PULMONARY PRINCIPAL BRONCHUS.

| | EPITHELIUM | | | GLANDS | | |
|---|---|---|---|---|---|---|
| ANIMAL | ACID | NEUTRAL | MIXED | ACID | NEUTRAL | MIXED |
| MC1 | 2+ | - | - | 2+ | - | 2+ |
| MC2 | 2+ | 1+ | 1+ | 2+ | 3+ | 2+ |
| MC3 | 1+ | 2+ | - | 1+ | 3+ | 1+ |
| MC4 | 2+ | 1+ | - | 1+ | 3+ | 1+ |
| MC5 | ND | ND | ND | ND | ND | ND |
| MC6 | 2+ | 1+ | - | 2+ | - | 2+ |
| MC7 | 1+ | - | - | 3+ | 2+ | 2+ |
| MC8 | 1+ | - | - | 3+ | 2+ | 2+ |
| MC9 | 2+ | - | - | 3+ | 1+ | 2+ |
| MC10 | 1+ | 1+ | 1+ | 2+ | - | 2+ |
| MC11 | 2+ | - | - | 3+ | - | 3+ |
| MC12 | ND | ND | ND | ND | ND | ND |
| MC13 | 2+ | - | - | 2+ | - | 2+ |
| MC14 | 2+ | - | - | 2+ | - | 2+ |
| MC15 | 3+ | - | - | 3+ | - | 4+ |
| MC16 | 1+ | - | - | 2+ | - | 2+ |
| MC17 | 2+ | - | - | 3+ | - | 3+ |

CAUDAL LOBAR BRONCHUS.

| | EPITHELIUM | | | GLANDS | | |
|---|---|---|---|---|---|---|
| ANIMAL | ACID | NEUTRAL | MIXED | ACID | NEUTRAL | MIXED |
| MC1 | 3+ | - | 1+ | 2+ | 1+ | 3+ |
| MC2 | 3+ | - | 1+ | 2+ | 2+ | 4+ |
| MC3 | 2+ | - | 1+ | 2+ | 2+ | 3+ |
| MC4 | 2+ | 1+ | - | 2+ | 3+ | 2+ |
| MC5 | 3+ | - | - | 2+ | 2+ | 3+ |
| MC6 | 4+ | 1+ | 1+ | 2+ | - | 3+ |
| MC7 | 2+ | 1+ | - | 2+ | 2+ | 3+ |
| MC8 | 3+ | - | - | 2+ | 1+ | 1+ |
| MC9 | ND | ND | ND | 3+ | 2+ | 4+ |
| MC10 | 2+ | - | - | 1+ | 2+ | 4+ |
| MC11 | 2+ | 2+ | 1+ | 1+ | 4+ | 2+ |
| MC12 | 2+ | - | - | ND | ND | ND |
| MC13 | 3+ | - | - | 3+ | 3+ | 3+ |
| MC14 | 3+ | - | - | 4+ | 2+ | 2+ |
| MC15 | 2+ | - | - | 2+ | 2+ | 2+ |
| MC16 | 3+ | - | - | 2+ | 2+ | 3+ |
| MC17 | ND | ND | ND | ND | ND | ND |

LARGE BRONCHIOLE.

| | EPITHELIUM | | | GLANDS | | |
|---|---|---|---|---|---|---|
| ANIMAL | ACID | NEUTRAL | MIXED | ACID | NEUTRAL | MIXED |
| MC1 | 2+ | - | 1+ | | | |
| MC2 | 1+ | - | 1+ | | | |
| MC3 | 1+ | - | 1+ | | | |
| MC4 | 2+ | 1+ | 2+ | | | |
| MC5 | 1+ | - | 2+ | | | |
| MC6 | 1+ | 1+ | 1+ | | | |
| MC7 | 2+ | 1+ | 1+ | | | |
| MC8 | 2+ | - | 1+ | | | |
| MC9 | 1+ | - | 2+ | | | |
| MC10 | 1+ | - | 1+ | | | |
| MC11 | 2+ | - | 2+ | | | |
| MC12 | 2+ | - | 1+ | | | |
| MC13 | 1+ | 1+ | 1+ | | | |
| MC14 | 1+ | - | 2+ | | | |
| MC15 | 2+ | - | 2+ | | | |
| MC16 | 1+ | - | 2+ | | | |
| MC17 | 2+ | - | 1+ | | | |

# VOLUME II

# AN ULTRASTRUCTURAL AND HISTOLOGICAL STUDY OF THE EPITHELIUM OF THE RESPIRATORY TRACT IN THE NEONATAL AND ADULT GOAT.

A THESIS SUBMITTED TO THE FACULTY OF VETERINARY MEDICINE, UNIVERSITY OF GLASGOW

FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

by

CHARLES K.B. KAHWA, BVM.

DEPARTMENT OF VETERINARY ANATOMY,
UNIVERSITY OF GLASGOW,



VOLUME II

## CONTENTS.

This volume contains the Figures referred to in the text of Volume I

# CHAPTER 1

Fig. 1.1 Nose.

The external nares appear as narrow slits (arrows). A narrow planum nasale (*) can be seen.

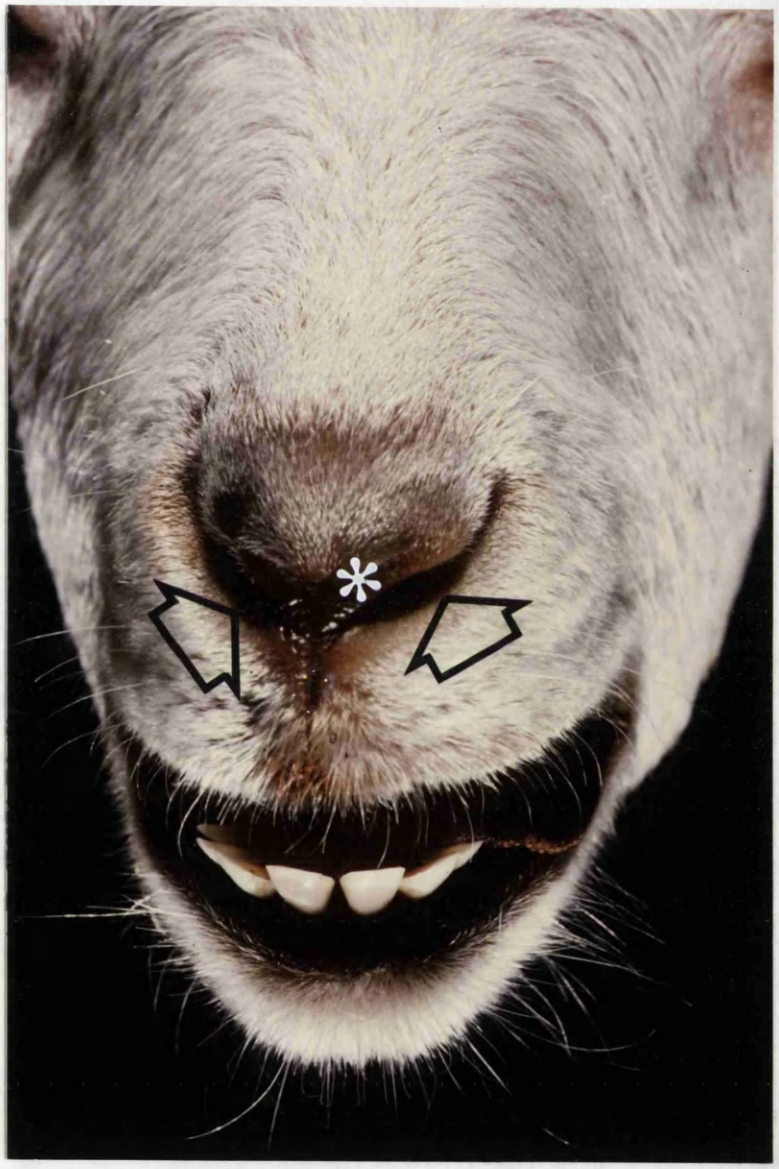


Fig. 1.2 Nasal cavity.

In this sagittal section of the head of an adult goat, the nasal septum has been removed so that the contents of the nasal cavity can be seen.

1. middle nasal concha.
2. dorsal nasal concha.
3. ventral nasal concha.
4. basal fold.
5. alar fold.
6. nasopharynx.

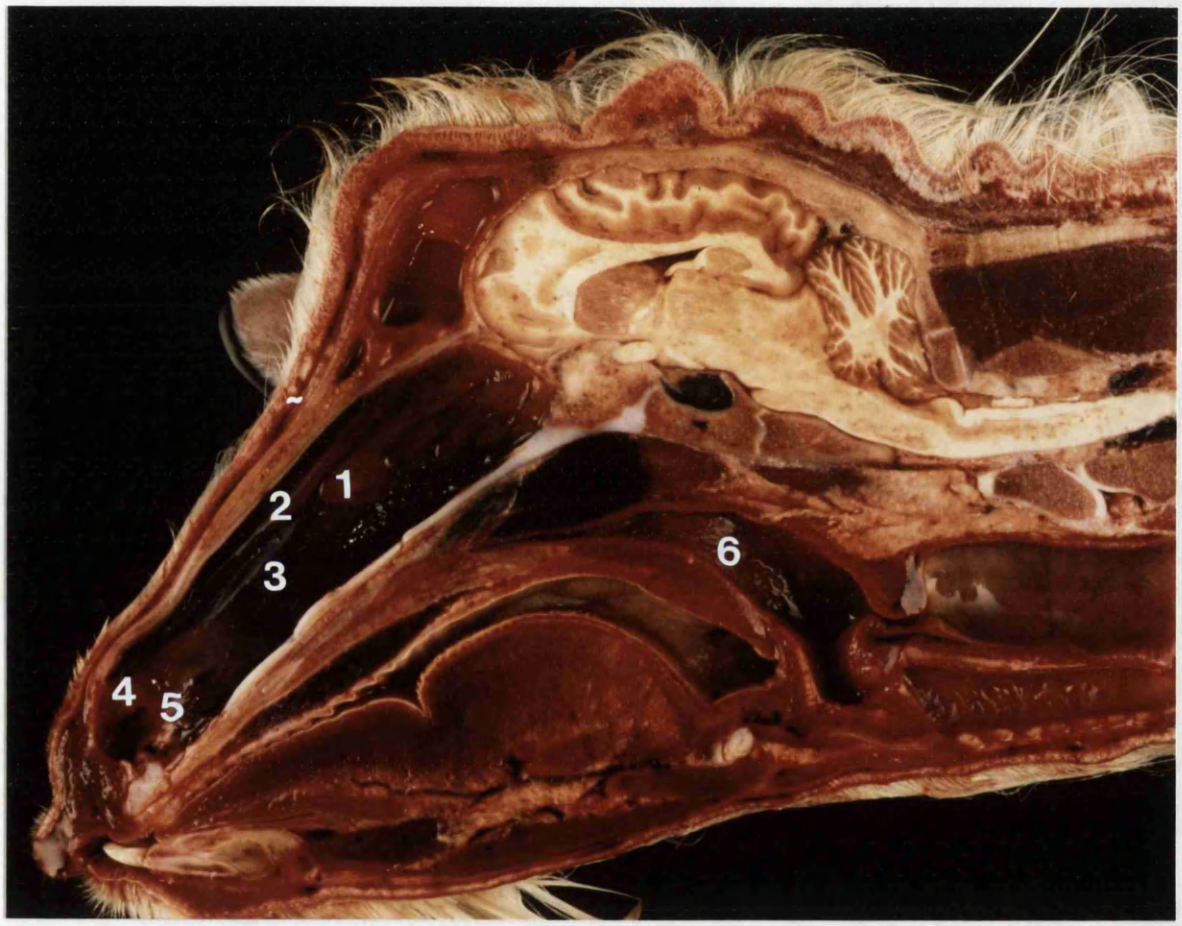
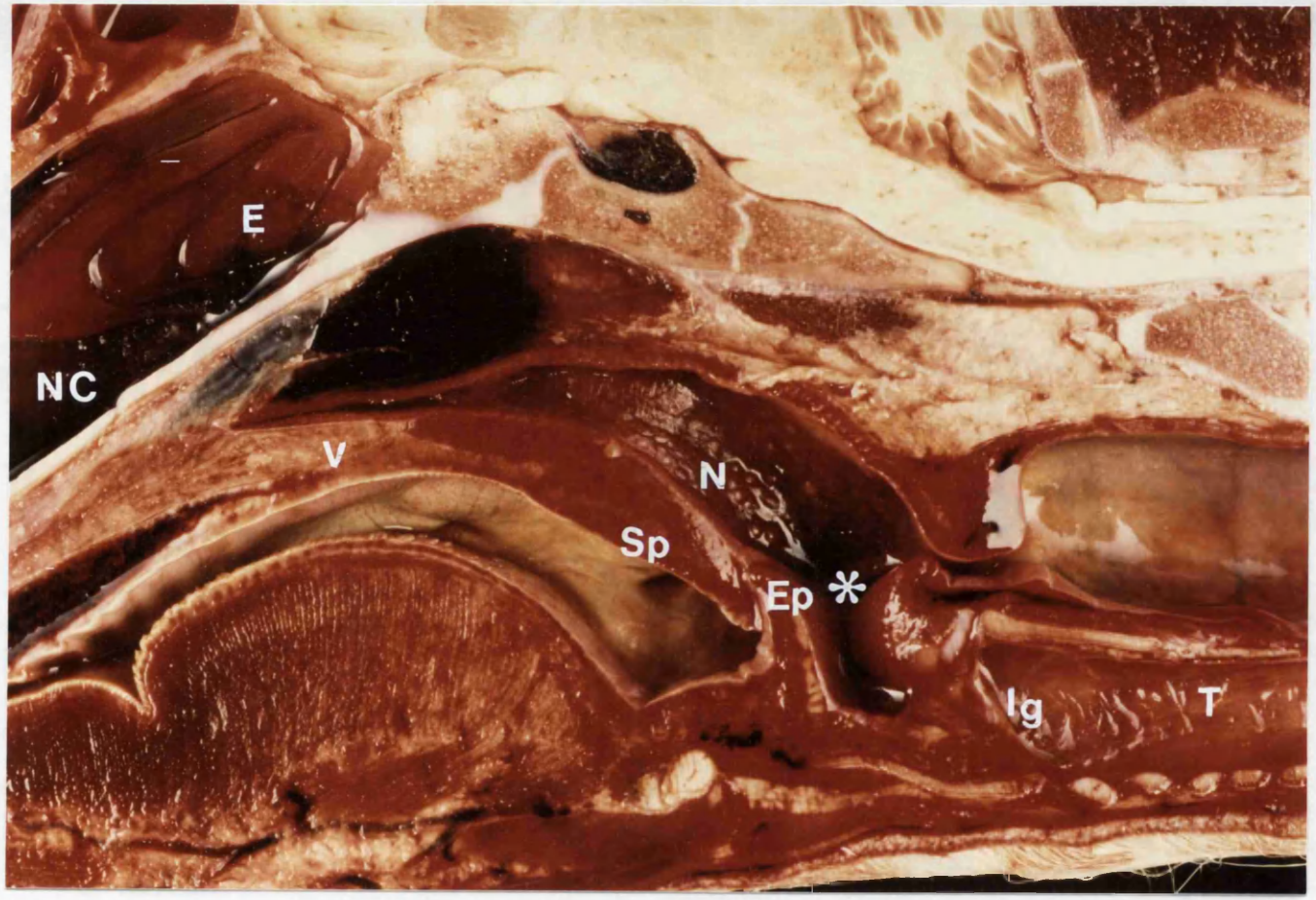

~
1
2
3
4
5
6

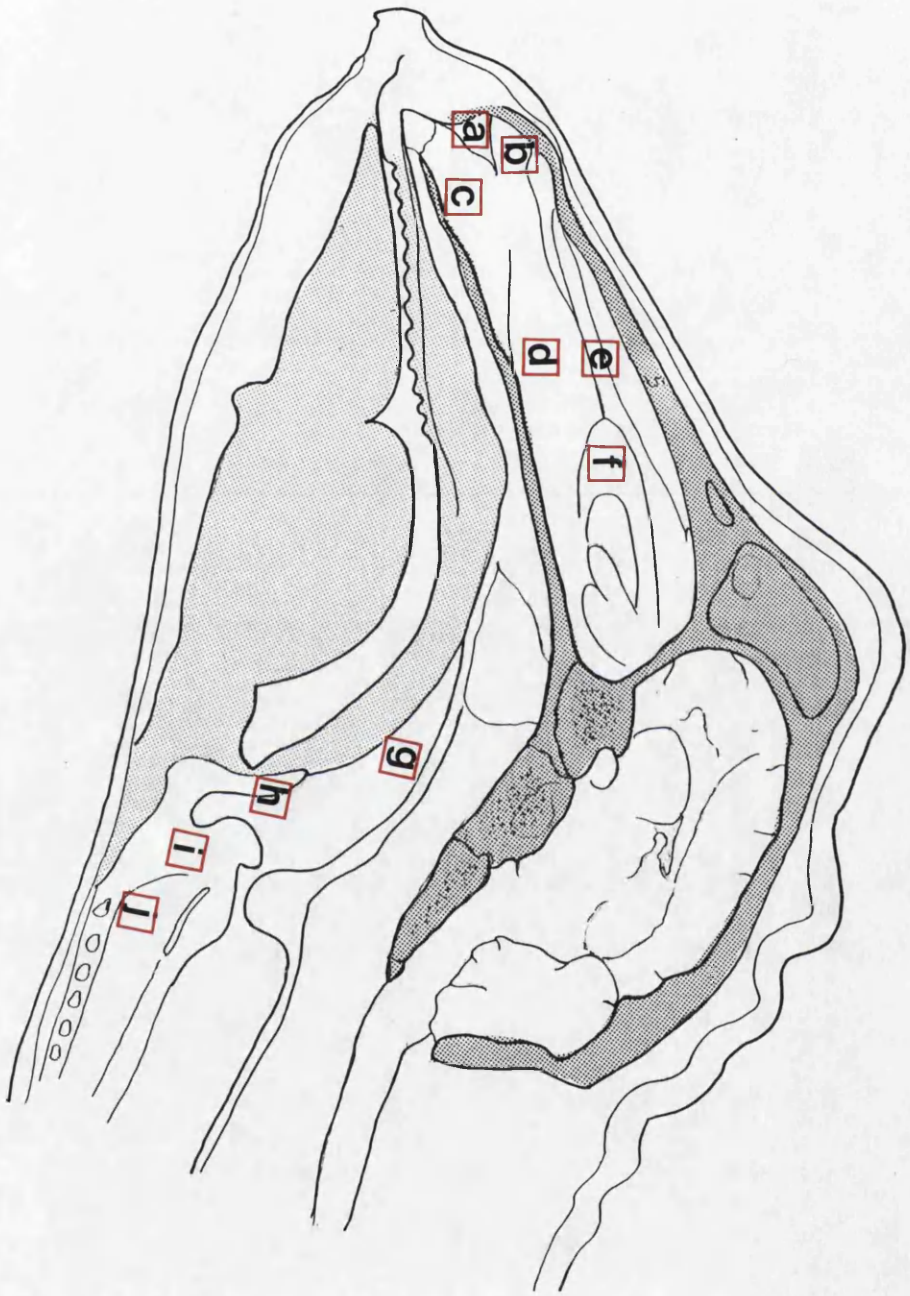
Fig. 1.3 Nasopharynx and larynx.
The internal choanae leading from the nasal cavity (NC) into the nasopharynx (N) lie at the level of the vomer (V).
Note the continuity of the nasopharyngeal airway with the larynx (L) and trachea (T) lumen via aditus laryngis (*).
Ethmoidal concha (E).
Infraglottic cavity (Ig).
Epiglottis (Ep).
Soft palate (Sp).


E
NC
V
N
Sp
Ep
*
Ig
T

# CHAPTER 2

Fig. 2.1 Diagram of a sagittal section of a goat head with the nasal septum removed to expose the contents of the nasal cavity. Sites where samples were taken for both LM and SEM are indicated:

a) Nasal vestibule.
b) Alar fold.
c) Basal fold.
d).Ventral nasal concha.
e).Dorsal nasal concha.
f).Middle nasal concha.
g).Nasopharynx.
h).Epiglottis.
i).Vocal fold.
j).Infraglottic cavity
Note: Sample site for the nasal septum is not indicated. The sample was taken at the same level as for the ventral nasal concha.


a
b
c
d
e
f
g
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i
l

Fig. 2.2 Diagram of the dorsal view of the goat lung.
Sample sites for both LM and SEM are indicated:

a).Dorsal cranial trachea.

b).Ventral cranial trachea.

c).Dorsal caudal trachea.

d).Ventral caudal trachea.

e).Extrapulmonary principal bronchus.

f).Caudal lobar bronchus.

g).Lung parenchyma to include:

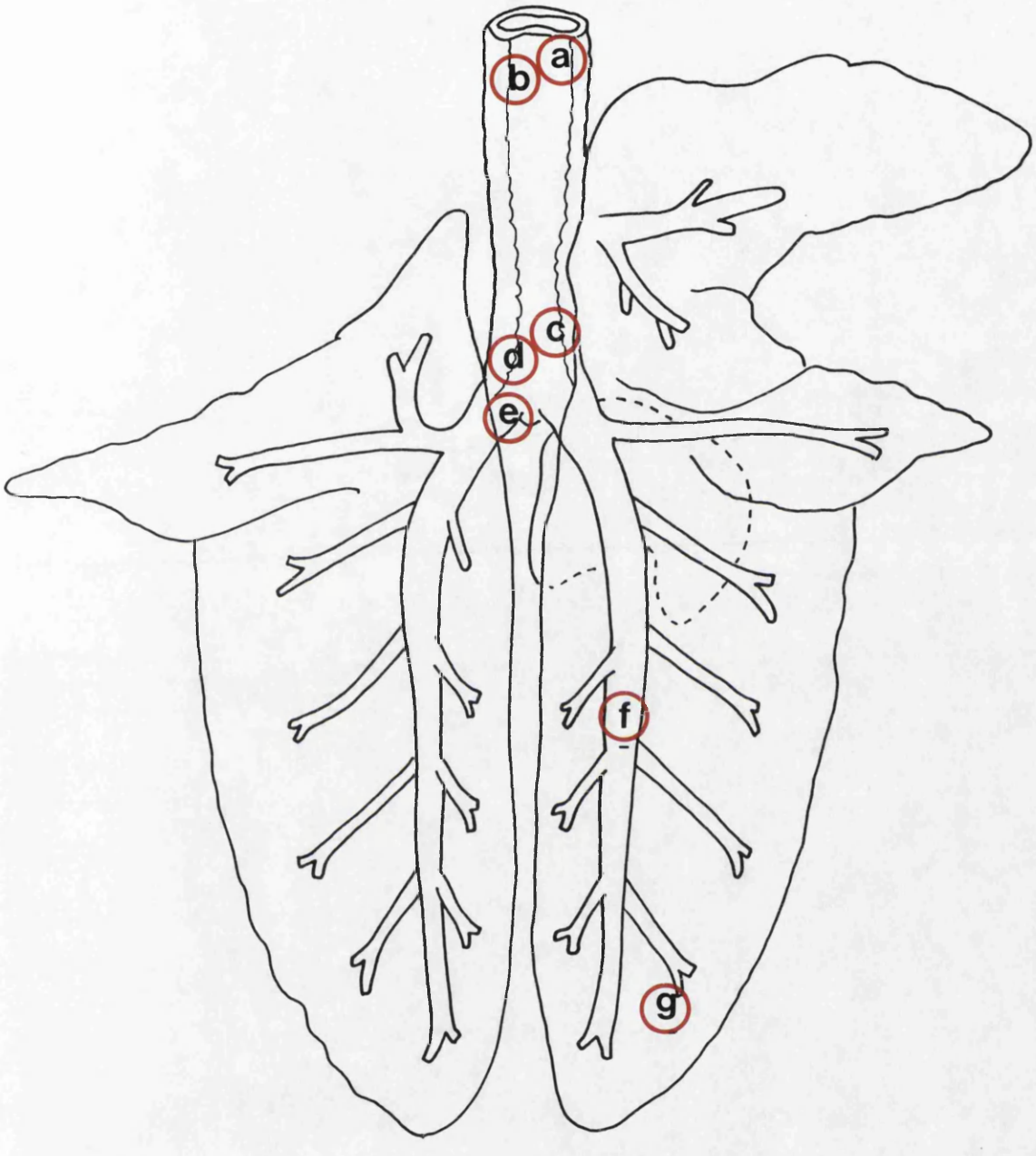
Large bronchiole.

Terminal bronchiole.

Respiratory bronchiole.

Alveolar duct and alveoli.

Samples for TEM were taken from site g) only.


a
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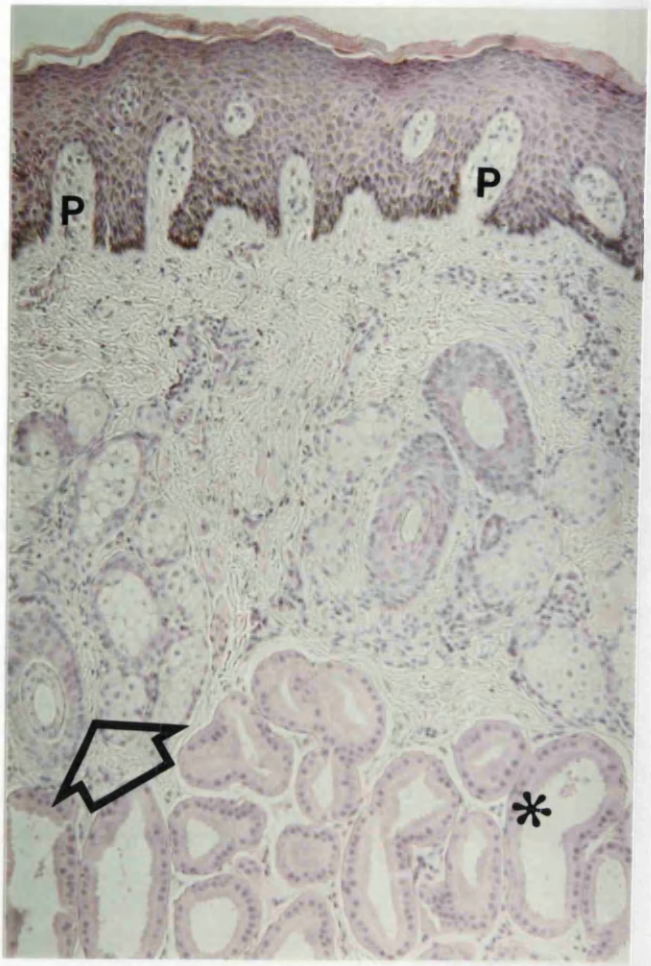
# CHAPTER 3

Fig. 3.1 Nasal vestibule (rostral region).
This region is lined by a keratinized squamous epithelium. Note the dermal papillae (P), abundant sweat glands (*) and hair follicle with associated sebaceous glands (arrow).
AB /PAS x 250.

Fig. 3.2 Alar fold (caudal region).
The epithelium is of stratified squamous non-keratinized type. Individual mucus-producing cells can be seen in the epithelium (closed arrows). Note submucosal gland duct opening onto the epithelial surface (open arrow).
AB /PAS x 250.


P
P
*

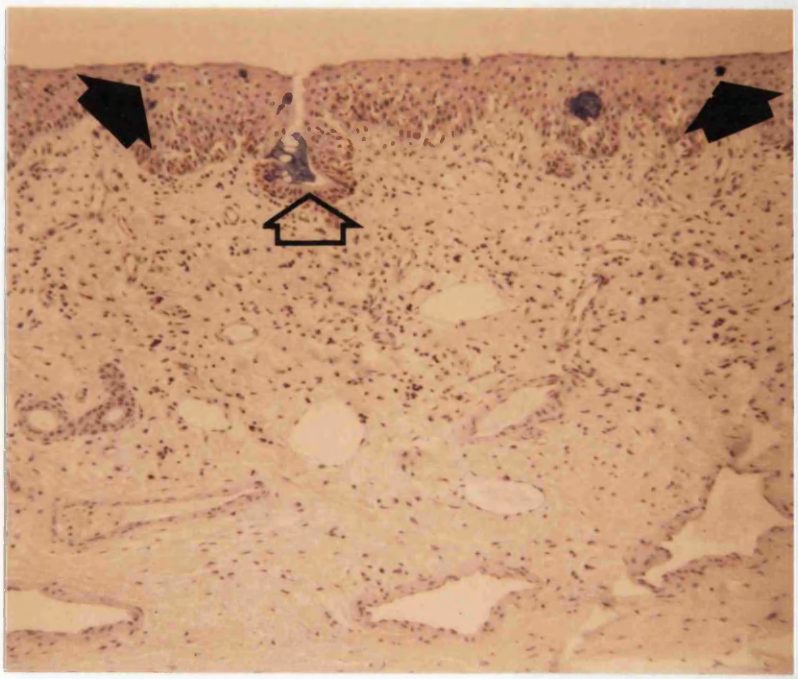
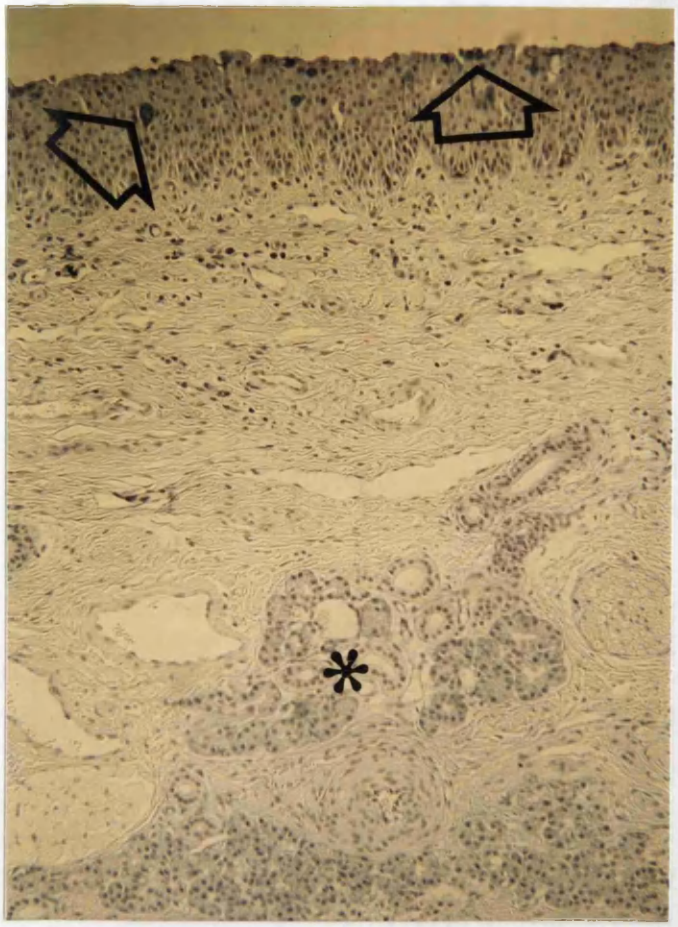


Fig. 3.3 Basal fold (caudal region).
Note the stratified cuboidal nature of the epithelium and the occasional individual mucus-producing cells exhibiting an acidic reaction (arrows).
Submucosal glands (*) are abundant; the majority stain negatively, indicative of their serous nature.
AB /PAS x 100.

Fig. 3.4 Middle nasal concha.
The lining epithelium is of the pseudostratified ciliated columnar type. Mucus-producing cells are numerous, being predominantly acidic in nature.
AB /PAS x 250.



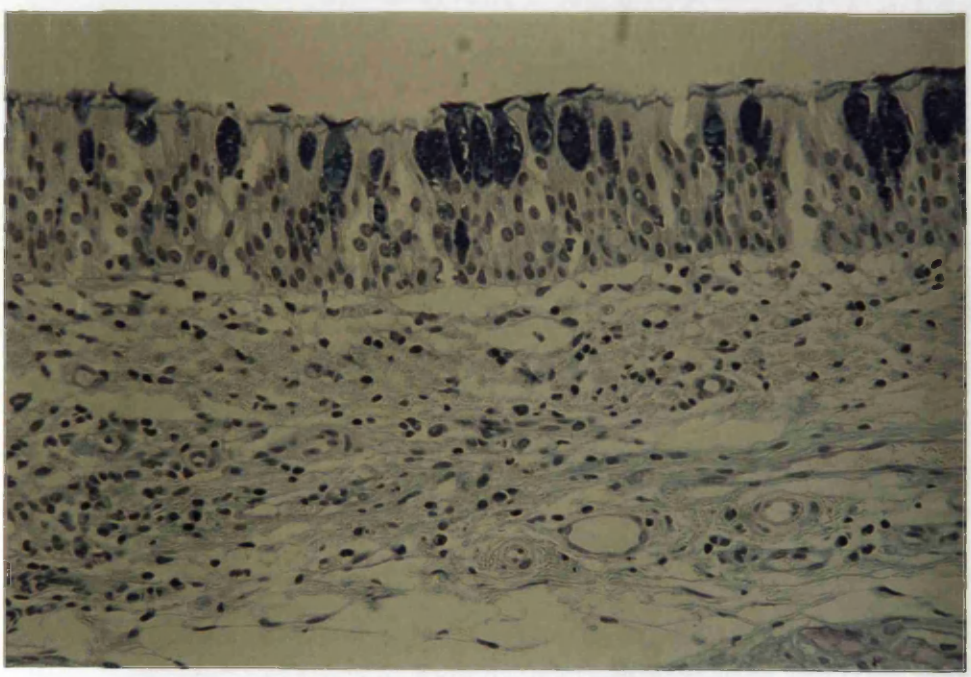
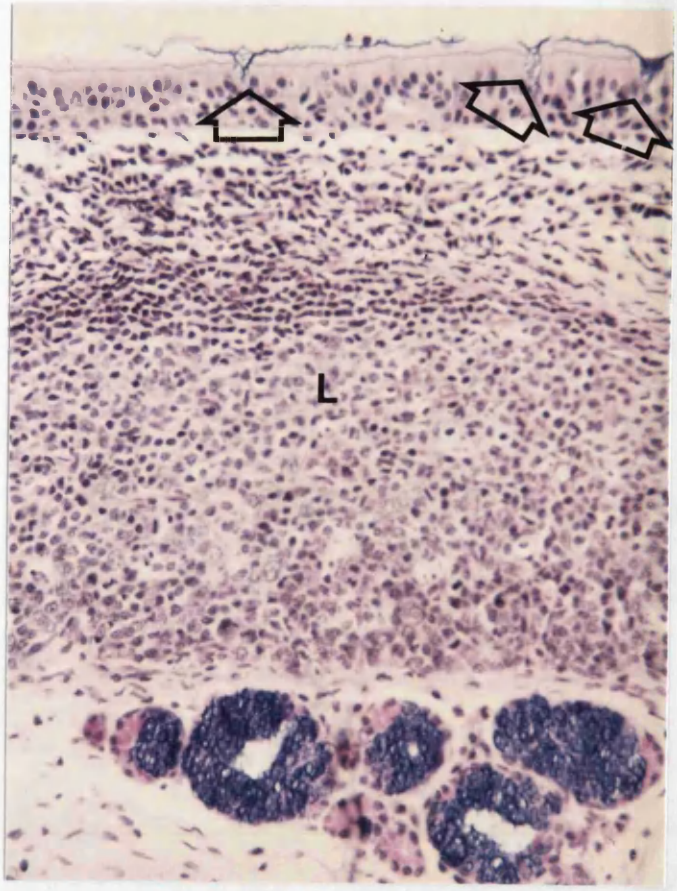


Fig. 3.5 Nasopharynx (transitional zone).
Note the aggregates of lymphoid tissue (L). Only occasional mucus-producing cells are seen within the lining epithelium (arrows).
Submucosal glands produce predominantly acidic mucosubstances.
AB /PAS x 250.

Fig. 3.6 Epiglottis.
Stratified squamous epithelium. Note the presence and appearance of the taste-buds (arrows).
AB /PAS x 250.


L

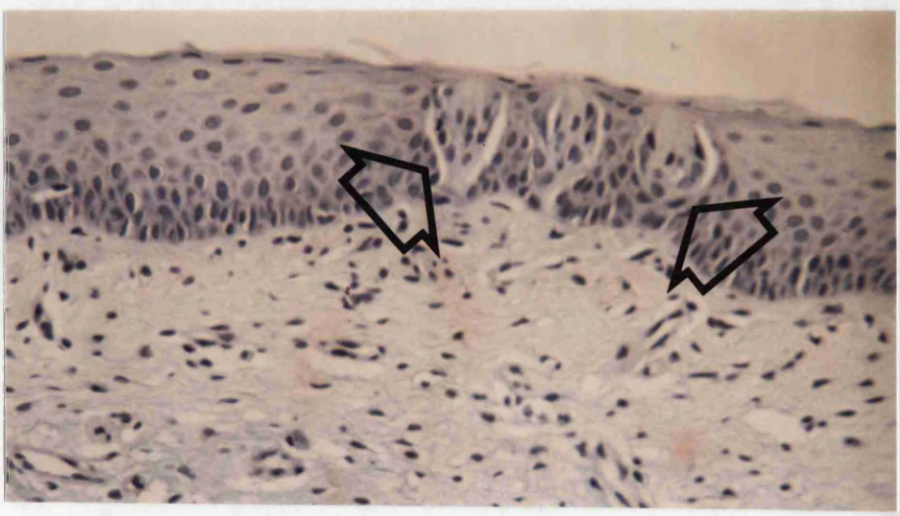
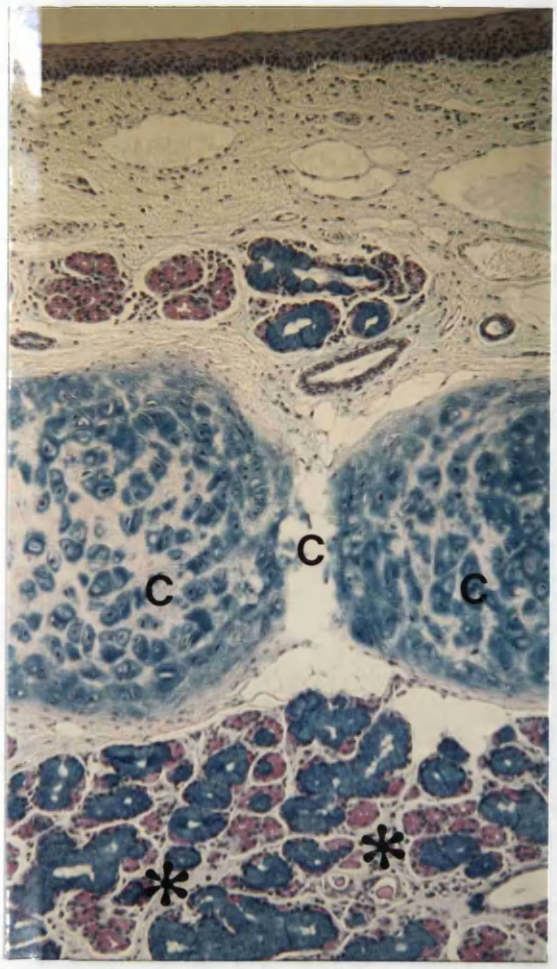


Fig. 3.7 Vocal fold (cranial region).
Stratified squamous epithelium.
Submucosal glands (*) exhibit equal amounts of both acidic and neutral mucosubstances. Note cartilage (C).
AB /PAS x 100.

Fig. 3.8 Trachea (dorsal cranial surface).
Submucosal gland duct opening into the gutter.
AB /PAS x 100.


C
C
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*
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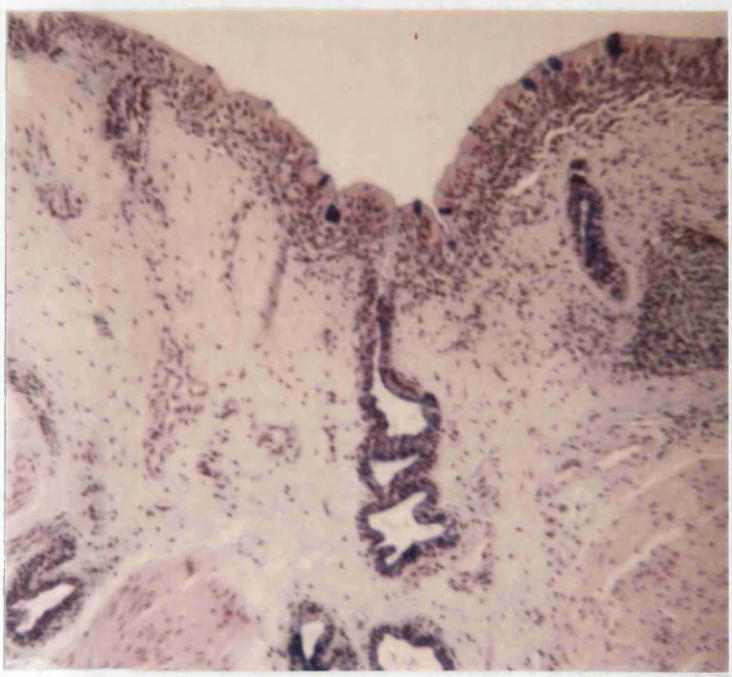
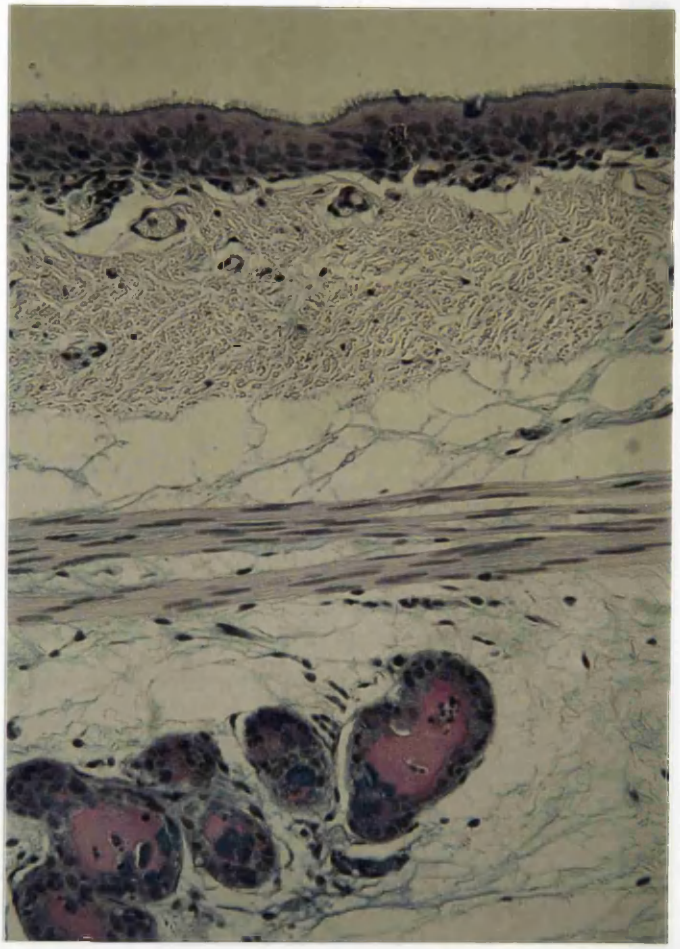


Fig. 3.9 Bronchus.
Pseudostratified ciliated columnar epithelium. Note that within the submucosal glands neutral mucosubstances predominate; only a few acidic mucosubstances are present.
AB /PAS x 100.

Fig. 3.10 Two respiratory bronchioles (R) can be seen arising from a terminal bronchiole (T). Note the presence of alveoli in the walls of the respiratory bronchioles (*).
H&E x 100.



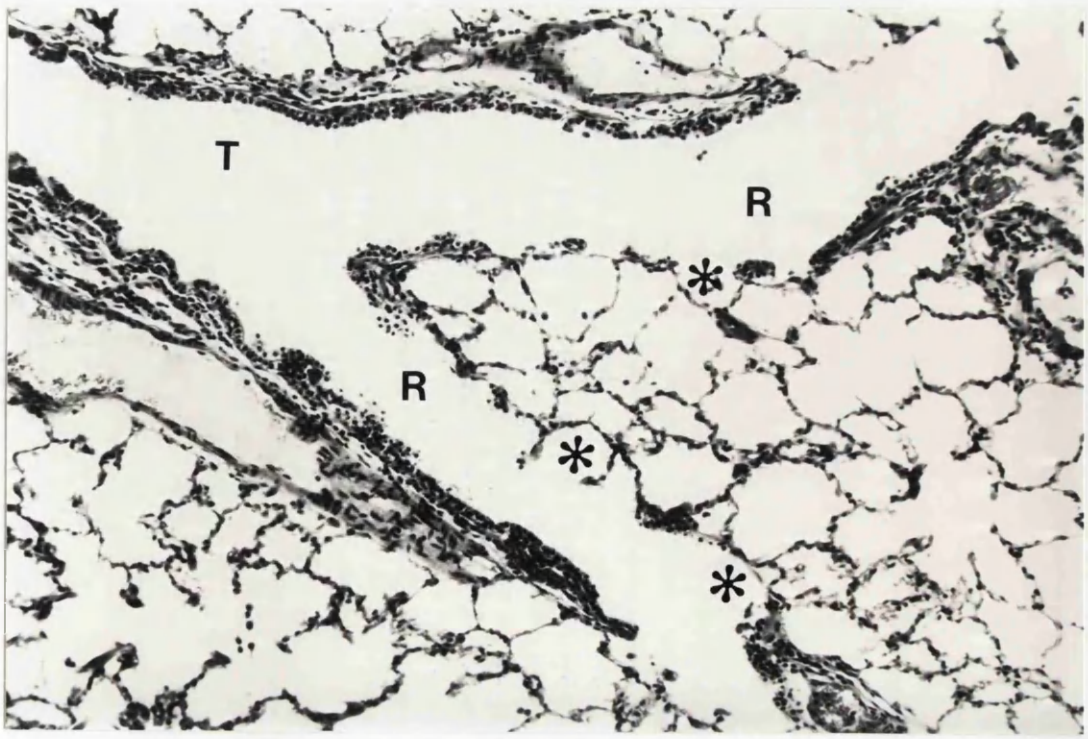
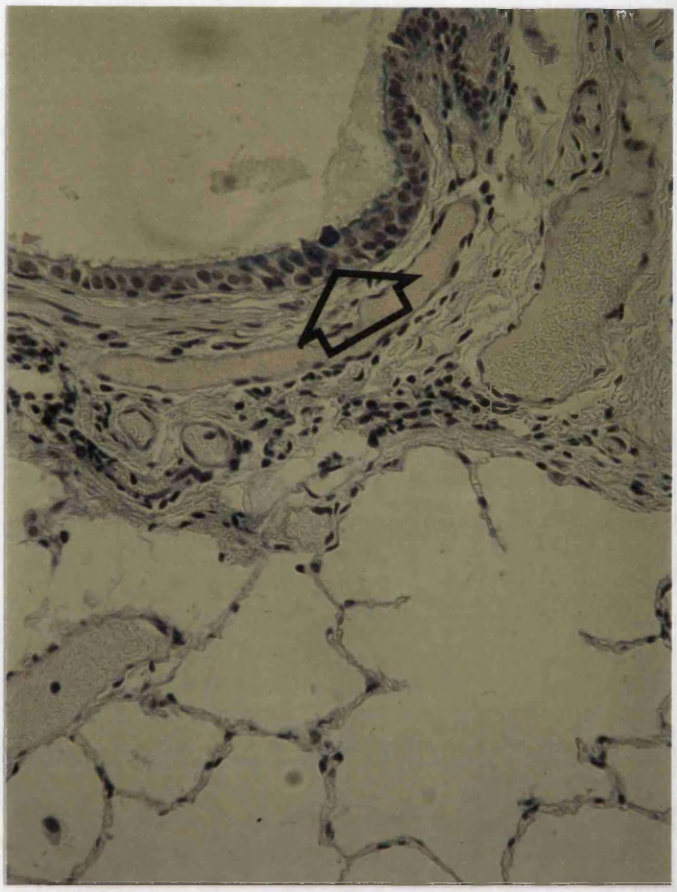

T
R
R
*
*
*

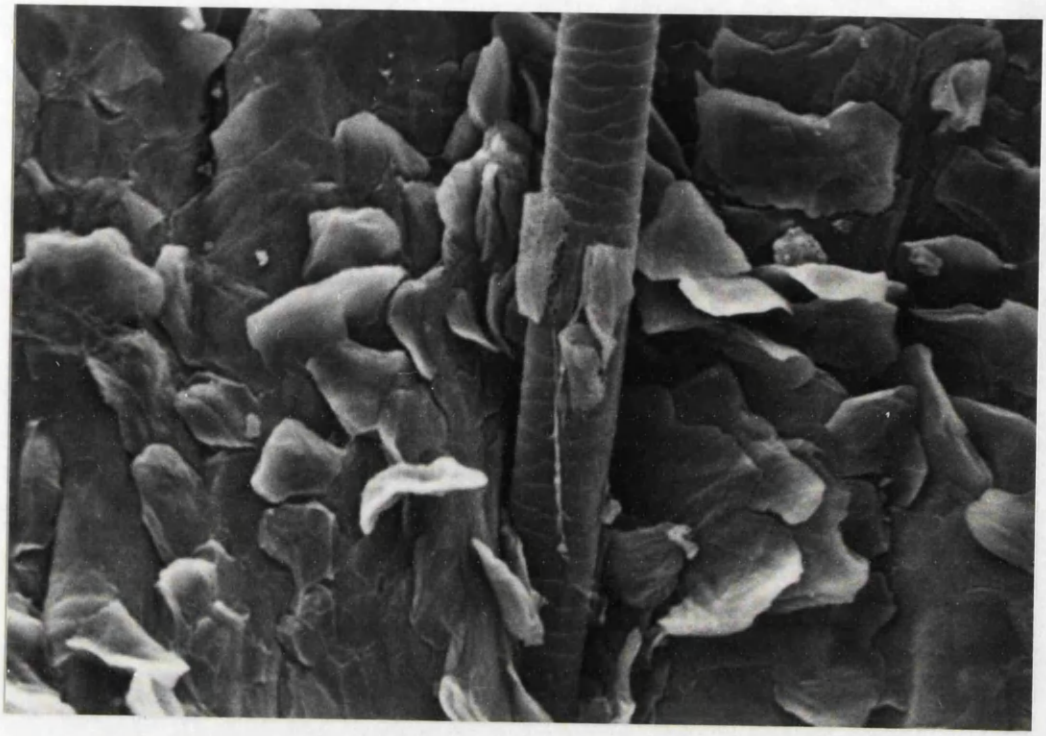
Fig. 3.11 Bronchiole.
Proximal to the terminal bronchiole, a few mucus-producing cells were observed in the lining epithelium (arrow).
AB /PAS x 250



# CHAPTER 4

Fig. 4.1 Nasal vestibule, (rostral region).
Squamous epithelium. Note hair shaft and desquamating cells.
SEM x 720

Fig. 4.2 Nasal vestibule, (middle region).
An occasional submucosal gland orifice is observed.
SEM x 720.



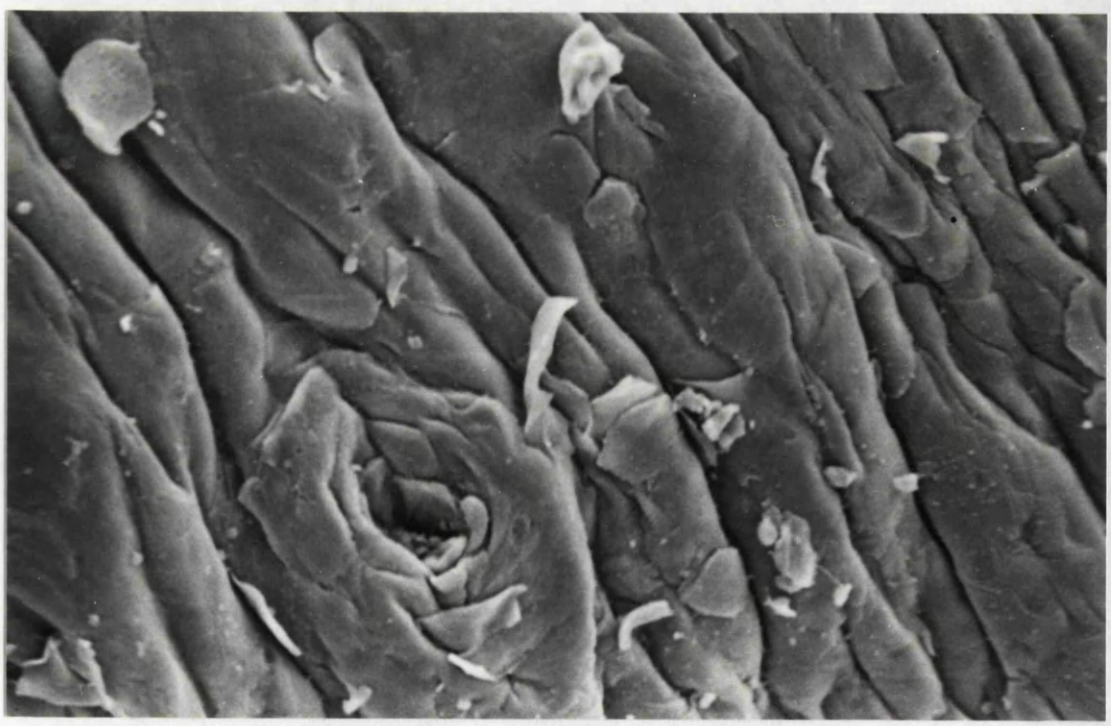
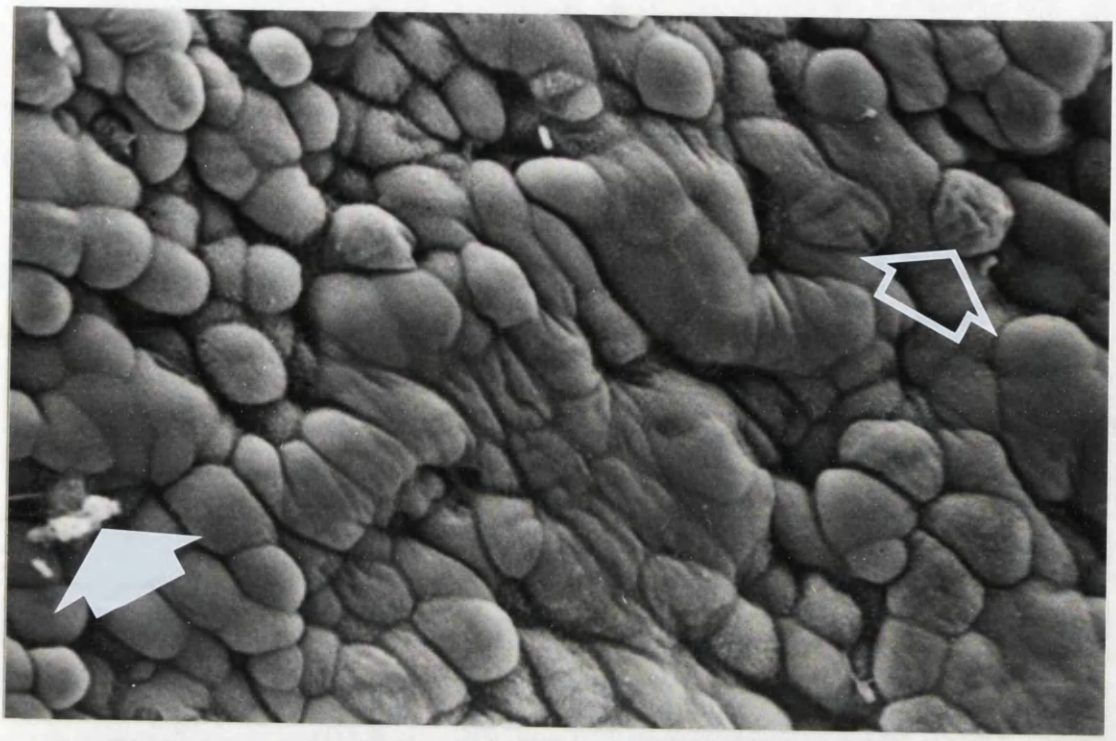


Fig. 4.3 Nasal vestibule (caudal region).
Note the spongy nature of the cells and their rounded borders, mucus being extruded from at least one surface cell (closed arrow) and a dying cell presenting a wrinkled apical surface (open arrow).
SEM x 1,440.

Fig. 4.4 Nasal vestibule (caudal region).
High power micrograph of Fig. 4.3. Note surface microvilli and microplicae.
SEM x 11,25



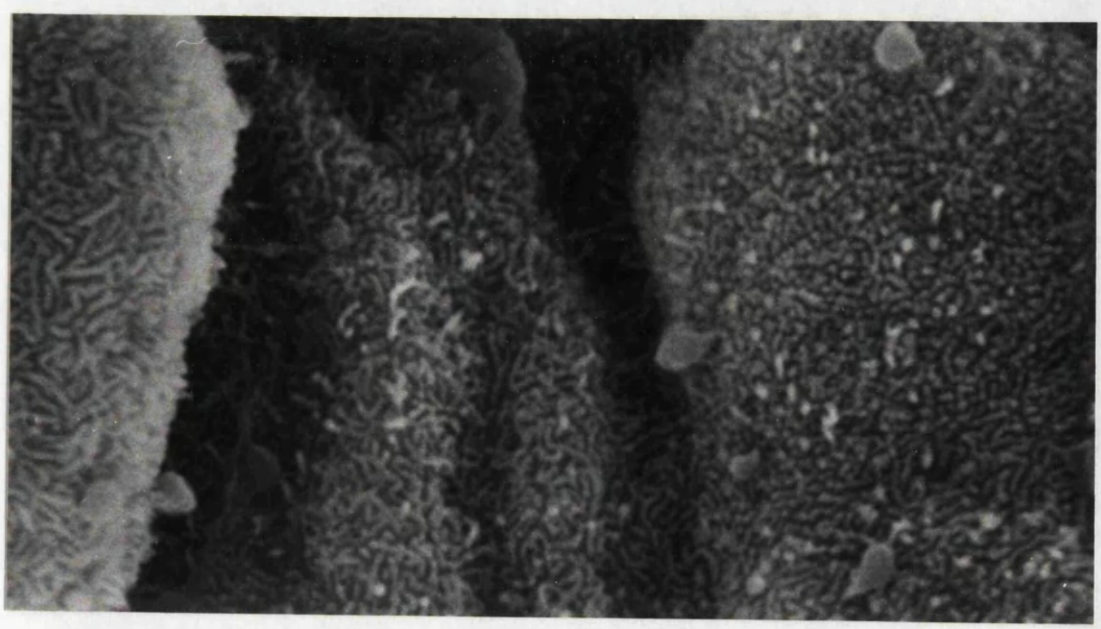
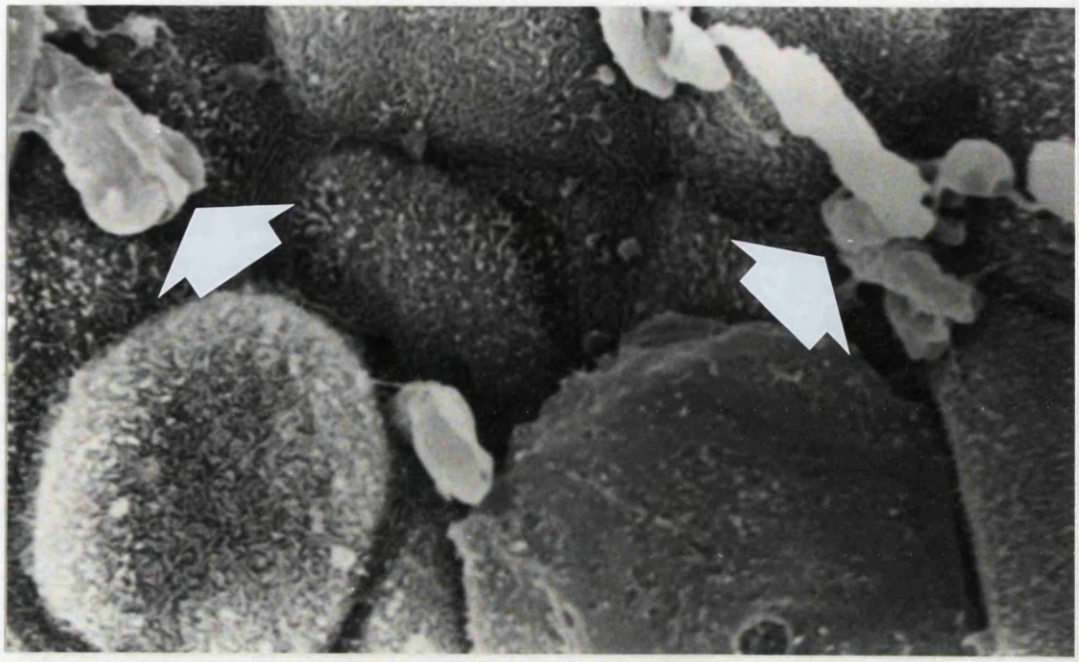


Fig. 4.5 Nasal vestibule (caudal region).
Note the production and extrusion of mucus (arrows) in this region.
SEM. x 5,600.

Fig. 4.6 Alar fold.
The epithelium is seen to changes from stratified squamous rostrally (R) to stratified cuboidal caudally (C). Note the appearance of mucus-producing cells in these caudal regions (arrow). Note the submucosal gland duct (*).
H&E x 250.



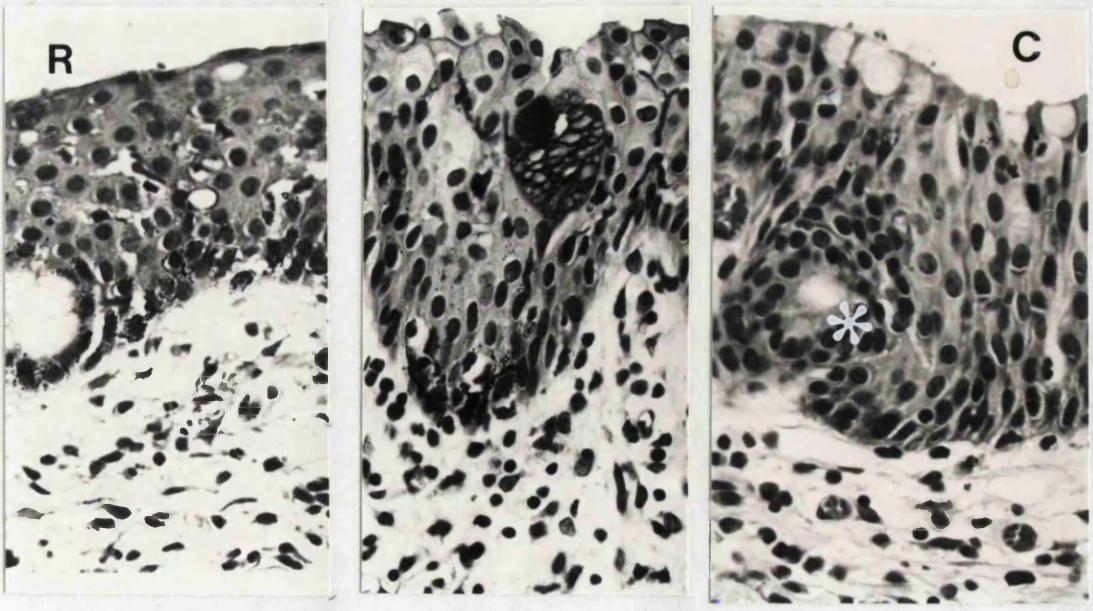

R
C

Fig 4.7 Alar fold.
The "cobblestone" nature of the epithelium is apparent. Note the distinct cell boundaries.
Submucosal gland orifice (*).
SEM x 1,440.

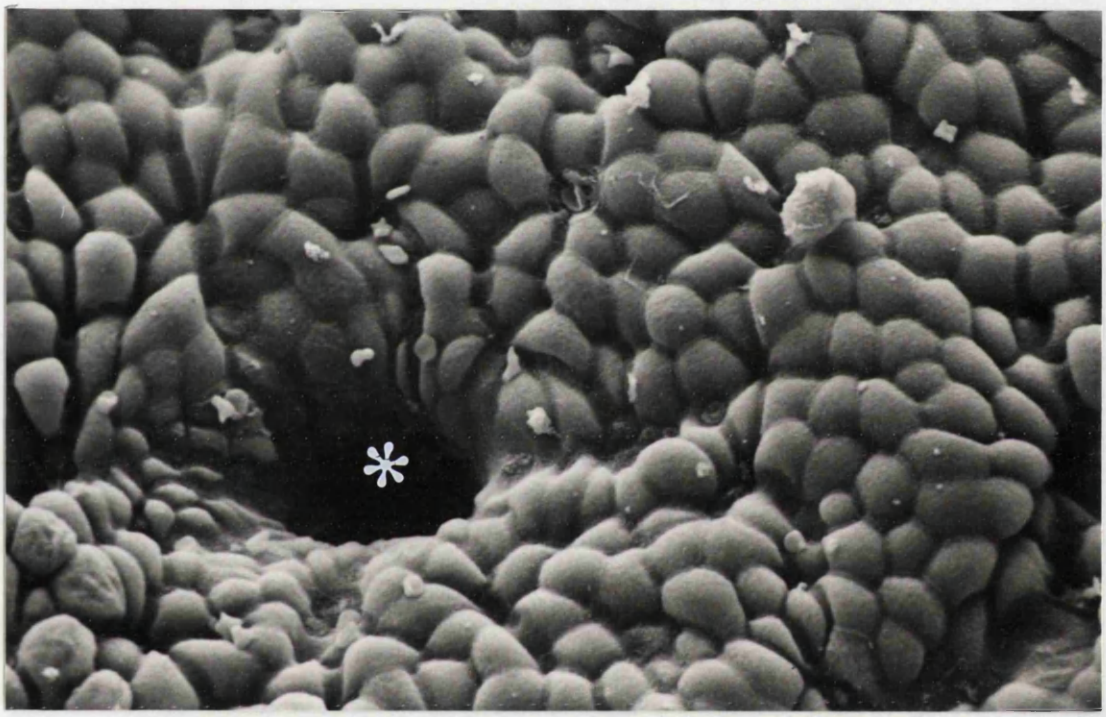


Fig. 4.8 Alar fold.
Densely packed surface microvilli cover the luminal surfaces of the cuboidal epithelial cells. Note the presence a number of mucus-producing cells (*) in this region.
SEM x 11,250.

Fig. 4.9 Alar fold.
Note the appearance of another cell type with prominent raised borders (arrows).
SEM x 1,440.

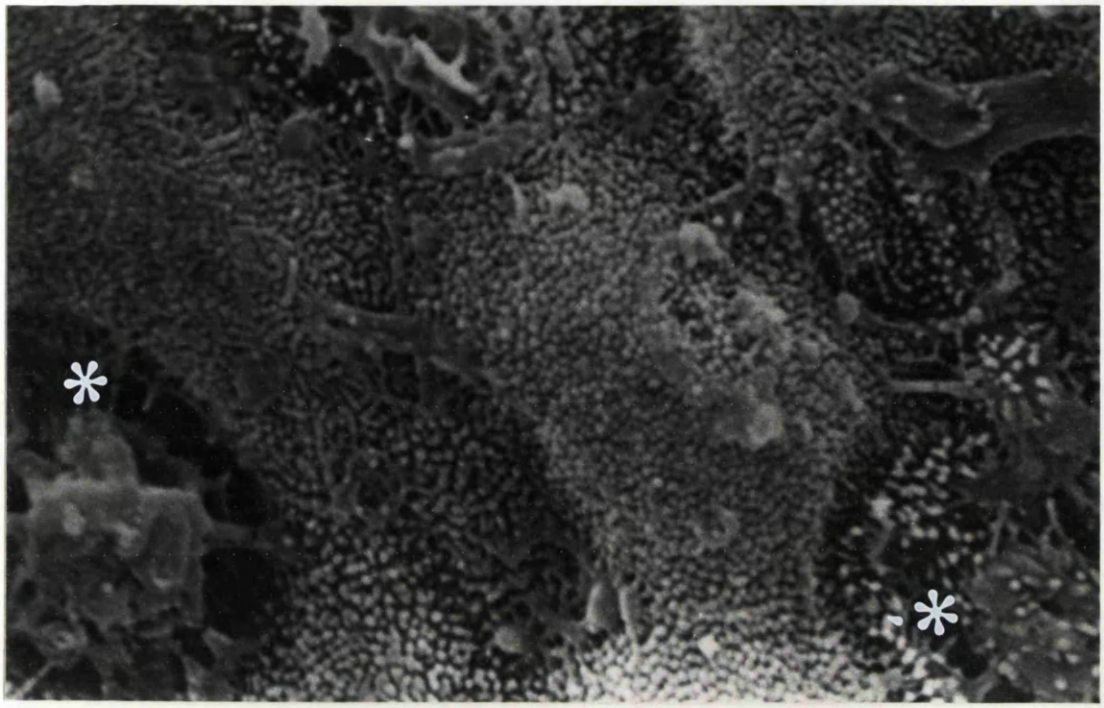



Fig. 4.10 Alar fold.

Mucus-producing cells (*) with depressed apical surfaces and a sparse population of surface microvilli are readily identifiable. Note their prominent borders.

SEM x 5,600

Fig 4.11 Alar fold.

Occasional submucosal gland orifices (*) are observed in this region. Note the two types of nonciliated microvillous cells (1 and 2).

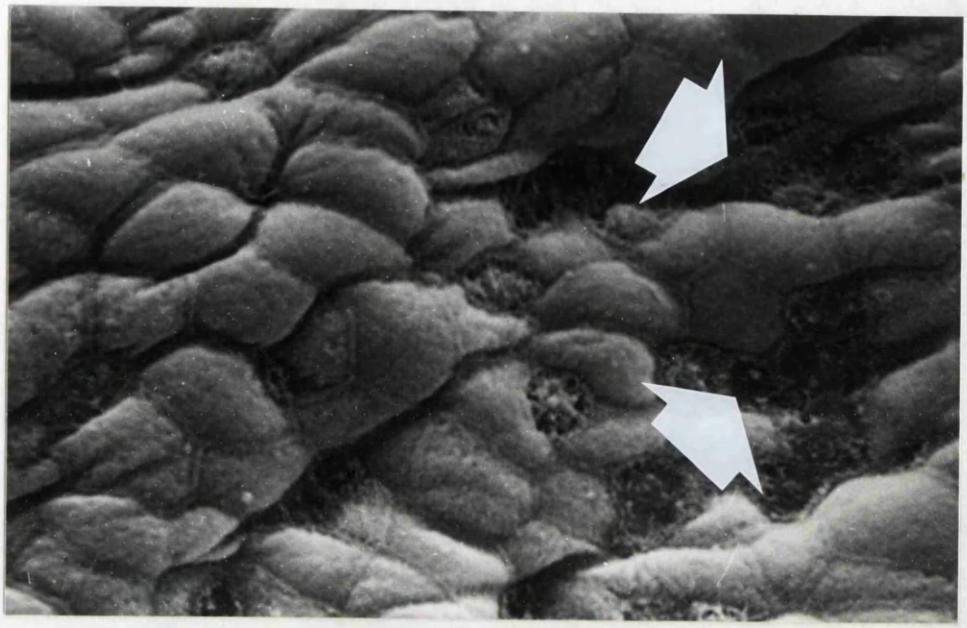
SEM x 5,600.


*
*


2
*
1

Fig 4.12 Basal fold.
Note the "cobblestone" appearance of the epithelial surface cells and their prominent cell boundaries. Mucus-producing cells can also be observed (arrows).
SEM x 5,600.

Fig. 4.13 Basal fold.
Mucus-producing cells.
Coalescing mucous granules may be seen through the plasmalemma (arrow).
SEM x 5,600.



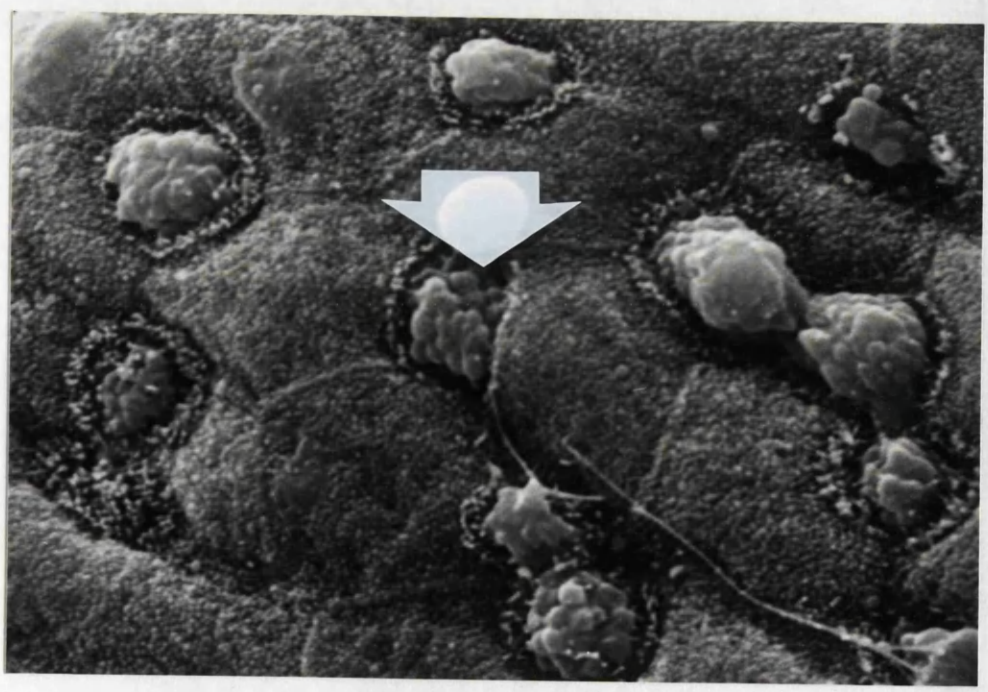


Fig. 4.14 Basal fold (transitional zone).
Note the abrupt transition from nonciliated to ciliated epithelium.
SEM x 2,800.

Fig. 4.15 Basal fold (transitional zone).
Note the appearance of regenerating ciliated cells at different stages of ciliogenesis (arrows).
SEM x 5,600.

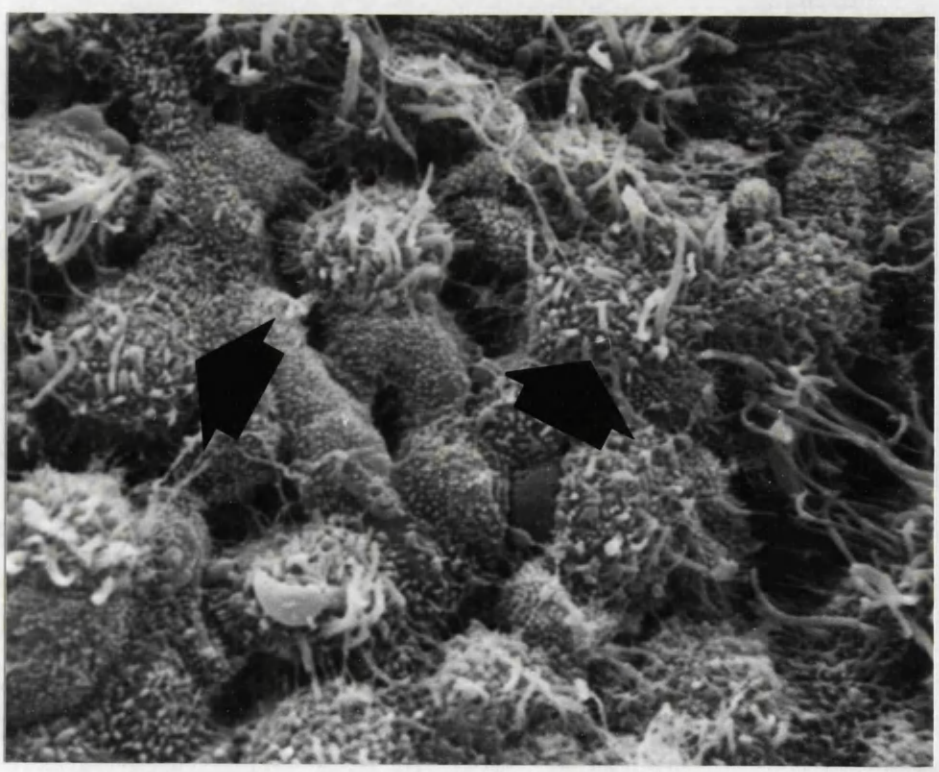
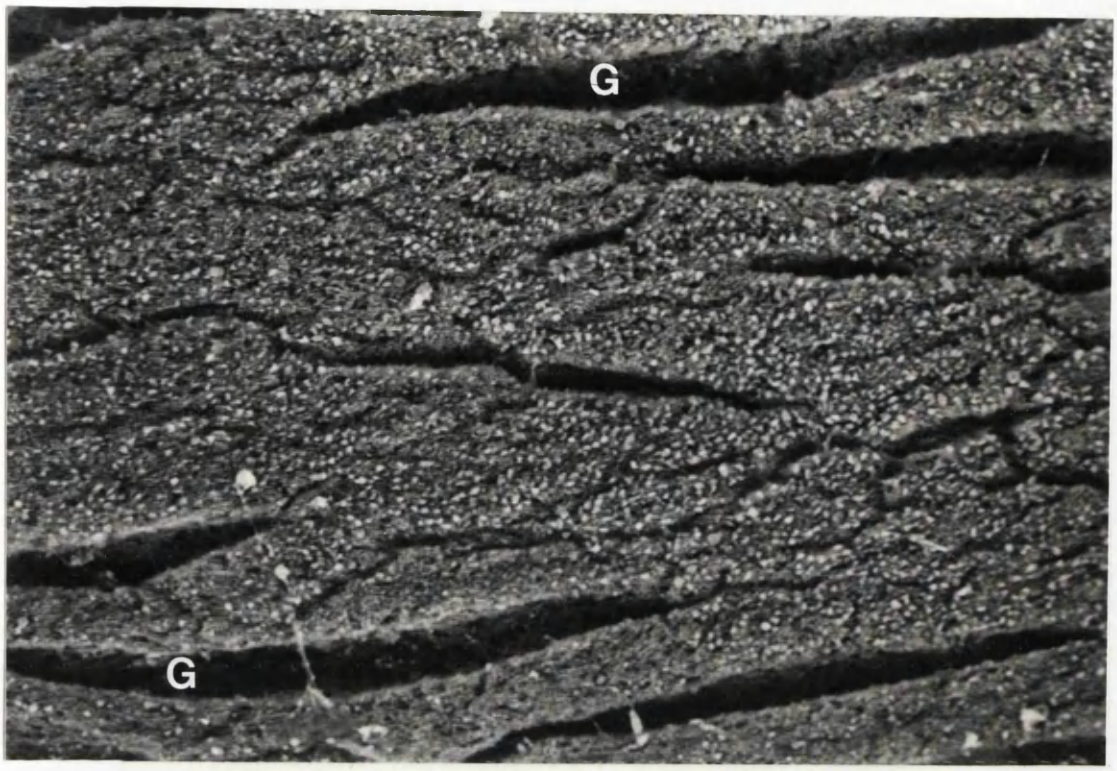


Fig. 4.16 Basal fold (caudal region).
Low power photomicrograph of the ciliated epithelial lining organised into longitudinal grooves (G). Note the large numbers of mucus-producing cells protruding beyond ciliary surface.
SEM x 360.

Fig. 4.17 Basal fold (caudal region).
Active mucus-producing cells with apical protuberances, distributed individually amongst ciliated cells.
SEM x 5,600.


G
G

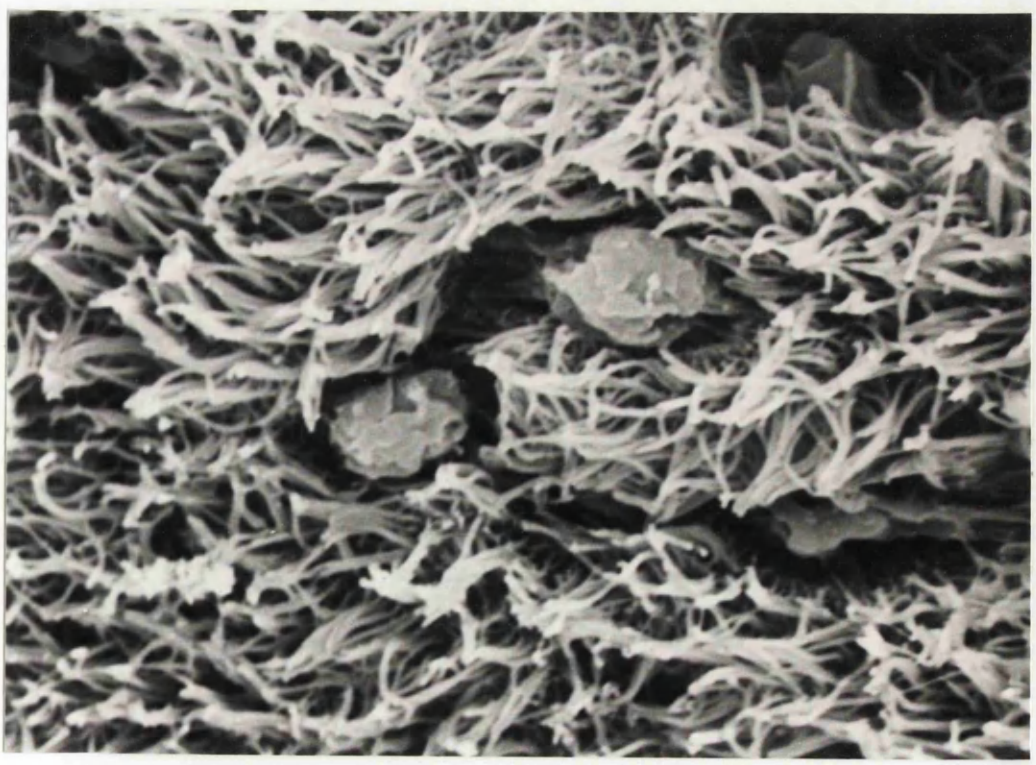


Fig. 4.18 Ventral nasal concha.
Ciliated epithelium. Note mucus-producing cells, exhibiting typical protuberances distributed individually amongst ciliated cells.
SEM x 2,800.

Fig. 4.19 Ventral nasal concha.
The slender cilia carried by ciliated cells frequently appear matted at their tips (arrows). Note microvilli on the apical protrusions of the mucus-producing cells.
SEM x 5,600.

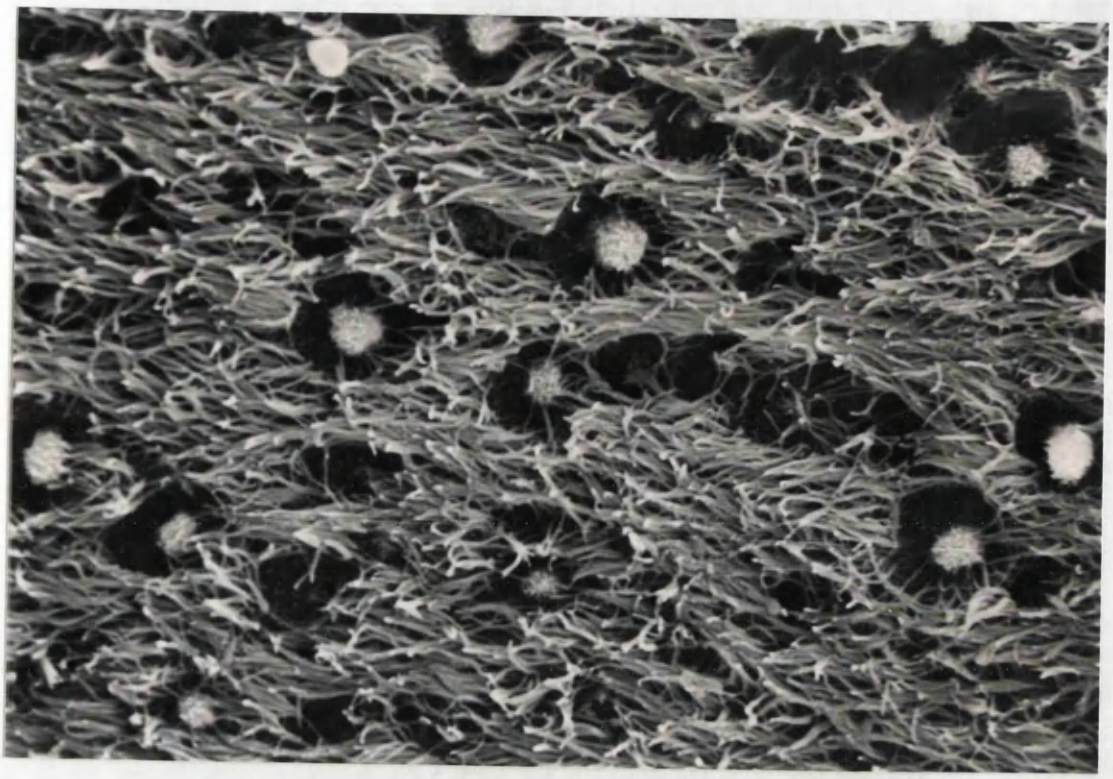
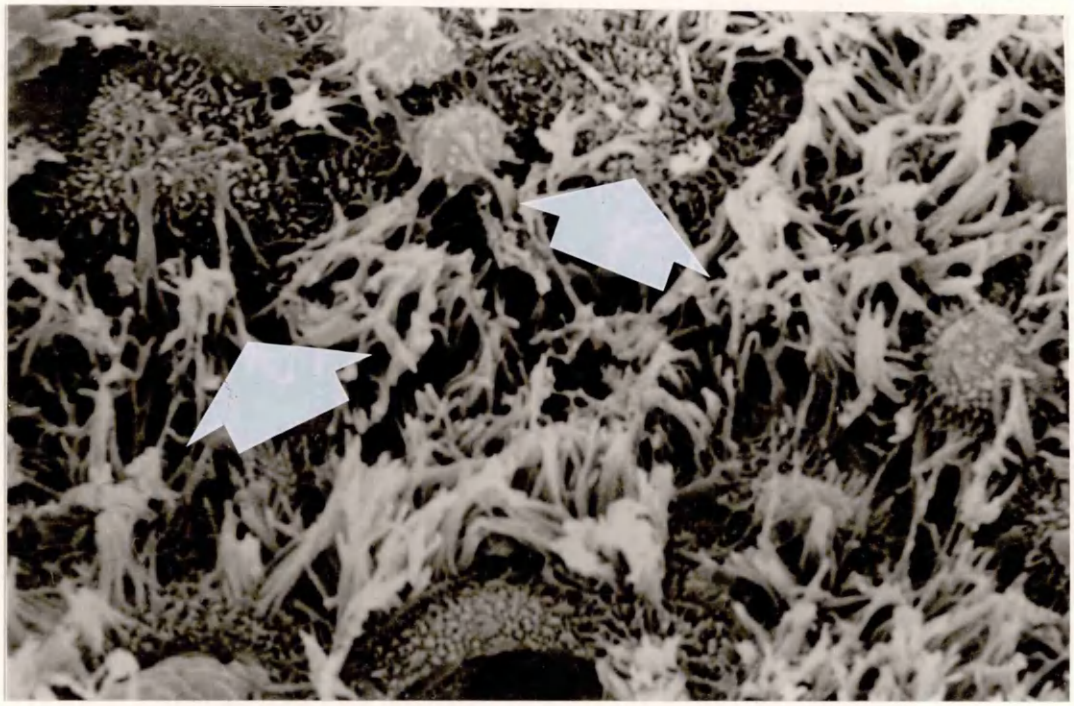


Fig. 4.20 Ventral nasal concha.
Ciliated cells carrying short, matted cilia of unequal lengths. These cells are considered to be regenerating ciliated cells (arrows).
SEM x 5,600.

Fig. 4.21 Ventral nasal concha.
Nonciliated microvillous cells with a thin layer of mucus on their apical surfaces (arrows) can be seen.
SEM x 2,800.



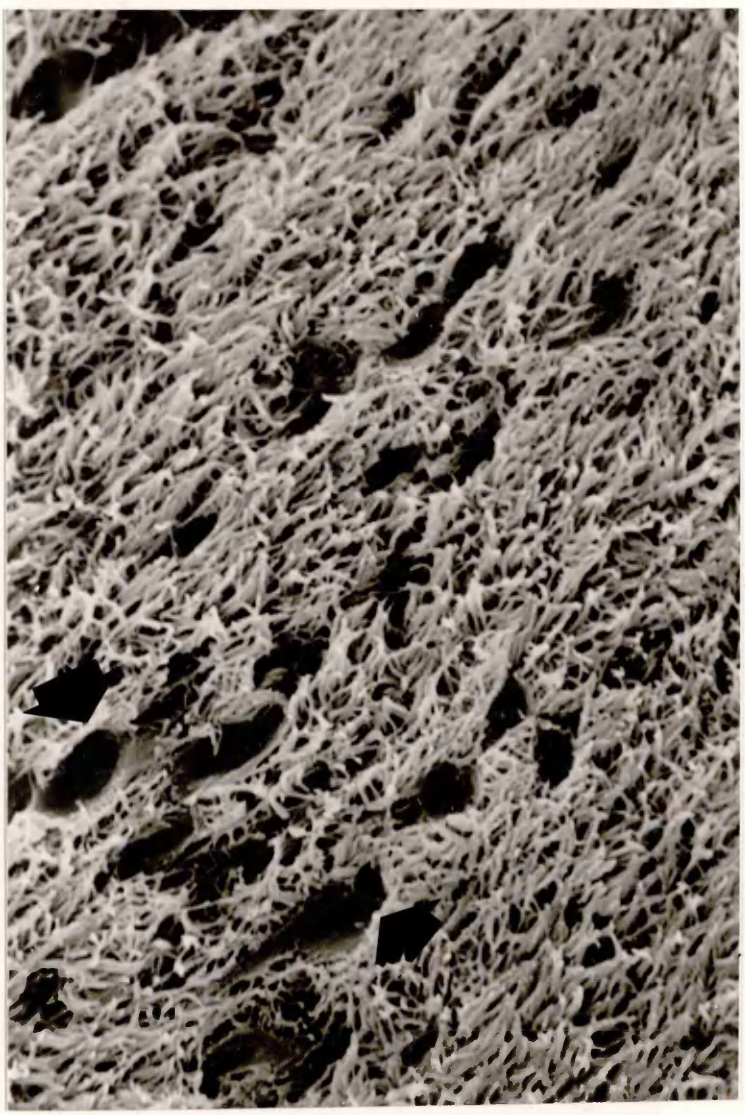
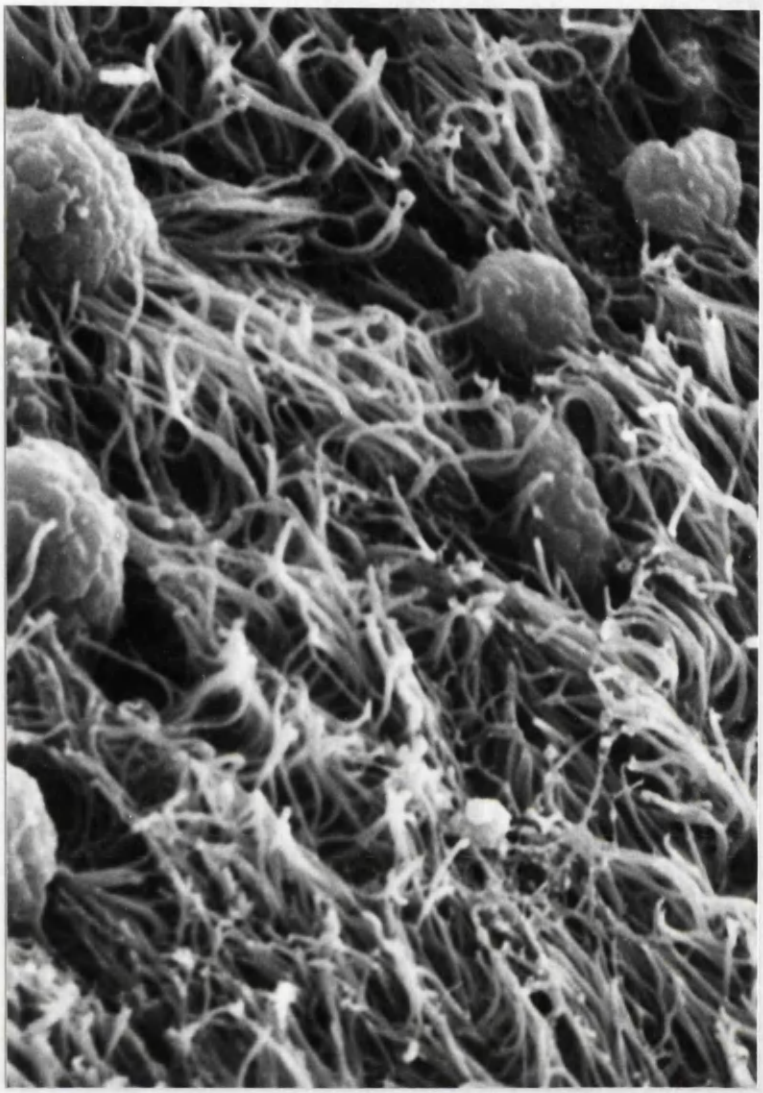


Fig. 4.22 Ventral nasal concha.
Mucus-producing cells are distributed individually amongst ciliated cells . Note the accumulation of mucous granules seen through the plasmalemma.
SEM x 5,600.

Fig. 4.23 Ventral nasal concha.
Patches of regenerating ciliated cells. Note the unequal lengths of their cilia.
SEM x 5,600.



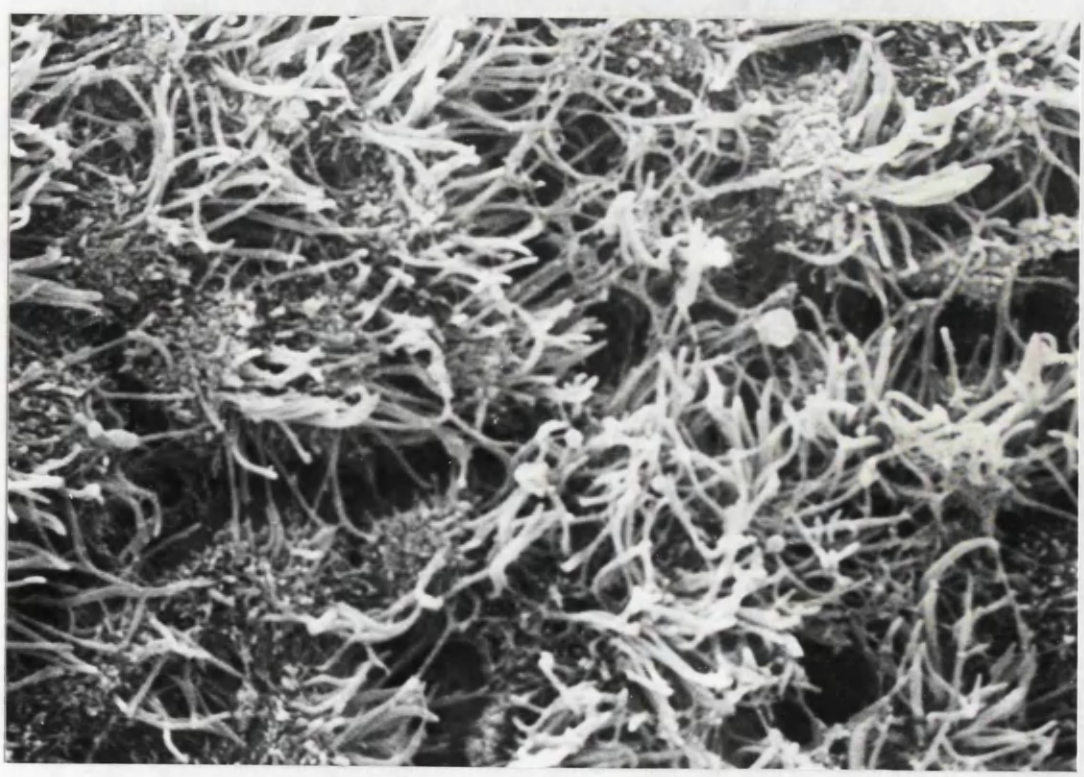
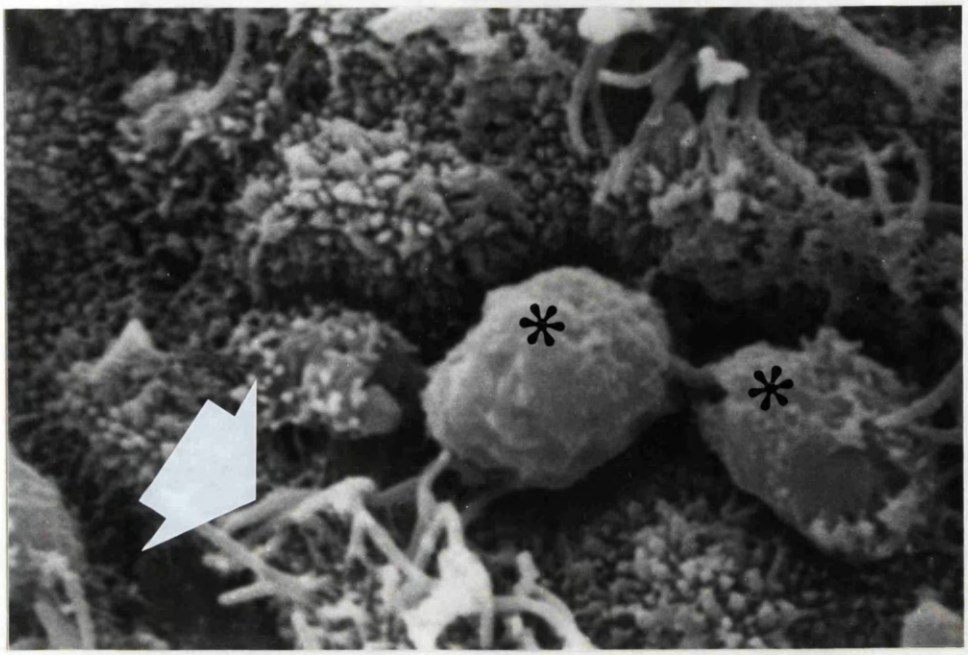


Fig. 4.24 Dorsal nasal concha.
A patch of nonciliated microvillous cells. Note mucus being discharged (*) at the surface. Regenerating ciliated cell (arrow).
SEM x 5,600.

Fig. 4.25 Dorsal nasal concha.
Submucosal gland orifice (*). Nonciliated microvillous cells are seen around the orifice.
SEM x 5,600.



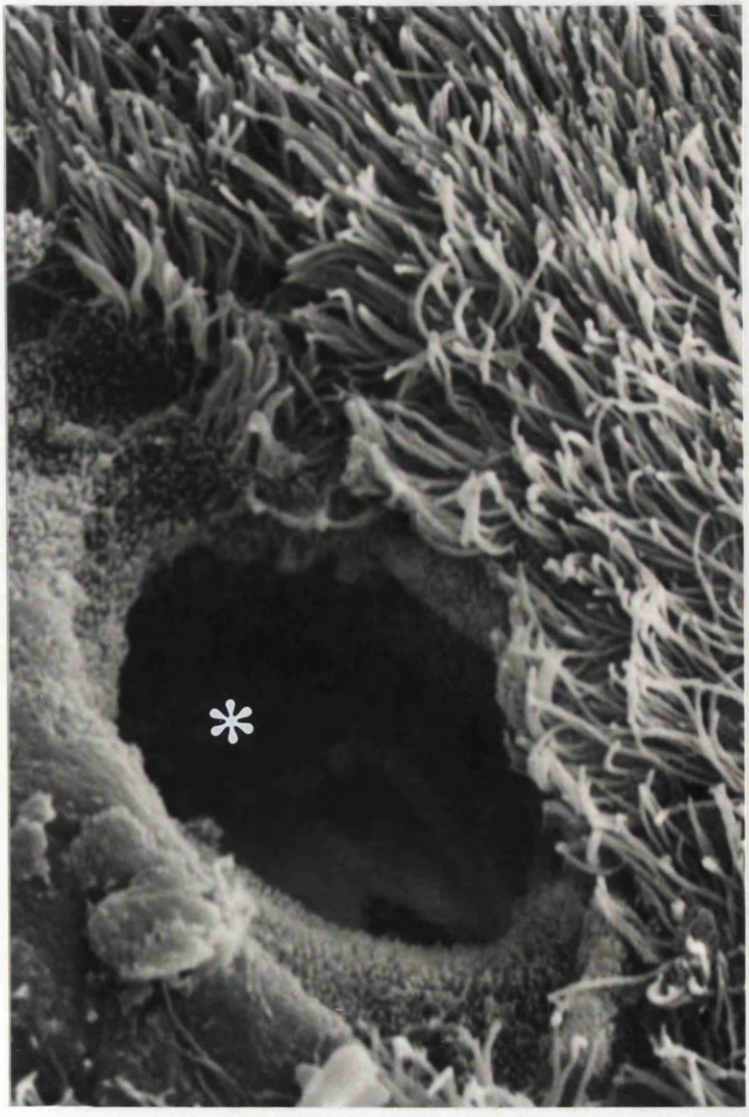
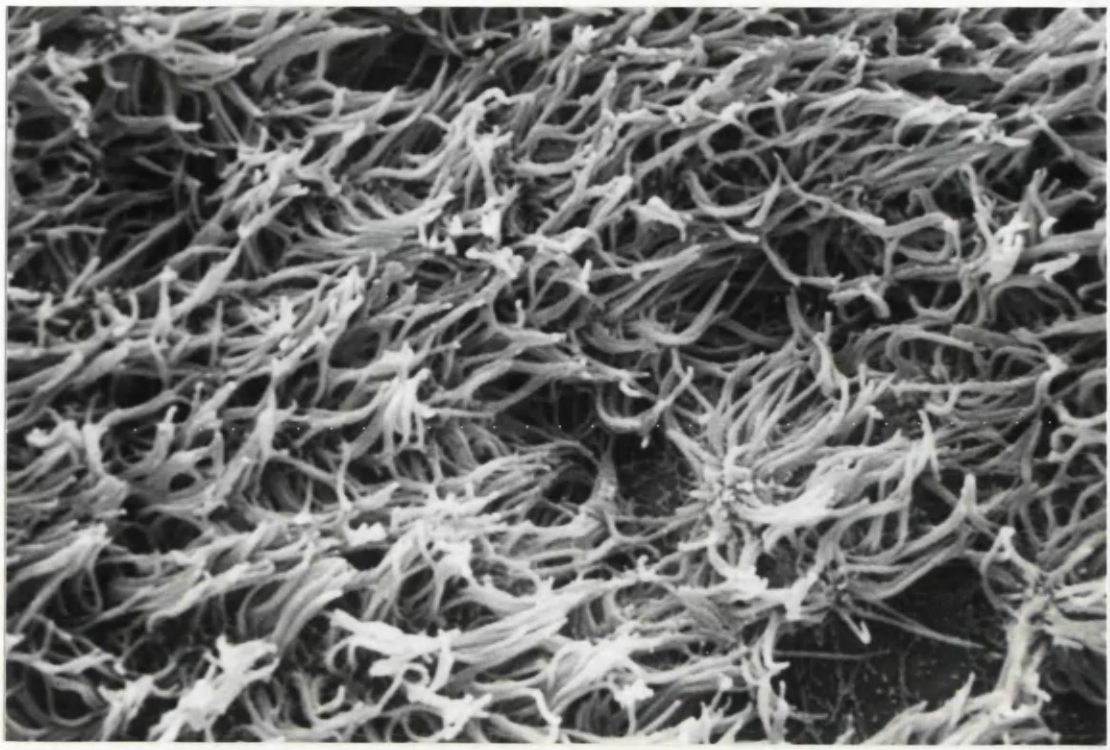


Fig. 4.26 Dorsal nasal concha.
Ciliated epithelium. Cilia appear matted and disorganised.
SEM x 5,600.

Fig. 4.27 Middle nasal concha.
At low power epithelial ridges (R) and gutters (G) are prominent.
SEM x 360.



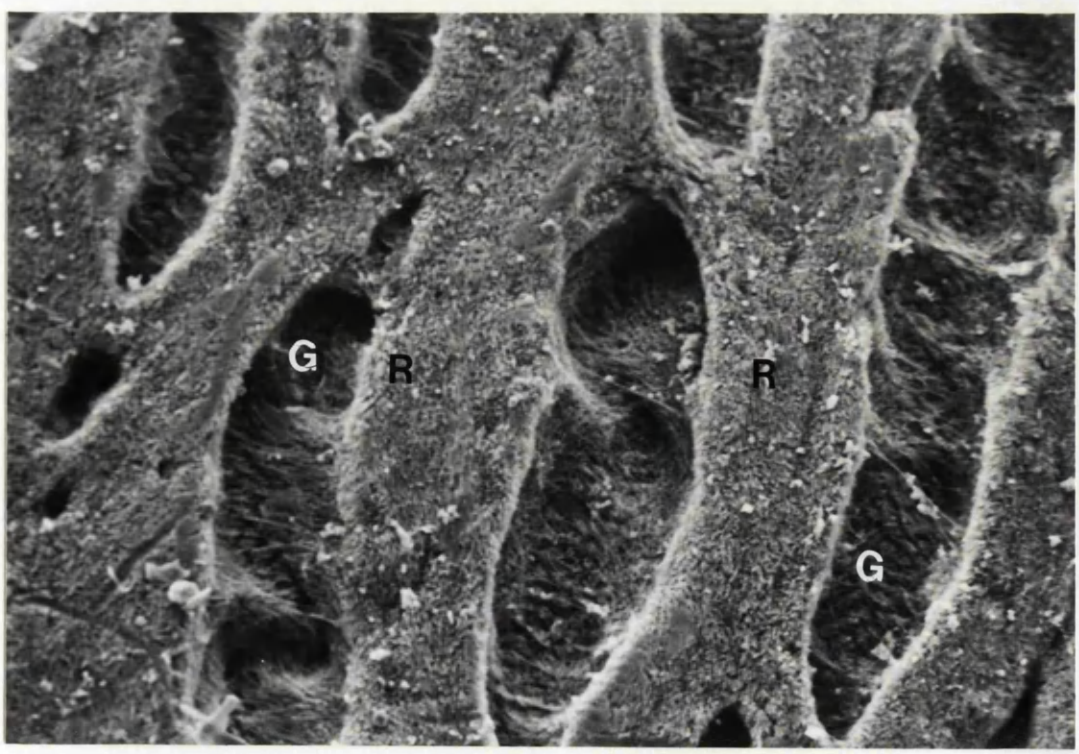
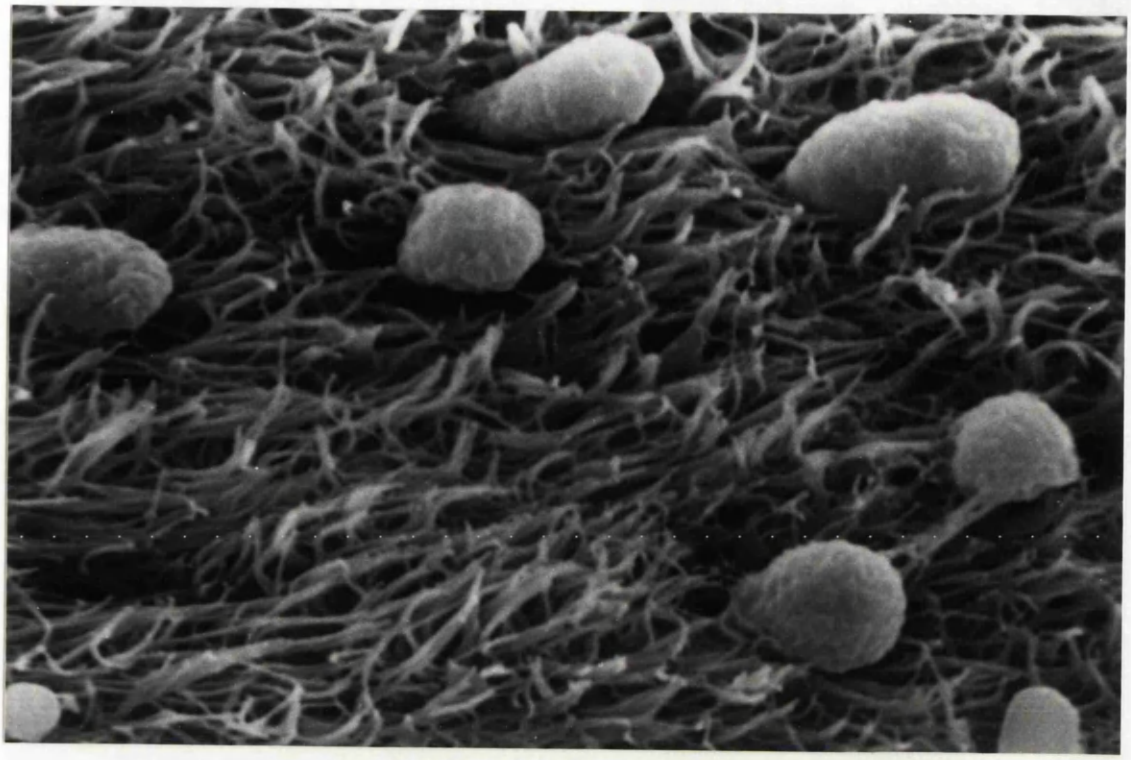

G
R
R
G

Fig. 4.28 Middle nasal concha.
Heavily ciliated epithelium
Note the individual mucus-producing cells scattered amongst the ciliated cells.
SEM x 5,600.

Fig. 4.29 Middle nasal concha.
Nonciliated microvillous cells showing relatively flat apical surfaces with a thin layer of mucus on top (*).
SEM x 5,600.



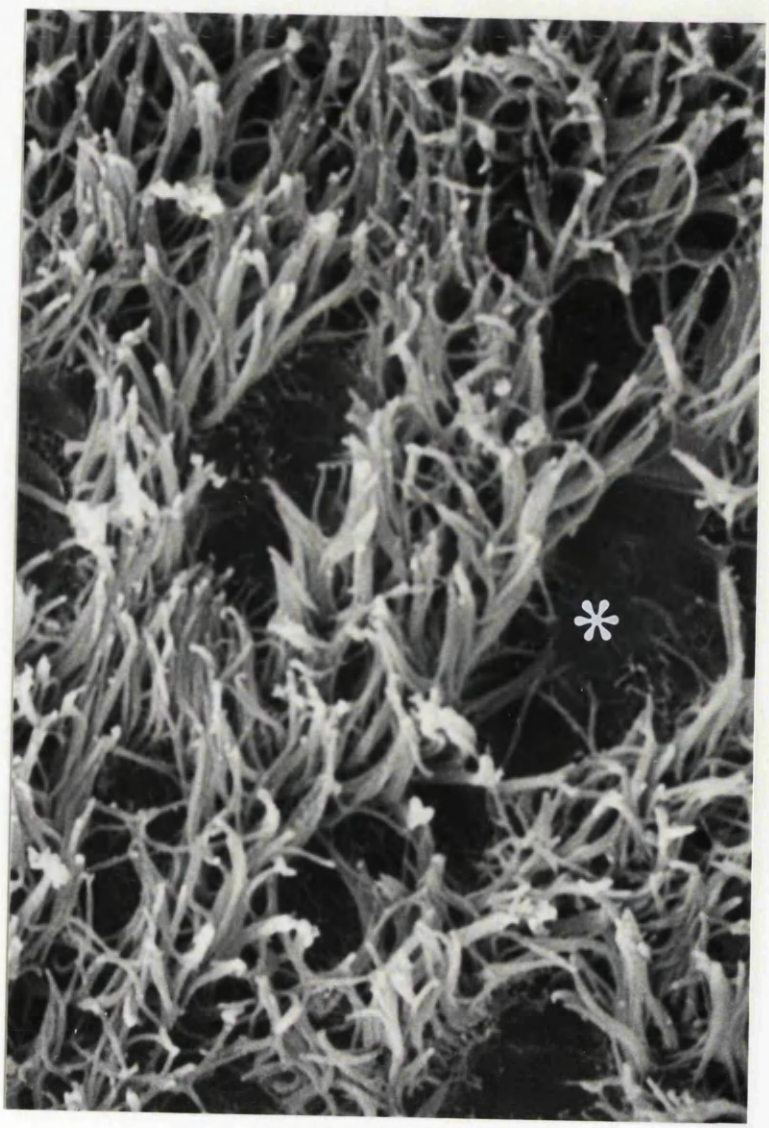
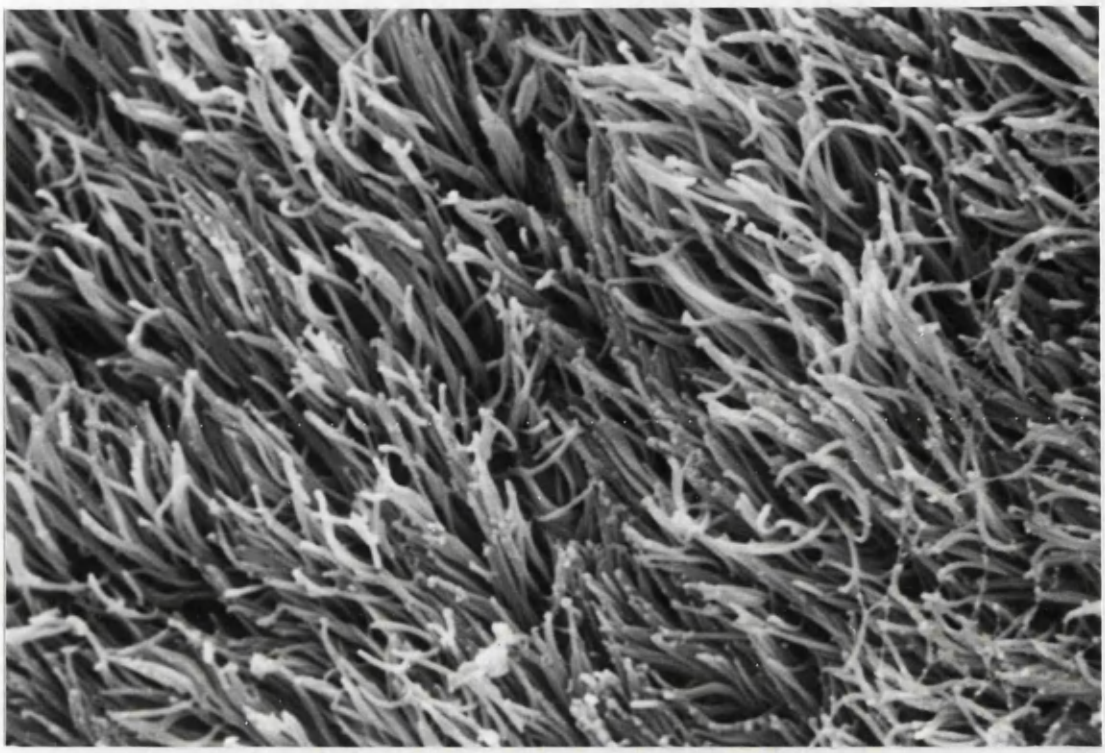


Fig. 4.30 Middle nasal concha.
Heavy cilial carpet with long, slender free-standing cilia.
SEM x 5,600.

Fig 4.31 Middle nasal concha.
Cilia appear matted and clumped. Note the nonciliated microvillous cell with microvilli entangled by mucus (*).
SEM x 11,250.



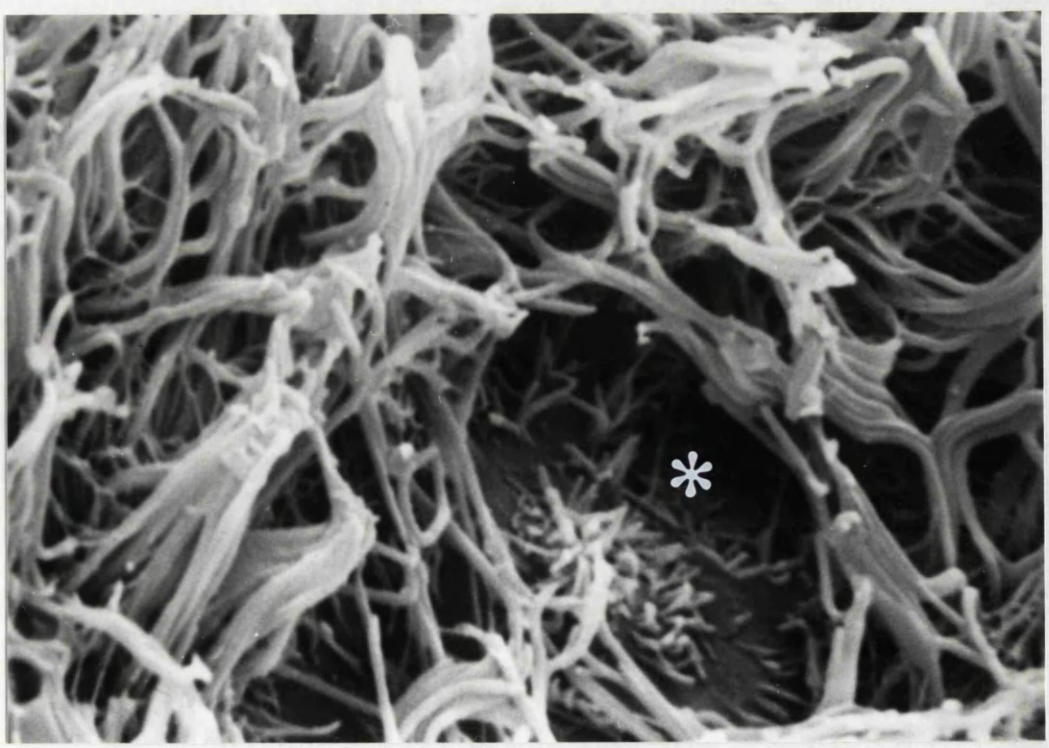
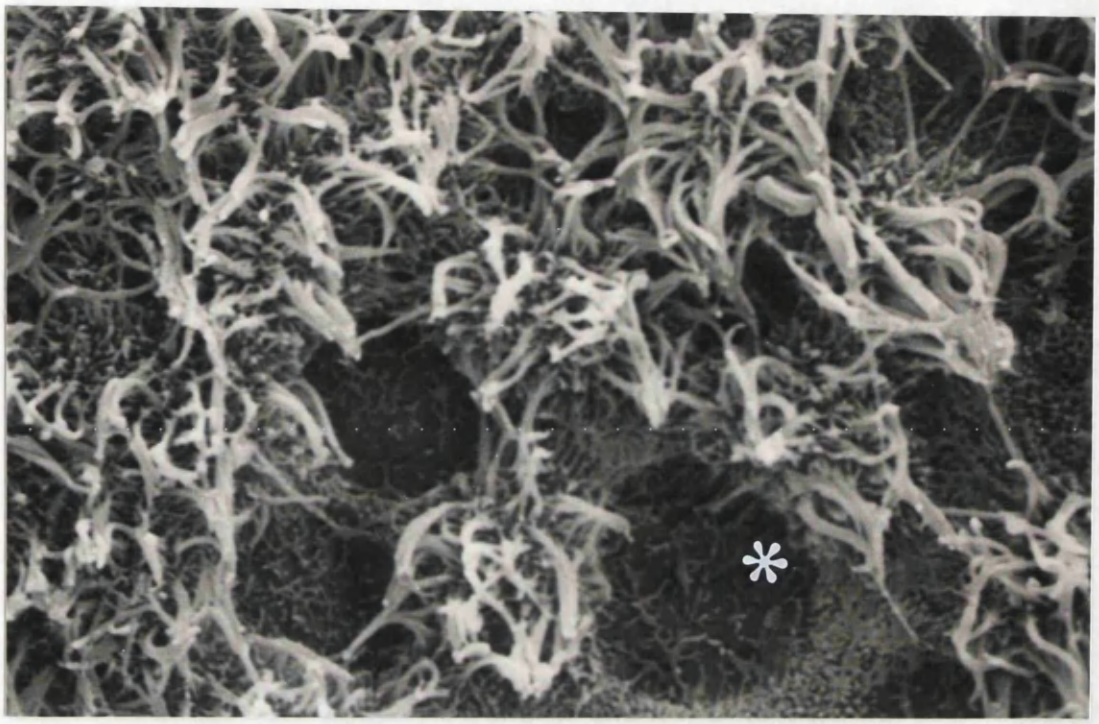


Fig. 4.32 Nasal septum.
Ciliated cells bear disorganised matted cilia.
Nonciliated microvillous cells with shallow apical surfaces and sparsely distributed surface microvilli (*) are also seen.
SEM x 5,600.

Fig. 4.33 Nasopharynx (rostral region).
Ciliated epithelium with a few scattered nonciliated microvillous cells.
SEM x 5,600.



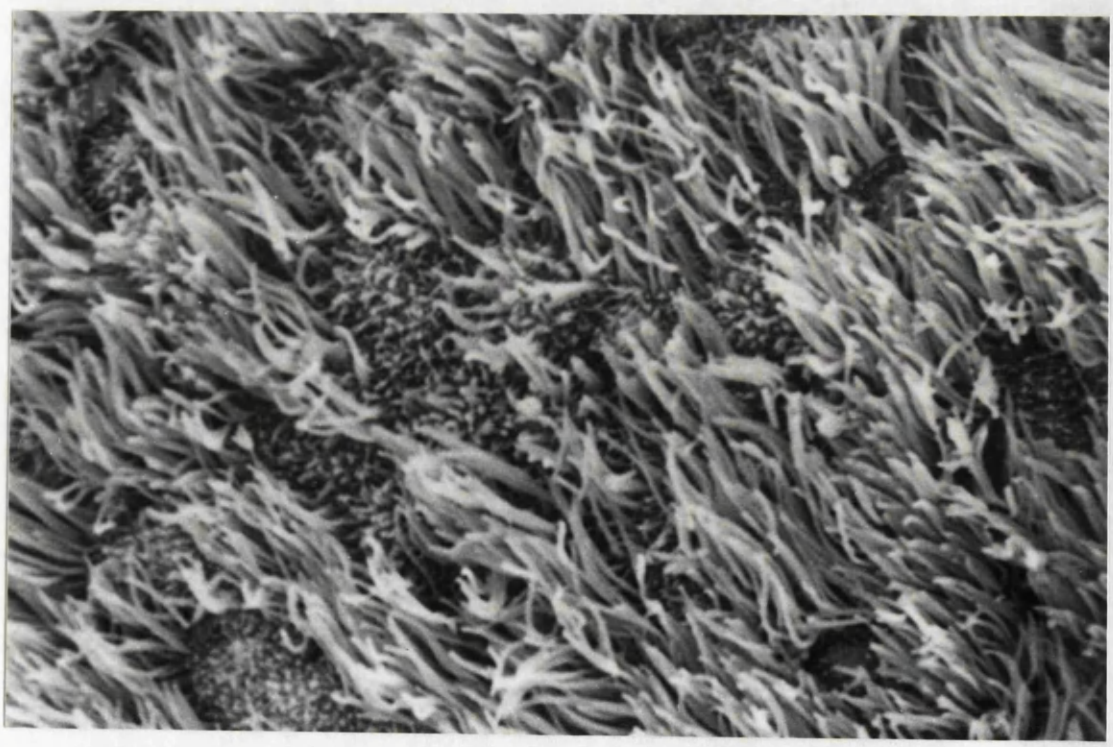


Fig. 4.34 Nasopharynx (rostral region).
Part of 'island' of nonciliated microvillous cells. Many of the latter are characterised by apical protuberances typical of mucus-producing cells.
SEM x 2,800.

Fig. 4.35 Nasopharynx (transitional zone).
The highly folded nature of the lining epithelium is characteristic of this region. Note the depth of the corrugations.
SEM x 90.

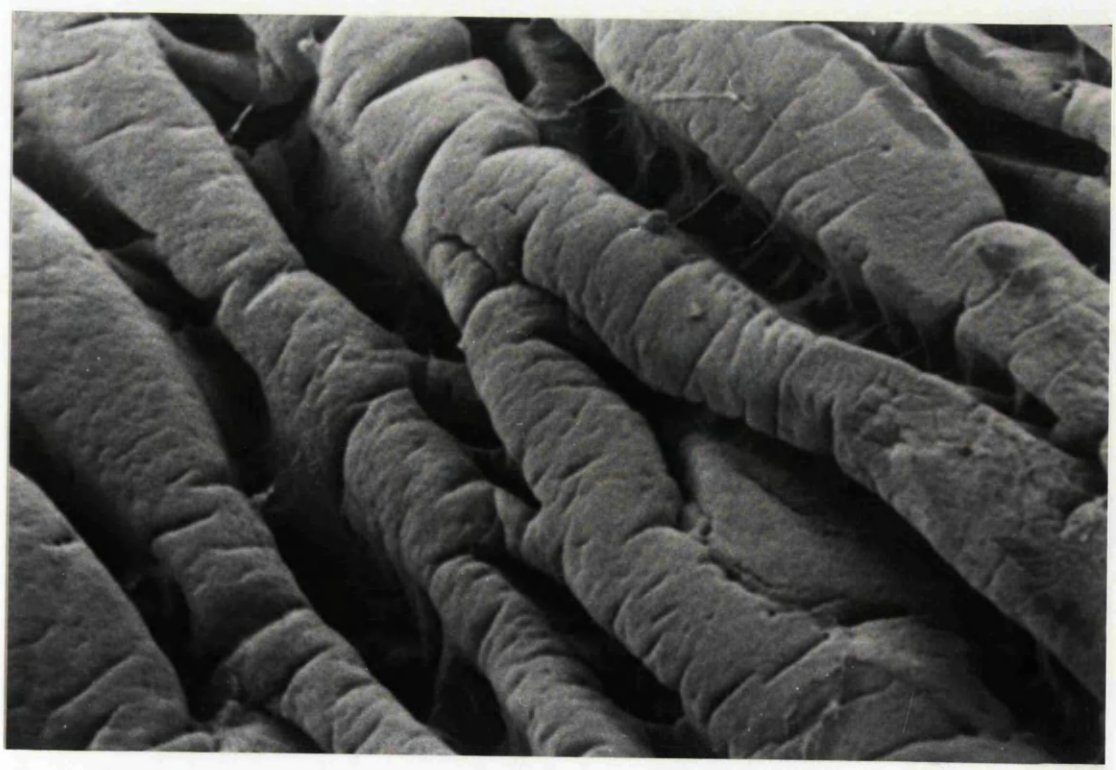


Fig. 4.36 Nasopharynx (transitional zone).
Nonciliated microvillous cells with slightly bulging apical surfaces and polygonal, or occasionally rounded, cell borders predominate in this region.
SEM x 5,600.

Fig. 4.37 Nasopharynx (transitional zone).
Nonciliated microvillous cells with slightly bulging apical surfaces. Note single ciliated cell (arrow) and mature mucus-producing cell with apical secretory granules (*).
SEM x 5,600

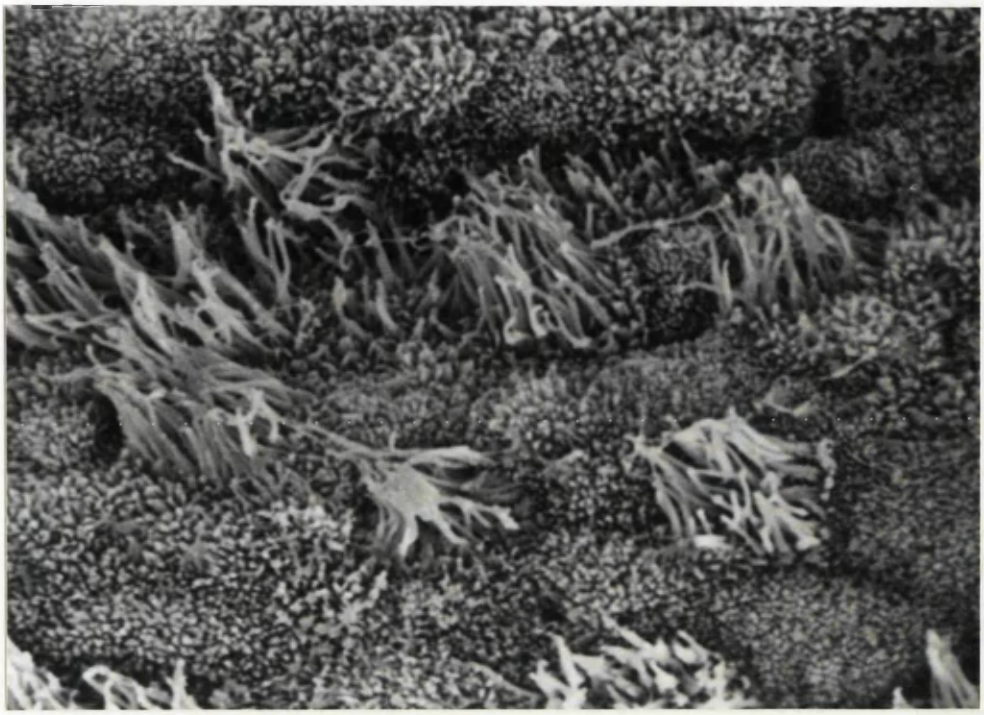


Fig. 4.38 Nasopharynx (transitional zone).
Further caudally within this region nonciliated microvillous cells predominate. Mature ciliated cells are rare here, although scattered regenerating ciliated cells (arrow) are not uncommon.
SEM x 2,800.

Fig. 4.39 Nasopharynx (transitional zone).
Mucous columns are seen to be extruded from an epithelium composed primarily of nonciliated microvillous cells.
SEM x 2,800.

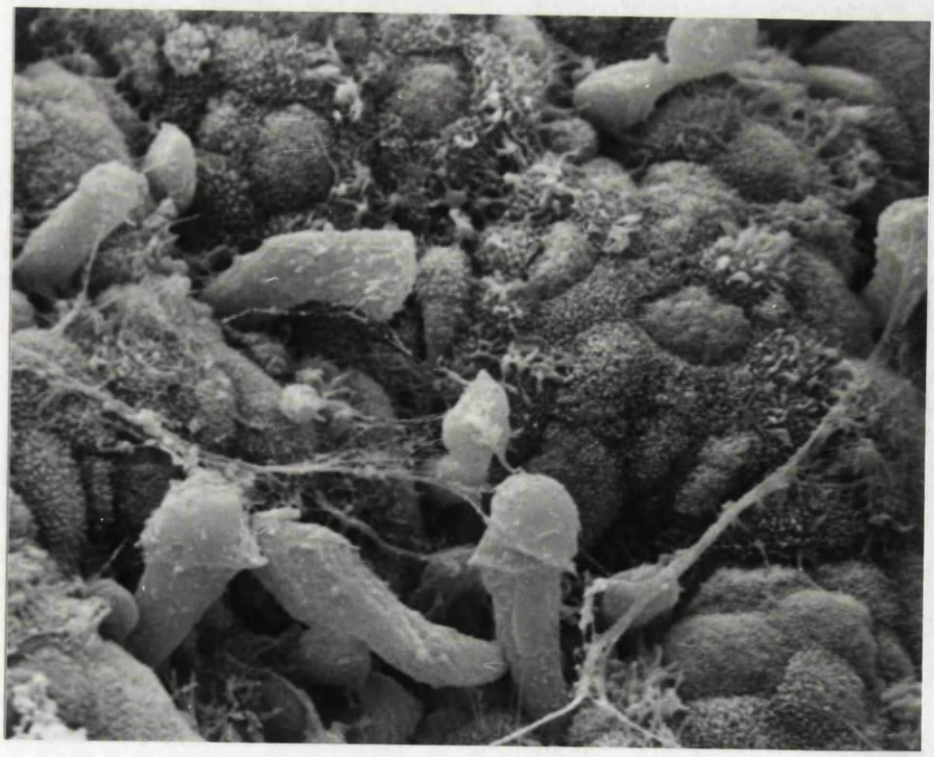


Fig. 4.40 Nasopharynx (transitional zone).
At higher magnifications the nonciliated microvillous cells are seen to present microvilli of varying lengths (1 and 2). A number of cells are identifiable as regenerating ciliated cells (*). Note the thick column of mucus being extruded from the apical surface of a mucus-producing cell (arrow).
SEM x 5,600.

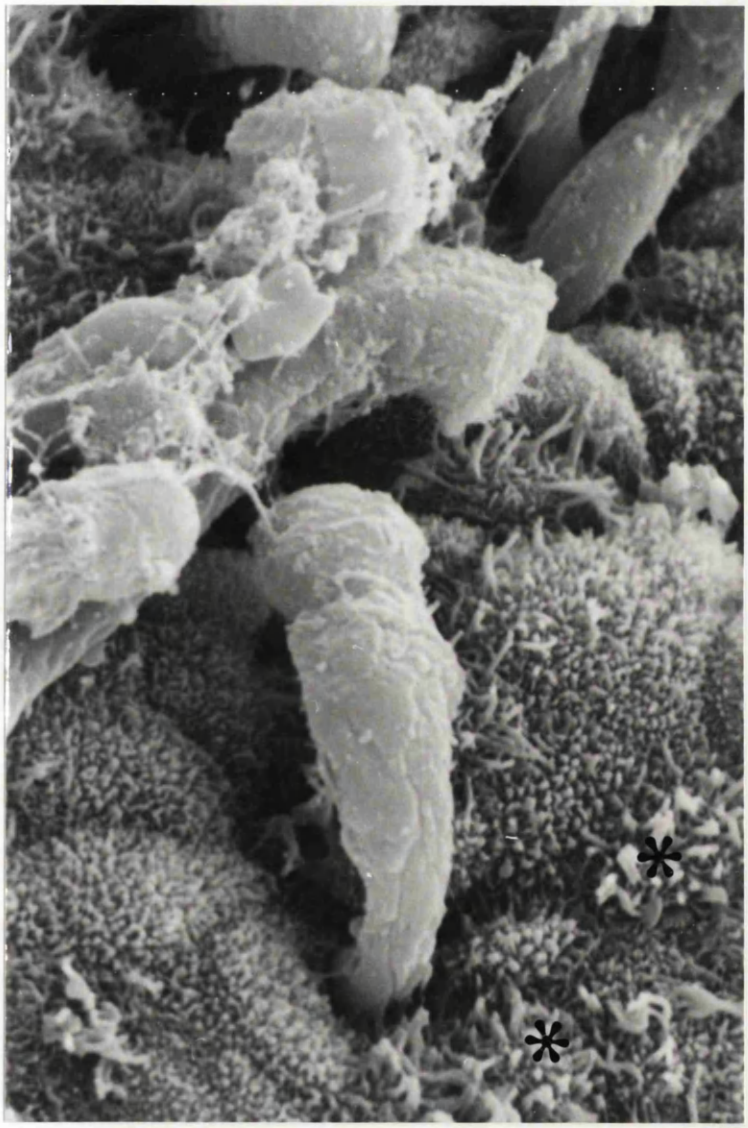
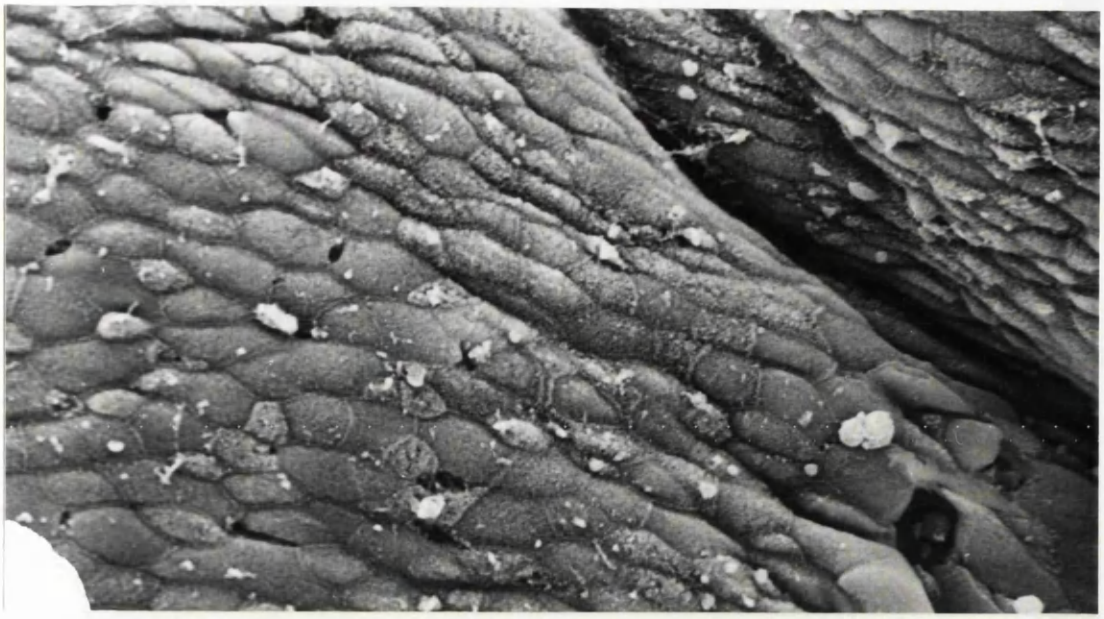


Fig. 4.41 Nasopharynx (transitional zone).
The caudal region of transitional zone is seen to be devoid of ciliated cells. Nonciliated microvillous cells are polygonal in outline and arranged in a 'paving-stone" manner.
SEM x 1,440.

Fig. 4.42 Nasopharynx (transitional zone).
Note that, in this caudal region of the transitional zone, microvilli on any individual cell appear uniform, in length although their length may differ from cell to cell.
SEM x 5,600.



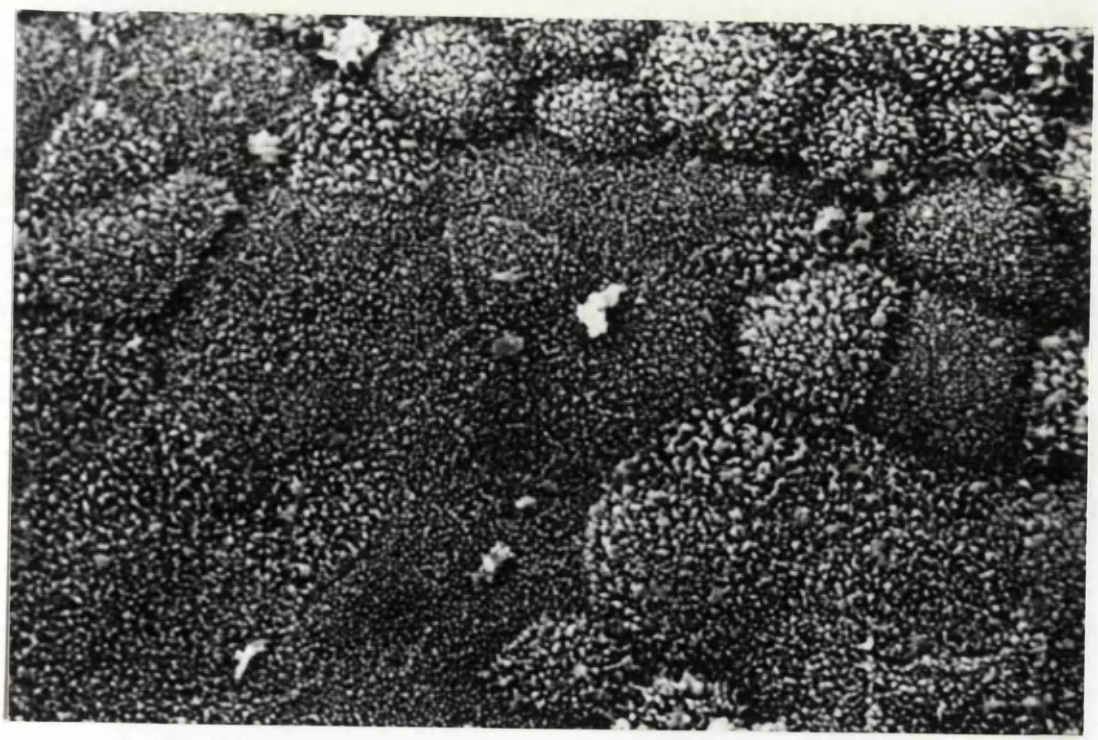


Fig. 4.43 Nasopharynx (transitional zone).
In the caudal region of this zone, nonciliated microvillous cells present distinct borders and evenly distributed surface microvilli. A cell can be seen lifting off from the epithelial surface. SEM x 5,600.

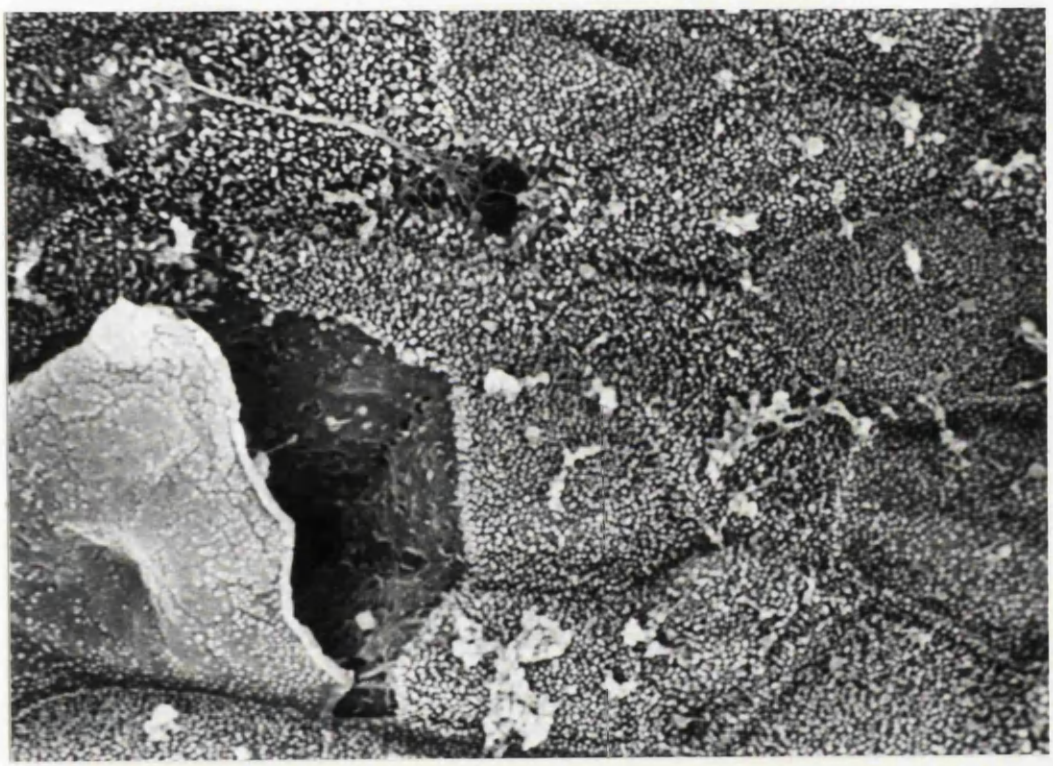


Fig. 4.44 Nasopharynx (caudal region).
Low, dome-shaped area characteristic of this region. Cells covering the dome appear smooth, whilst those on the periphery are seen to be wrinkled (*).
SEM x 360.

Fig. 4.45 Nasopharynx (transitional zone).
Exaggerated intercellular spaces can be seen between the covering surface cells of one of the dome-shaped areas in this region.
SEM x 1,440.

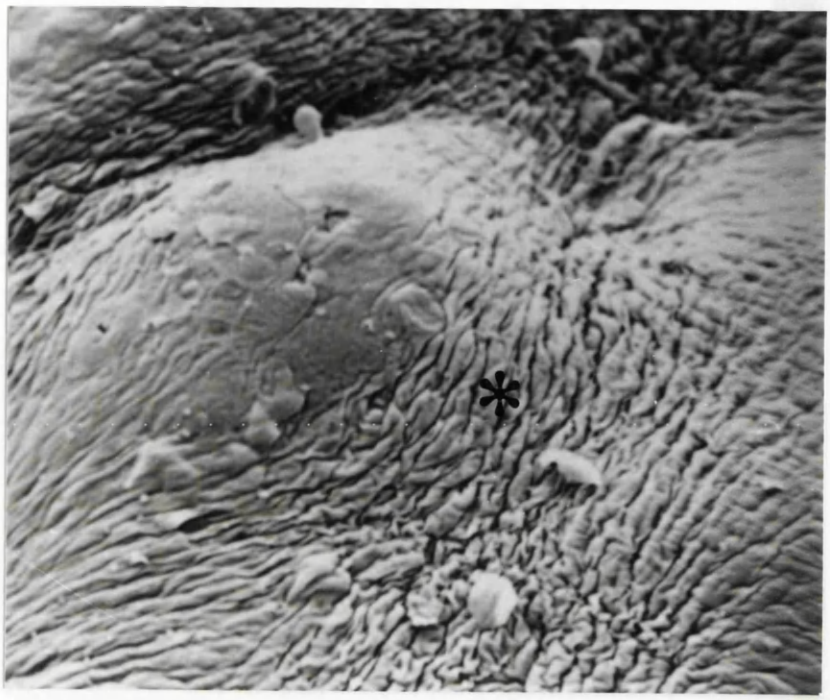


Fig. 4.46 Epiglottis.
The laryngeal surface is lined by squamous cells. Some cells are seen detaching from the surface. A submucosal gland orifice (*) can be seen.
SEM x 720.

Fig. 4.47 Epiglottis.
Squamous cells show surface microplicae. Note the presence of a taste bud (arrow).
SEM x 5,600.

Inset: Taste bud.
Numerous sensory hairs seen protruding through the pore; a few secretory granules can also be seen.
SEM x 5,600.

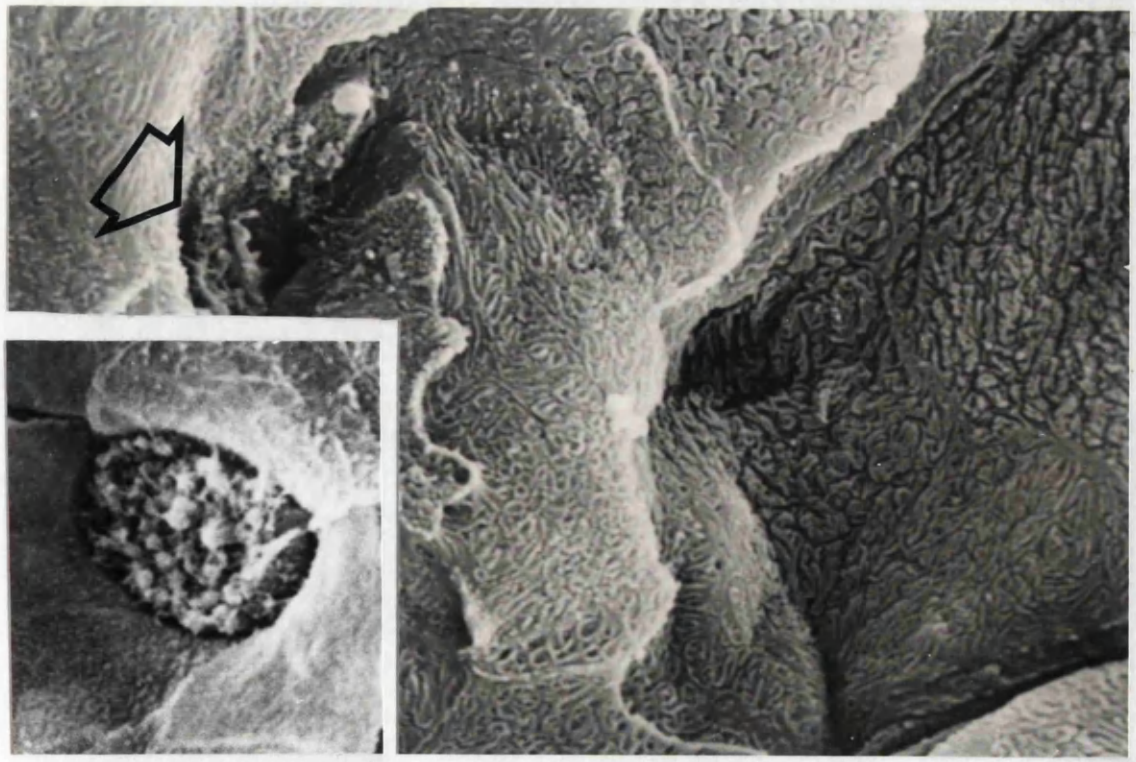
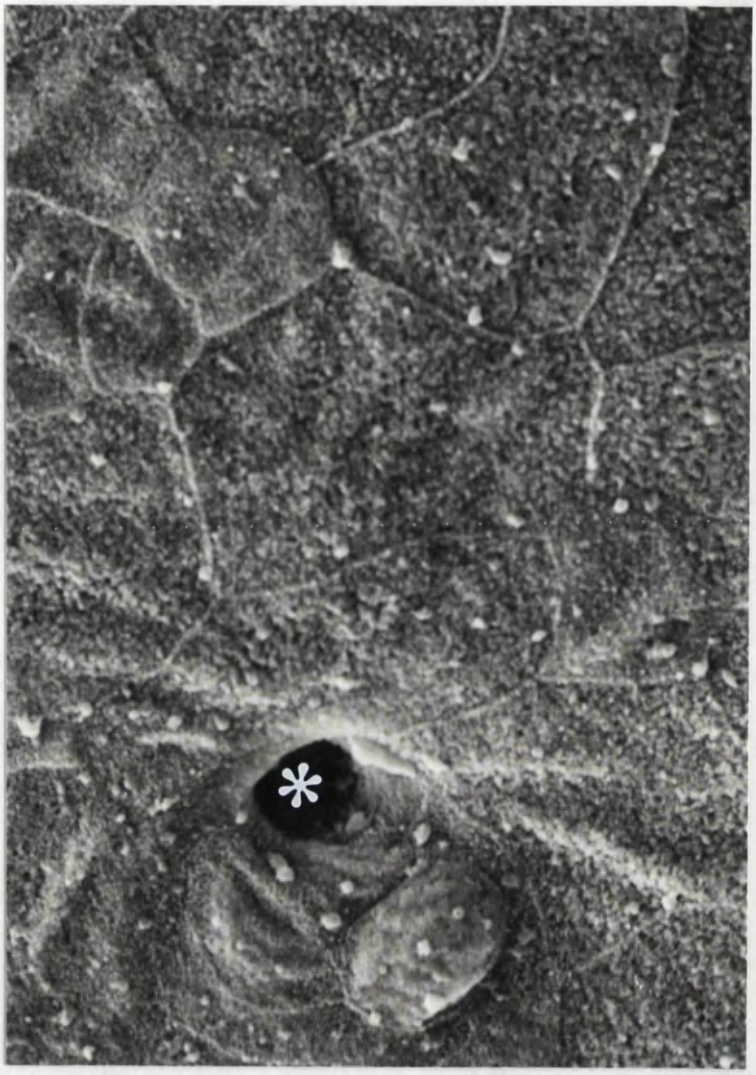


Fig. 4.48 Vocal fold.
The epithelium is lined by flattened nonciliated microvillous cells with distinct cell borders.
A submucosal gland orifice (*) can also be seen.
SEM x 1250.

Fig. 4.49 Vocal fold.
In a few individuals regenerating ciliated cells (arrow) were also seen.
SEM x 2,800.



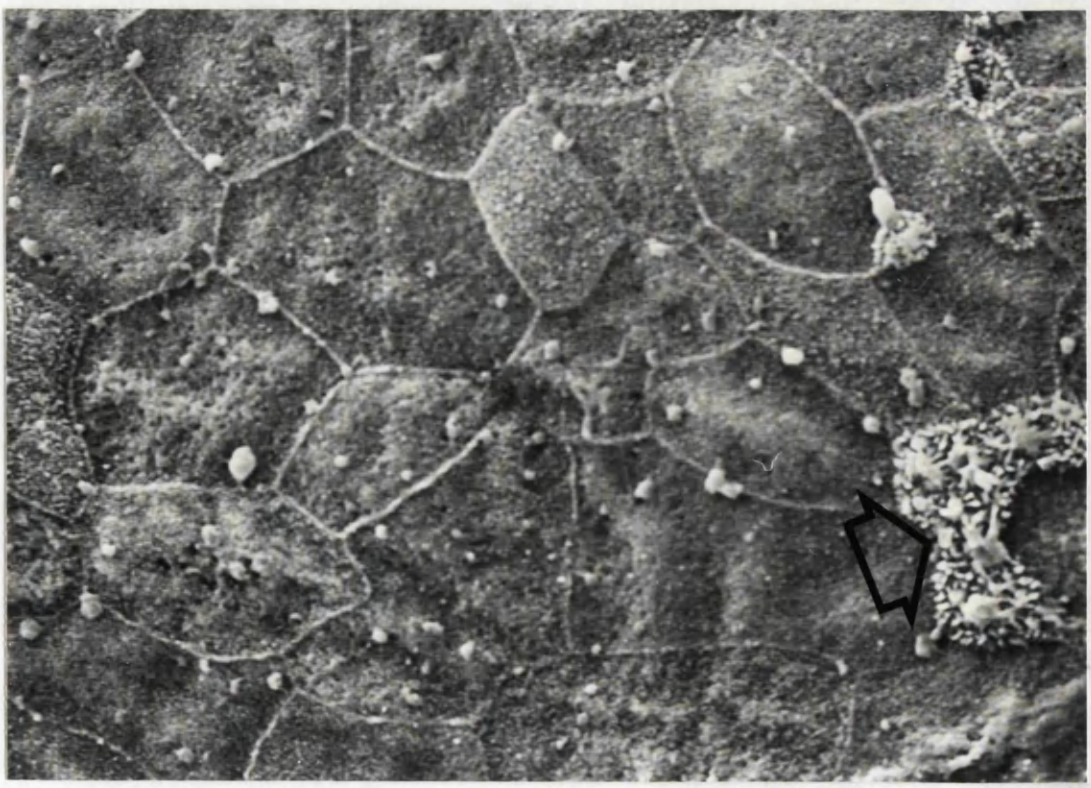



Fig. 4.50 Vocal fold.
Note the sharp border between nonciliated and ciliated epithelia. A few cells have wrinkled apical cell surfaces and seem to be detaching from the layer beneath (arrow). Note also the opening of the submucosal gland which appears to be covered by a sheet of mucus.
SEM x 2,800.

Fig. 4.51 Vocal fold (ciliated region).
Within the ciliated region, ciliated cells (C), nonciliated microvillous cells (N), and regenerating ciliated cells (R) can be seen. Note the presence of surface microvilli between the cilia of the regenerating ciliated cell.
SEM x 5,600.

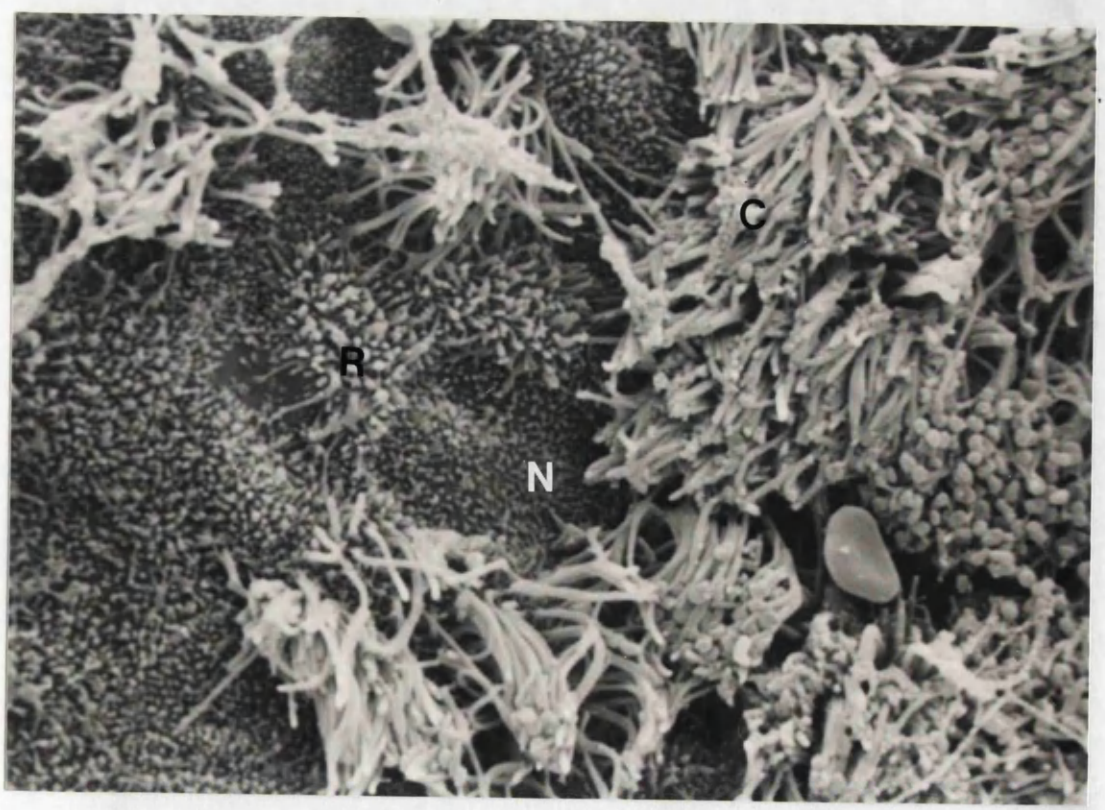
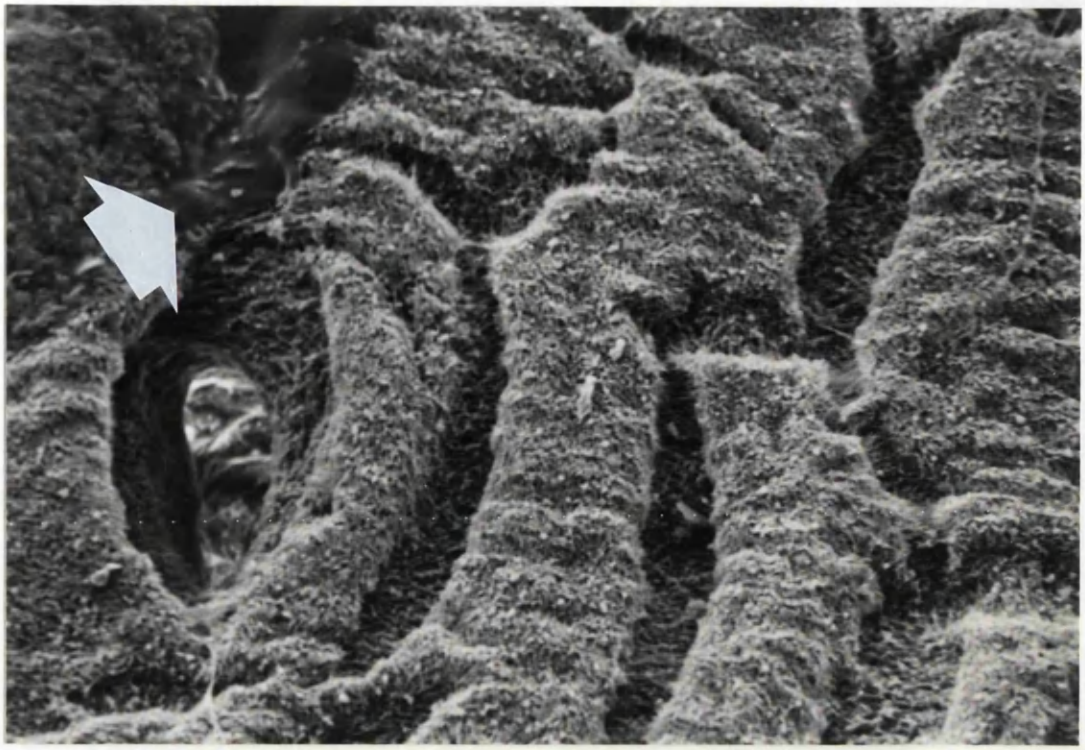

C
R
N

Fig. 4.52 Infraglottic cavity.
The folded nature of the lining mucosa is clearly visible. A thin sheet of mucus (arrow) can be seen covering part of the surface.
SEM x 360.

Fig 4.53 Infraglottic cavity.
At higher magnifications the heavily ciliated nature of the lining epithelium is observed. Note the shallow gutters (*).
SEM x 720.



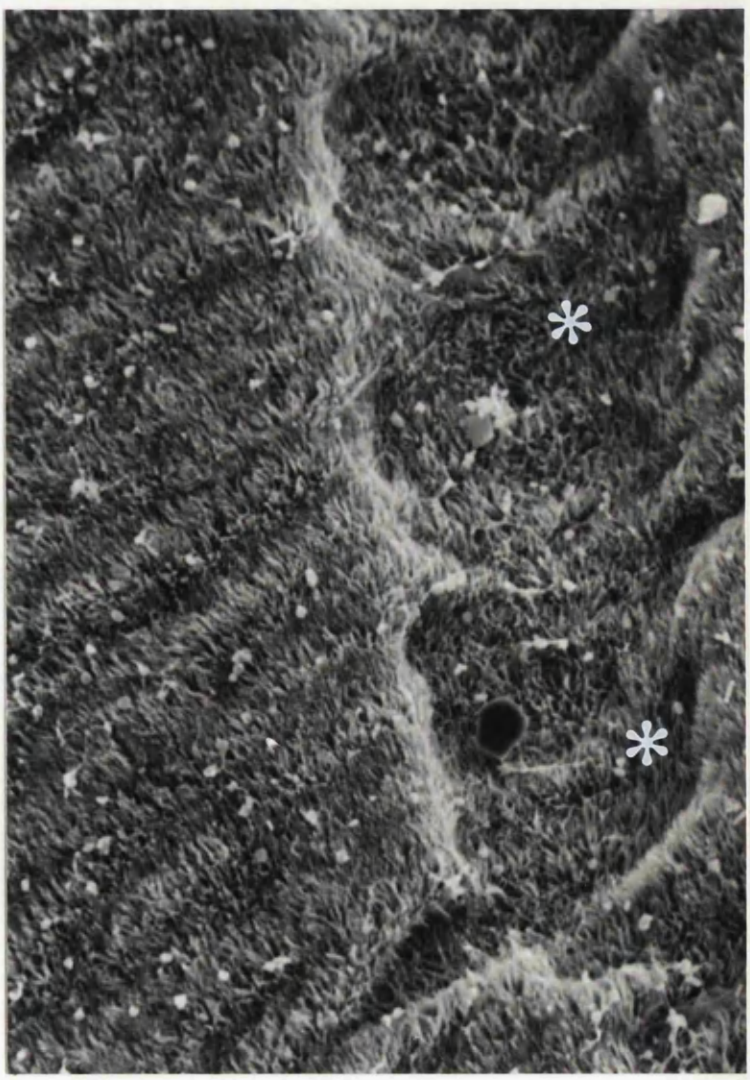


Fig. 4.54 Infraglottic cavity.
In two individuals, the folded mucosa lining the cranial region of the infraglottic cavity was lined by a squamous epithelium.
SEM x 180.

Fig. 4.55 Trachea (cranial dorsal surface).
The mucosa shows high folds and deep intervening gutters.
SEM x 360.

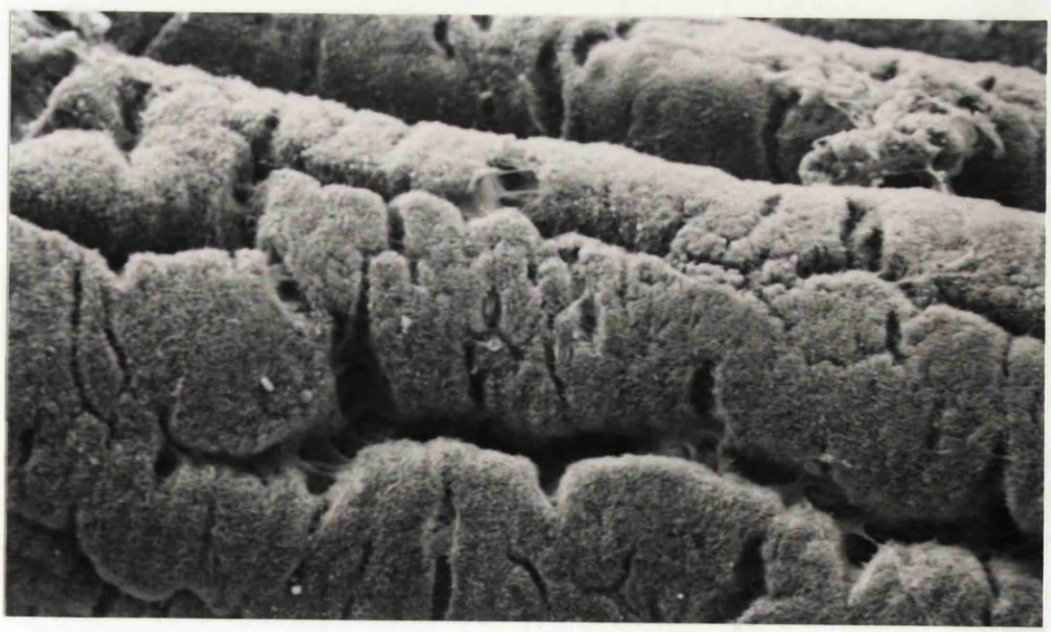
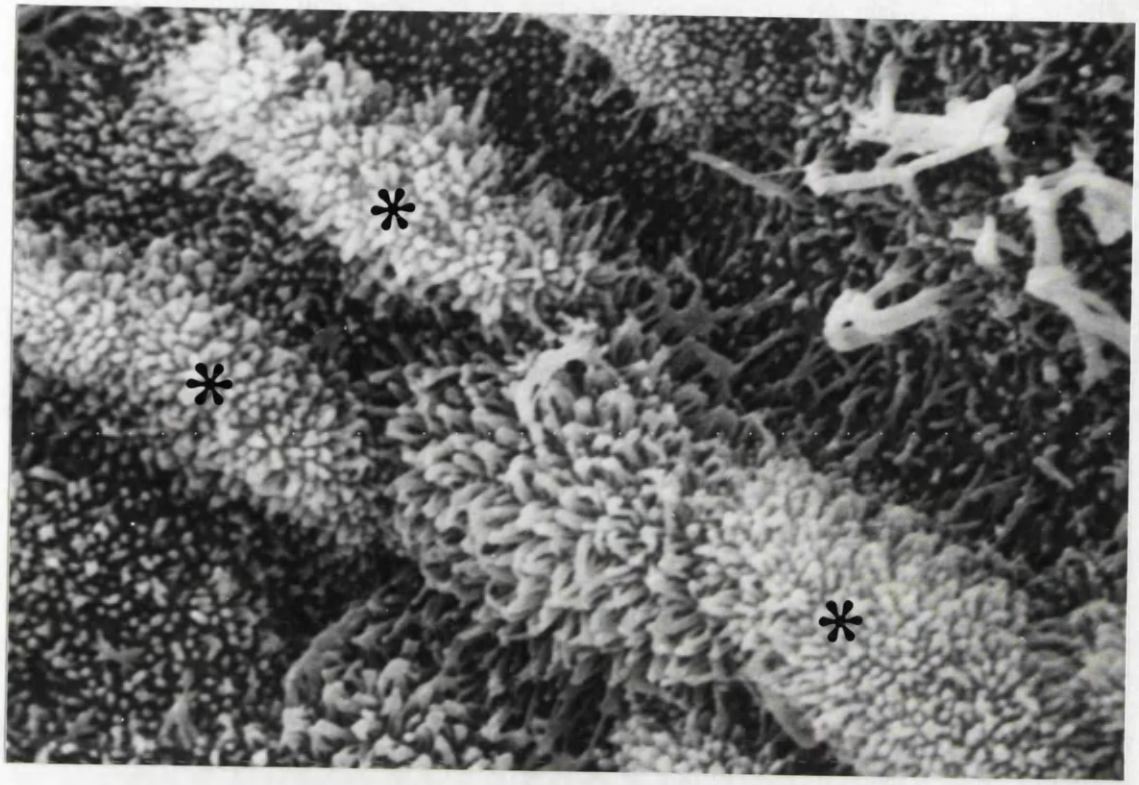


Fig. 4.56 Trachea (cranial dorsal surface).
Note the complete ciliation of the lining epithelium. Mucus-producing cell (arrow).
SEM x 5,600.

Fig. 4.57 Trachea (cranial dorsal surface).
Nonciliated microvillous cells (*) and regenerating ciliated cells (arrow) Note that the density and length of the microvilli differ from cell to cell.
SEM x 11,250.

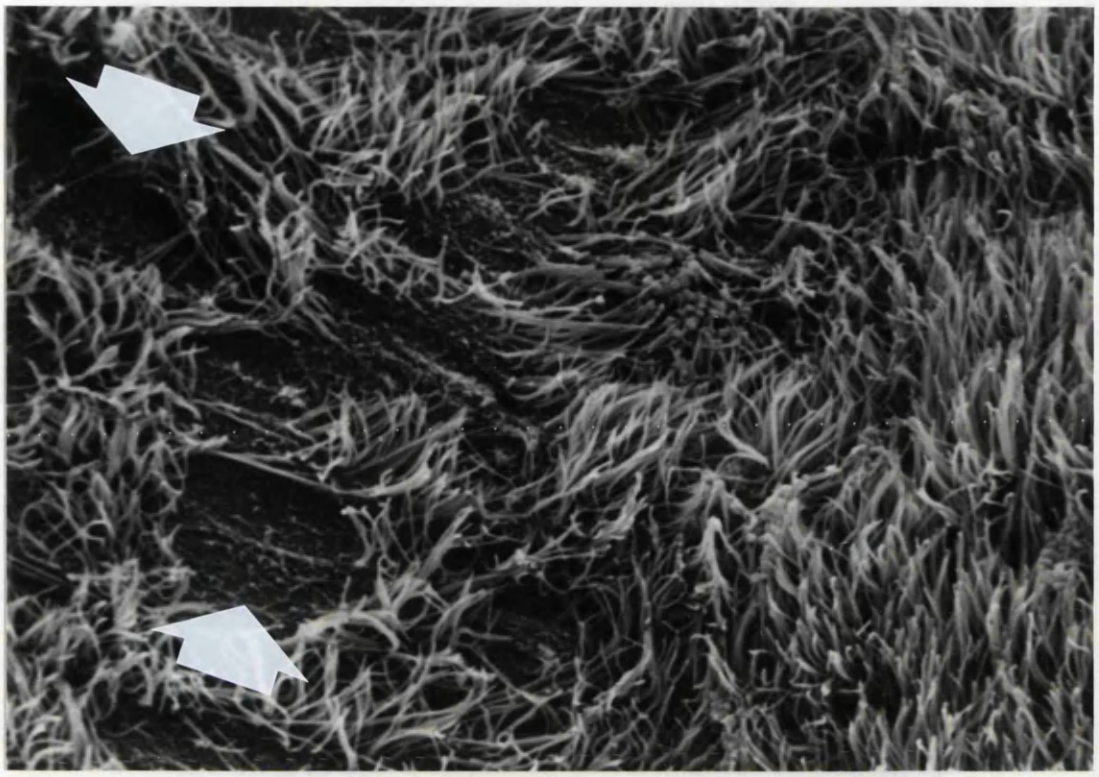
Fig. 4.58 Trachea, (cranial dorsal surface).
Note that the exposed part of the gutter (G) has relatively fewer ciliated cells . Submucosal gland discharging mucus at the orifice (*). Nonciliated microvillous cells are seen around the gland orifice.
SEM x 360.




G
G

Fig. 4.59 Trachea (cranial dorsal surface).
Nonciliated microvillous cells with flat to shallow apical surfaces (arrows). Microvilli are very sparsely distributed over the cell surface.
SEM x 2,800.

Fig. 4.60 Trachea (cranial ventral surface).
Shallow gutters give the mucosa a characteristic pattern.
SEM x 180.



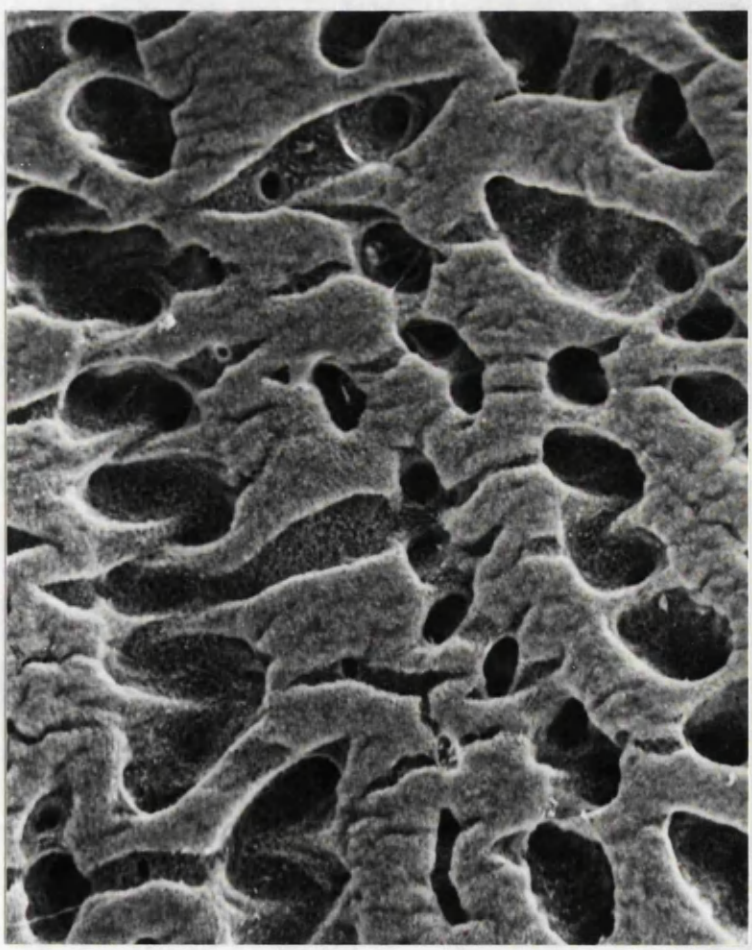
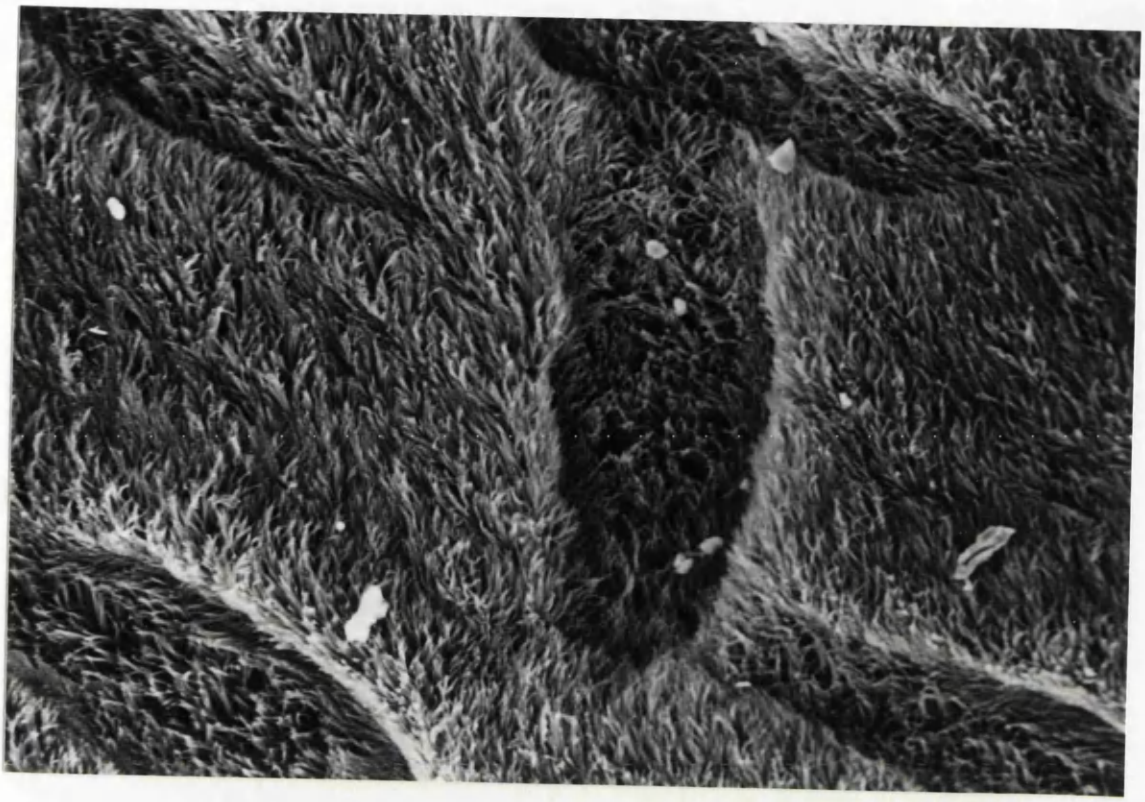


Fig. 4.61 Trachea (cranial ventral surface).
Higher magnifications show the heavily ciliated nature of the lining epithelium.
SEM x 1,440.

Fig. 4.62 Extrapulmonary bronchus.
Nonciliated microvillous cells with rounded apical cell surfaces. Note a film of mucus on the apical surface (arrow).
SEM x 2,800.



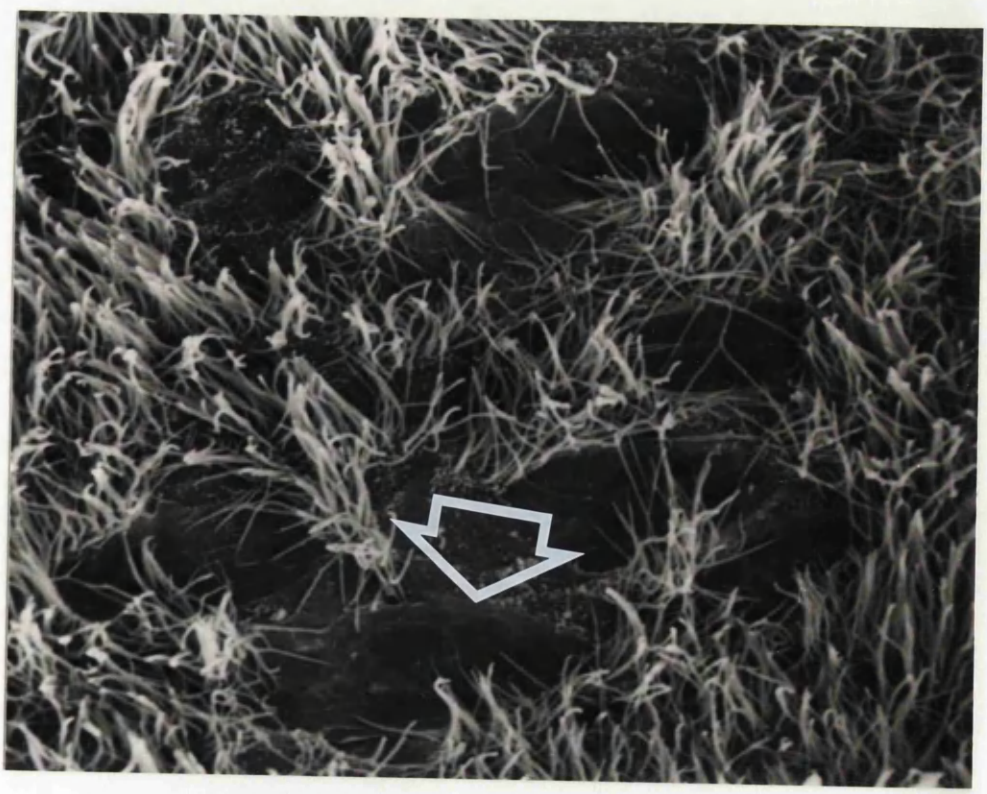
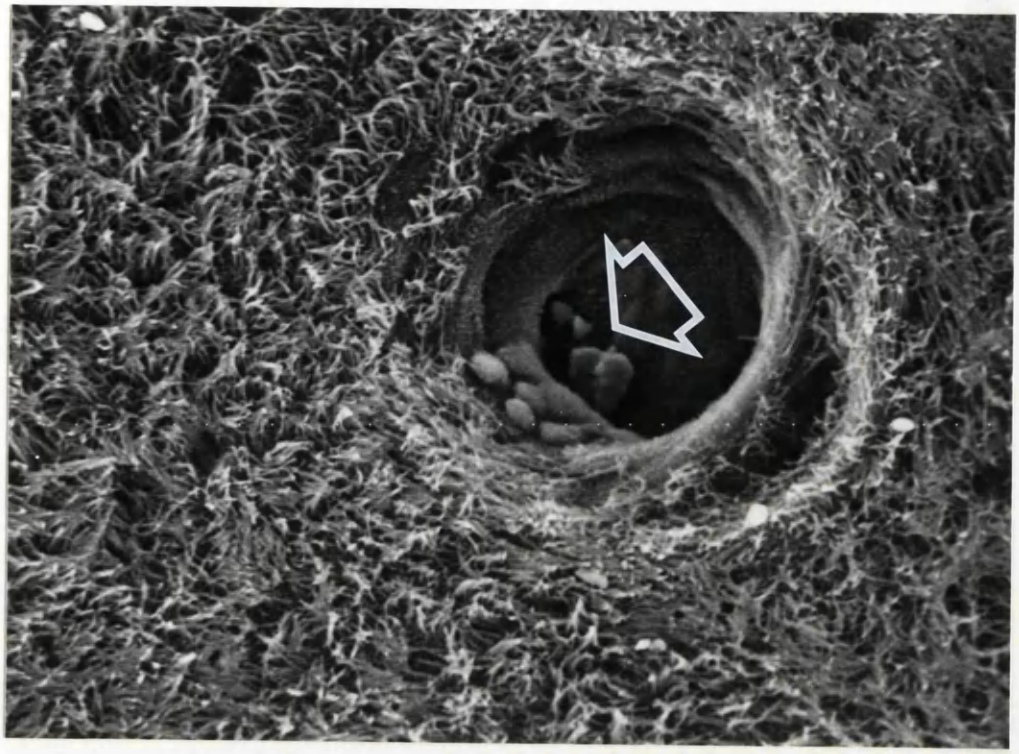


Fig. 4.63 Caudal lobar bronchus.
Submucosal gland orifice surrounded by nonciliated microvillous cells. Note mucus droplets (arrow).
SEM x 1,440.

Fig. 4.64 Caudal lobar bronchus.
Straight and slender cilia with curved tips (arrows).
SEM x 2,800.



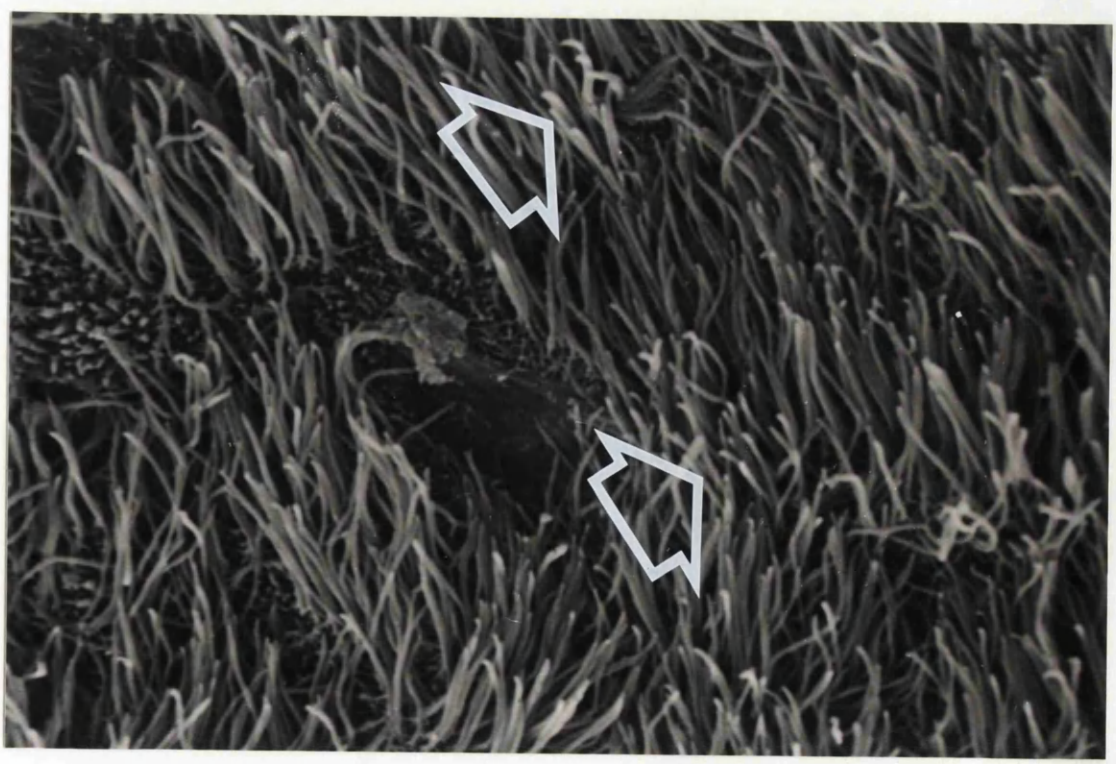
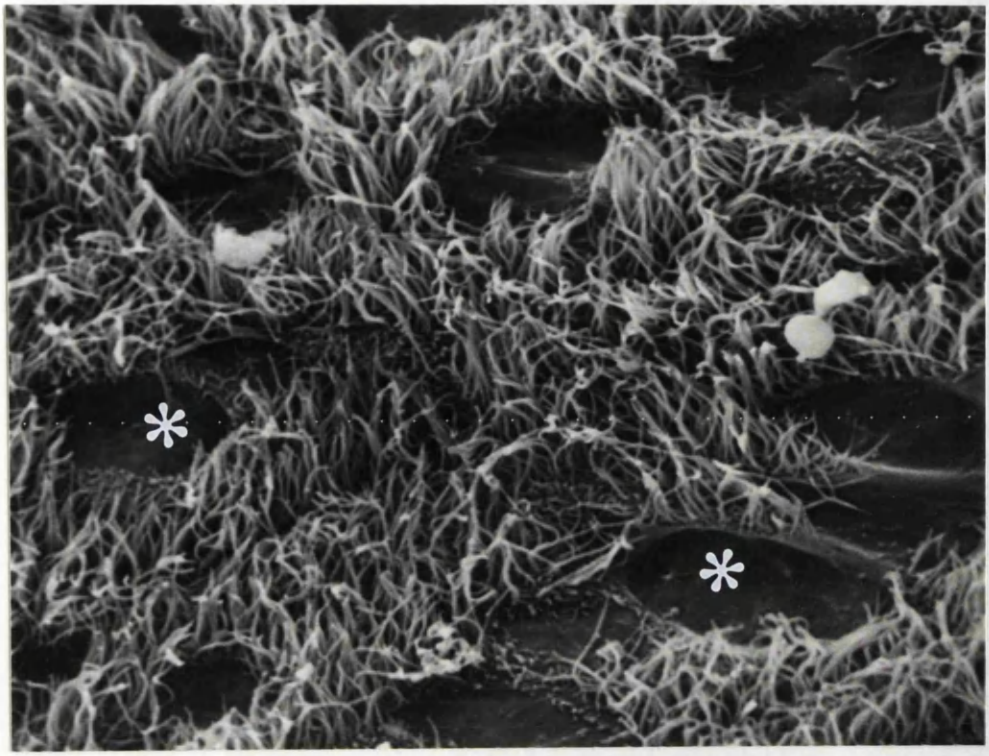


Fig. 4.65 Small bronchus.
Cilia appear matted. Note the depressed apical surfaces of the mucus-producing cells (*).
SEM x 2,800.

Fig. 4.66 Bronchiole.
Relatively short, straight and slender cilia. Note the nonciliated bronchiolar epithelial cells (*).
SEM x 2,800.



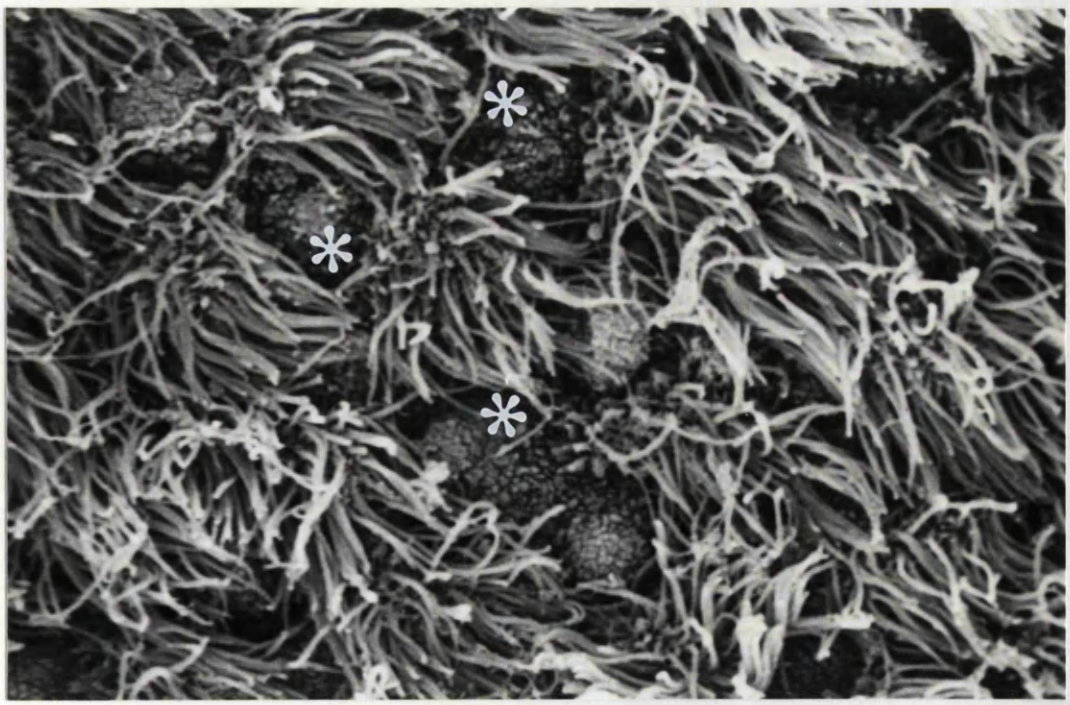
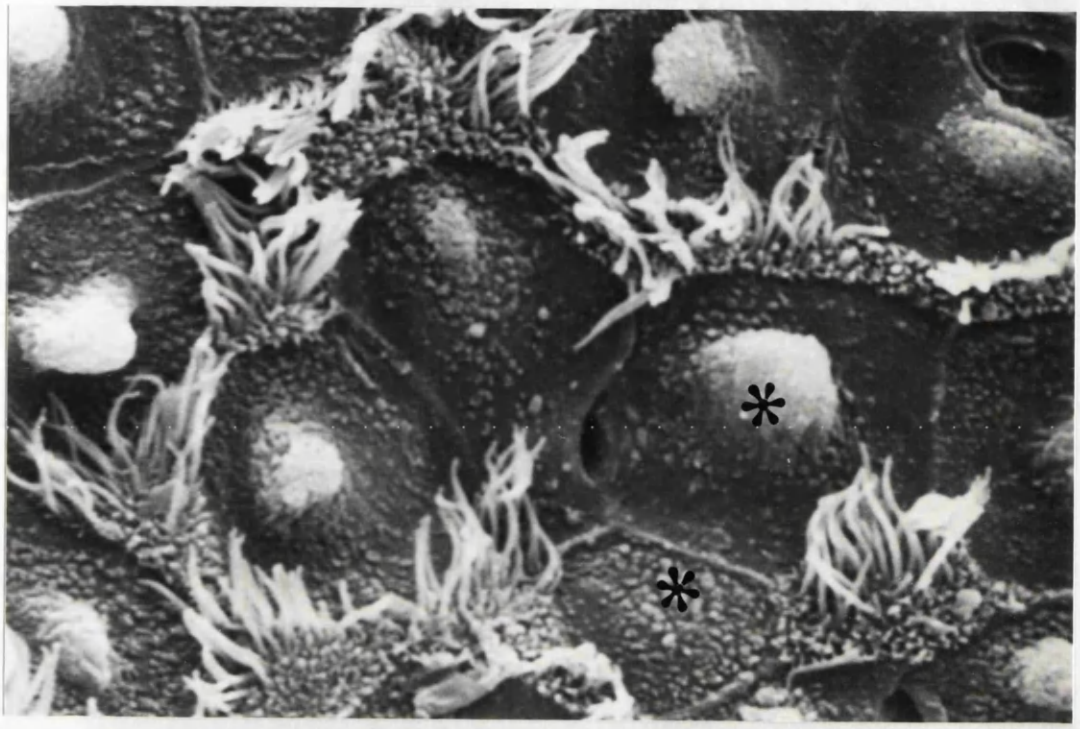


Fig 4.67 Bronchiole.
Clara cells with raised pentagonal boundaries (*).
Note their flattened periphery and small low
central bulge.
SEM x 5,600.

Fig. 4.68 Bronchiole
Clara cells with flattened apical surfaces bearing
short, stubby microvilli (*).
SEM x 5,600.



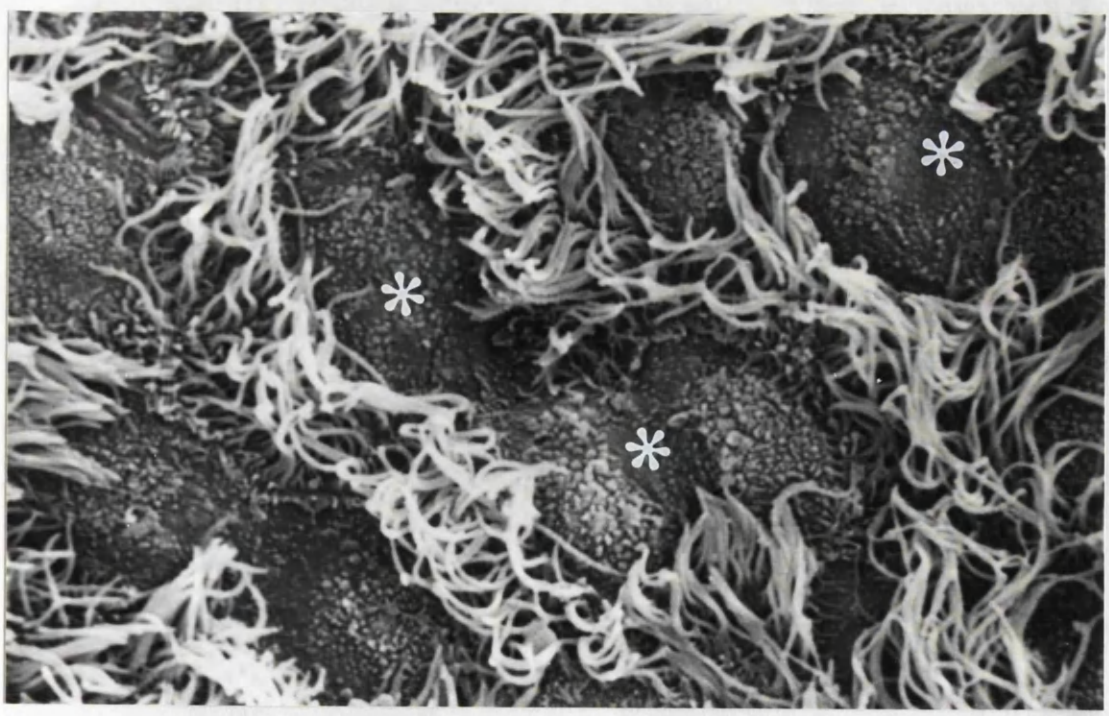


Fig 4.69 Respiratory bronchioles.
Two respiratory bronchioles (R) can be seen arising from a terminal bronchiole (T). The former are characterised by the presence of shallow alveoli (*) in their walls.
SEM x 360.

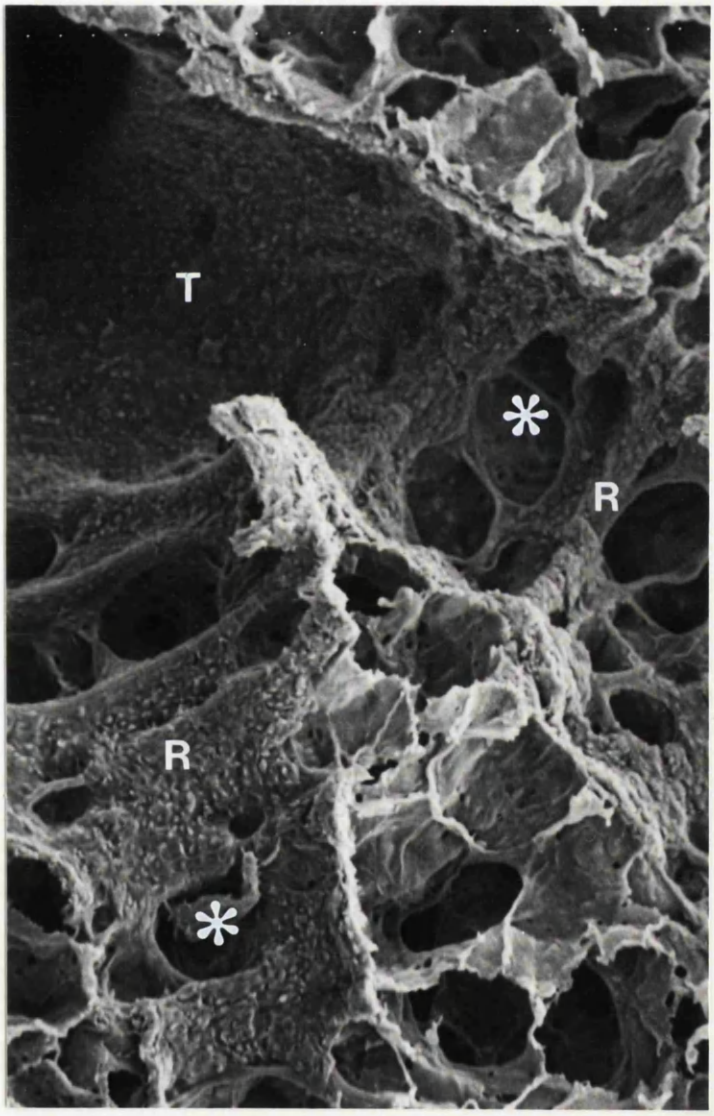
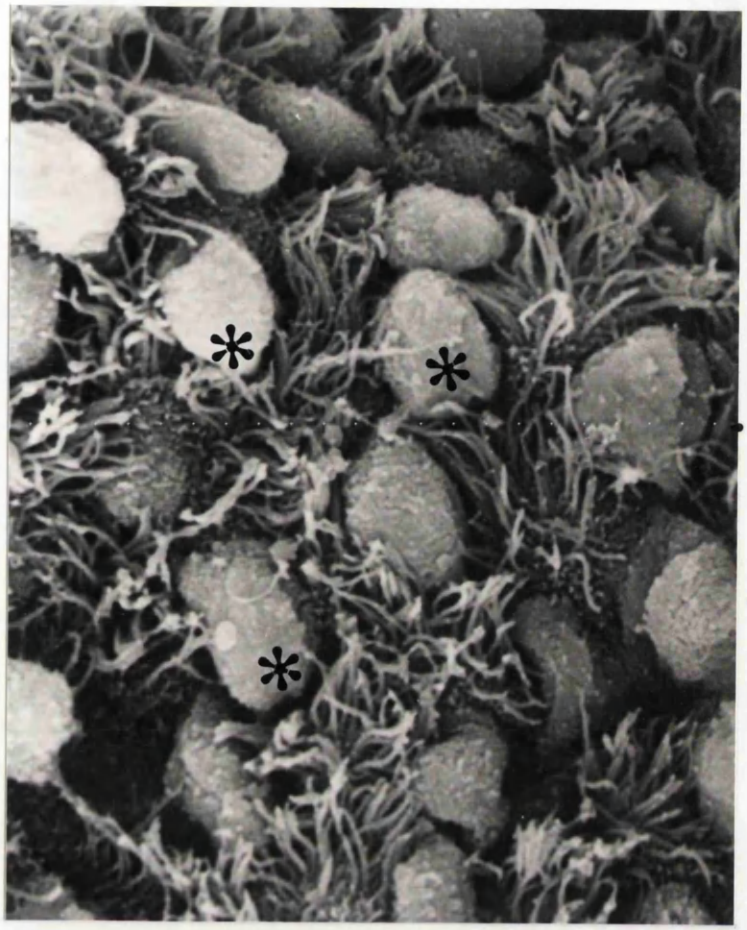

T
R
R

Fig. 4.70 Respiratory bronchiole.
Note the presence of numerous Clara cells (*), their apical surfaces raised into a dome. Ciliated cells appear squeezed in between the Clara cells.
SEM x 5,600.

Fig. 4.71 Respiratory bronchiole.
Clara cells occasionally exhibit 'withered' apical protuberance (arrow). Note clumping of cilial tips.
SEM x 5,600.



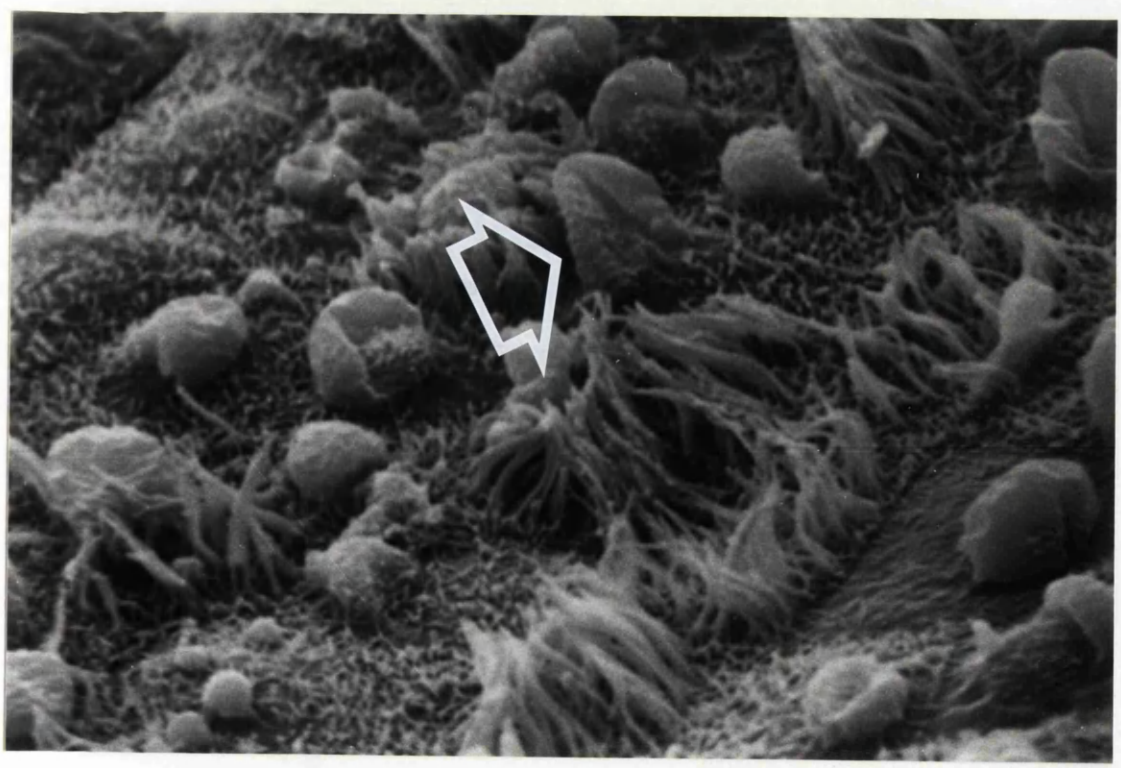
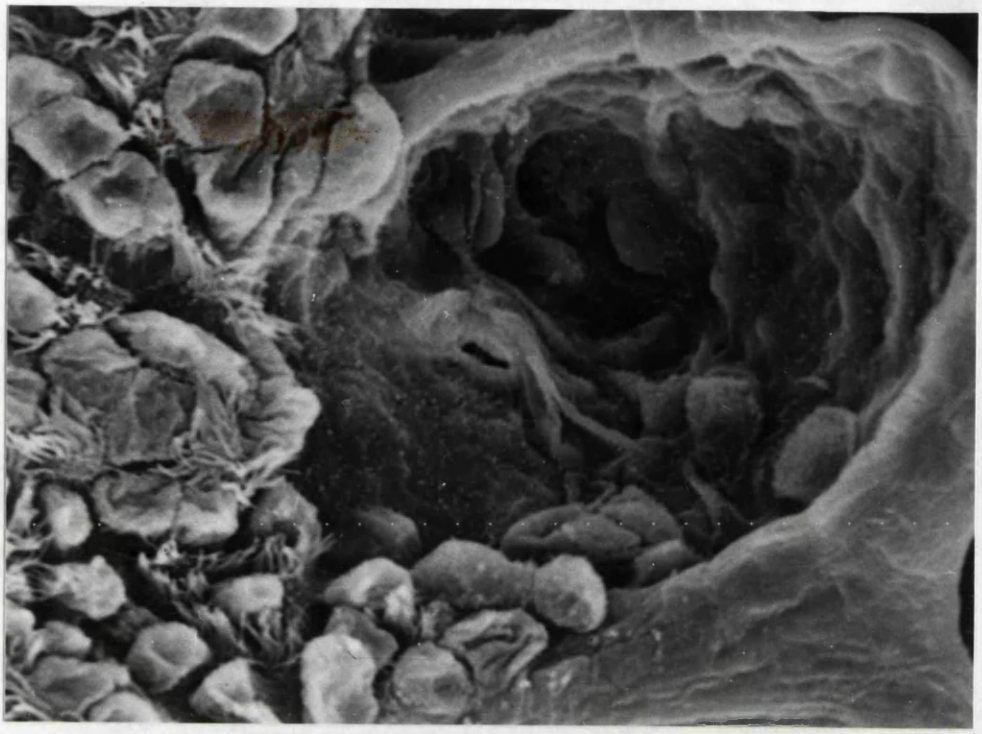


Fig. 4.72 Respiratory bronchiole / alveolar border.
The junction between the respiratory bronchiolar epithelium and the alveolar epithelium is clearly demarcated (arrows).
SEM x 2,800.

Fig. 4.73 Alveolar membrane.
Note alveolar Type I cell (*), alveolar Type II cell (open arrow) and alveolar pore of Kohn (closed arrows).
SEM x 11,250.



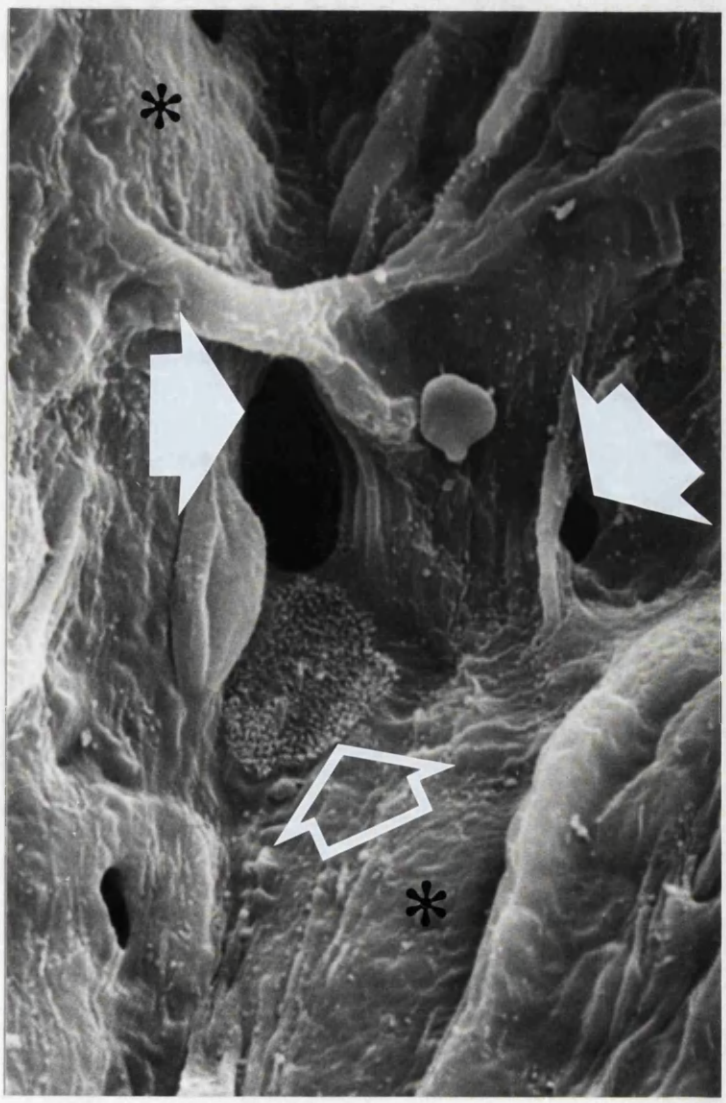
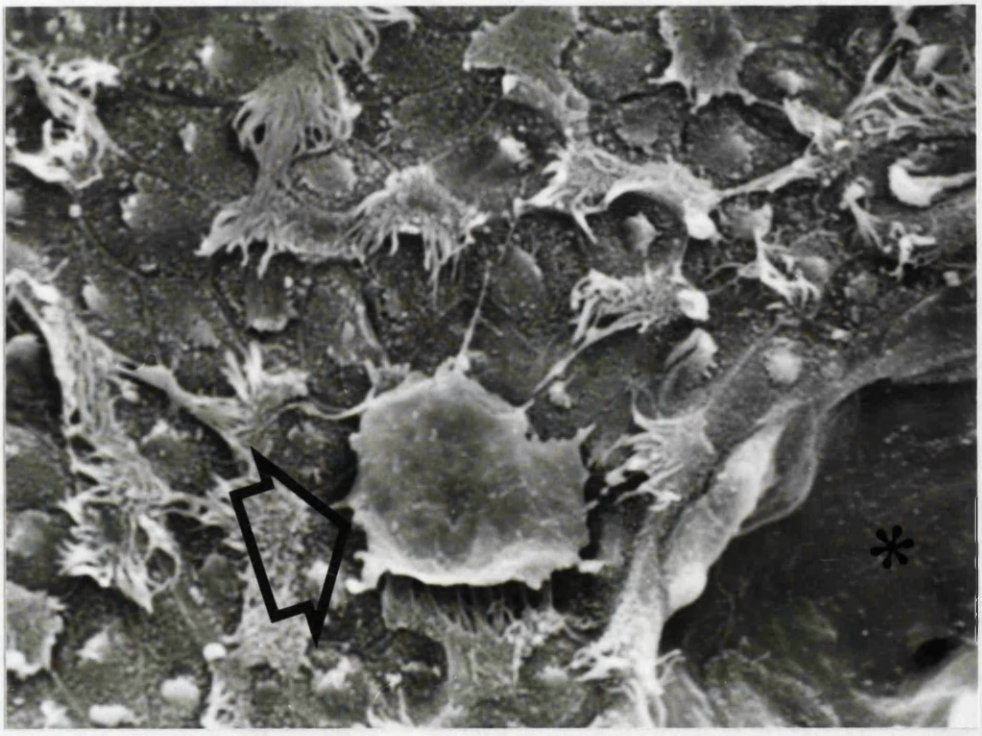


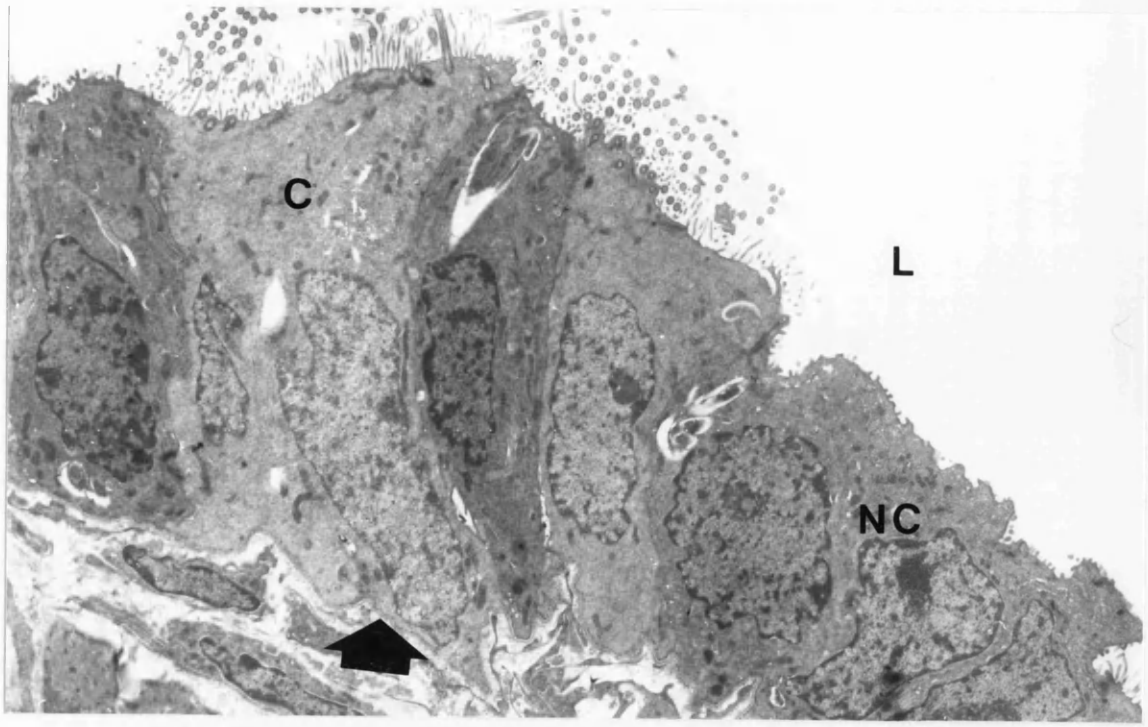
Fig. 4.74 Respiratory bronchiole.
Note the presence of an alveolar macrophage (arrow) lying on the epithelial surface.
Alveolus (*).
SEM x 2,800.



# CHAPTER 5

Fig. 5.1 Terminal bronchiole.
Simple columnar epithelium composed of ciliated cells (C) and nonciliated bronchiolar epithelial (Clara) cells (NC) resting on a prominent basal lamina (arrow).
Airway lumen (L).
TEM x 5,400.

Fig. 5.2 Terminal bronchiole.
In more distal regions of the terminal bronchiole, epithelial cells are cuboidal in shape. The nonciliated bronchiolar epithelial cell (NC) exhibits a more electron-dense cytoplasm than the adjacent ciliated cell (C). Intracytoplasmic inclusion bodies are seen in the ciliated cell (closed arrow).
Airway lumen (L); basal lamina (open arrow).
TEM x 8,000.


C
L
NC

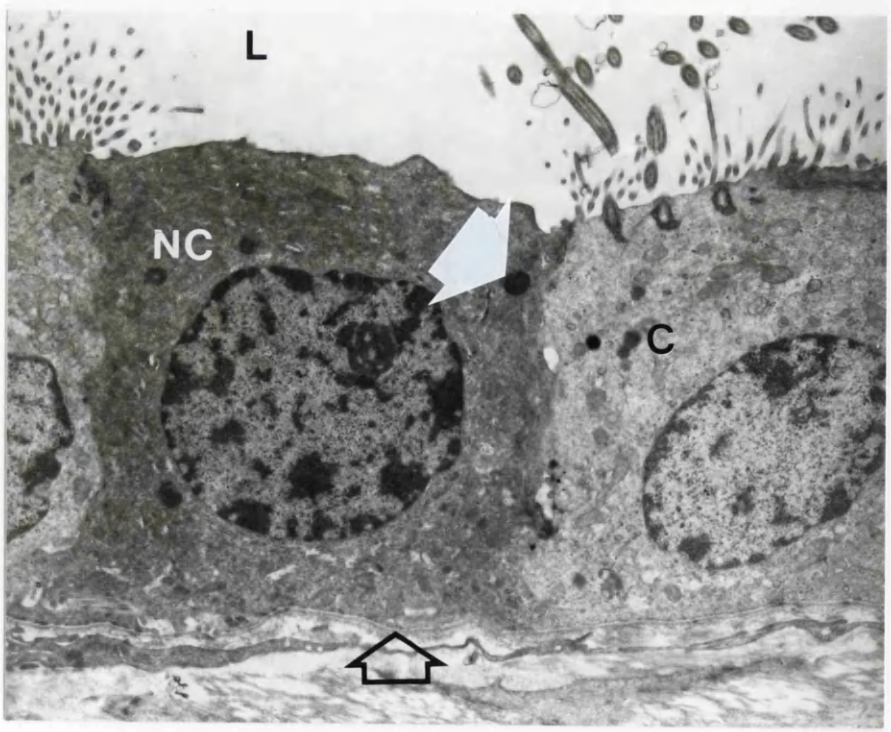

L
NC
C

Fig. 5.3 Ciliated cell.

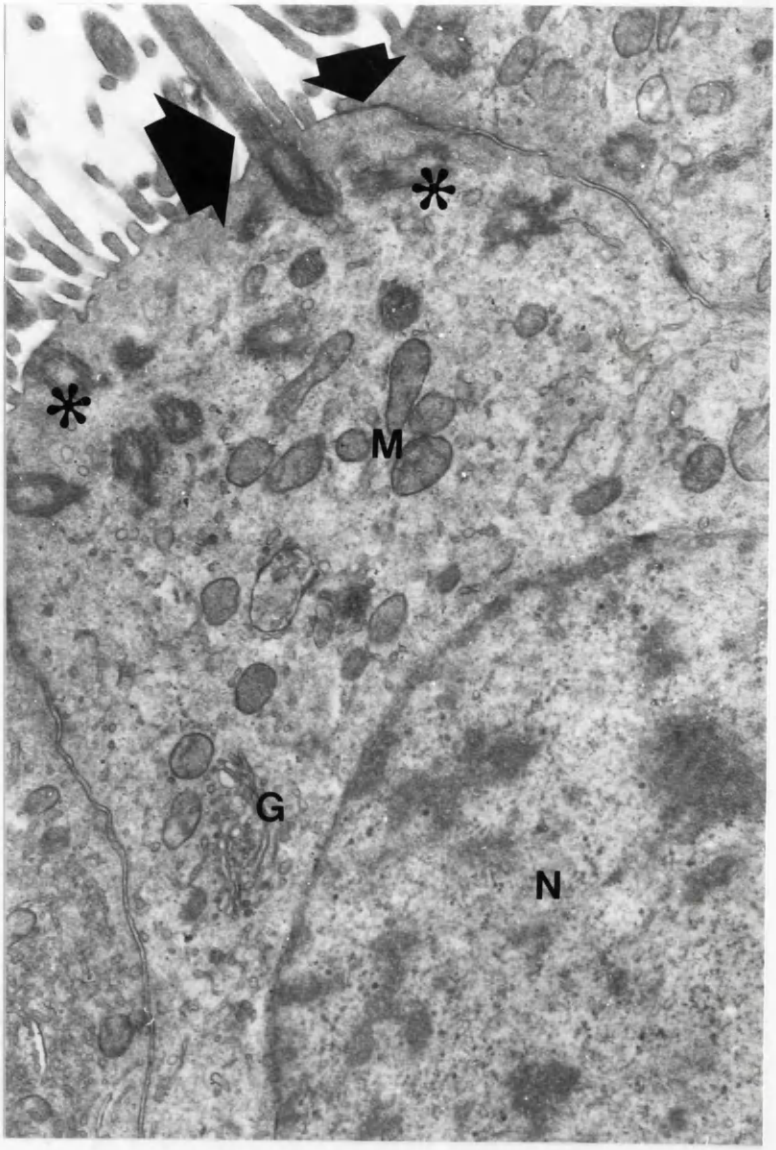
Note cilium (arrow) and basal bodies (star) and numerous microvilli on luminal surface. Part of the nucleus (N) is visible, as is a prominent Golgi body (G) and many mitochondria (M). Tight junctions (small arrow) can be seen between adjacent cells.

TEM x 20,000.

Fig.5.4 Developing ciliated cell.

Numerous microvilli on the apical surface. Basal bodies (*) are seen just below the luminal surface. A dense homogeneous granule (open arrow) and multivesicular body (closed arrow) are present.

TEM x 40,000.


M
G
N

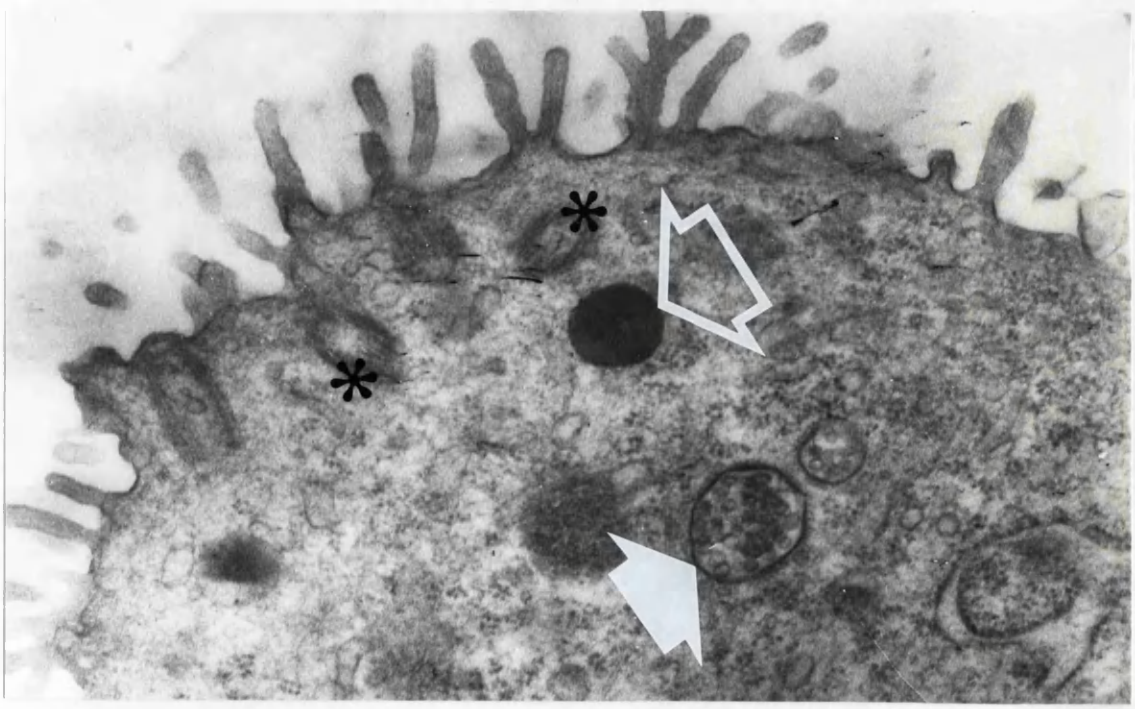
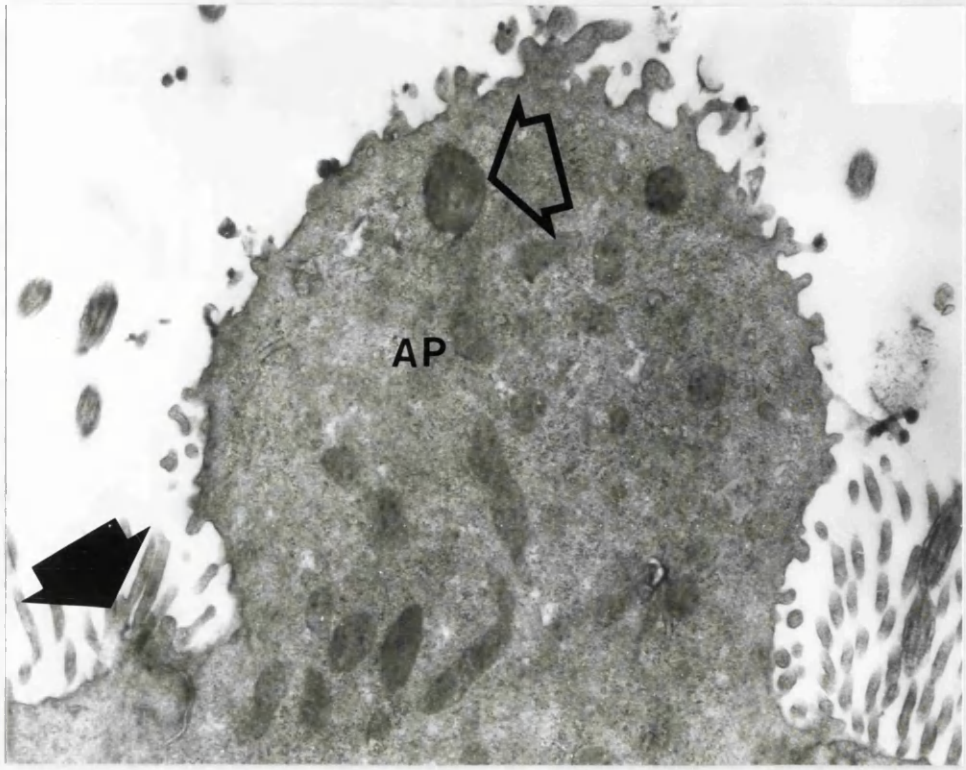


Fig. 5.5 Terminal bronchiole.
Nonciliated bronchiolar epithelial (Clara) cell.
Note the characteristic apical protuberance (AP).
Secretory granules (open arrow) are visible as is the raised intercellular boundary and surface microvilli (closed arrow).
TEM x 13,400

Fig. 5.6 Clara cell.
Numerous profiles of smooth endoplasmic reticulum in the apical region of the cell (arrow).
Note the short, stubby surface microvilli (*).
Airway lumen (L).
TEM x 28,000.


AP

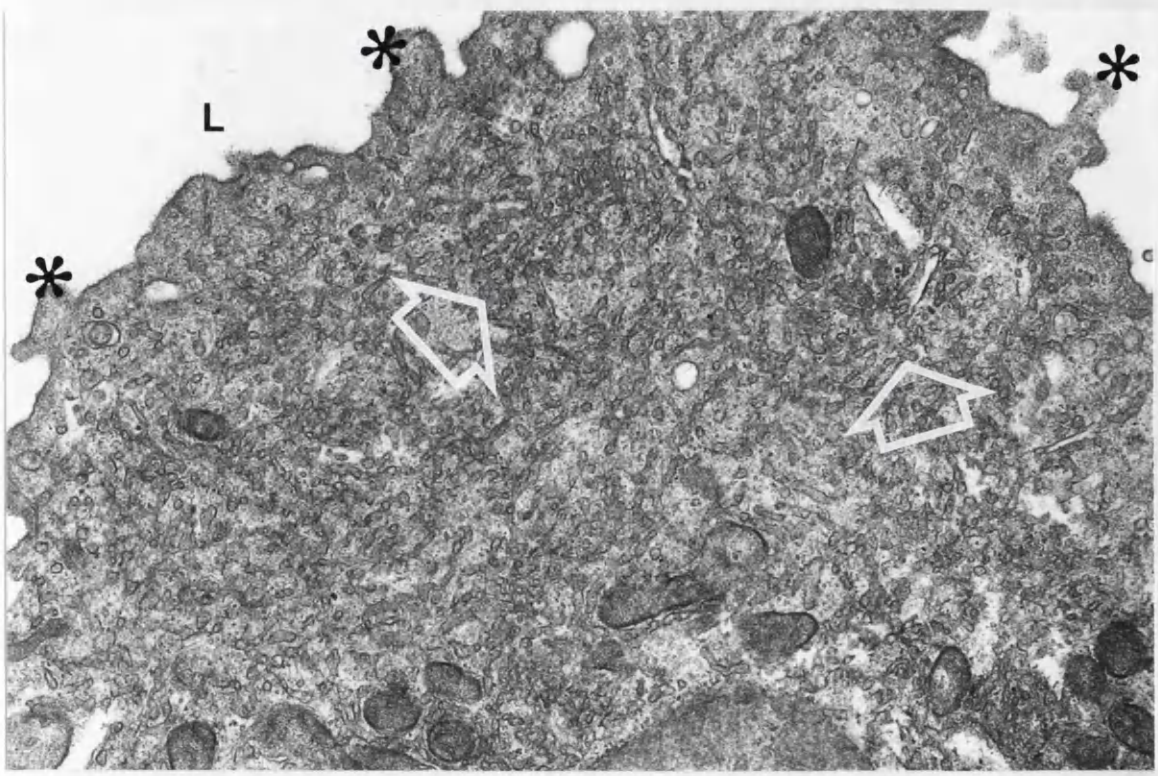
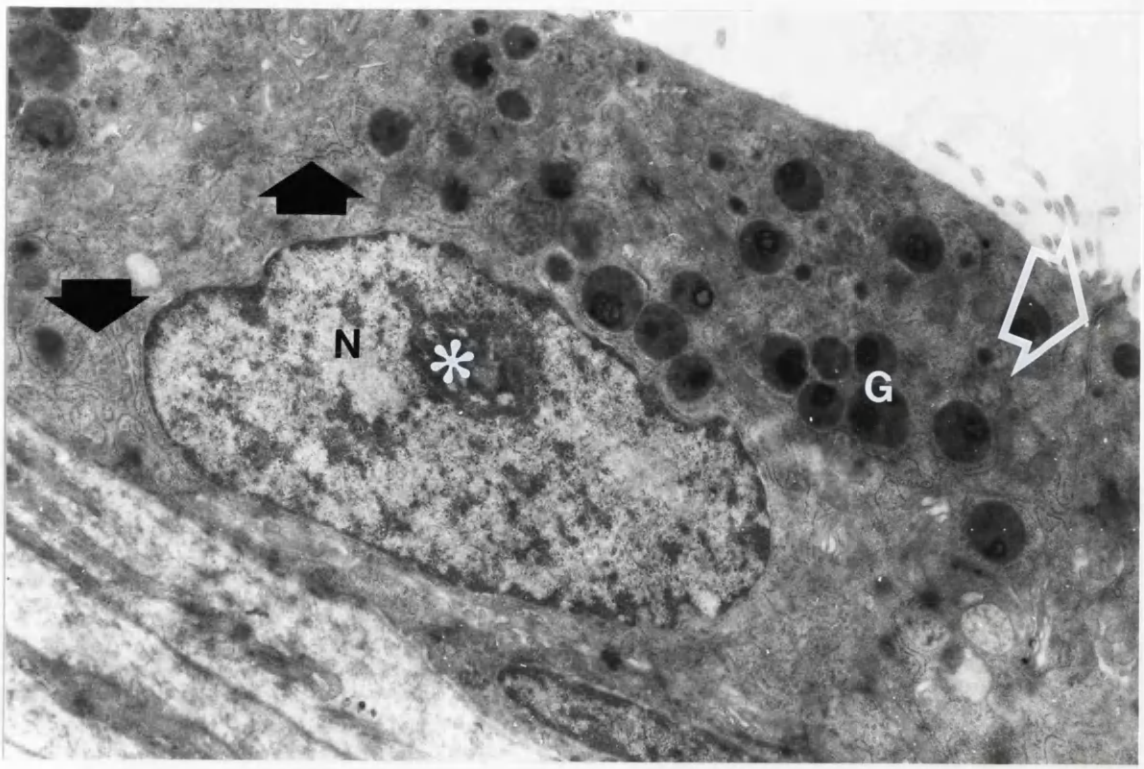

L

Fig. 5.7 Mucus-producing cell.
Numerous heterogeneous secretory granules (G). A large nucleus (N) with a prominent nucleolus (star). Note tight junctional complexes (open arrow). Microvilli are seen on the luminal surface. Profiles of rough endoplasmic reticulum are distributed throughout the cytoplasm (closed arrow).
TEM x 10,000.

Fig. 5.8 Alveolar membrane.
Note the protruding alveolar Type II cell (*), blood capillary (C) and cytoplasmic extensions of alveolar Type I cell carrying a few surface microvilli (small arrow).
Alveolar lumen (L).
TEM x 5,400.


N
G

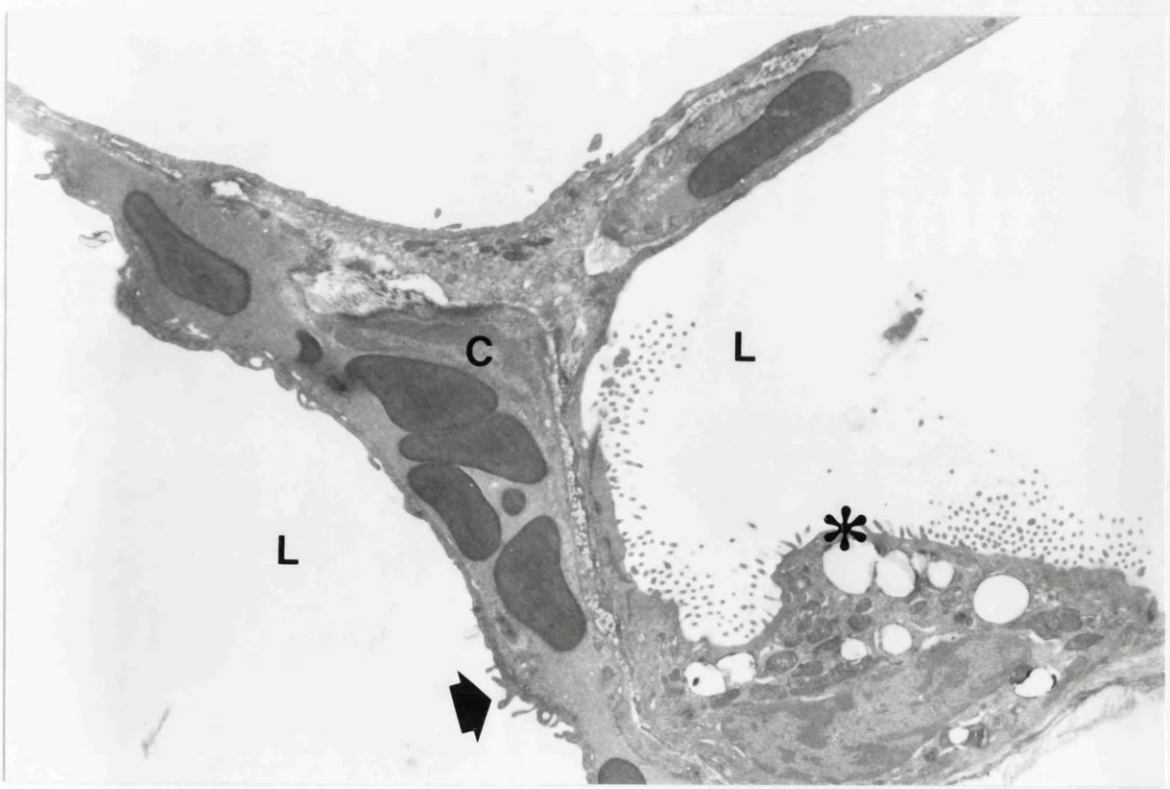
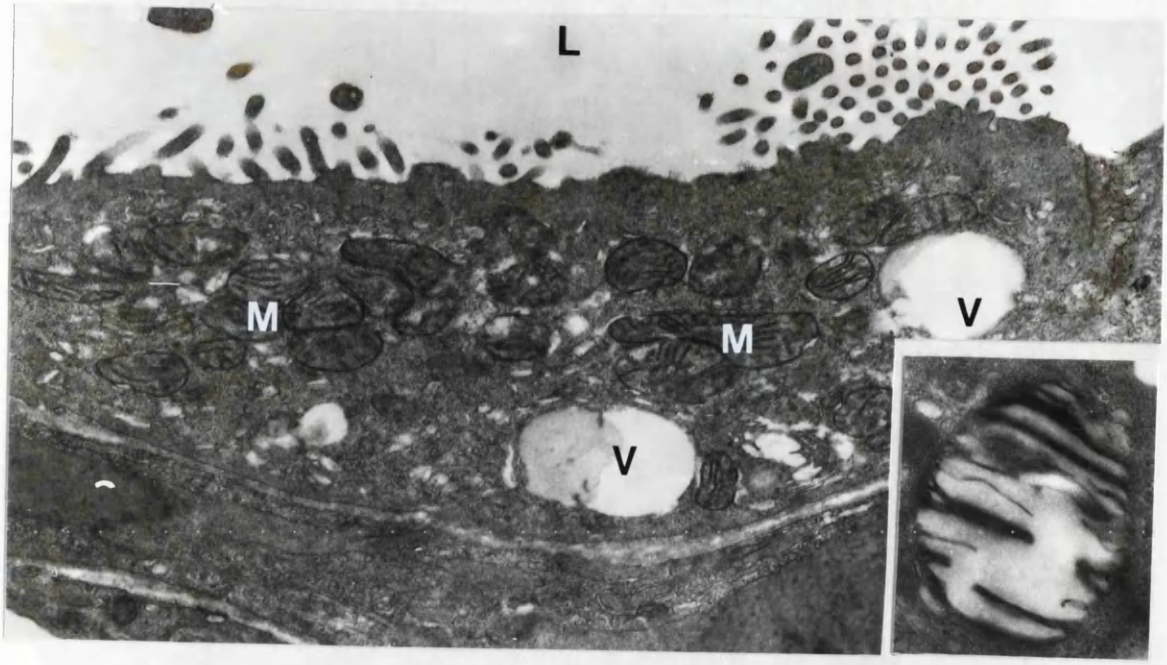

C
L
L

Fig. 5. 9 Alveolar Type II cell.
Note the numerous surface microvilli projecting into the alveolar lumen (L). Large lipid vacuoles are seen (V) and large well formed mitochondria are also visible (M).
TEM x 20,000.

Inset: Osmiophilic lamellated inclusion body.
TEM x 40,000).

Fig. 5.10 Alveolar Type II cell.
The cell dramatically protrudes into the alveolar lumen (L). Desmosomal attachments with adjacent alveolar Type I cells can be seen (arrow). Numerous microvilli are seen protruding from the cell surface.
TEM x 16,000.


L
M
M
V
V

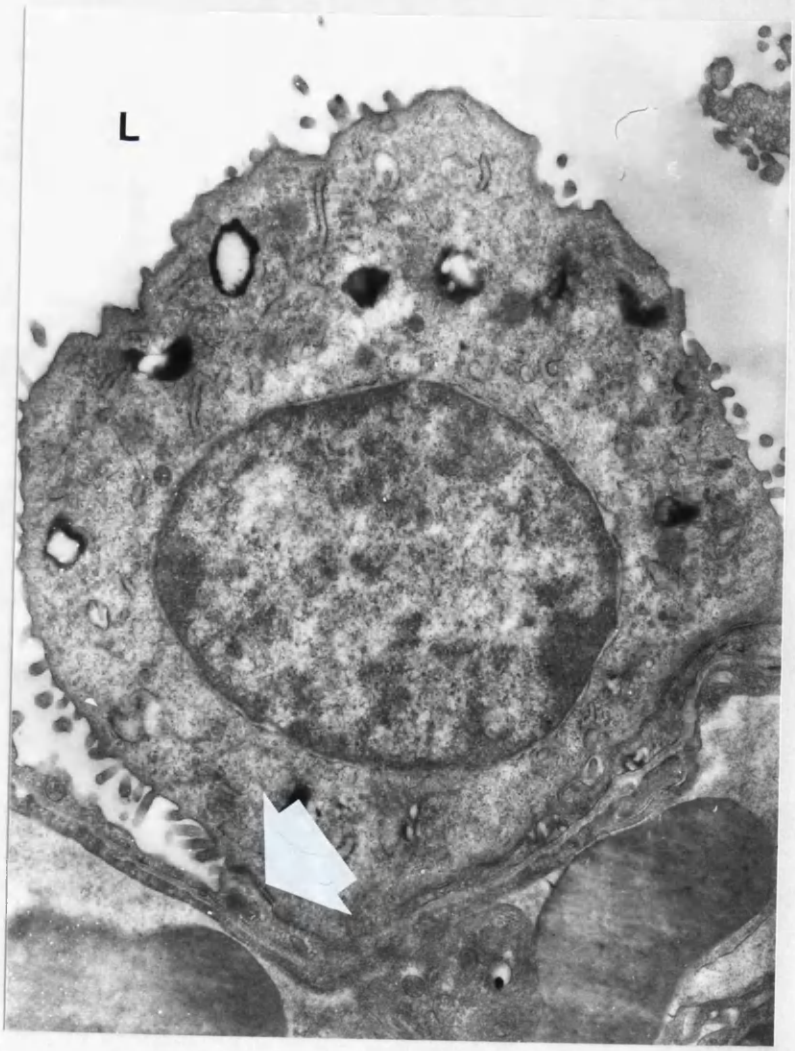
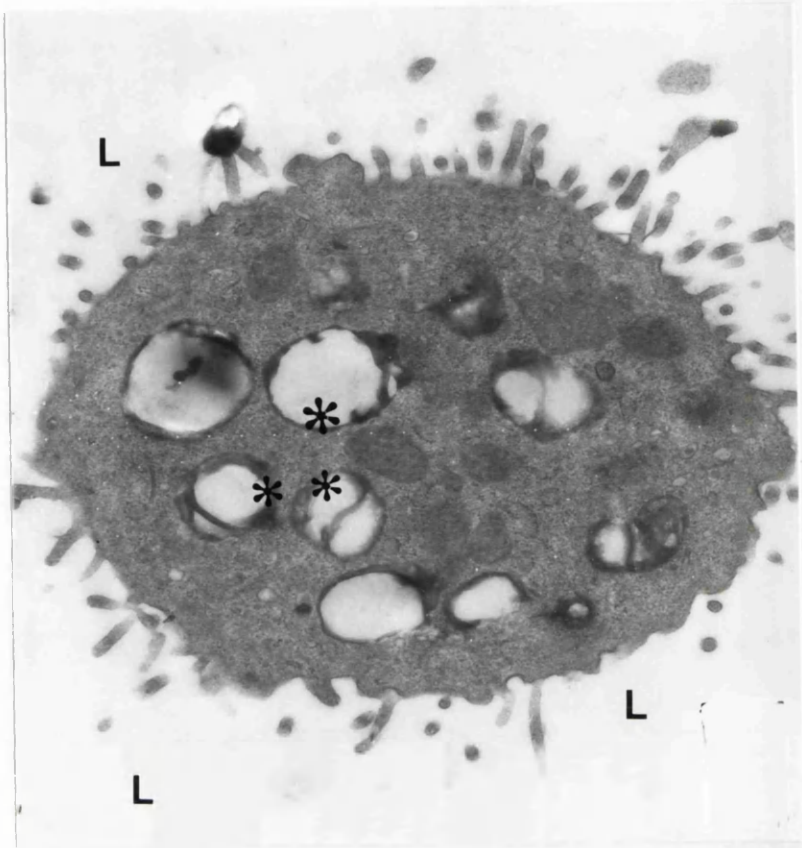

L

Fig. 5.11 Alveolar Type II cell.
A cross-section through the apical portion of an alveolar Type II cell similar to that seen in Fig. 5.10. Note its apparently unattached and 'free' position in the alveolar lumen (L).
Osmiophilic lamellated inclusion bodies (*).
TEM x 20,000.

Fig. 5.12 Alveolar membrane.
Smooth luminal surface of alveolar Type I cell (open arrow). Collagen fibres (*) and fibroblast process (F) lie between alveolar Type I and blood capillary endothelial cells, these structures forming the blood / gas barrier.
Alveolar lumen (L); capillary lumen (C); basal lamina (closed arrow).
TEM x 40,000.


L
L
L

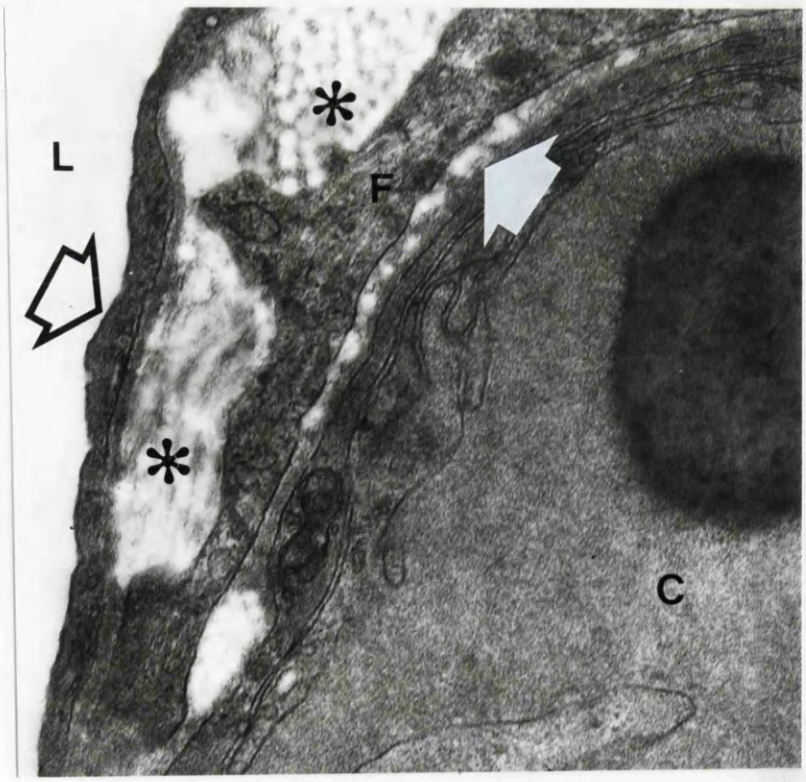
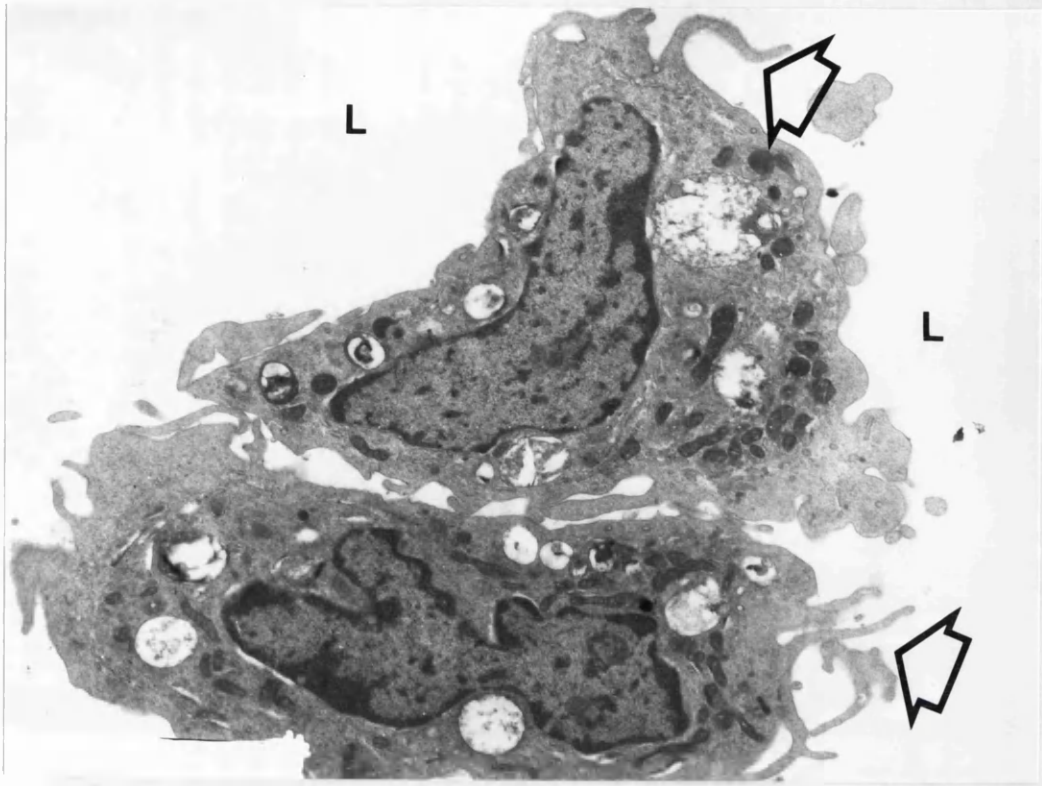
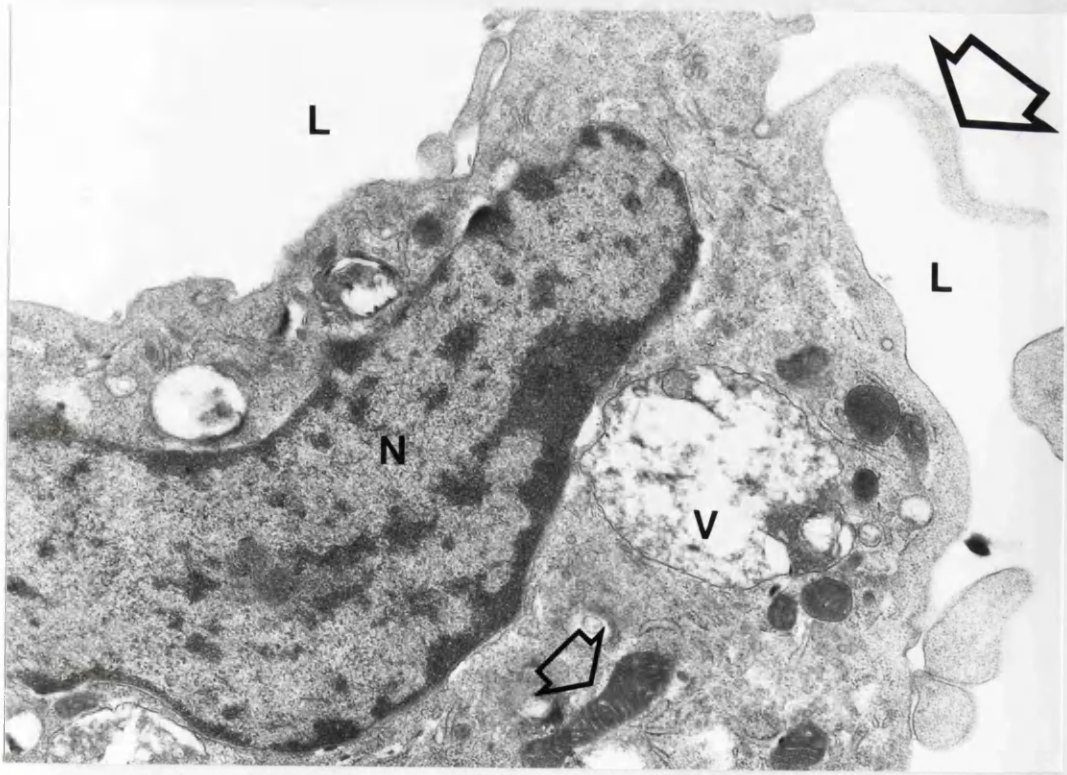

L
F
C

Fig. 5.13 Two alveolar macrophages.
Note large extent of smooth cell surface and long pseudopodia-like extensions (arrows).
Alveolar lumen (L).
TEM x 10,000.

Fig. 5.14 Alveolar macrophage.
Note the large nucleus (N), ellipsoid mitochondria (small arrows), large membrane-bound vesicle (V) and long pseudopodia-like extension (arrow). Alveolar lumen (L).
TEM x 20,000.

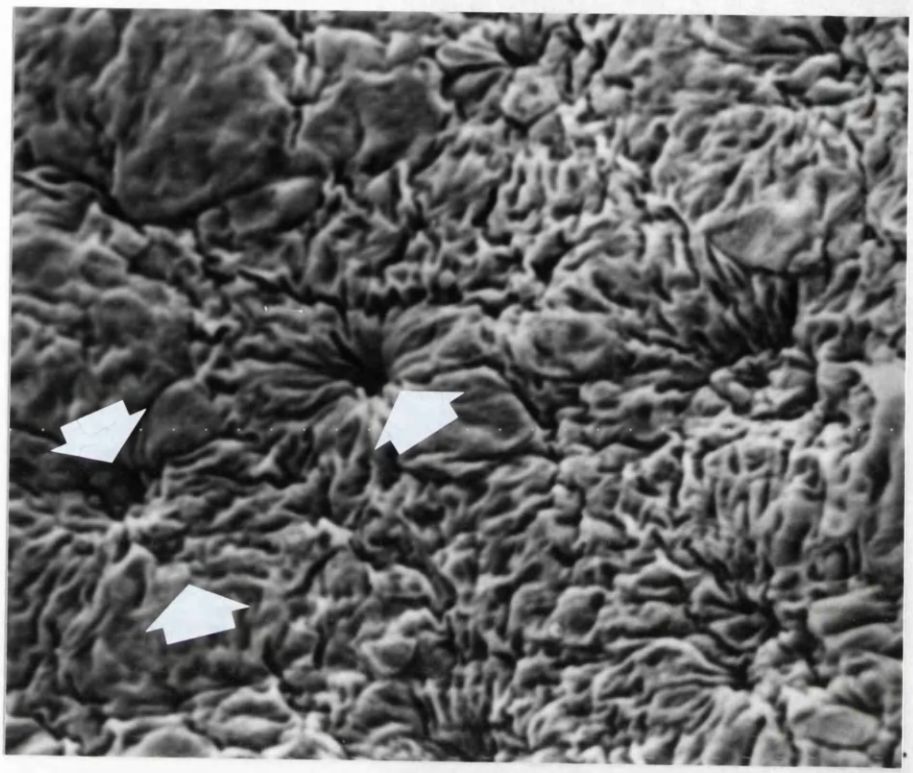

L
L


L
L
N
V

# CHAPTER 6

Fig. 6.1 Nasal vestibule (Caudal region), 3-day-old kid. Note surface squamous cells exhibiting folded apical surfaces and the presence of numerous submucosal gland orifices (arrows). SEM x320.

Fig. 6.2 Nasal vestibule, 2-day-old. Individual squamous cells present microplicae on their apical surface. SEM x 11,250.



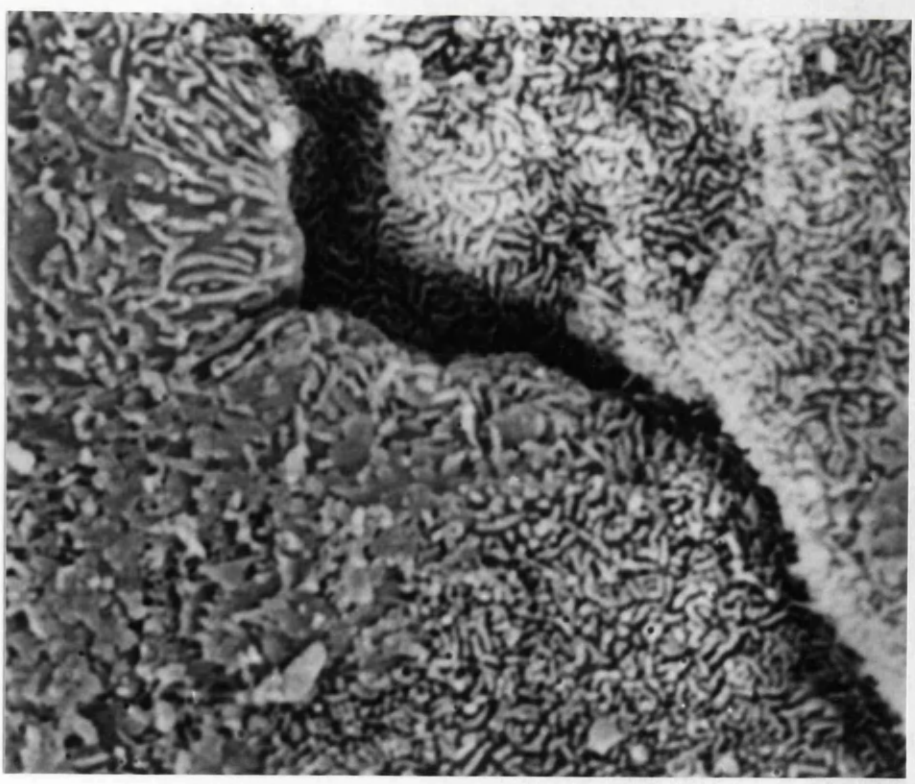
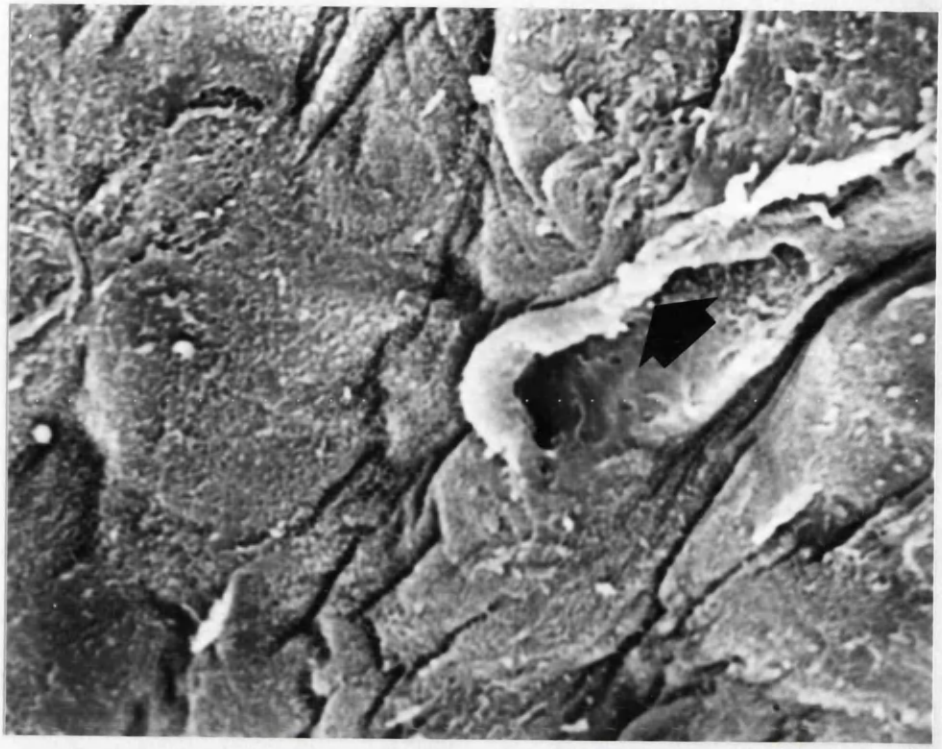


Fig. 6.3 Nasal vestibule (caudal region), 2-day-old kid. Occasional mucus strands were observed in this region (arrow).
SEM x 2,800.

Fig 6.4 Nasal vestibule, 9-day-old kid.
Note the smooth nature of the squamous cells covering one of the dome-shaped areas typical of this region. These areas usually exhibit a central pore (*).
SEM x 1,440.



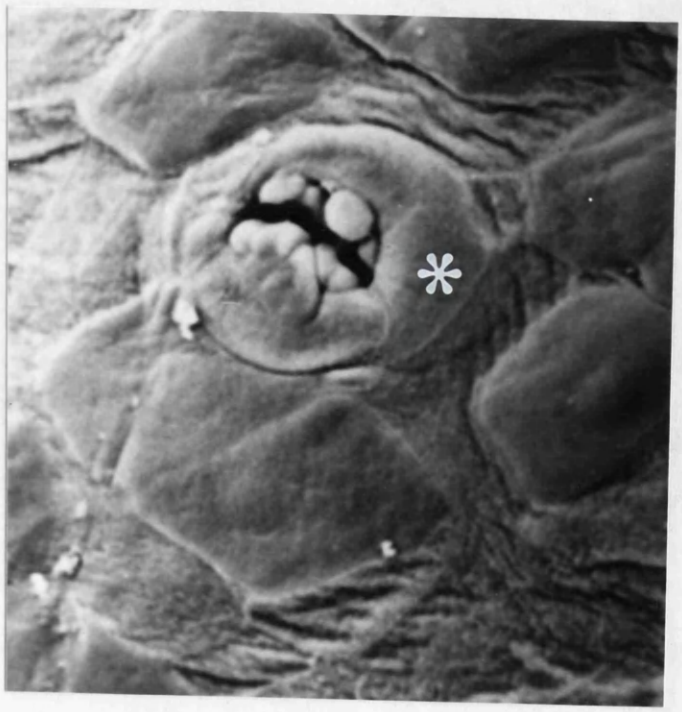
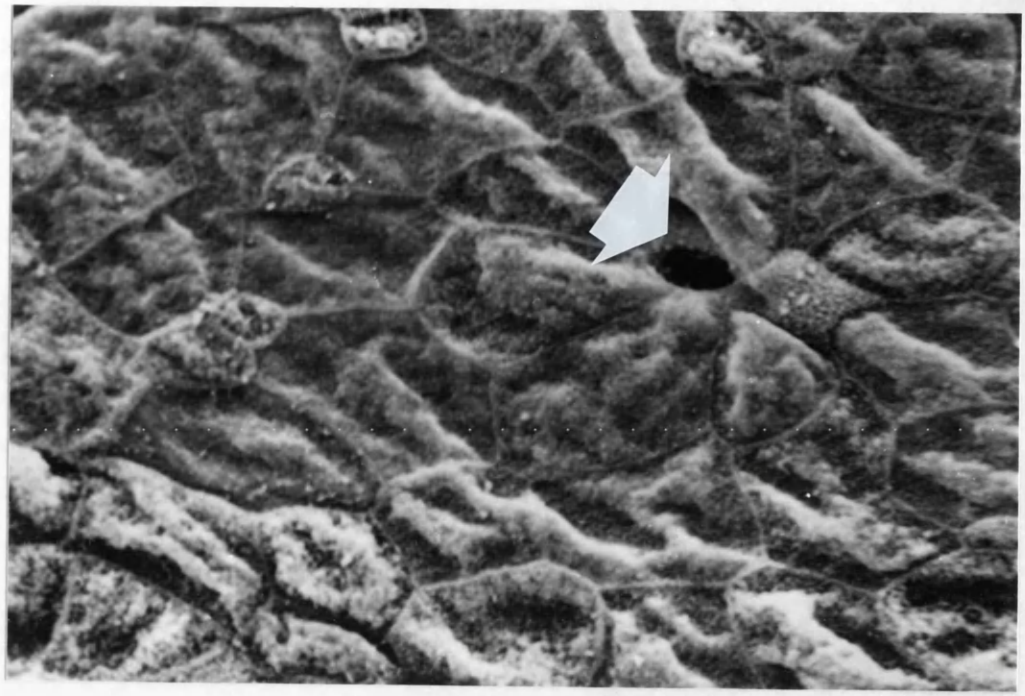


Fig. 6.5 Alar fold(rostral region), 3-hr-old kid.
The narrow rostral zone of the fold is seen to be lined by a stratified squamous epithelium. A submucosal gland orifice is visible (arrow).
SEM x 2,800.

Fig. 6.6 Alar fold (middle region) 3-day-old kid.
Apical cells are seen to present dimples or folds (arrows) on their luminal surfaces.
SEM x 2,800.



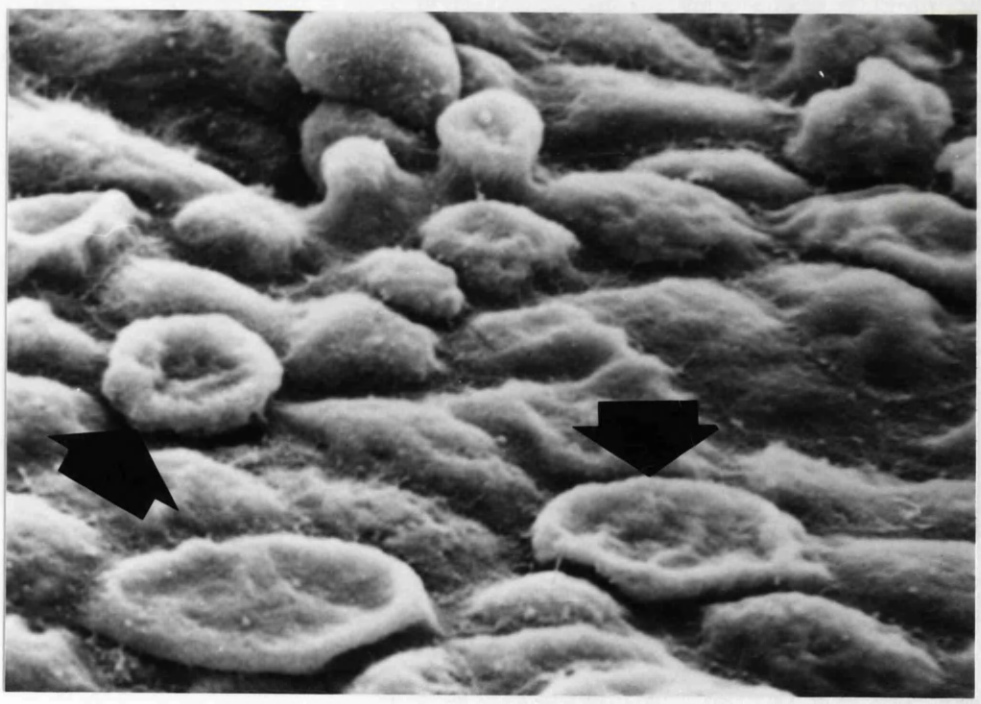
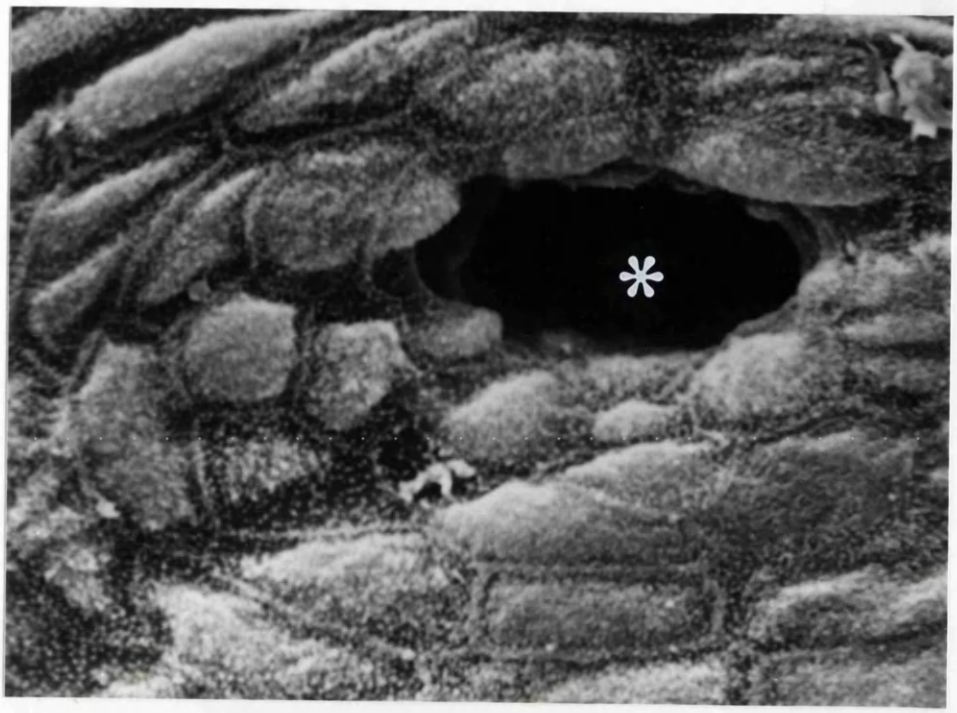


Fig. 6.7 Alar fold (middle region),3-hr-old kid.
A submucosal gland orifice (*) surrounded by numerous nonciliated microvillous cells with distinct cell borders.
SEM x 5,600.

Fig. 6.8 Alar fold ( caudal region), 3-hr-old kid.
The caudal region of the fold is seen to be heavily ciliated even in the new-born. Mucus-producing cells (arrows) are numerous.
SEM x 720.



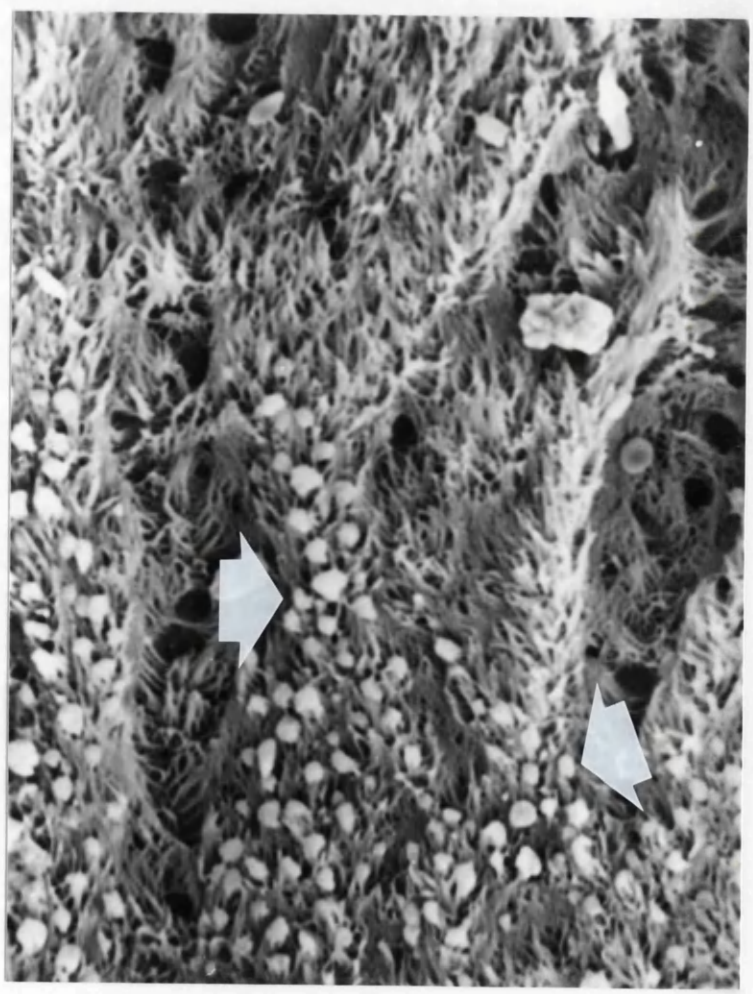
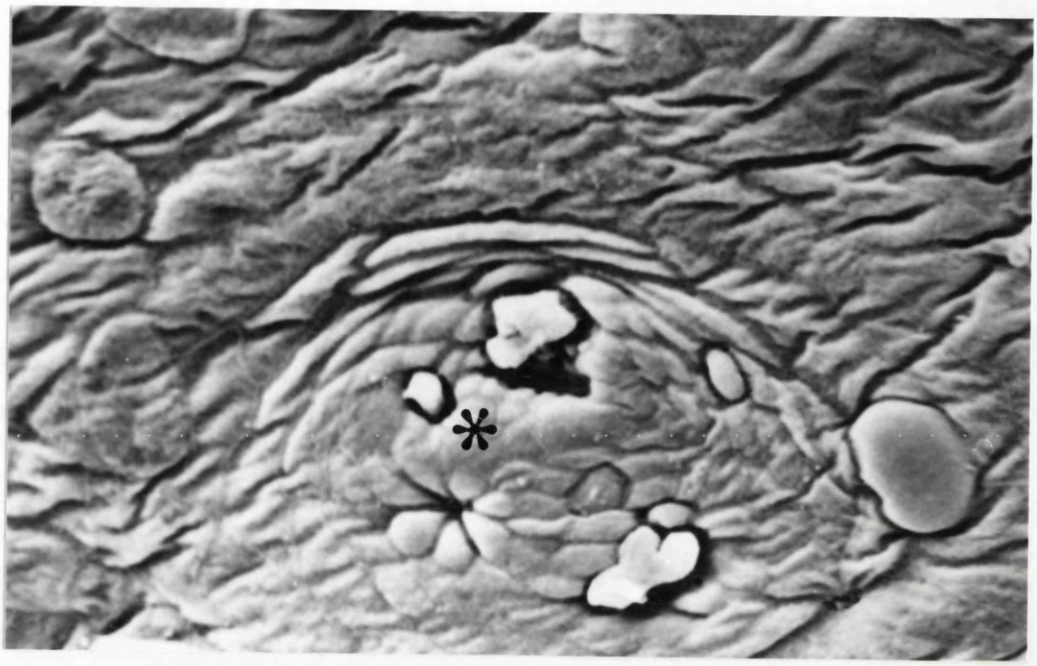


Fig. 6.9 Alar fold (rostral region), 3-hr-old kid.
Domed areas (*), similar to those observed in the nasal vestibule, are also seen in this region.
SEM x 1,440.

Fig. 6.10 Basal fold, 7-day-old kid.
Caudal to the narrow rostral zone of the squamous epithelium, the apical cells appear "spongy", with depressed or wrinkled surfaces.
SEM x 2,800.



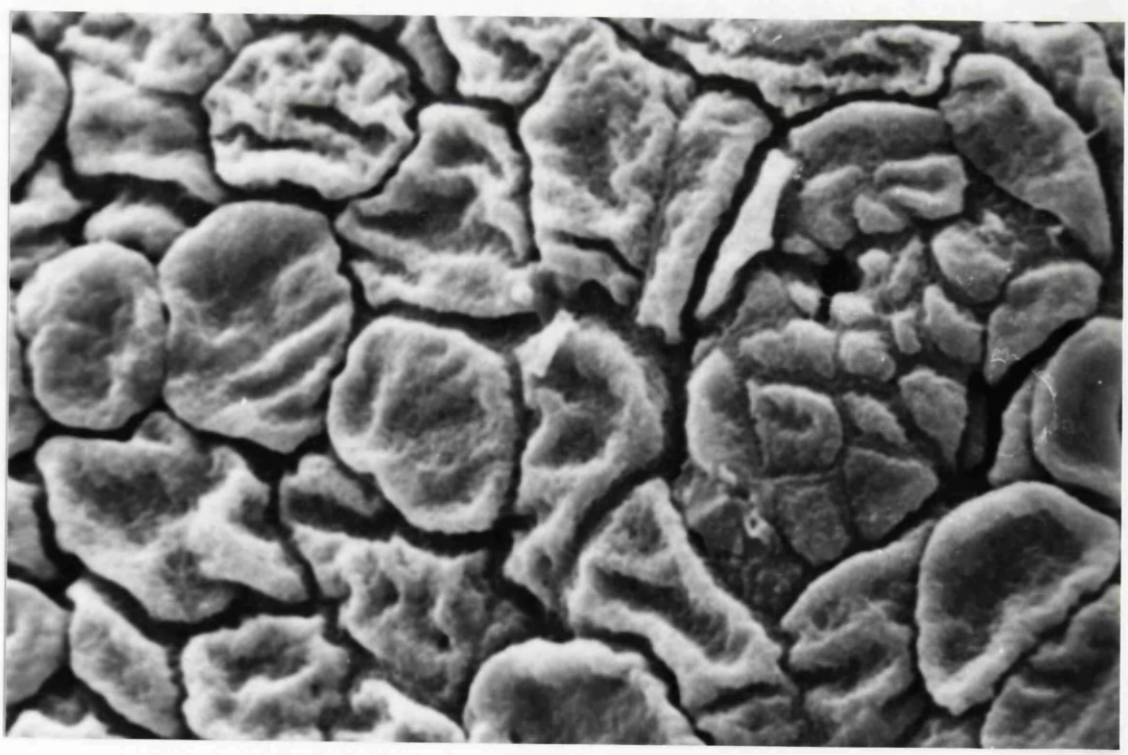
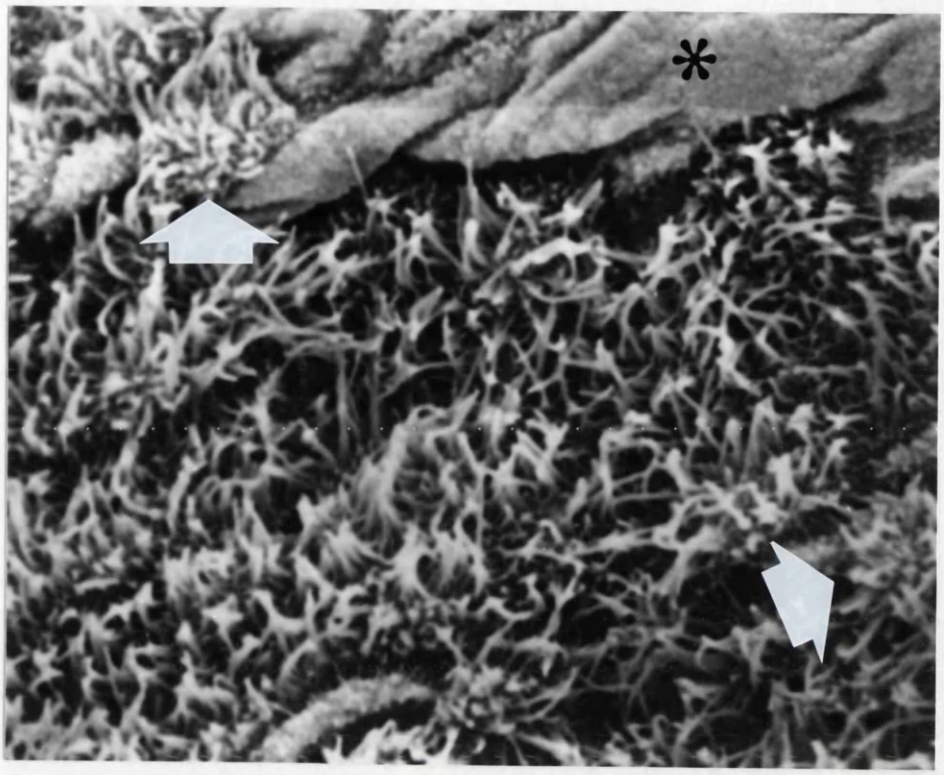


Fig. 6.11 Basal fold (caudal region), 3-hr-old kid.
Note a patch containing numerous regenerating ciliated cells (arrows) together with a few nonciliated microvillous cells (*).
SEM x 2,800.

Fig. 6.12 Basal fold, (caudal region), 3-day-old kid.
The caudal region of the fold is completely ciliated in kids of all ages. A patch of nonciliated microvillous cells, apparently devoid of regenerating ciliated cells, can be seen (*).
SEM x 1,440.



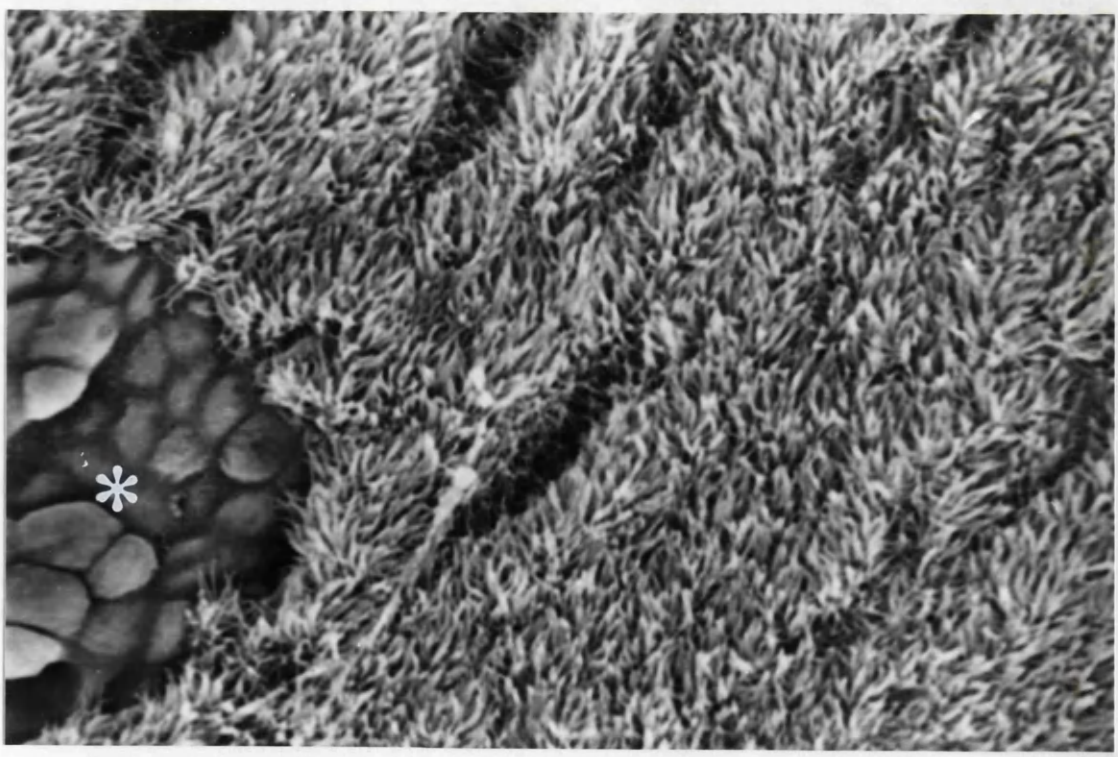


Fig. 6.13 Basal fold (caudal region), 2-day-old kid.
A patch of nonciliated microvillous cells. Note the presence of a few regenerating ciliated cells (*) scattered amongst numerous nonciliated microvillous cells.
SEM x 5,600.

Fig. 6.14 Ventral nasal concha, 3-day-old kid.
Numerous mucus-producing cells presenting typical apical protuberances(*) are observed within the ciliated epithelium.
SEM x 2500.

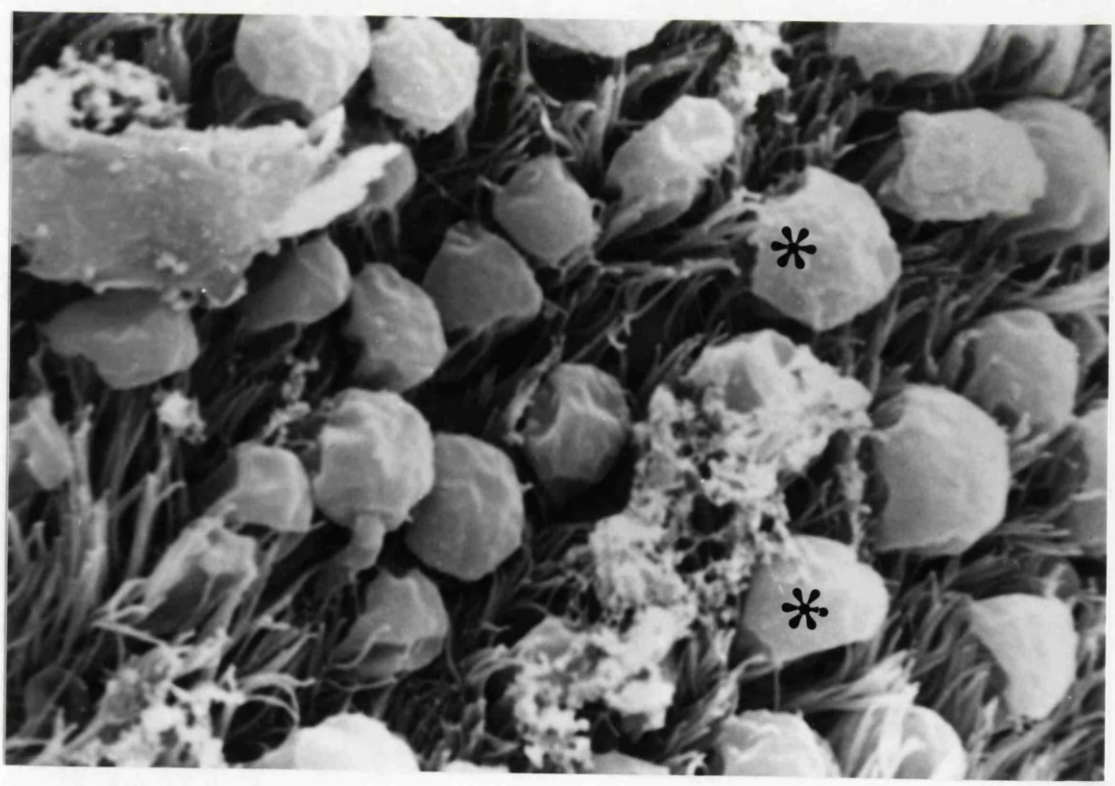
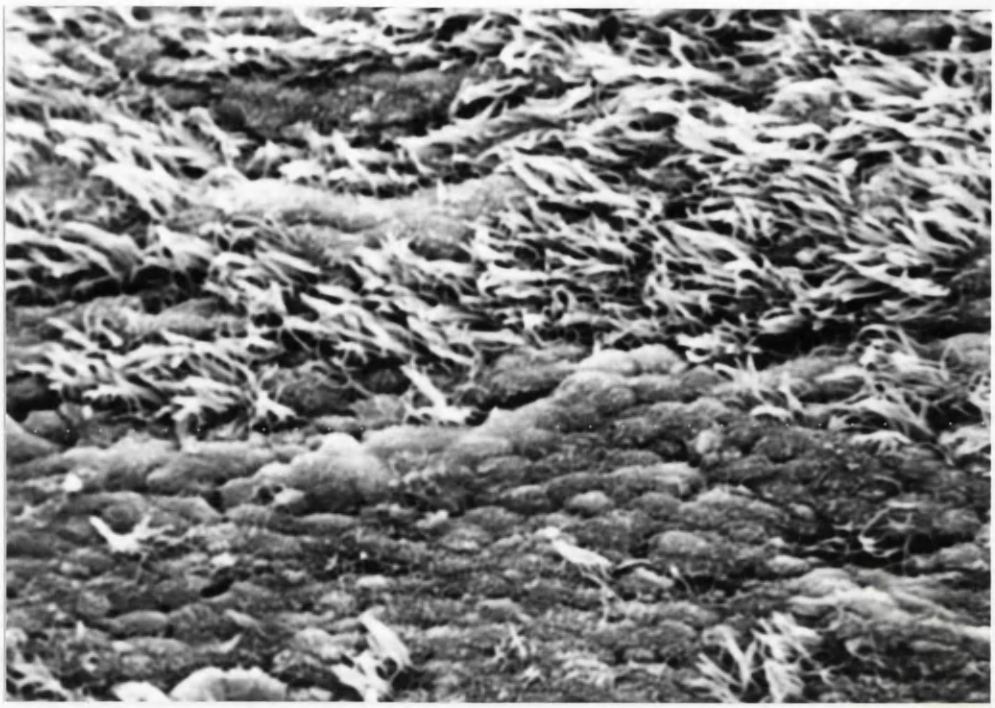


Fig. 6.15 Middle nasal concha, 3-hr-old kid.
This region is seen to be poorly ciliated.
SEM x 1,440.

Fig. 6.16 Middle nasal concha, 3-week-old kid.
The region is seen to have a dense cilial carpet with the cilia being frequently matted (arrow).
Submucosal gland orifice (*).
SEM x 2,800.



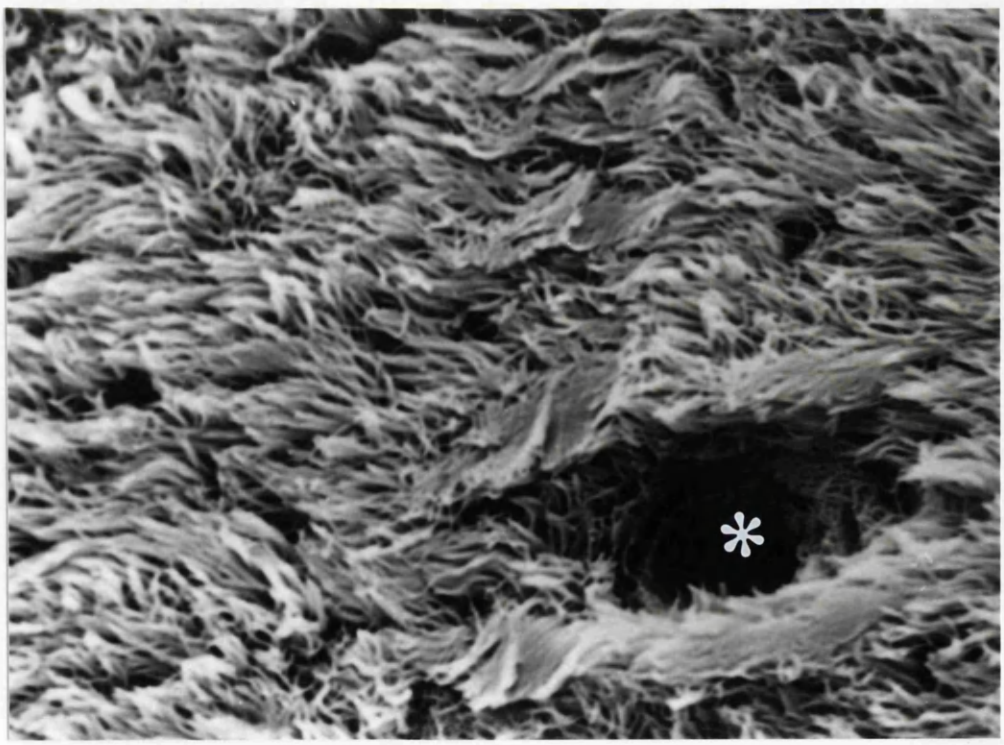
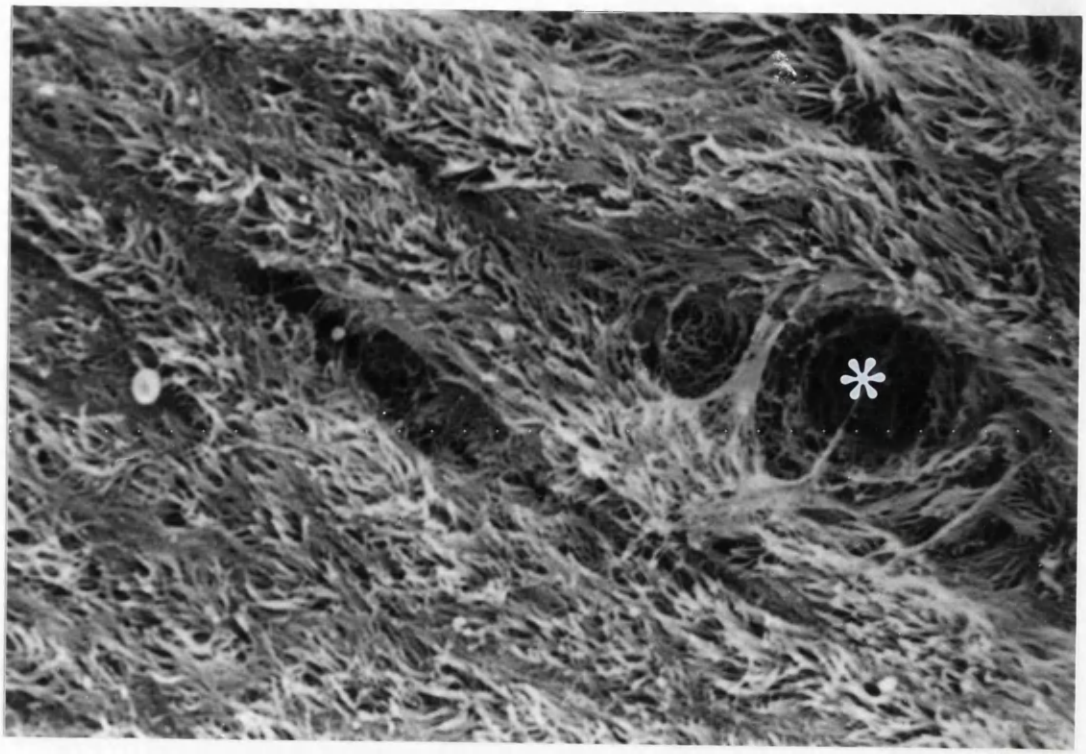


Fig. 6.17 Nasal septum, 12-hr-old kid.
The epithelium is heavily ciliated and the cilia matted. A submucosal gland orifice (*) and mucus strands (arrow) can be seen.
SEM x 1,440.

Fig. 6.18 Nasal septum, 3-week-old kid.
The epithelium presents a "moth eaten" appearance similar to that observed in the adult goat. Numerous nonciliated microvillous cells and a few regenerating ciliated cells (arrows) can be seen.
SEM x 2,800.



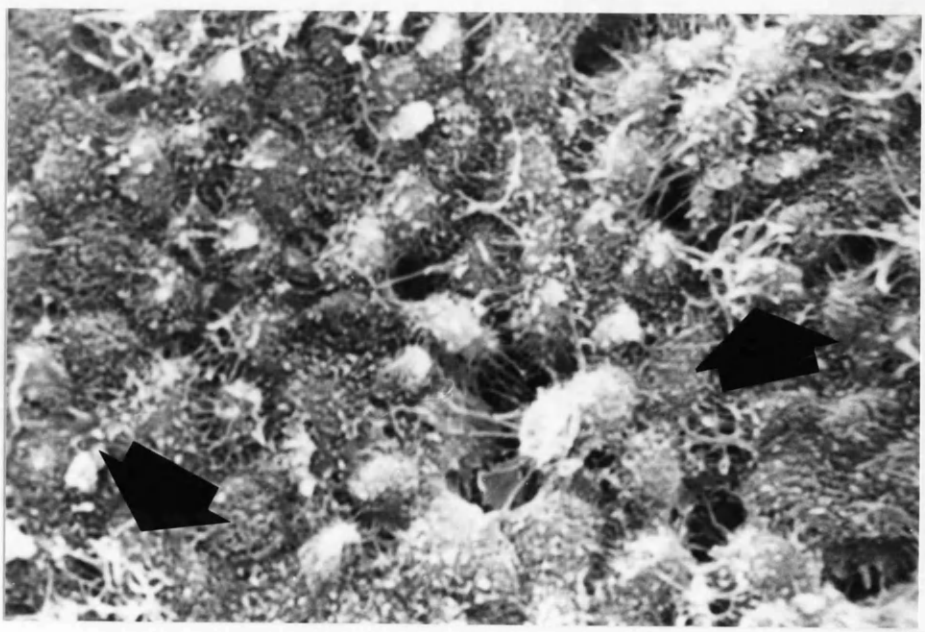
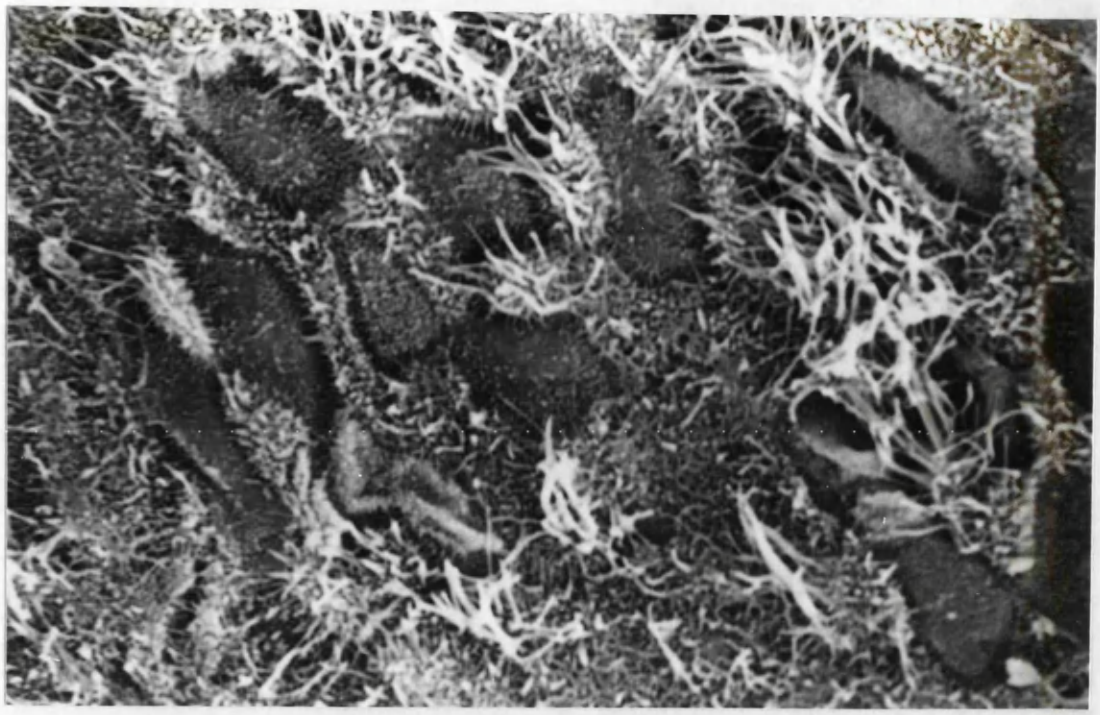


Fig. 6.19 Nasopharynx (rostral region), 2-day-old kid. The degree of ciliation is seen to be relatively poor in the 2-day-old kid with nonciliated microvillous cells being numerous. SEM x 2,800.

Fig. 6.20 Nasopharynx (rostral region), 2-day-old kid. An occasional patch of densely ciliated cells is seen in this 2-day-old kid. Such a heavily ciliated epithelium is characteristic of kids of older ages. SEM x 2,800.



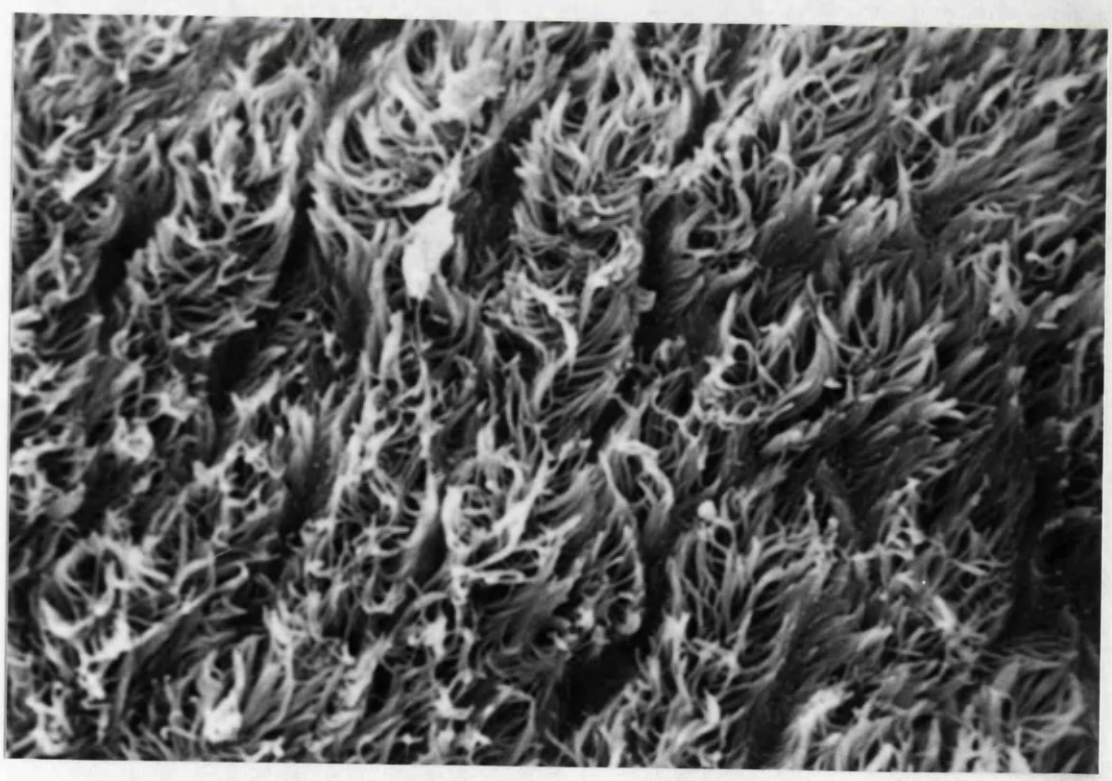
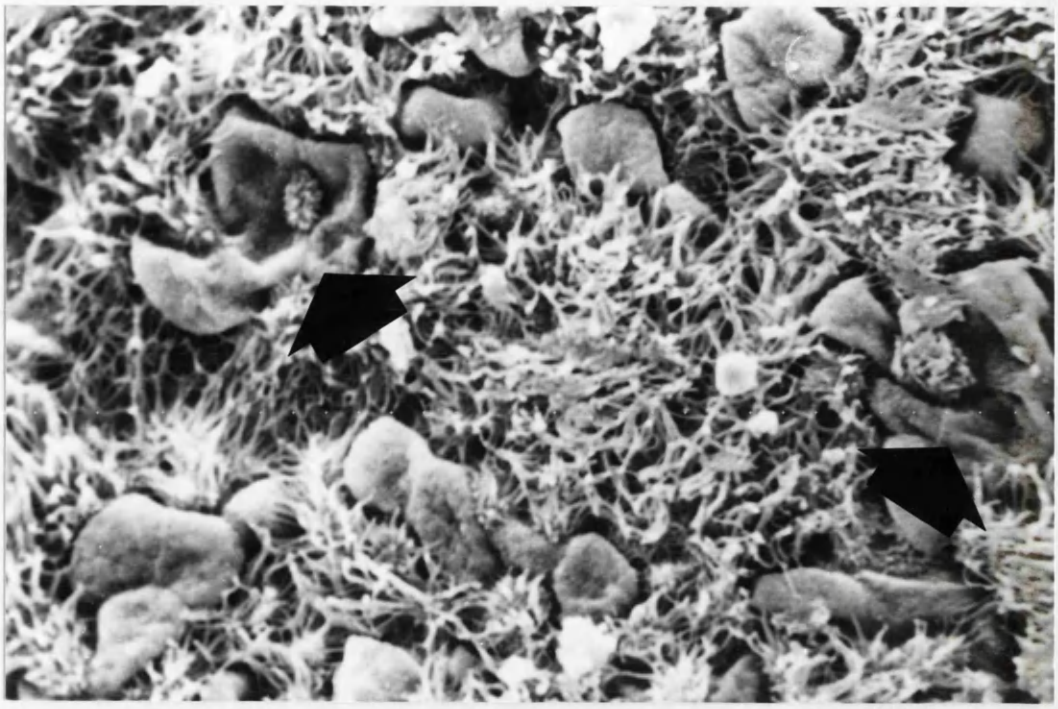


Fig. 6.21 Nasopharynx (transitional zone), 2-day-old kid. Caudal to the ciliated region, numerous regenerating ciliated cells and a few ciliated cells can be seen. Nonciliated microvillous cells present dimples on their luminal surfaces (arrows).
SEM x 2,800.

Fig. 6.22 Nasopharynx, (transitional zone), 15-day-old kid.
Nonciliated microvillous cells are seen present aggregations of short surface microvilli.
SEM x 11,250.



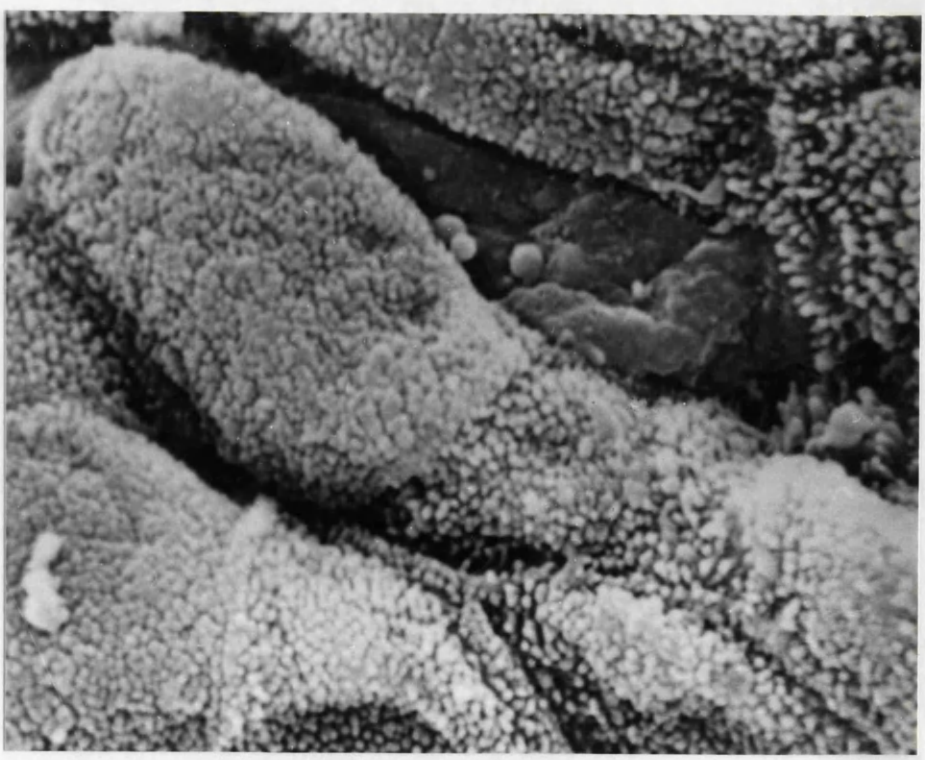
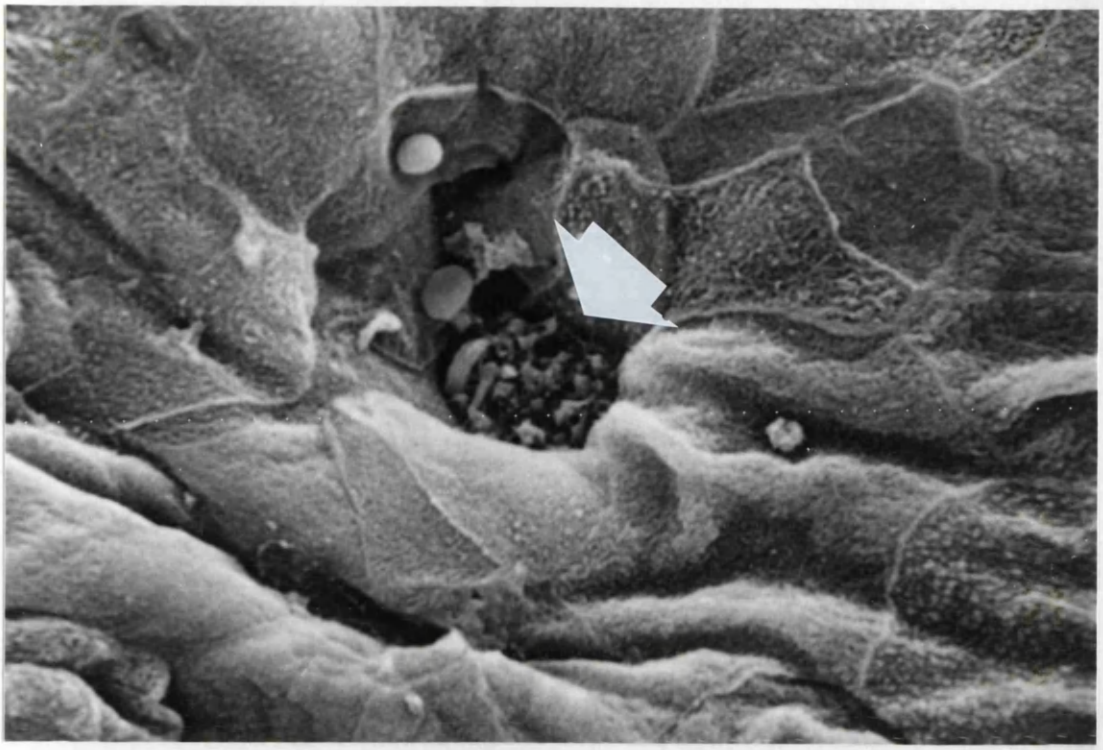
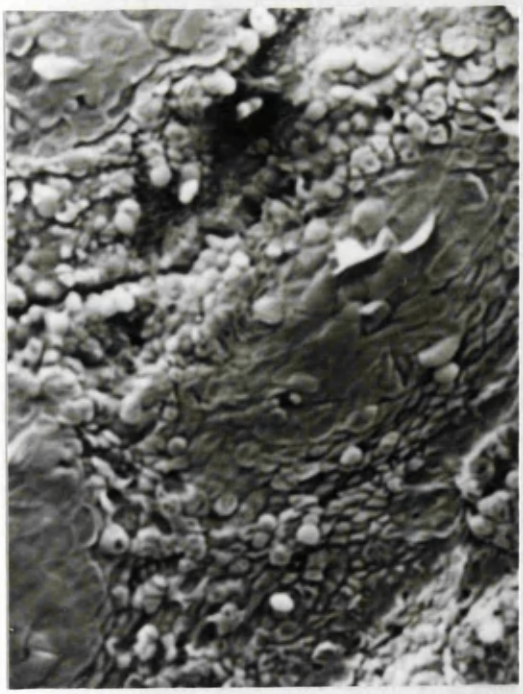


Fig. 6.23 Epiglottis, 5-day-old kid.
Squamous cells line the laryngeal surface. A taste bud is seen, with some sensory hairs protruding through the pore (arrow).
SEM x 2,800.

Fig. 6.24 Vocal fold, 7-day-old kid.
Circumscribed, dome-shaped areas.
(a) These are lined by smooth flattened squamous cells. Such areas frequently exhibit a central pore.
SEM x 360.
(b) At higher magnifications, many of those cells around the periphery of the dome-shaped areas are seen to present a pitted appearance.
SEM x 1,440.





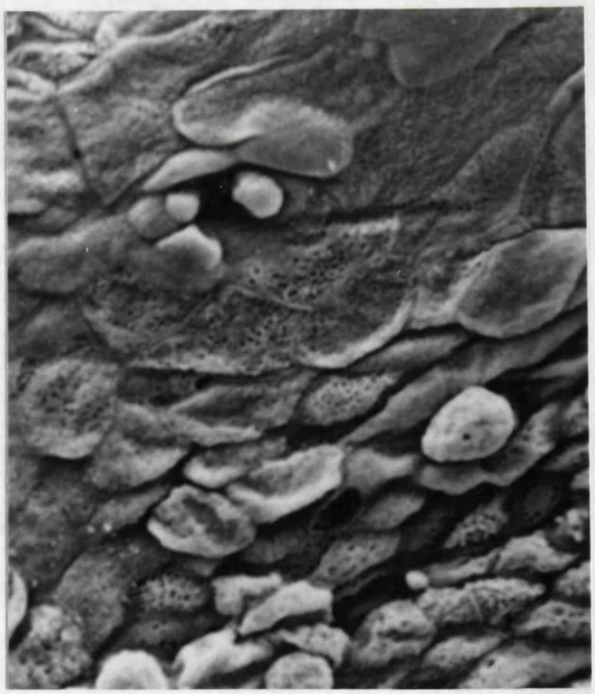
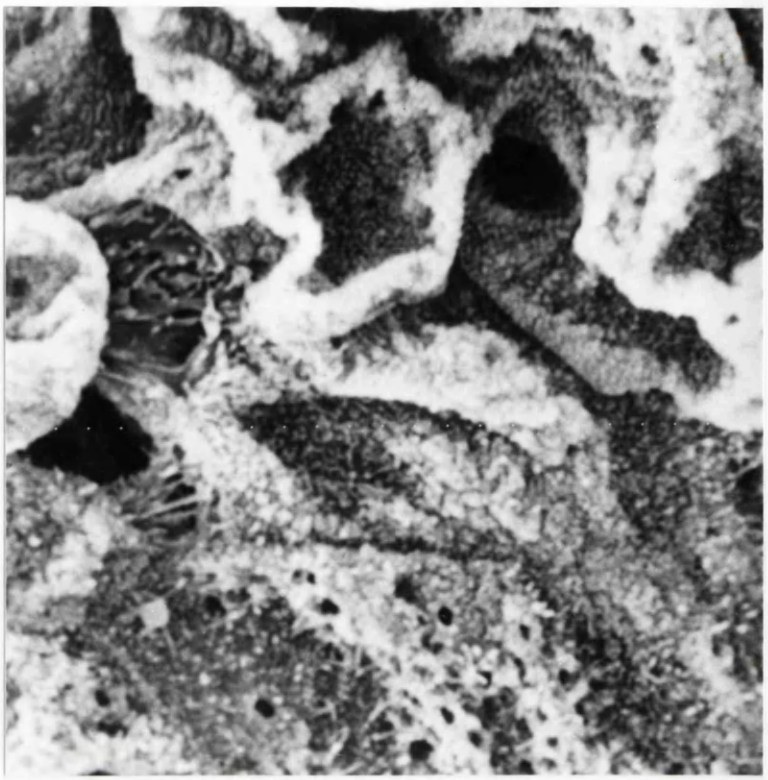


Fig.6.25 Vocal fold, 7-day-old kid.
The spongy nature and pitted surface of the peripheral cells shown in Fig. 6.24 can be better appreciated at higher magnifications.
SEM x 5,600.

Fig. 6.26 Vocal fold (transitional zone), 3-week-old kid.
Mature ciliated cells, numerous regenerating ciliated cells and nonciliated microvillous cells are characteristic of this region.
SEM x 2,800.



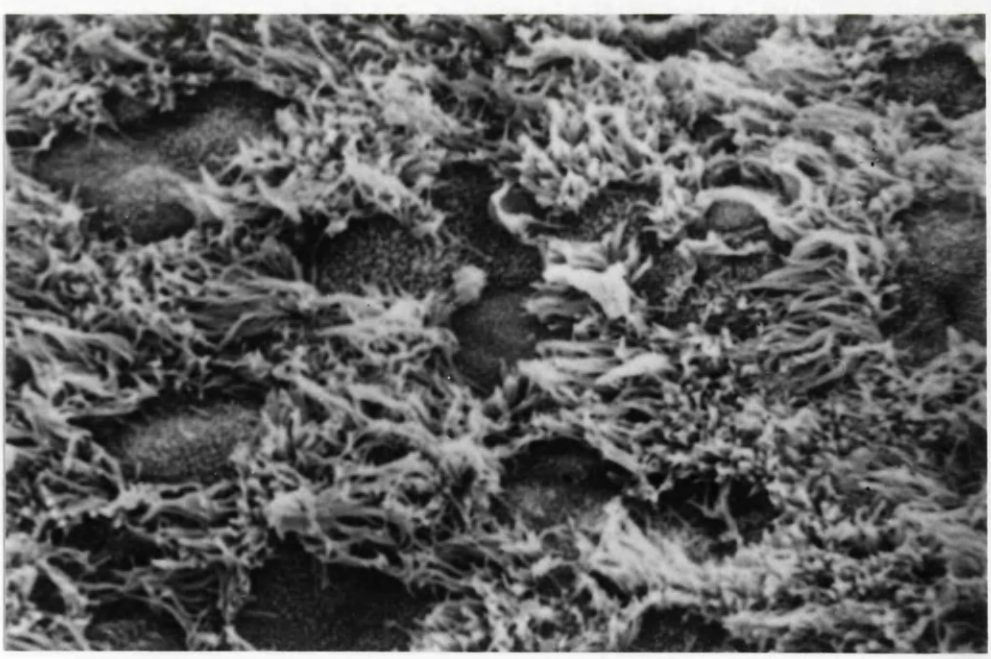


Fig. 6.27 Vocal fold, 15-day-old kid.
A patch of normal nonciliated microvillous cells.
SEM x 2,800.

Fig. 6.28 Infraglottic cavity, 2-day-old kid.
Individual nonciliated microvillous cell (*)
presenting, a large wrinkled apical surface.
SEM x 5,600.

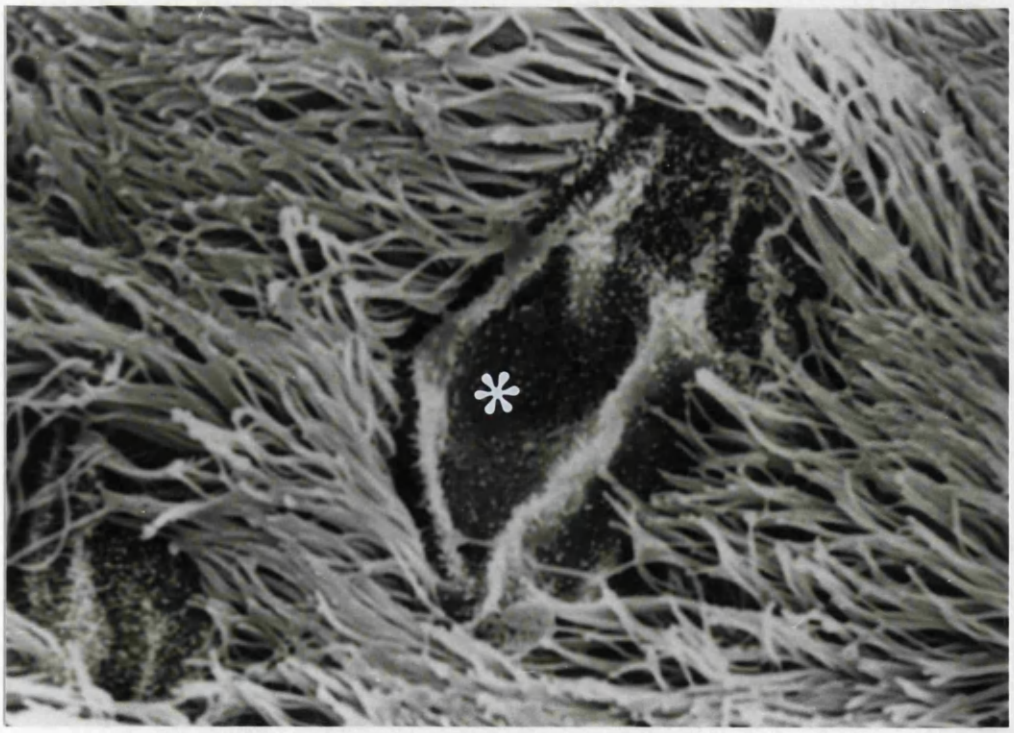


Fig. 6.29 Trachea, 2-day-old kid.
A patch composed of numerous nonciliated microvillous cells and regenerating ciliated cells (arrows).
SEM x 2,800.

Fig. 6.30 Trachea, 5-day-old kid.
Nonciliated microvillous cells with a characteristic wrinkled luminal surface are seen distributed individually amongst ciliated cells. These are characteristic in the new-born to 15-day-old kid.
SEM x 2,800.

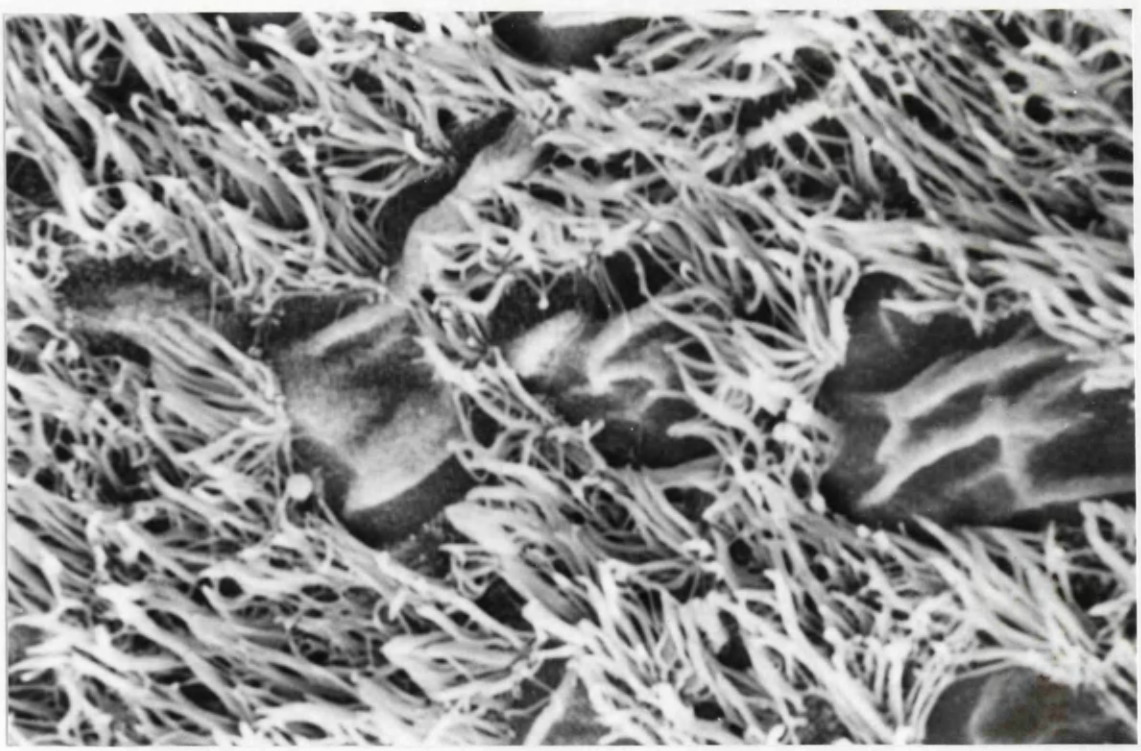
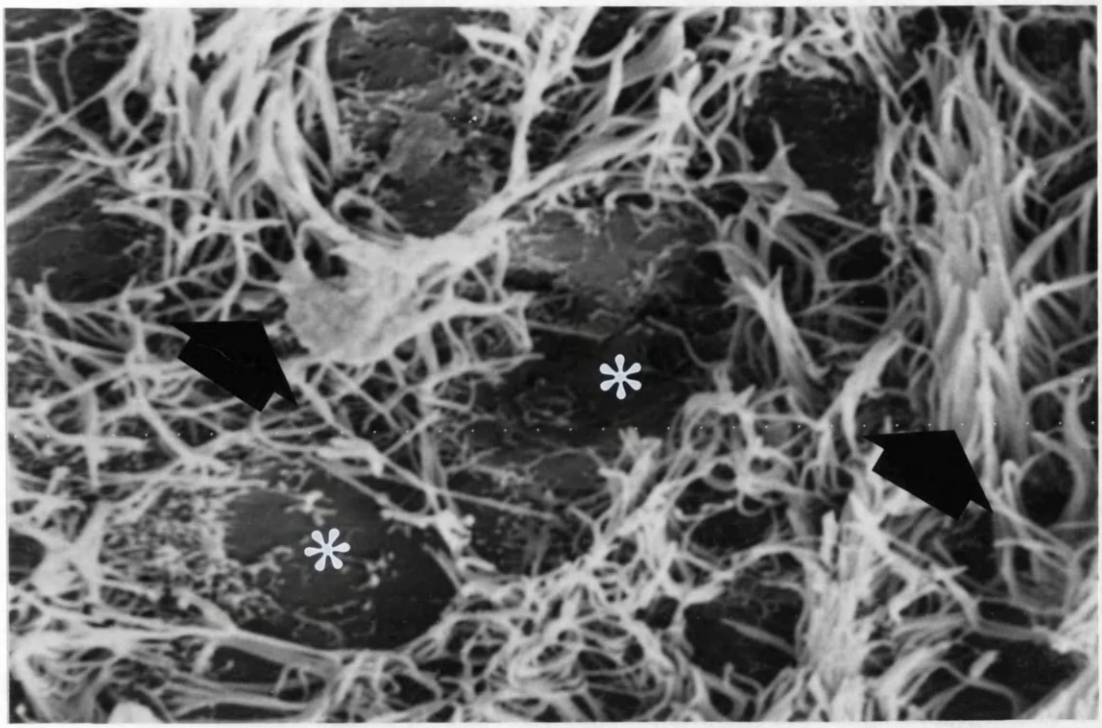


Fig. 6.31 Caudal lobar bronchus, 2-day-old kid
Mucus-producing cells (*), characterised by depressed luminal surfaces with a scarce population of surface microvilli, are numerous. Ciliated cells present matted cilia (arrows). SEM x 5,600.

Fig. 6.32 Lung Parenchyma, 9-day-old kid.
A respiratory bronchiole characterised by shallow alveoli (arrows) is seen in a 9-day-old kid. Note that the lung parenchyma in general presents an appearance similar to that seen in the adult goat. SEM x 180.



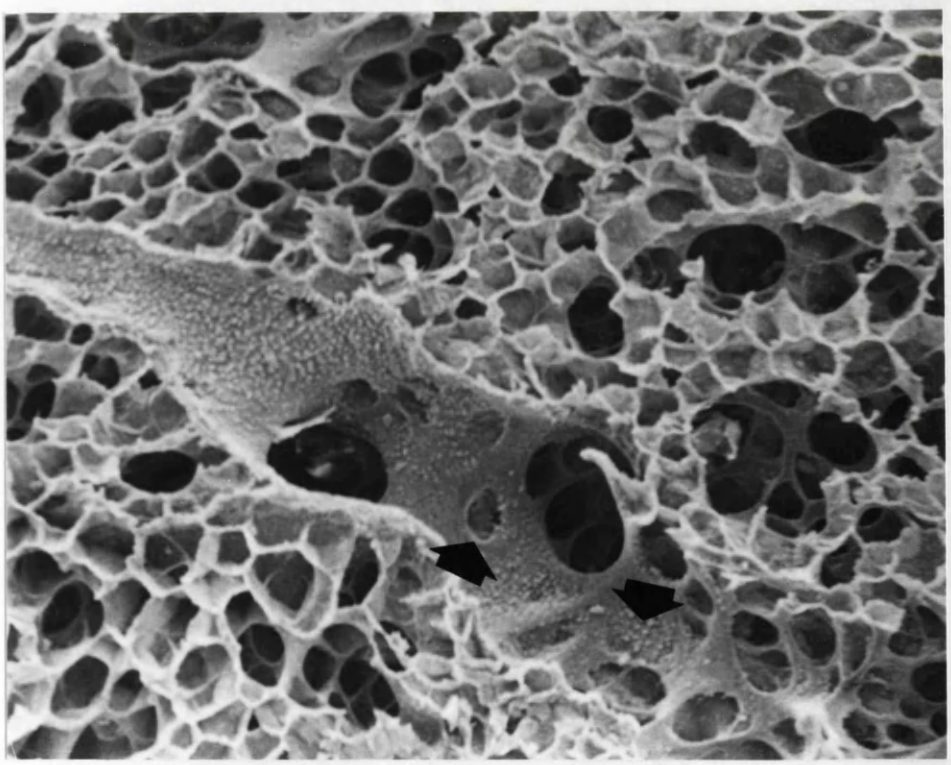
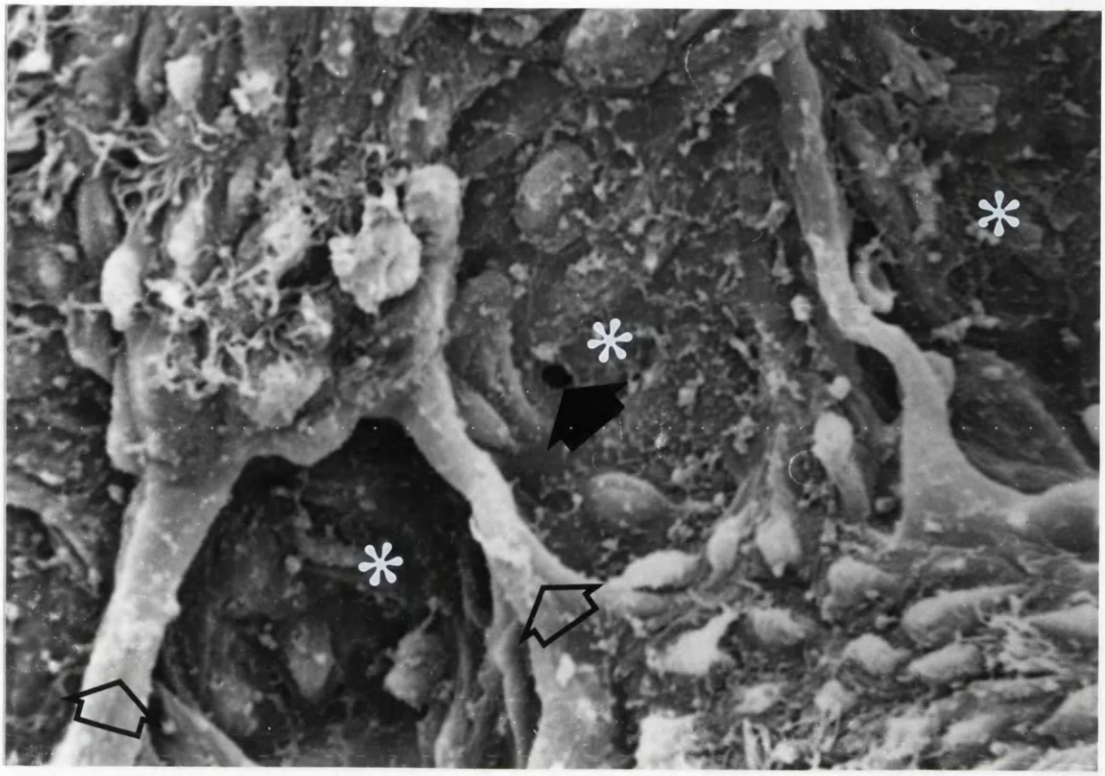


Fig. 6.33 Respiratory bronchiole, 3-day-old kid.
Shallow alveoli (*) are defined by obvious raised ridges (open arrows). An alveolar pore can also be seen (closed arrow).
SEM x 2,800.

Fig. 6.34 Respiratory bronchiole, 3-day-old kid.
The epithelium is composed of numerous nonciliated bronchiolar epithelial (Clara) cells presenting characteristic apical protuberances and ciliated cells with poorly developed cilia.
SEM x 2,800.



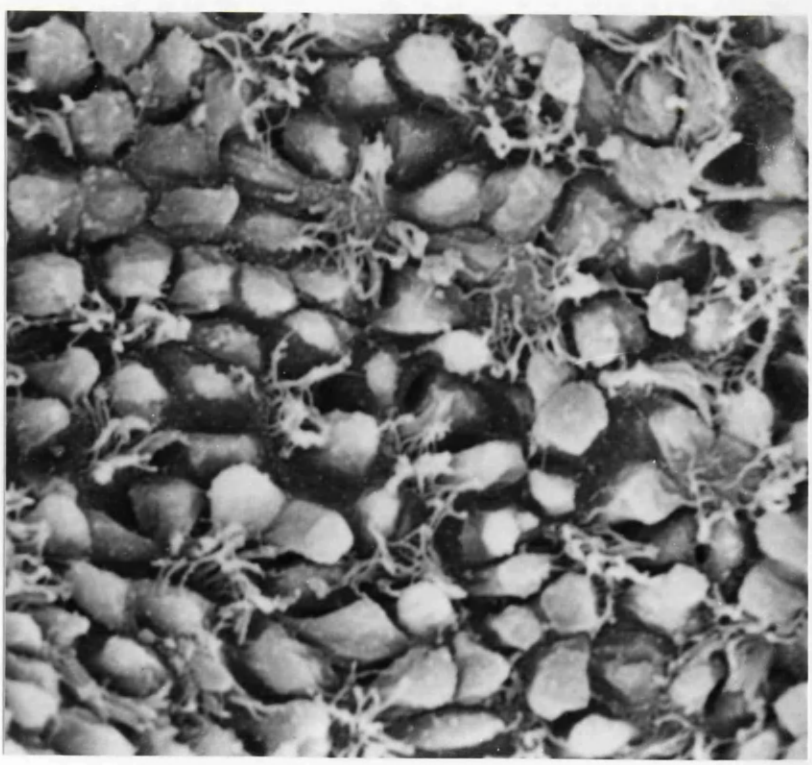
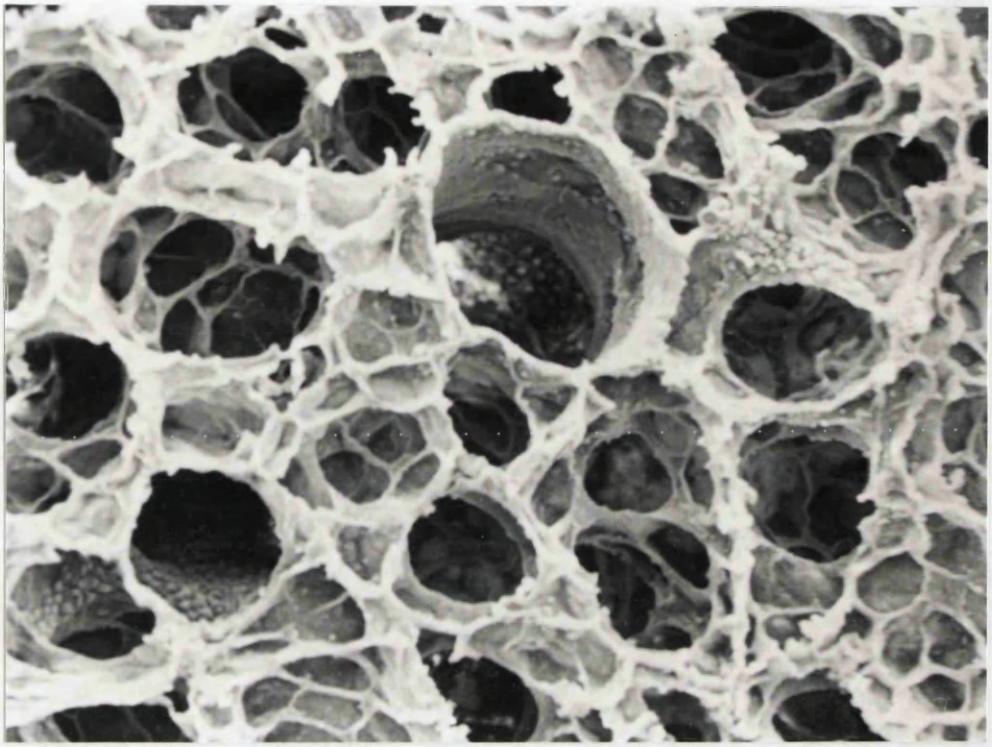


Fig. 6.35 Lung parenchyma, 12-hr-old kid.
The alveoli present similar appearance to those observed in the adult goat. Note that alveolar pores are very rare.
SEM x 360.

Fig. 6.36 Alveolus, 5-day-old kid.
In very young kids, alveoli are usually lined mainly by Type II cells (*), as illustrated. Such alveoli are, however, occasionally observed in older kids.
SEM x 5,600.



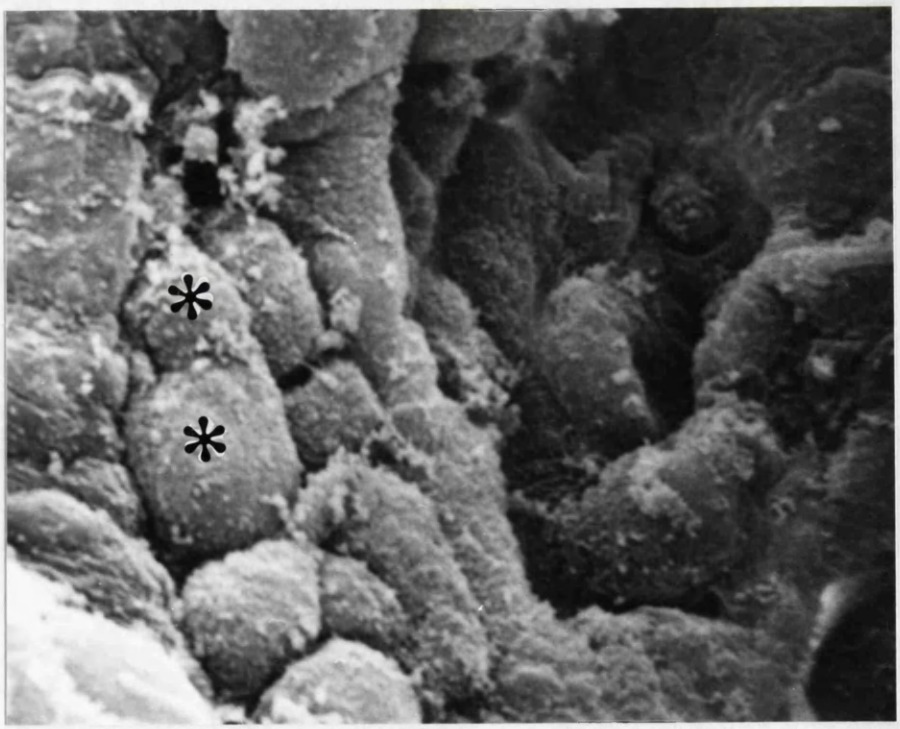
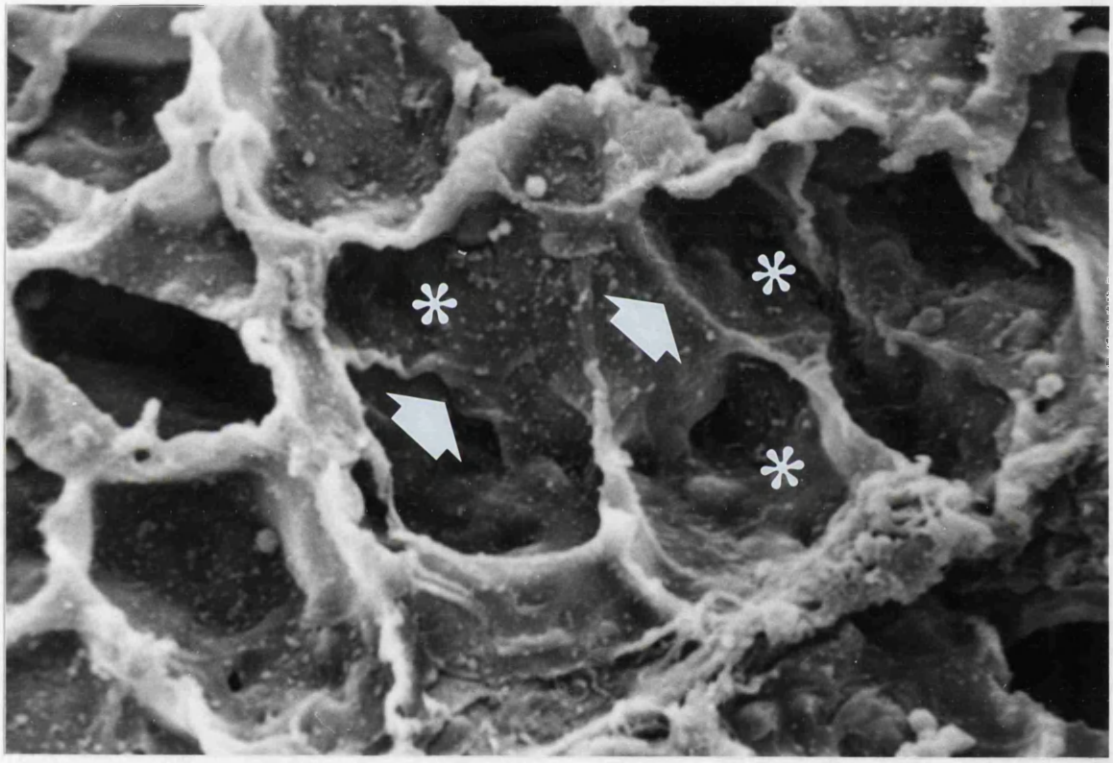


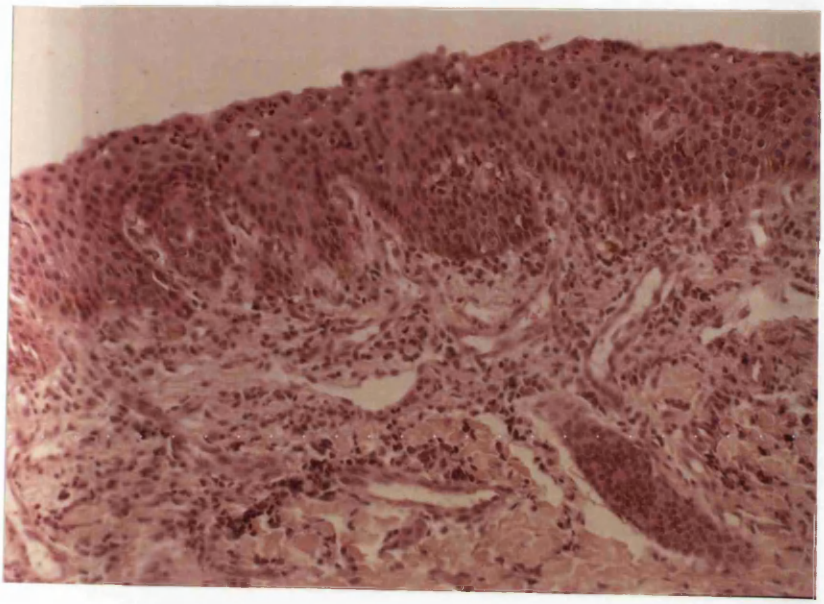
Fig. 6.37 Alveolar sac, 3-day-old kid.
Low septa (arrows) are seen subdividing a large alveolar sac into shallow alveoli (*).
SEM x 1,440.



# CHAPTER 7

Fig. 7.1 Nasal vestibule.
The epithelium and dermis are seen to be infiltrated by inflammatory cells.
H&E x 250.

Fig. 7.2. Ventral nasal concha.
Note the numerous surface mucus-producing cells in the epithelium (E). A thick mucous blanket (B) lies on the luminal surface.
AB /PAS x 250.



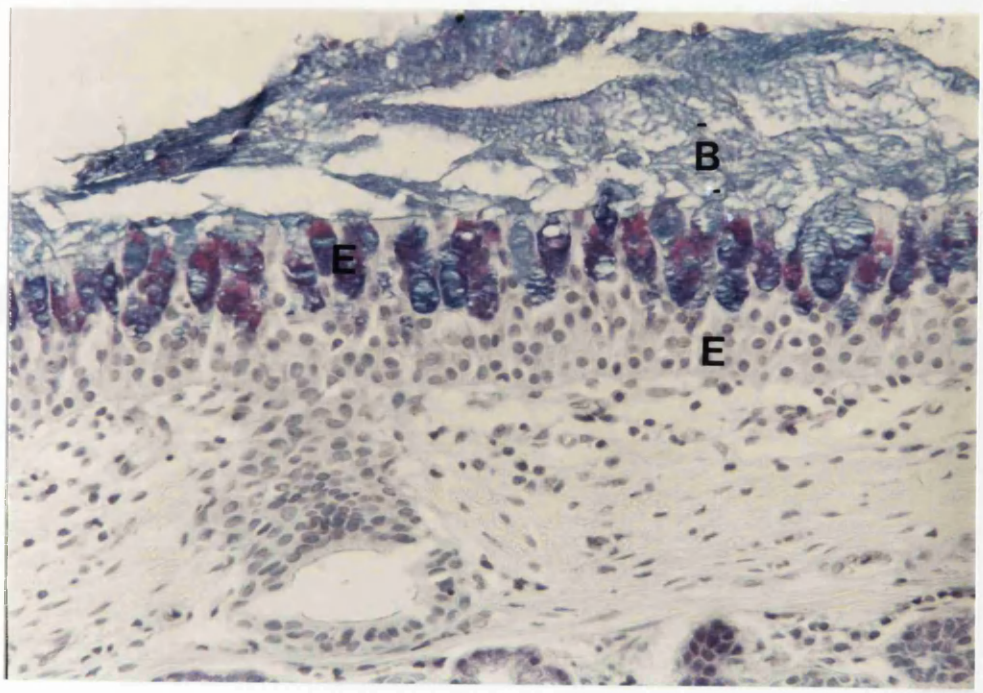
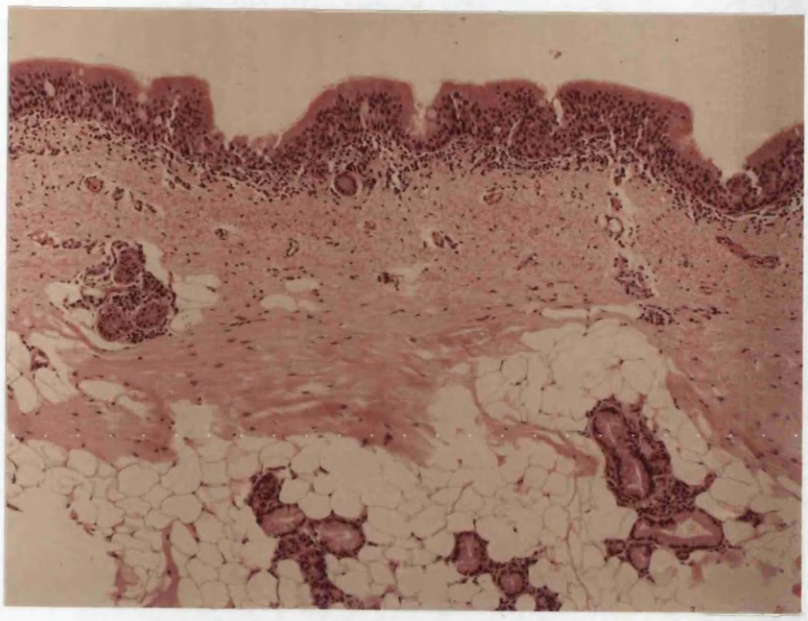

B
E
E

Fig. 7.3. Trachea.
A mild degree of inflammation, with some inflammatory cells in the lamina propria.
H&E x 100.

Fig. 7.4 Small bronchus.
The lumen is seen to be blocked by inflammatory exudate composed of mucus, debris and inflammatory cells.
H&E x 100.



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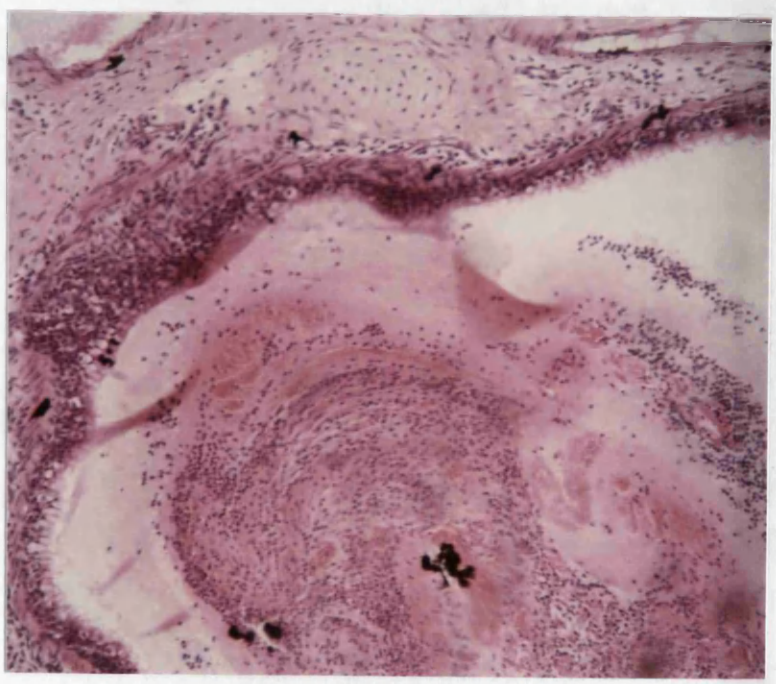

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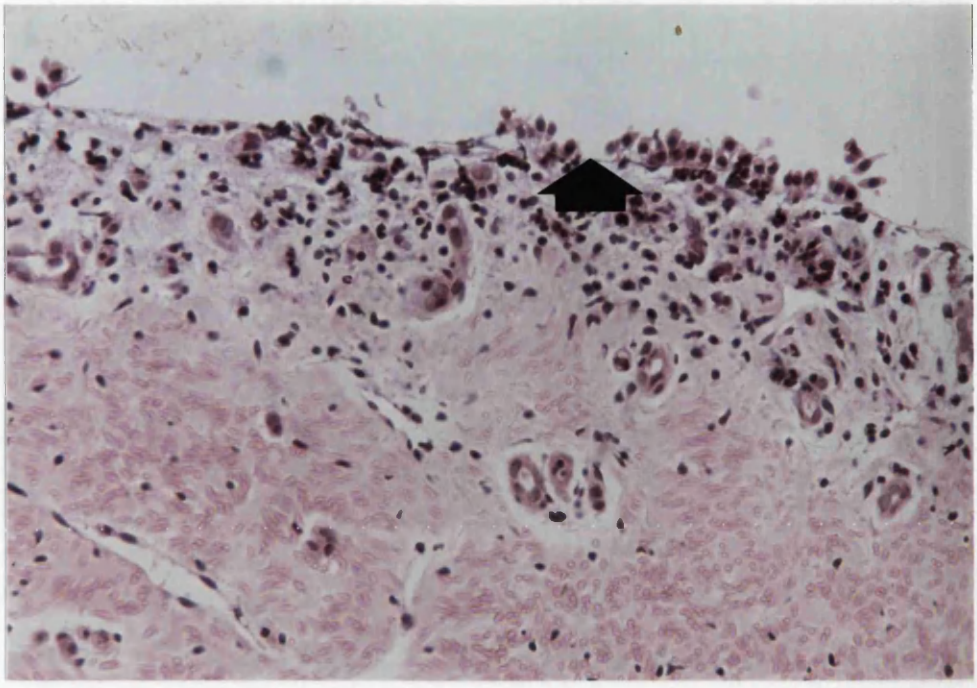
Fig. 7.5 Ventral nasal concha.
Most of the epithelial cells have desquamated, leaving an intact basal lamina (arrow) and a few basal cells.
Note an infiltration of mononuclear cells.
H&E x 250.

Fig. 7.6 Nasopharynx.
Disrupted lymphoepithelium. Lymphocytes can be seen passing through the ruptured epithelium (arrow).
H&E x 250.



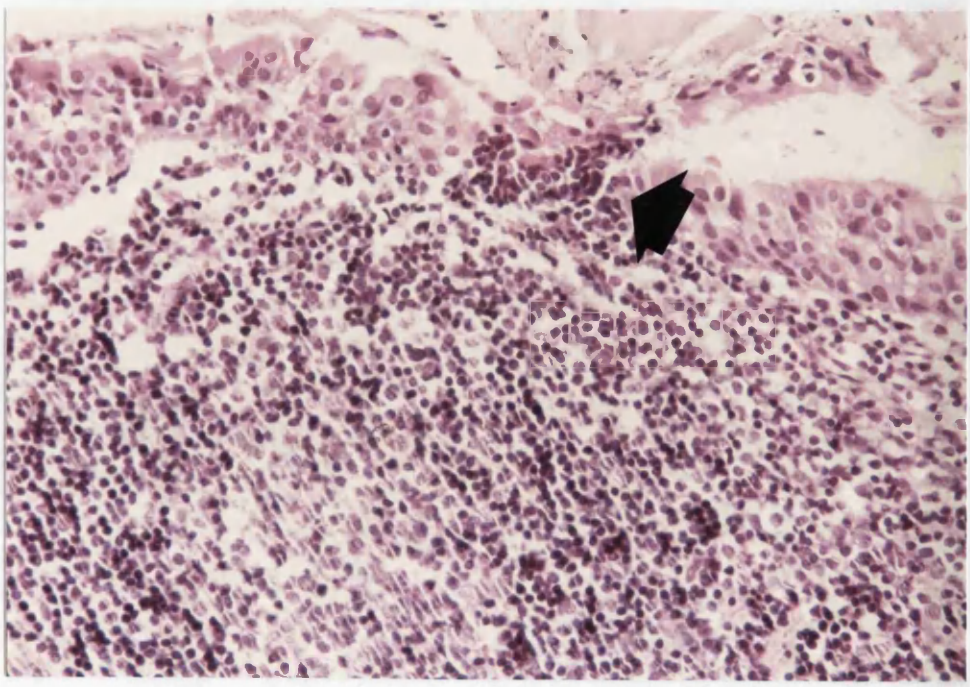
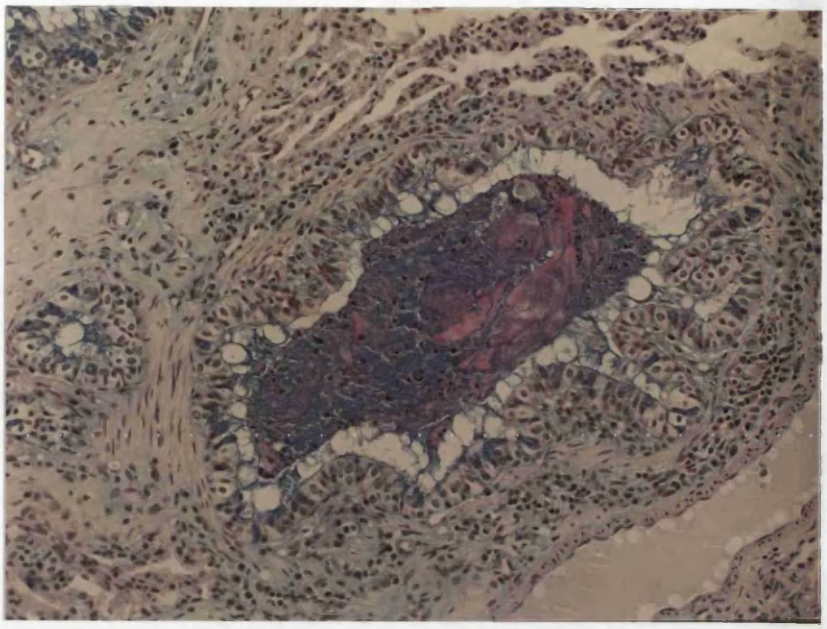


Fig. 7.7 Small bronchiole.
The lumen is blocked by inflammatory exudate composed of neutrophils and osmiophilic material. Note the presence of mucus-producing cells in the lining epithelium.
H&E x 250.

Fig. 7.8 Lung parenchyma.
There is extensive alveolar collapse. Note the accumulation of inflammatory exudate in the respiratory bronchiole (*).
H&E x 250.



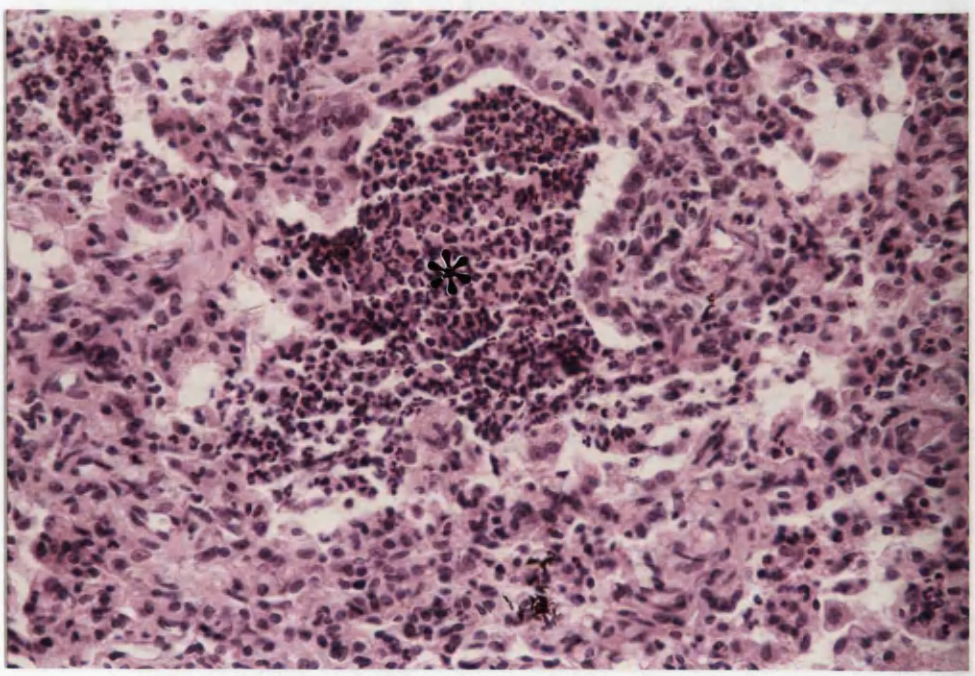


Fig. 7.9 Bronchiole.
The lumen is blocked by plugs of mucus, debris and inflammatory cells. Note the numerous numbers of mucus-producing cells.
AB /PAS x 250.

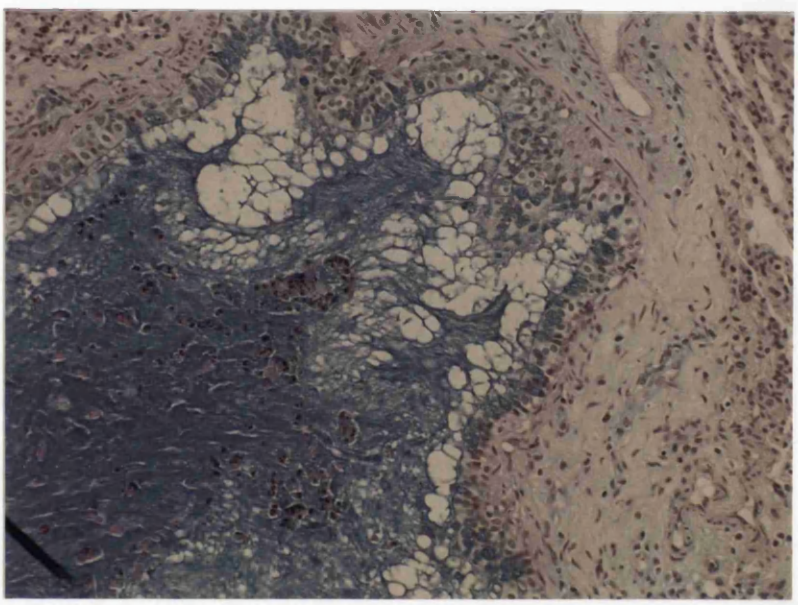
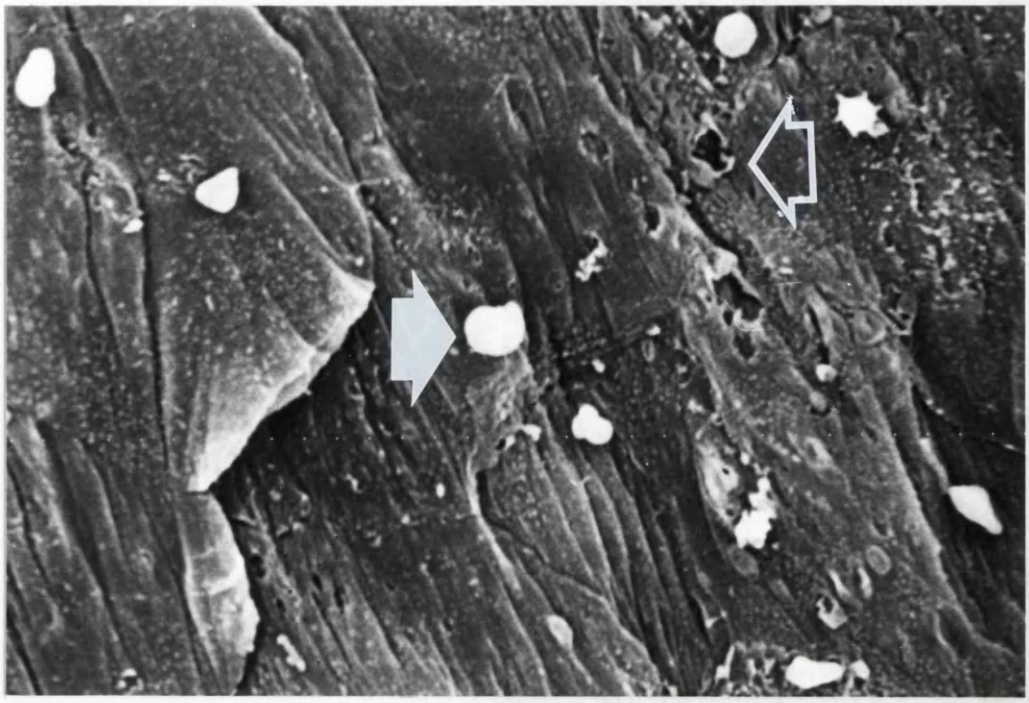


Fig. 7.10 Nasal vestibule.
Note the presence of micro-organisms lying on the luminal surface (closed arrow). Some squamous cells have pores (open arrow) on their luminal surface.
SEM x 5,600.

Fig. 7.11 Nasal vestibule.
High power micrograph of Fig. 7.10. Note the pores on the luminal surface of squamous cells. Cells presenting such pores were seen to increase in number towards the alar fold.
SEM x 11,250.



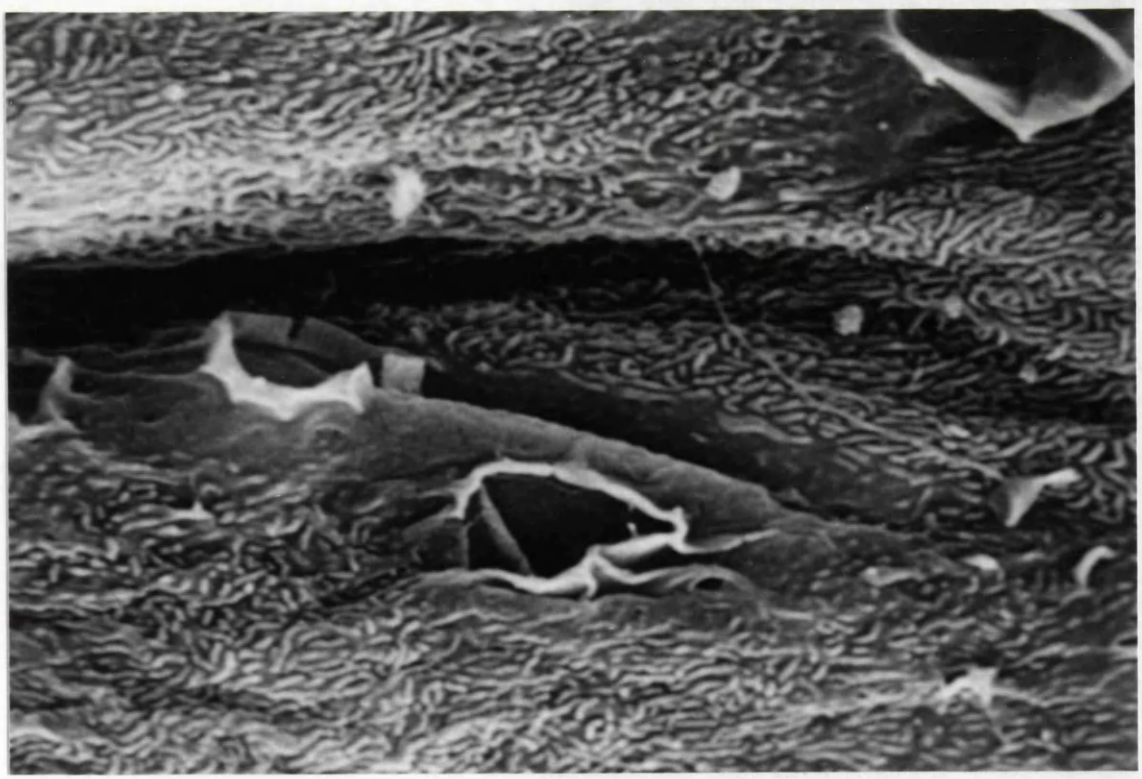


Fig 7.12 Alar fold.

(a) Mucus strands, micro-organisms and debris are seen lying on the surface.

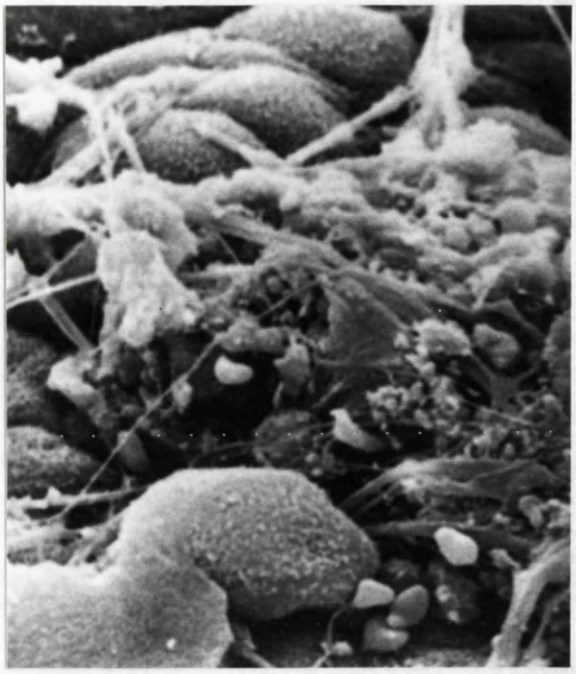
(b) In the caudal region, mucus strands were seen being extruded (arrow).

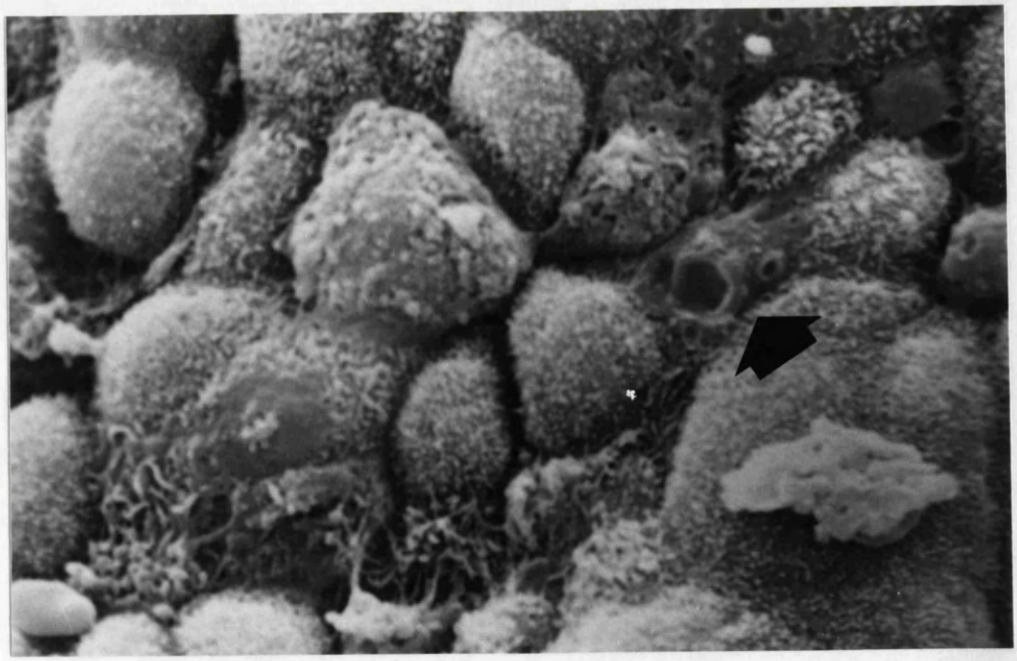
SEM (a) x 2,800, (b) x 5,600.

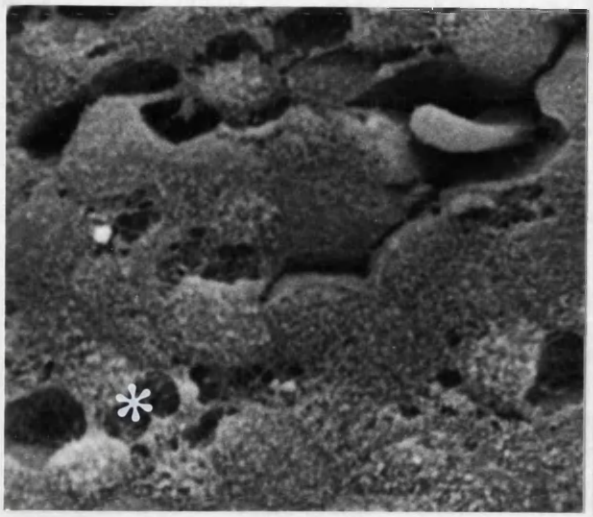
Fig. 7.13 Basal fold.

Mucus-producing cells are seen actively producing mucus (arrow).

SEM x 5,600.







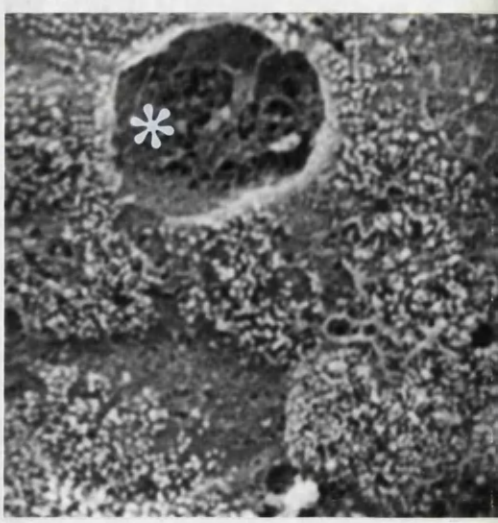
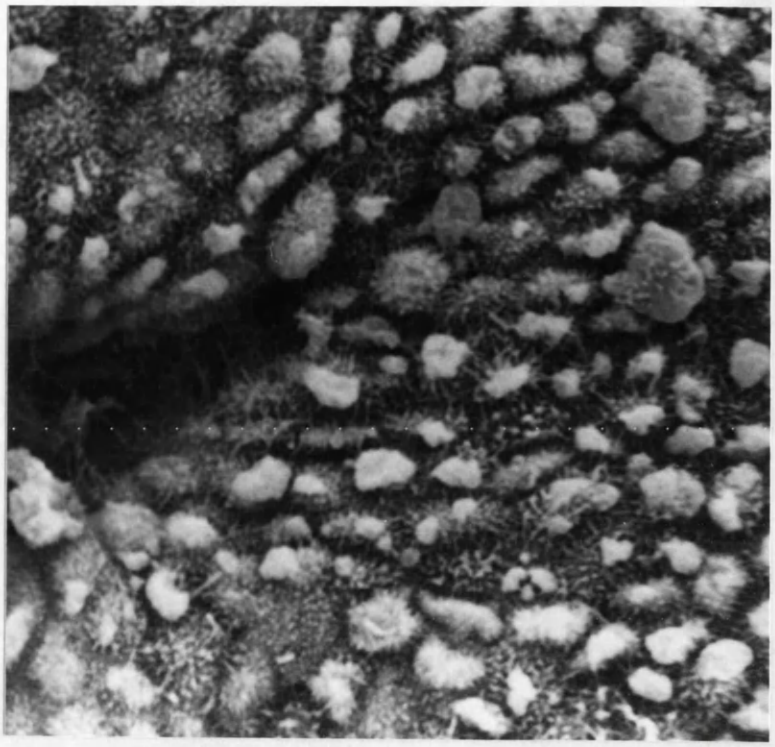


Fig. 7.14 Nasal septum.
The epithelium is completely devoid of cilia. Note discharged (*) and discharging mucus-producing cells.
SEM (a) x 2,800, (b) x 5,600

Fig. 7.15 Ventral nasal concha.
A patch consisting of numerous nonciliated microvillous cells. Small numbers of mature and regenerating ciliated cells are present. SEM x 2,800.

Fig. 7.16 Dorsal nasal concha.
Extensive deciliation is evident. Most of the cells are of the nonciliated microvillous type, possibly mucus-producing cells.
SEM x 1,440.

Fig. 7.17 Epiglottis.
The epithelial surface is seen to be extensively covered by a sheet of mucus (*). Individual squamous surface cells are seen in the exposed region, along with a few micro-organisms (arrows).
SEM x 1,440.



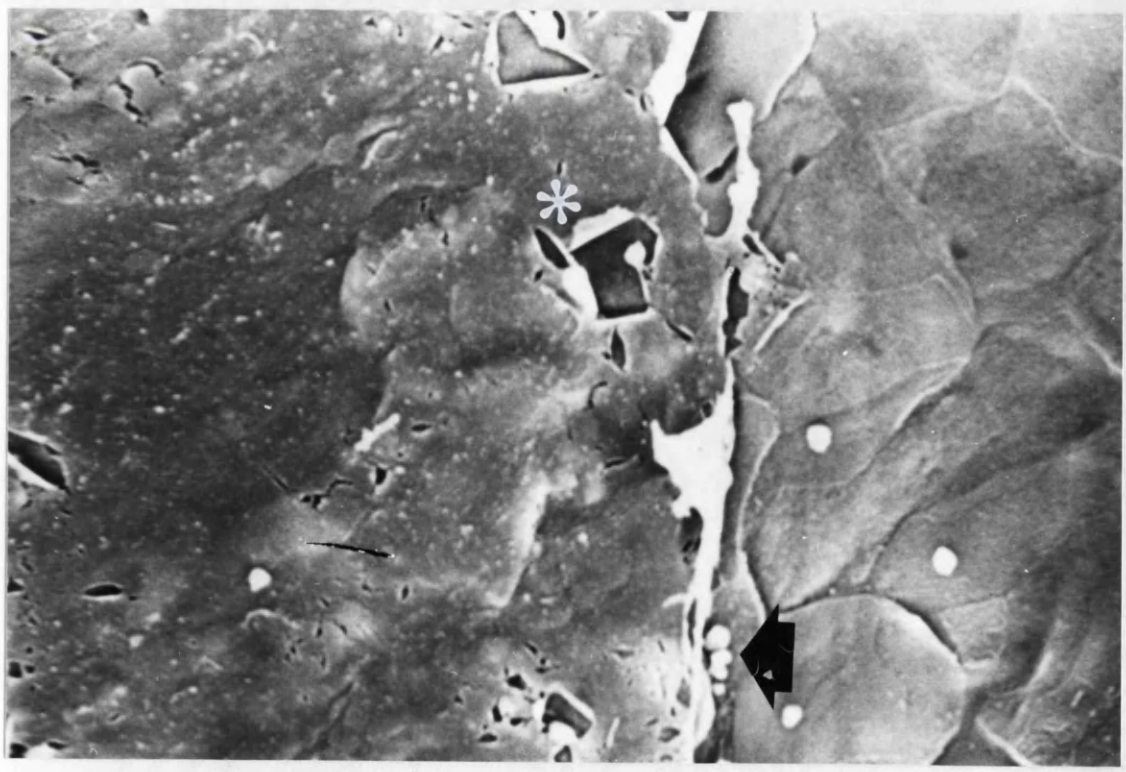


Fig. 7.18 Trachea.
Narrow clefts between mucosal folds are plugged with mucus and debris.
SEM x 360.

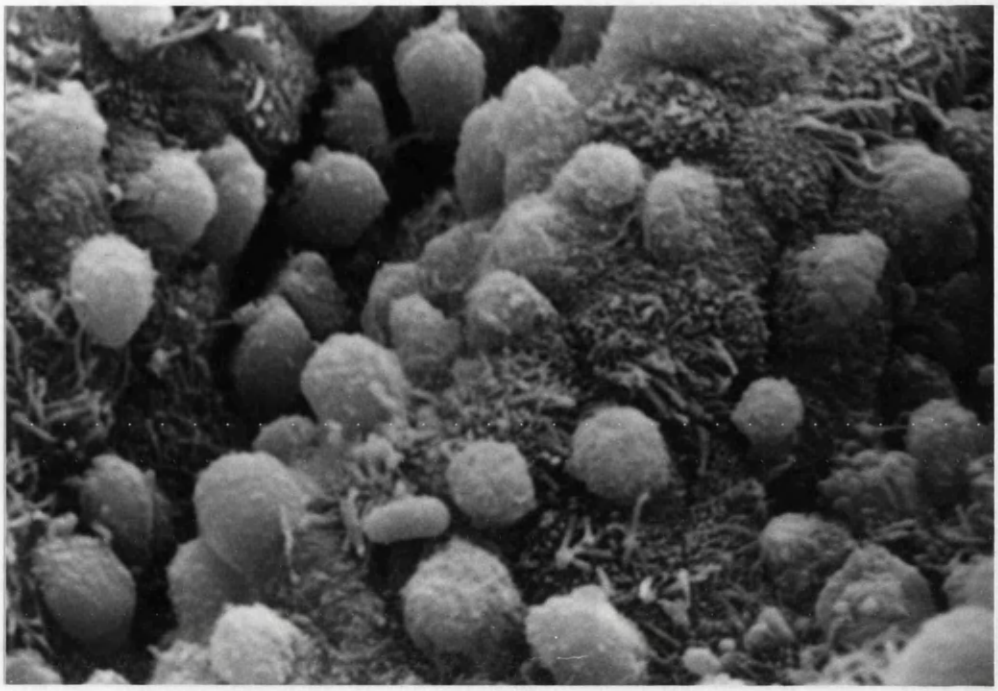
Fig. 7.19 Trachea.

Numerous highly active mucus-producing cells are seen. Note that mucus is being extruded in long columns.

SEM x 2,800.

Fig. 7.20 Bronchiole.
Note that stubby microvilli are not evident on Clara cells. Also note the poor degree of ciliation.
SEM x 5,600.

Fig. 7.21 Alveoli.
The alveolar surface is extensively covered by a sheet of amorphous material.
SEM x 2,800.



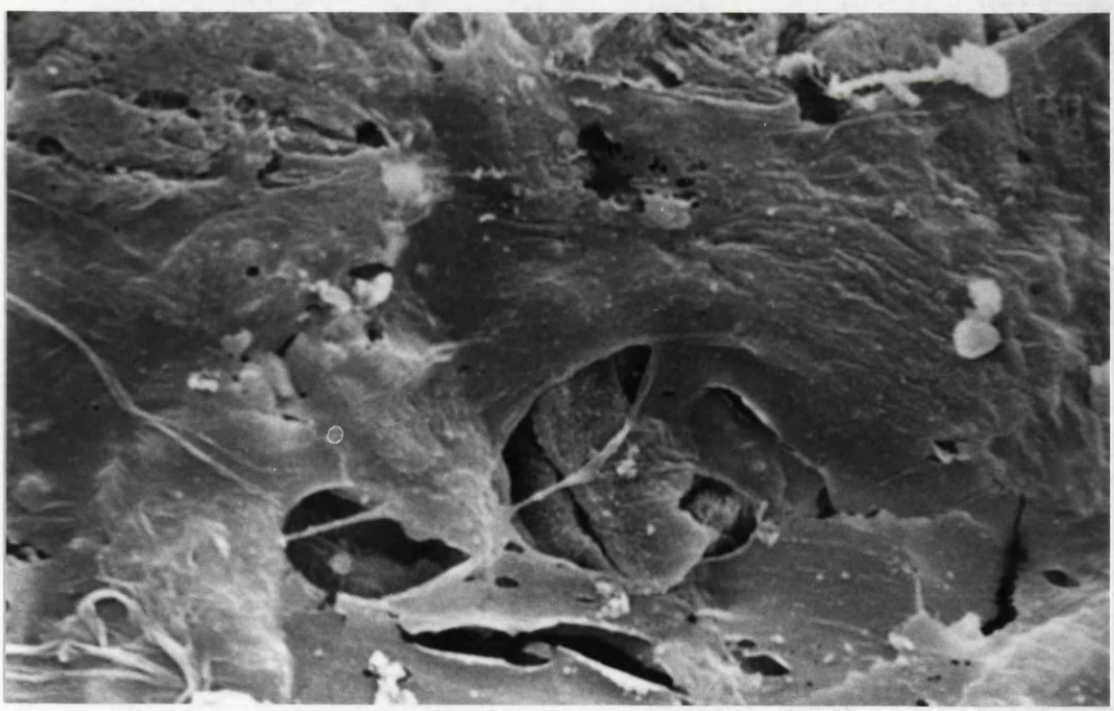


Fig.7.22 Alveoli.
Note the bulging blood capillaries.
SEM x 5,600.

Fig. 7.23 Alar fold.
Numerous apical cells are seen to be desquamating, leaving numerous depressions on the luminal surface.
SEM x 1,440.

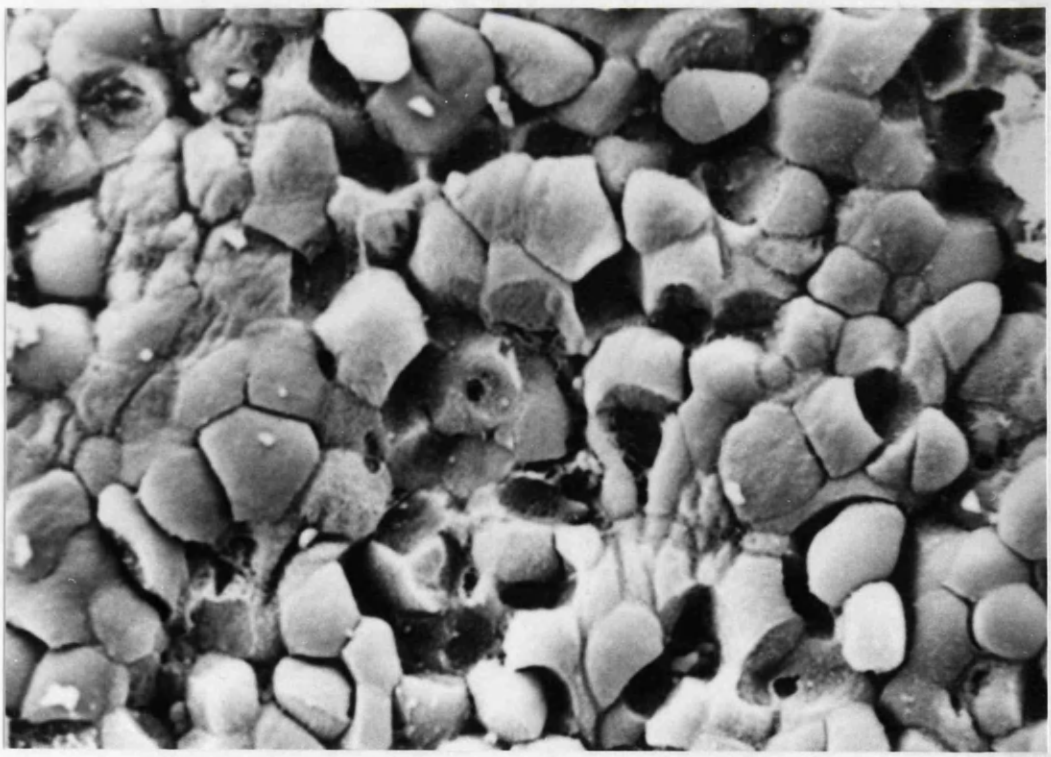
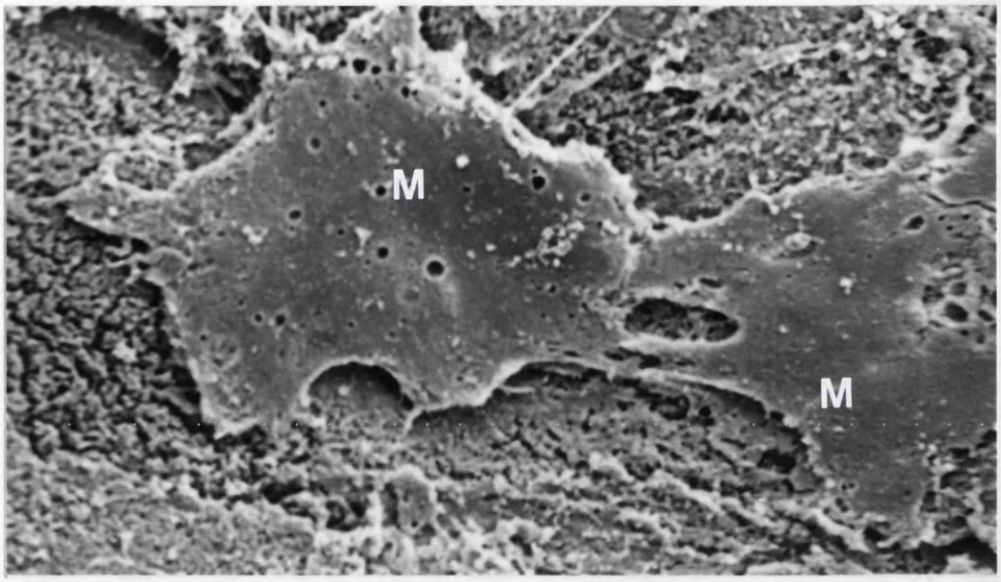


Fig. 7.24 Ventral nasal concha.
Extensive areas are seen to be covered by a mucous blanket (M).
SEM x 180.

Fig. 7.25 Dorsal nasal concha.
Mucus, debris and inflammatory cells are seen lying on the epithelial surface.
SEM x 1,440.


M
M

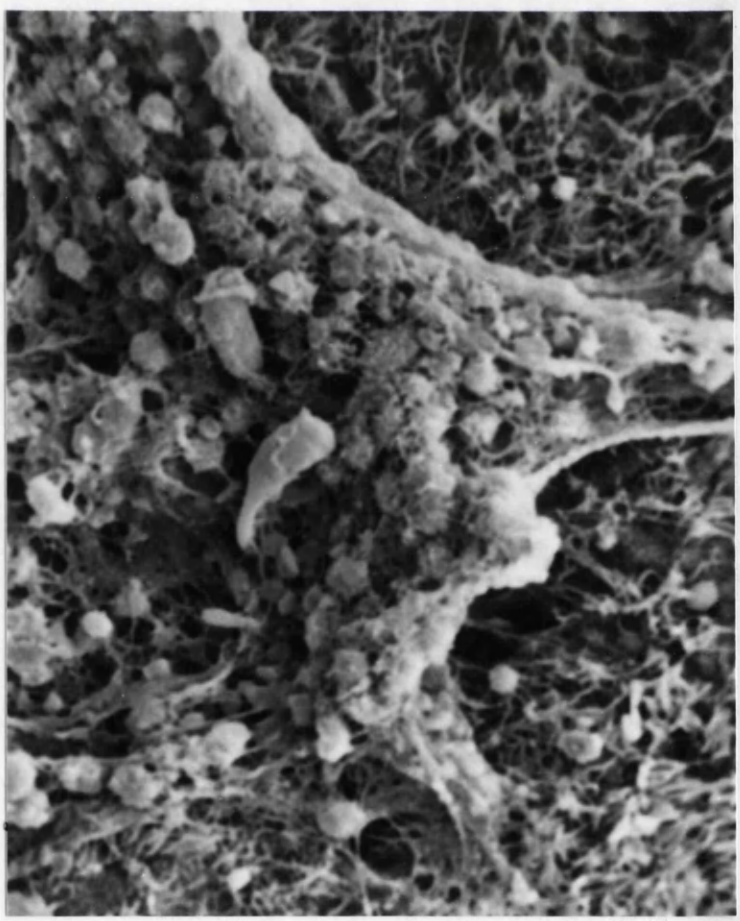


Fig. 7.26 Larynx (infraglottic cavity).
Extensive areas of epithelial desquamation are seen. Note both eroded (arrow) and intact epithelium (*).
SEM x 180.

Fig. 7.27 Trachea.
Extensive deciliation is observed, with ciliated cells being only poorly ciliated.
Note the mucous plug in the submucosal gland opening.
SEM x 5,600.

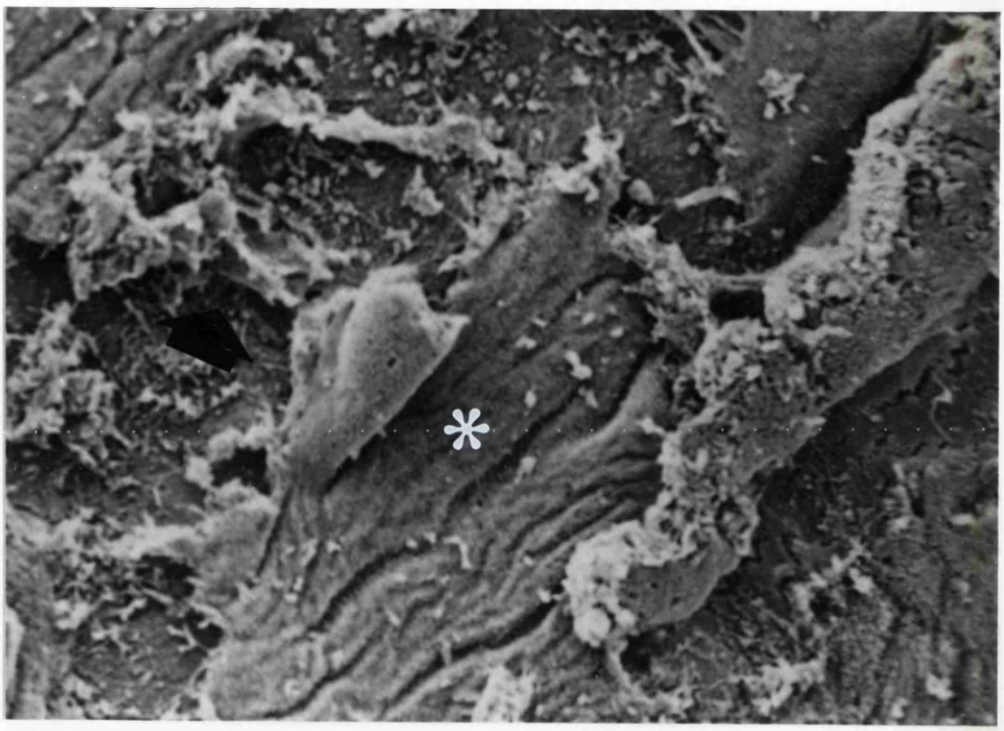


Fig. 7.28 Small bronchi.
A brush cell, with tall, spiky, densely organised, uniform microvilli (arrow), amongst ciliated cells.
SEM x 5.600.

Fig. 7.29 Bronchiole.
Note the presence of flocculent material covering most of the epithelial surface.
SEM x 1440.

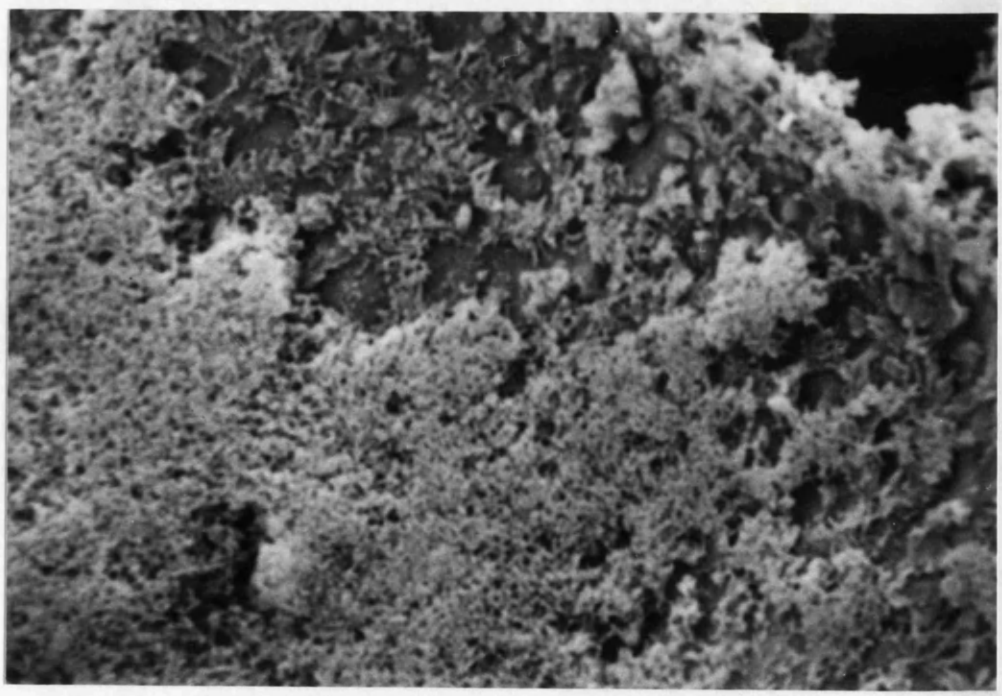
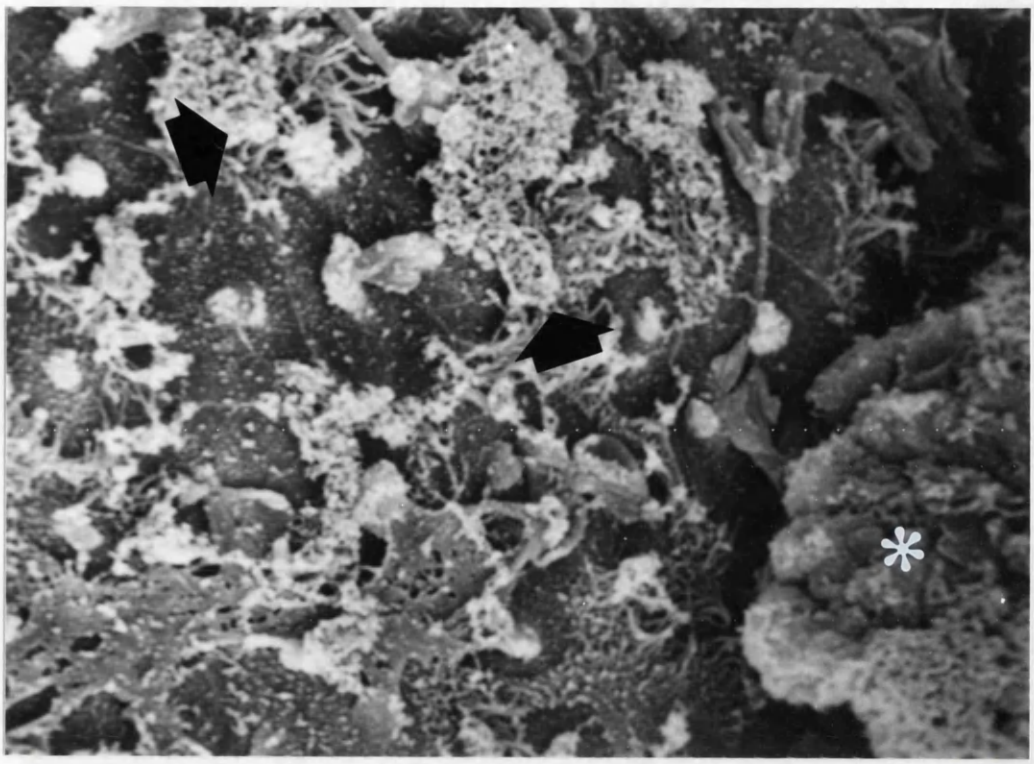


Fig. 7.30 Bronchiole.
Note the presence of flocculent material matting the cilia (arrows). Most Clara cells present a flattened apical surface and a few have collapsed apical protuberances. Cellular inflammatory exudate is also seen (*).
SEM x 2,800.

Fig. 7.31 Alveolus.
Note the aggregation of alveolar Type II cells.
SEM x 2,500.



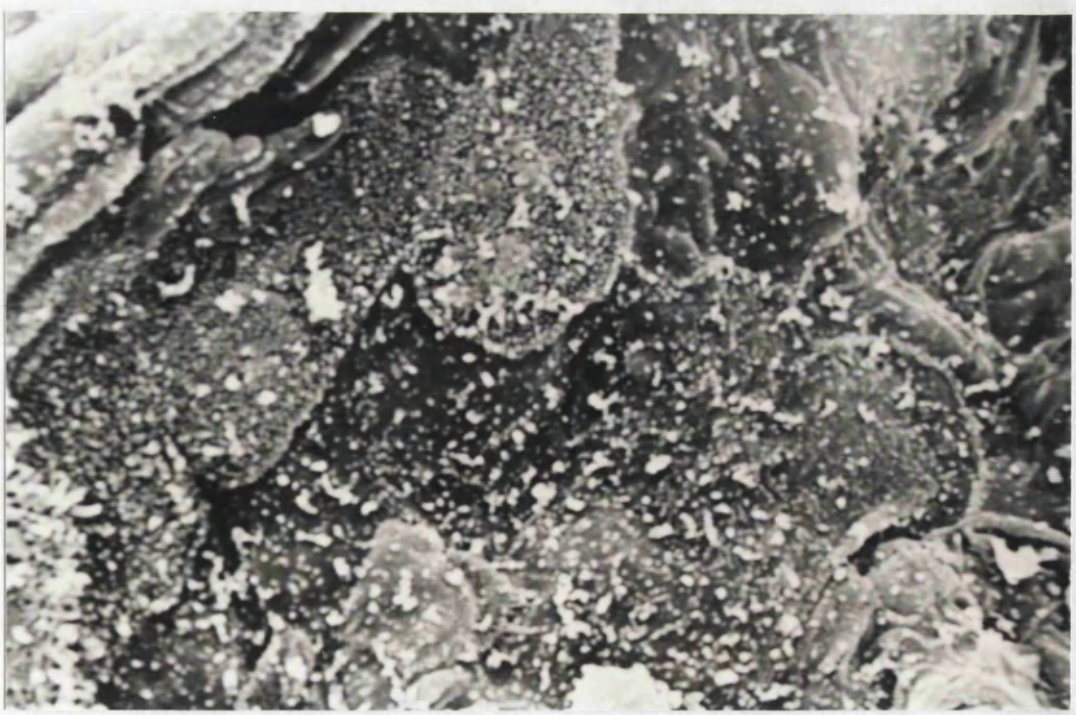


Fig. 7.32. Alveolar Type II cell.
A number of pores are seen on the luminal surface. Note that the central region of the luminal surface is sparsely populated by surface microvilli as opposed to the densely populated periphery.
SEM x 20,000.