SCANNING ELECTRON MICROSCOPIC STUDY OF THE LOWER RESPIRATORY TRACT IN CATTLE

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In memory of

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CONTENTS

LIST OF ABBREVIATIONS

LM = Light microscopy

NBF = Neutral buffered formalin

NI = Not immune

SEM = Scanning electron microscopy

SPF = Specific pathogen free

TEM = Transmission electron microscopy

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SUMMARY

The surface characteristics of the bovine lower respiratory tract were studied with the use of the scanning electron microscope (SEM). The first step in the investigation was to become familiarized with the methods of SEM. Thereafter two different projects were designed and performed.

The first project was to assess the pattern of ciliated cells in normal one week old calves and compared this with the pattern in adult cattle. Two groups of animals, one calves and the other adult cattle, were studied and none of them had gross morphological evidence of pulmonary disease. The trachea was examined as well as bronchi, bronchioles and alveoli in both the cranial and caudal lobes of the righ lung. In general in both groups, the lumenal surface of the large airways was completely covered by cilia apparently forming an efficient "mucociliary escalator". However, in the adult cows some areas of ciliated cells were found devoid of cilia, and these were considered to be abnormal. The non-ciliated cells in this jpart of the lower respiratory tract were not easily identified unless they were discharging secretion. In small bronchi, non-ciliated cells were more evident and based on the fact that these cells were present sometimes as frequently as the ciliated cells, they were thought likely to be epithelial secretory cells, either mucous or serous cells.

The bronchioles had many non-ciliated cells and almost no significant ciliated cells capable of forming a complete ciliary carpet. Type I and Type II alveolar epithelial cells, as well as alveolar macrophages, were identified in both groups of animals. Pores of Kohn were found in the alveolar walls in all the animals and considered to be normal. No brush cells were found. Distinctive respiratory bronchioles were not seen and there was a relatively sudden transition from terminal bronchioles to alveolar ducts.

After the normal pattern of the surface morphology was established for normal bovine lower respiratory tract, a second investigation was designed. This was done to assess the changes that could be observed with SEM on the surface of the respiratory tract of calves infected with Dictyocaulus viviparus and calves vaccinated against lungworm and experimentally infected with D. viviparus. Ten Friesian cross calves were divided into three groups which received different treatments. Group 1 comprised two calves vaccinated with Dictol as recommended by the manufacturers. Group 2 comprised four calves experimentally infected with approximately 5,000 infective larvae of D. viviparus and Group 3 comprised four calves vaccinated with Dictol at the same time as Group 1 and challenged orally with the same dose of D. viviparus larvae at the same time as Group 2. One calf from Group 1, Group 2 and Group 3 was killed on 15, 25, 35 and 45 days after challenge.

The changes observed in the luminal surface of the trachea, as well as the bronchi, bronchioles and alveoli of both the cranial and the caudal lobes of the right lung were recorded on the pleural surface of the lungs. In Group 2 the infected calves appeared with a variety of pathological changes as the infection progressed. These changes were mainly described as adult parasites and eggs in the bronchi, a severe cellular infiltration of the lung with worm eggs and aspirated larvae occupying the lumena of the alveoli.

Intercurrent infection was diagnosed occurring on the surface of the conducting airways, where microorganisms were found colonising the tips of the cilia. In addition, areas devoid of cilia and extruded cells were observed and considered to have resulted from viral infection.

The relative proportions of epithelial ciliated cells and non-ciliated cells were also affected at these levels. The surface of the conducting airways of the calves in Group 3 were slightly modified. The lung showed infiltration of immune cells, lymphocytic nodules implanted in the lung parenchyma in close relation in close relation to bronchioles and small bronchi, was the most relevant finding.

The SEM results produced three dimensional pictures which strikingly illustrated the modified epithelium and the reactions present during the course of prepatent and patent lungworm infection in susceptible and immune calves.

SECTION ONE

INTRODUCTION

REVIEW OF THE LITERATURE

INTRODUCTION

During the past 25 years studies using transmission and scanning electron microscopy have provided much information about the fine structure of the cells in the conducting airways and respiratory tissue of the lungs.

The discovery in the early 1960s, of the important physiologic role of lung surfactant led the way to the recognition of the lung as an active metabolic organ. It is now clear that the lung has important metabolic functions, influencing the whole body. The structure and function of alveolar tissue, both the epithelium and the interalveolar septum has been detailed on several occasions but less attention has been paid to the epithelium of the conducting airways. The multiplicity of cell types now known to be present in the epithelium of the airways in different species of animals has raised new questions about cell kinetics, relationships and functions. As a result of the increase in knowledge about these cell types in the lung and the renewed interest in the structure and function of these cells in relation to pulmonary defense mechanisms more answers will be found in the near future.

Understanding the different functions of the cells of the lung has now become possible with the availability of methods which study individual lung cells in culture as well as *in situ* and more recently by examining the interactions that can occur between the cells.

Systematic morphologic studies of the respiratory system in numerous species has been the subject of many recent publications. In them the object was to investigate and compare the morphologic features of developing and mature lung, in commonly used domestic and laboratory animals as well as man, in order to establish structural and functional relationships when possible. Until recently, very few comparative anatomic studies had been performed and in almost none of them is the ox included. There is a tendency to extrapolate from one species to another, however it has been

found that although the same epithelial cell types are found in most species their detailed morphology and frequency distribution varies. In addition, there are some aspects of the distribution of the airways that are also species specific and some caution should be taken when comparing structures.

Knowledge about the respiratory system in the ox is important not only for reasons of comparative biology but also because respiratory disease is very prevalent in this species. In the past 20 years as the numbers of cattle kept on the farm unit has increased under economic pressures there has been a concomitant rise in the prevalence of respiratory illness. Respiratory infections are a significant problem all over the world, in countries involved in intensive beef or dairy production. The total economic impact of the diseases results from their high morbidity and ability to cause death. All age groups of cattle have their own particular problems. The serious respiratory diseases of adult cattle include fog fever, farmer's lung, diffuse fibrosing alveolitis and reinfection with lungworm. In young grazing calves parasitic bronchitis and pneumonia caused by the lungworm Dictyocaulus viviparus, is very important. The indoor calf pneumonias represent a major common and economic problem from which many infectious agents have been isolated.

The availability of the scanning electron microscope (SEM) has provided an important tool that has been extensively used to study the surface of the respiratory passages and pulmonary alveoli, in several species. The use of SEM has brought about a major advance in our understanding and appreciation of the three dimensional organisation and surface topography of tissues and organs. The instrument is particularly useful for studying the respiratory system because of its ability to examine large surface areas rapidly at either low or high magnifications with greater depth of focus at equivalent magnifications than any other light or electron optical system. The large area that can be examined, the intrinsic depth of focus, and the relatively simple specimen preparations, make the SEM very useful for the examination and evaluation of

normal and abnormal lung structure. In particular, SEM has provided information about the size, shape and density of surface structures characterising various respiratory cell types. It has also usefully been applied to demonstrate the architecture of alveoli and their relationship to each other and to bronchioles. Since there have been few studies with SEM on the respiratory system of the ox it seemed useful to conduct the study described in this thesis. This study involved the examination of the epithelial surface of the lower respiratory tract of normal calves and normal adult cattle to provide more information about the morphology of this region. The objective was to describe and characterise the distribution of the ciliated cells forming the mucociliary apparatus, to assess differences in the surface related to age in normal bovine animals and then to associate these morphological patterns with pathological conditions such as lungworm infection.

REVIEW OF THE LITERATURE

The scanning electron microscopy (SEM) has been extensively used to study the surface of the airways and respiratory acini of the lungs (Andrew, 1979). By definition the SEM reveals the surface topography of a specimen. Although it has been used for a number of years by scientists to study inorganic material e.g. steel, bioligists have only recently developed techniques for preparing non-conductive soft tissue for examination in the SEM (Nowell and Tyler, 1971). One of the most useful characteristics of this instrument is the ability, not only to examine a large surface area, but to do this rapidly. Weibel (1963) had estimated that the lung from a human adult has 95 m^2 of air-tissue interface. Examination of a significant amount of this surface by transmission electron microscopy (TEM) is not feasible. Pease (1964) has estimated that it would require seven and one half years of continuous TEM use to photograph 1 cm^2 of ultrathin sectioned material. This comparison emphasised the usefulness of an instrument capable of being applied to study the morphology of large surfaces. Apart from the large surface area that can be examined by SEM it is possible to examine longitudinal or oblique conducting airways and assess the relative proportions of different cells and their distributions on a surface (Nowell and Tyler, 1971). It has been reported in a review of microscopic studies on the respiratory tract that 13 cell types (Table 1) have been identified in the epithelium (Breeze and Wheeldon, 1977).

A recent review (Tyler, 1983) provided detailed information on the physicochemical nature of airways secretions. The anatomy, pharmacology and physiology of airways and their secretion in health and disease were reported. Comparative morphologic aspects of the airways from various species were presented by Tyler (1983) but most of the information was based on TEM and light microscopy (LM) studies and was not confirmed by SEM. Although SEM is limited to the examination of surfaces it is very useful for studying distal airways as large blocks can be examined at very low or high magnification with high resolution and the view of the

COMMON CELL TYPES IN NORMAL LUNGS

Epithelial cells	Gland
Airways	mucous
Ciliated	serous
Goblet	
Epithelial serous	Alveoli
Brush	Type I pneumocytes
K (including neuroepithelial body cells)	Type II "
Basal	Type III "
Intermediate	Alveolar macrophage
Special type	
Non-ciliated bronchiolar secretory (CLARA)	

Connective tissue cells

- Globule leukocyte
- Lymphocyte
- Plasma cell
- Subepithelial mast cell
- Connective tissue mast cell
- Eosinophil
- Cartilage

Table 1: List of different cell types, according to Breeze and Wheeldon (1977).

surface provides much of the information in a readily comprehended form. In addition, material prepared for SEM can be processed for examination by TEM or LM. Carr (1981) described procedures for examining samples from SEM by TEM or LM on 1μ sections after embedding the sample in resin. In addition there have been studies made on several species by LM and TEM and they have described differences in the comparative anatomy of the lungs.

In humans, the lung at birth is not a small version of the adult lung (Reid 1972). Alveoli develop after birth, increasing in number until the age of eight years and then increasing in size until growth of the chest wall is finished. The known pattern of growth of the human lung has facilitated the interpretation of the pathogenesis of certain pathological changes. Bastacky (1983) described the structure of human airways by a combined study using SEM and airways dissection. He examined and mapped the luminal surface of a single airway from the point where it had its origin from the main stem bronchus through large and small bronchi to bronchioles and continuing to alveolar ducts and to alveoli as far as the pleura. He obtained lobes of human lung by surgery. They were inflated with air, allowed to deflate and then reinflated with fixative. The airway was dissected and examined with a stereomicroscope, then the samples were processed for SEM and the airway mapped at different magnifications. The interesting areas were then excised and processed for TEM. He described a unit airway as that continuous luminal surface consisting of one branch of the airway at each bifurcation. Preliminary studies suggested that alveoli varied in shape, size and entrance width; all of which were a function of the position of the alveoli along the airway. The transition from ciliated conducting airway epithelium to squamous respiratory epithelium was quite abrupt. The surface of alveoli in the hydrated state were quite smooth, tended to be round and had surfactant occluding almost all the pores of Kohn.

Jeffery (1983) proposed a classification (Fig. 1) for all the surface epithelial types on the basis of a review of their features as determined by light and electron microscopy. The classification

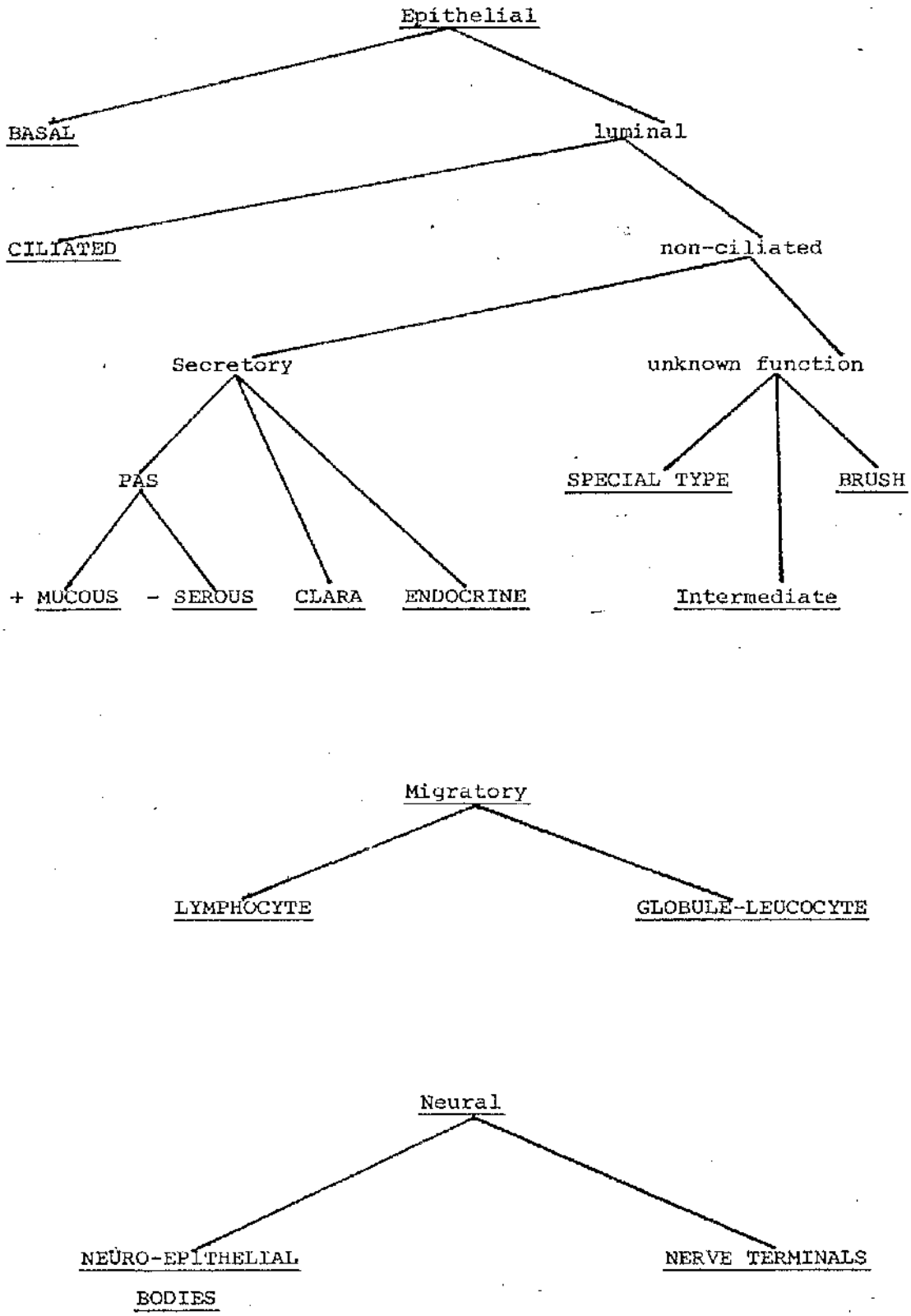


Fig. 1. A classification of respiratory tract surface epithelial cells as proposed by Jeffery 1983.

was based on morphologic features and took into consideration the occurrence and species distribution of each cell type. Jeffery (1983) said that many, but not all, of the epithelial cells were found in humans. Ciliated, mucous, Clara non-ciliated bronchiolar, and basal cells were consistently identified in all mammals as judged by light microscopy and transmission electron microscopy. Not many complete studies had been done by SEM, therefore there was a shortage of information in this respect. In his conclusion he suggested that any one cell type might perform more than one function and that further research would confirm or refute these hypotheses.

Jeffery and Reid (1975) had made a systematic survey of the ultrastruture of the cell types at different airway levels in specific pathogen-free (SPF) rats. The main objective was to establish how far proximally Clara cells were found. The study was done using TEM to examine five extrapulmonary levels and five intrapulmonary levels. The distribution and frequency of each epithelial cell type, epithelial thickness and depth of the ciliary layer were assessed and were expressed as a percentage of the total epithelial cells counted. Nearly 50% of the cells at the level of the upper trachea and distal bronchioles were non-ciliated but at the lower part of the trachea, extrapulmonary bronchus, intrapulmonary bronchus and small bronchus only 40% were non-ciliated. Taking all levels together 22% of the epithelial cells were serous and this was the most frequent cell type at all airway levels except the upper part of the trachea. The serous epithelial cells and the Clara cells could not be distinguished from each other by TEM and were counted as one cell type. Goblet cells made up less than 1% of the total at any level. The intermediate cells were most numerous at the upper part of the trachea and significantly fewer were found in the distal parts. Basal cell decreased progressively distally. Migratory cells, lymphocytes and globule leucocytes were considered together in these counts. Lymphocytes were more numerous in extrapulmonary airways. Both lymphocytes and globule leucocytes, were greatest in the upper segment of the

trachea, and their concentration progressively decreased in the extrapulmonary and intrapulmonary bronchus.

In the mouse the conducting airways were studied by Pack et al. (1980). A quantitative light microscopic and TEM investigation was made at different airway levels since it was considered that this had not been carried out in the mouse previously. They tried to establish the distribution of cell types in the airways and to determine whether the cells containing mucus occurred in the primary bronchi or smaller airways. Previous workers had shown that the mucous cells of the mouse occurred in larger numbers in the lower trachea than the upper trachea, especially at the level of the carina. Whether this increase in the mucous cell population persisted in the primary bronchi was unknown. The investigation was done using MFI strain (S.P.F.) mice. The epithelium of the conducting airways was sampled at five levels from the trachea to the distal bronchi and three sections from each level were then observed by LM and TEM. The authors concluded that, contrary to what was found in other species, the majority of the cells (50-60%) were Clara cells. Mucous producing tissue was infrequent although epithelial mucous cells occurred in large numbers at the carina and primary bronchi. In their observations they pointed out that the paucity of mucous cells in the trachea and primary bronchi and their absence from the axial airways had been done only in SPF animals and when stock mice were examined some mucus-containing cells were more frequently seen at every level. On the other hand, no mucous or serous cells or submucosal glands were seen in the SPF animal at the intralobular airways. On the morphological basis, as judged by TEM, Pack et al. (1980) recognized three distinctive forms of Clara cels. On occasion they also observed cells which were apparently transitional types between these three types of Clara cells and also between Clara cells and mucous or ciliated cells. It was suggested that the transforming cell type might indicate a role for the Clara cell as a basic developmental cell in the generation of the other cell types. Although the Clara cell is generally thought to be a secretory cell type characteristic of the epithelial lining of bronchioles, some investigators (Plopper,

1983) have shown that in some species non-ciliated cells closely resembling those in the bronchioles, the Clara cells can be found in the epithelial lining of proximal cartilaginous airways i.e. trachea and bronchi.

In 1975, Castleman et al, studied the morphology of the intrapulmonary airways in three species of monkeys by SEM and TEM. Their results indicated that there was general similarity between the mucosal structure of the bronchi and the respiratory bronchioles in the three different monkeys and man. There were, however, differences in the number of generations of terminal bronchioles and differences in their epithelial lining. The objective of the study was to find a model for experimental studies on human pulmonary disease processes. There were some differences described by McLaughlin et al. (1961) between the distal airways of monkeys compared with rats and pigs, who have very short respiratory bronchioles; however, cats and dogs have long well developed respiratory bronchioles. McLaughlin et al (1961) described the main subgross anatomical features of the lungs of various mammals and had grouped them into three distinctive subgross lung types, according to which the monkey was considered to be included in the second group with the cat and the dog. This group was characterised by the absence of secondary lobules, with an extremely thin membranous pleura. The most distal airways were composed of numerous well developed respiratory bronchioles leading into large alveolar ducts. The monkeys were an extreme example of this, possessing only short, large, and well alveolarised bronchioles which terminate in lengthy alveolar ducts. The diameter of the distal portion of these inflated bronchioles were 0.5 mm. Castleman et al. (1975) pointed out in their conclusion that the terminal bronchioles in macaques were lined by pseudostratified columnar epithelium. They could not find non-ciliated cells similar in morphology to bronchial brush cells reported by Watson and Brinkman (1964). An unusual form of ciliated cell containing membrane-bound inclusions was described by Castleman et al. (1975), they were often observed in one of the species of monkeys. This cell was suspected to have a secretory

function. In addition, whereas non-ciliated cells containing secretory droplets, comparable to human Clara cells were observed in bronchioles of bonnet and stumpystail monkeys, no such cells were obsrved in the rhesus monkeys which are usually considered to be similar to man. They suggested that non-ciliated bronchiolar epithelial cells with a merocrine secretory function were not normally present in rhesus lungs or that they were extremely scarce in comparison to the other macaques. In both, man and macaques alveoli were seen as outpockets from the respiratory bronchiolar wall and these alveoli increased in frequency as more distal areas were reached. Cuboidal cells although continuous in the initial portions of respiratory bronchioles becomes dispersed between squamous epithelial cells in more distal areas. In distal areas of the respiratory bronchioles cells were observed containing osmiophilic lamellar bodies. These cells were identical in morphology to type II alveolar epithelial cells. It could be that type II cells lining respiratory bronchioles have a similar function and lower surface tension in the distal airways (Castleman et al., 1975).

In a recent report six morphologically distinctive granule containing secretory cells were described in the sheep lungs (St. George et al., 1983). These cells were referred to as four mucous cell types (M1-M4), serous cells (S.C.) and Clara cells (C.C.). The cells were classified on the basis of their airway distribution and their ultrastructure. Mariassy and Plopper (1983) examined airways generation in sheep lung by microdissection, and then examined by LM tissue samples embedded in resin. Based on differences in cell morphology, staining properties and distribution, eight major cell groups were recognised and quantified. The four new mucous cell categories similar to those of St. George et al. (1983), were referred as M1, M2, M3 and M4; ciliated cells, basal cells, Clara cells and serous cells were also described. They said that the serous cells were restricted to submucosal glands. The tracheal epithelium had the most cells per unit length, primarily due to large numbers of basal cells. Basal cells were found in the epithelium of airways without cartilage or

glands. The goblet cells, M1, M2, M3 and M4 in proximal airways were a constant finding and M4 goblet cells (containing sulphated glycoproteins) were present in glands of the proximal airways and in the epithelium lining the airways without gland. The most distal airways were lined by Clara cells and ciliated cells and were free of glands, basal cells and goblet cells.

There was no correlation between submucosal structure, generations of airway branching and epithelial cell populations. They found a number of generations of intrapulmonary airways lacking cartilage, which had submucosal glands, basal cells, mucous cells, and ciliated cells. They also found basal and mucous cells in the bronchioles which also contained Clara cells. In the most distal bronchioles these were only secretory cells present. They concluded that while a distribution pattern of cell populations in the sheep tracheobronchial tree was clearly related to airway generations, there was no distict relationship between the distribution of epithelial cell types and the submucosal structures (glands and cartilage).

The tracheal epithelium differed between cartilaginous and non-cartilaginous regions, in height and in the abundance of basal cells. The epithelium was lower and basal cells fewer in non-cartilaginous regions. The epithelium of the extrapulmonary bronchi and that of a few proximal intrapulmonary, cartilaginous bronchi resembled the epithelium of the trachea. The first 20 generations of airways in both lobes had about the same number of cells. The density decreased in the distal airways in the cranial lobes, which had fewer total airway generation in the axial pathway, compared with the caudal lobes. The basal cells were about 25% of the population in the trachea and decreased progressively in more distal generations. The percentage of ciliated cells was between 35 and 65% being lowest in the trachea and the most distal airways. The proportion of secretory cells, within the epithelial population was approximately the same in all the cartilaginous airways and increased in the distal non-catilaginous airways.

The rabbit was the only other species in which a similarly detailed study has been performed (Plopper et al., 1980). In contrast to the sheep, which has been between 20 and 23 generations of cartilaginous airways with submucosal glands, only the five most proximal generations of intrapulmonary airways of the rabbit had cartilage and most of these lacked glands. Goblet and basal cells were not as extensively distributed in rabbit airways as in sheep and the wide variety of epithelial cell types in proximal airways was not present. Less detailed studies in the hamster (Kennedy et al., 1978) rat (Jeffery and Reid, 1975) and mouse (Pack et al., 1980) have shown similar differences when compared to the sheep. As in the mouse, the predominant secretory cell lining all airway generations in the rabbit was the Clara cells (Pack et al., 1980). Mucous goblet cells were rarely observed in this species. The serous cells were the major secretory cell in the proximal airways of the rat (Jeffery and Reid, 1975). On the other hand, the mucous cells had the same role in the hamster, being the main secretory cell (Kennedy et al., 1978). Clara cells lined distal airways in all of these species.

Basal cells also had interspecies variation in distribution and abundance. More than 60% of the airways in the sheep had basal cells, whereas they were found in less than a third of proximal airways in the rabbit (Plopper et al., 1980). The rat (Jeffery and Reid, 1975) and hamster (Kennedy et al., 1978) apparently have an arrangement similar to the rabbit. The density of basal cells in the most proximal airway generations of the sheep, trachea 28.5% and primary bronchus 18% (Mariassy and Plopper, 1983) were similar to those in the rabbit, trachea 28% and primary bronchus 27% (Plopper et al., 1980); the rat, trachea 27% and bronchus 27% (Jeffery and Reid, 1975) and the hamster, trachea and bronchus 20% (Kennedy et al., 1978). The mouse however has lower densities of basal cells, trachea 8%, primary bronchus 3.5% (Pack et al., 1980). The findings on cell distributions in the sheep when compared with other species suggested differences in airways function between species (Mariassy and Plopper, 1983).

It seems likely that the composition of the mucociliary blanket would be different in the different species. In addition, variation in the abundance of secretory cell types and glands in different airways generations of the same species suggested that the lining varies within the tracheobronchial tree itself (Mariassy and Plopper, 1983). They inferred that the differences in the extent of basal cells within the tracheobronchial tree of rabbit and sheep may indicate a difference in potential for responding to epithelial injury by infectious or toxic agents. Basal cells were thought to be a primary source of new cells for epithelial replacement in proximal airways (Gordon and Lane, 1974; Kauffman, 1980). The sheep not only had greater numbers of airways generations with progenitor cells, but also had a greater abundance of these cells in distal airways than did the rabbit (Mariassy and Plopper, 1983). A number of factors may influence airway epithelial repair in response to injury including degree of injury number of progenitor cells present, their rate of mitosis and the rate of differentiation of daughter cells. They also emphasized the necessity for clarification of the effect of progenitor cell abundance on the epithelial repair process following injury.

Although there have been many detailed studies on the nature and distribution of epithelial cells, in the airways of several species, using the TEM and LM, there is not much information about the character of the surface epithelium of these airways seen by SEM.

Nowell and Tyler (1971) conducted a SEM study on the surface morphology of mammalian lungs on four horses and six hamsters, to determine the features of the airways and compare a variety of methods of preparing pulmonary tissues for SEM. The lungs from horses and hamsters were perfused via the airways with fixative, using cacodylate buffered, glutaraldehyde of physiologic osmolality and cacodylate buffered formaldehyde-glutaraldehyde. After dehydration by graded solutions of ethanol, some were air dried and some were dried by the critical point drying methods (CPD) using carbon-dioxide. In addition, hamster lung was prepared by freezing

in liquid propane at -175°C after thoracotomy. The frozen lung was then sectioned and put in absolute ethanol at -84°C and subsequently dried by CPD. The specimens that were fixed with aldehyde fixation and follow by CPD were most useful because the mucous blanket and surfactant were removed. The air-drying from ethanol resulted in extensive shrinkage and loss of ultrastructural detail. It was concluded that this did not merit further use in studies of pulmonary tissue. Studies that concerned the shapes or relative distribution of tissues components could be accomplished using the rapid freeze methods followed by freeze substitution and CPD. Using those methods, Nowell and Tyler (1971) presented low and high magnification photomicrographs showing different components of pulmonary tissue at different levels but without giving a proper correlation of the position of the segments studied in the two species. They described in their results alveoli opening directly from bronchioles in the hamster, finding ciliated and non-ciliated cells in hamster bronchioles. They also described the type II alveolar epithelial cells in both the horse and the hamster with openings and depressions associated with the discharge of pulmonary surfactant. The other elements found were alveolar macrophages adjacent to pores in the interalveolar septum or the cut surfaces of interalveolar septa.

Tyler et al. (1971) studied the surface morphology of hypoplastic and normal lungs from newborn lambs by SEM. Two normal lambs were examined after being delivered by cesarian section, at full term gestation. Prior to delivery, the trachea of the lambs was clamped to prevent loss of fluid from the airways and the heart was arrested by injection of potassium chloride into the umbilical vein to prevent absorption of lung fluid into the circulation. After the lungs were removed from the thoracic cavity, they were perfused with buffered 10% formalin and kept in the fixative for 72 hours. The lungs were then cut into slices and some tissues were taken for LM. Selected pieces of lung were processed for SEM, adjacent to those examined for LM and the CPD method of drying was used. An alveolar duct space was described as a space between the terminal bronchioles and the alveoli and the bronchiolar space was

the space within airways smaller than 0.5 mm, lined by cuboidal or columnar epithelium and not containing cartilage or glands. This was established according to the sites selected by LM. They considered that the lung parenchyma of the newborn lamb appeared similar to those from other species that had been examined before in hamster and horse (Nowell and Tyler, 1971).

In the lungs of the newborn lambs all the typical structure could be readily identified. Very general information about the appearance of a perfused lung was presented. The alveoli were described as uniform in size and well expanded within a well organised parenchyma containing bronchi, bronchioles, alveolar ducts and alveoli. Five or more alveoli appeared to open into each alveolar duct. Free cells were also described which were considered to be alveolar macrophages, sitting on the alveolar surface. The surface of the interalveolar septa was described as thin and cleanly cut. Little information was given about the mucous membrane of the airways. However ciliated cells interspersed with the non-ciliated cells at the bronchiolar level and at the termination of these into an alveolar duct were mentioned. The ciliated cells looked poorly ciliated. In the same study type II alveolar epithelial cells were seen and were present in the alveolar ducts and in the alveoli.

In 1972, Greenwood and Holland carried out an investigation on mice with the purpose of describing the surface characteristic of the respiratory tract, from the nares to the pulmonary alveoli using the SEM. In preparation for SEM examination of the respiratory tract, the trachea was exposed below the larynx and the lungs were perfused intratracheally. First they were lavaged with 0.1 M-sodium cacodylate buffer and then in the same way perfused for fixation with 2.5% glutaraldehyde. Specimens from trachea, major bronchi, secondary bronchi and pulmonary parenchyma were obtained after fixation, dehydrated in graded alcohols and allowed to air-dry or were dried by CPD. The specimens were then coated with gold-palladium and examined at 20 kV. Greenwood and Holland (1972) pointed out that their findings indicated that the surface

structural data were best obtained using SEM. They were able to define cellular orientation and relative distribution of varied cell types. In the trachea of the mouse, they observed two main cell types and referred to the area containing them as microvillus-covered respiratory epithelium and ciliated epithelium. The ciliated cells were described as cells arranged in a group interspersed between numerous non-ciliated cells. Hansell and Moretti (1969) using TEM also noted the abundance of the non-ciliated cells and concluded that these cells were mucous secreting in nature. Later, and also by TEM, Pack et al. (1980) pointed out that SPF mice had few mucous cells in their tracheas. However they added that stock mice had more mucous containing cells in the trachea and that these were more frequently seen at every level. Plopper (1983) also emphasised that in some species of mice, non-ciliated cells closely resembling bronchiolar Clara cells, could be found in the trachea and bronchi. The capacity of microvillus-covered cells and ciliated cells in the normal trachea and bronchi to undergo regeneration was suggested by SEM observation of the same authors (Greenwood and Holland 1972). Previous workers had studied the events following mechanical injury to tracheal epithelium and noted that after removal of overlying ciliated cells, folded processes of remaining cells straightened to form microvilli. Four days after injury immature cilia and microvilli uniformly covered the cell surface and one day later a mixture of mature cilia, immature cilia and microvilli were present. The varied length of microvilli, the presence of cilia tufts projecting from the surface of predominately microvillus-covered cells and marked differences in ciliary length between adjacent cells and cells in the same area were observed in the trachea as well as in bronchi. For Greenwood and Holland (1972) this finding characterised the normal respiratory tract. They also described in normal pulmonary alveoli fixed in inspiration, the surface of three distinct cell types; the smooth surface of the type I alveolar epithelial cells lining the alveolar ducts, alveolar sacs and alveoli. The large type II alveolar epithelial cell surface covered with short microvillous projections. The third cell type observed was the alveolar macrophage, visualised lying in situ on the epithelial surface.

the epithelial surface. The plasma membrane appeared ruffled due to undulating waves of activity. The brush cell was not reported.

In the dog the SEM was used to establish and record the normal surface topography of the respiratory tract from the trachea to the alveoli (Wright et al., 1983). Studying the distribution of the ciliated cells in the tracheobronchial tree they established differences related to age in the dogs. They observed that in the newborn puppies the dorsal wall of the trachea was completely ciliated while the lateral and ventral walls showed patches without cilia. After five days of age the whole tracheal wall was found to be completely ciliated. The lower large airways i.e. the bronchi of the newborn puppies were uniformly poorly ciliated but complete ciliation was achieved by two days of age. In the same study, large and small bronchioles of the newborn animals had fewer cilia and although the number of ciliated cells had increased by two days, a complete carpet of ciliated cells was never observed regardless of the age of the dog. Ciliated cells were not seen in the respiratory bronchioles of any of the 25 animals investigated in this study, corroborating the results presented by Plopper et al.(1980) who established by TEM that 97% of the cells present in the bronchioles of dogs were non-ciliated and the remaining cells were poorly ciliated.

The bovine lung was studied using the SEM by Mariassy et al. (1975). This work on the bovine animal was preceded by a TEM investigation carried out by Epling (1964) and Rybicka et al. (1974). At this time some useful observations were made by McLaughlin et al. (1964) on the anatomy of the bovine lung when they compared the bovine lung with those of other species.

McLaughlin et al. (1964) reported the subgross anatomical differences of specimens from laboratory animals and domestic animals, including cattle. The bovine specimens were obtained from abbatoirs, inflated and washed with water to clear them of blood and then injected with latex. After solidification of the latex, the tissues were fixed for 48 hours. This treatment was followed

by quick freezing and sectioning to obtain specimens which were studied under the dissecting microscope. The authors tabulated and grouped the data obtained into three distinctive sub-gross lung types, (Table 2). The cow, the sheep and the pig possessed what was arbitrarily designated as sub-gross lung Type I. The lungs of animals in Group I, were characterised by the presence of extremely well developed secondary lobules due to the marked interlobular septa and the thick pleura. The most distal airways were composed of numerous terminal bronchioles leading either directly into alveolar ducts, or into alveolar buds proximal to the junction with the alveolar duct. They considered that each of the secondary lobules constituted a small individual lung because of their extreme development.

Epling (1964) described the ultrastructure of the blood-air barrier in normal cattle. Attention was focussed on the TEM features of the alveolar epithelium and the alveolar wall. Type I and Type II alveolar epithelial cells were characterised for the first time in the bovine lung and the findings were the same as those of previous investigators studying other mammalian species. Rybicka et al. (1974) made a more comprehensive study and described some of the TEM features of bovine lung, again, this report concentrated on the alveolar septum. Type I and Type II alveolar epithelial cells were identified and illustrated by these workers. Alveolar macrophages were also found, although only occasionally.

Observations based on a SEM study were made by Mariassy et al. (1975) who described some characteristic of the bovine lung. They proposed, and illustrated several distinctive anatomic features of the lung surface that might be important for an understanding of pathophysiological responses. Twelve animals were studied, they were adult beef cattle, of different sexes, coming from an abbatoir. Tissues were prepared for SEM by perfusion of the airways with Karnovsky's fixative. After dehydration in alcohol and CPD, the tissues were double coated with silver and gold before examination with an ETEC AUTOSCAM. Their main observations came from the lower intrapulmonary respiratory tract

Subgross Lung Types found in Seven Species of Mammals.

	Subgross Type		
	Group I Cow, Sheep, Pig.	Group II Monkey, Cat, Dog.	Group III Horse, Man
Lobulation	Extremely well developed	Absent	Imperfect development
Pleura	Thick	Thin	Thick
Terminal bronchioles	Present Predominant distal airways	Absent	Present
Respiratory bronchioles	Infrequently observed Extremely poor development	Present Very well developed	Present Poorly developed

Table 2. A classification of some mammals based on the subgross anatomy of their lungs, from McLaughlin et al. (1964).

as they did not examine the trachea or large bronchi. The bronchi were identified by their large luminal diameters, the thick band of smooth muscle and their dense connective tissue as well as the presence of glands and plates of cartilage. The epithelium described was composed of ciliated and non-ciliated cells. In the bronchi the ciliated cell was considered to be the predominate cell type over the majority of the surface but there were interspersed single-non-ciliated cells. The ciliated cells were described as cells with a surface containing dense aggregations of long uniform cilia, with microvilli distributed around their bases. The mucosal surface of the non-ciliated cells were observed to be covered by many short microvilli; these cells were considered not to protrude into the lumen. Similar non-ciliated cells with few microvilli and large pits or pores opening into the surface were seen and considered to be mucous goblet cells. In their descriptions, Mariassy et al. (1975), considered that these pores sometimes contained secretory material fixed in situ and that an actively secreting non-ciliated cell had very few microvilli in contrast to the quiescent non-secreting cells which were covered by a dense population of short stubby microvilli. Brush cells were not described.

The bronchiolar mucosa, described by Mariassy et al. (1975) was composed of approximately equal numbers of ciliated and non-ciliated cells. The ciliated cells were described as having slightly shorter cilia than those in the bronchi and it was noticed that the bronchiolar ciliated cells had shorter but more numerous microvilli when compared with their bronchial equivalent. The luminal surface of the non-ciliated bronchiolar cells were described as having an oval perimeter and a granular surface with few short microvilli. It was not confirmed whether or not the non-ciliated cells were Clara cells. They did not determine the role of the bronchiolar non-ciliated cells or their resemblance to Clara cells of other species. They confirmed the observations made by McLaughlin et al. (1964) on the paucity of respiratory bronchioles and, when they were present, their short length with few alveoli. The proportion of non-ciliated cells seemed to have

increased in the respiratory bronchioles. Mariassy et al. (1975) added that in respiratory bronchioles ciliated cells became few in number, had shorter cilia, which were less abundant and more disorderly at these other levels. Differences were found between the non-ciliated cells of the bronchioles and those in the most distal part of the bronchioles. The non-ciliated cells at the bronchiolar junction with the respiratory epithelium were more prominent in their apical protrusions. Three cells were described in the alveoli. Most of the alveolar surface was observed to be covered by the flattened, smooth surfaced squamous (Type I) alveolar epithelial cells. The remainder of the surface contained the second cell present which was the granular Type II alveolar epithelial cell. According to Mariassy et al. (1975) these cells usually lined only a small portion of the alveolar surface, the cell body protruded into the alveolar lumen and were covered by many microvilli in contrast to the Type I alveolar epithelial cells which had only a few small projections scattered on its surface.

The openings or pits on the surface of the Type II alveolar epithelial cells as described by Nowell and Tyler (1971) in the horse, were rarely seen by Mariassy et al. (1975) in cattle. In these animals the Type II alveolar epithelial cells were particularly numerous in alveoli adjacent to interlobular septa and in alveoli close to bronchioles. A prominent junction was also seen at the boundaries between epithelial cells. The third cell, the pulmonary alveolar macrophage was rarely seen. Interalveolar pores of Kohn were not often found and they interpreted that this was to be considered typical for that age group of animals.

Newhouse et al. (1976) considered in their review on lung defence mechanisms that one of the most important functions of the lung, aside from its role in gas exchange and metabolism, was the provision of an essential biological barrier between animals and their environment. Green et al. (1977) also subscribed to the view that the respiratory membrane is exposed to a large volume of air containing contaminants each day and considered that the infectious agents, chemical toxins, mineral dusts and immunogenic particles

contained in this air would find a variety of pulmonry defence mechanisms which could prevent these agents damaging the vulnerable tissues. The extent to which these lung defences could be overcome by the inhaled foreign materials was thought by Green et al. (1977) to determine the appearance of respiratory disease. The lung protected by these interlocking non-specific and specific defence systems could be heavily exposed to inhaled substances without suffering damage since the material could be removed physically or neutralised by the mechanisms described by Green et al. (1977).

There have been several studies by TEM and SEM of the damage produced in pulmonary tissue by different types of injurious agents, however, there have been very few studies on the damage produced in the bovine respiratory tract by these agents.

In the rat, Hijita (1977) studied the lung injury induced by Bleomycin sulphate, therapeutic drug effective against squamous cell carcinoma, malignant lymphoma and testicular tumours. Unfortunately pulmonary injury eventually resulting in diffuse lung fibrosis was reported as a serious complication in patients receiving large doses of the drug. Hijita (1978) using SEM had followed the pathogenesis of Bleomycin induced lung injury. The changes observed depended on the route of administration. In the case of administration via bronchioles and alveolar lumen, damage to epithelial cells was the most severe. Through a quantitative study by SEM, the authors detected swellings and exscoriations on the attenuated cytoplasm of Type I cells. At this stage also destruction of Type II cells was noticed; necrosis of the cytoplasm and increased secretion of lamellar bodies from Type II cells was commonly observed. The third epithelial cell, the alveolar brush cell, was studied in this experiment. Although in control animals, the alveolar brush cells had a slight tendency to increase in number with age, there was a remarkable increase in number brought about by the Bleomycin treatment. The proliferation of alveolar brush cells within a vascular ring was considered to constitute a functional unit. Microvilli of Type II epithelial cells in close proximity to the alveolar brush cells seemed to fuse

with each other. It seemed that Type II epithelial cells with highly fused mircovilli, represented an intermediate cell between alveolar brush cells and Type II epithelial cell. Adenomatous hyperplasia of the bronchiolar epithelium was also recognised as a toxic effect of Bleomycin. The increased number of the bronchiolar brush cells was commonly reported to occur near the bronchiolar junction; this phenomenon was considered to represent alveolar bronchiolisation.

Organ cultures of hamster trachea have been used extensively to study the pathogenicity of Mycoplasma pneumoniae. Using the SEM, Gabridge et al. (1977) observed the luminal surface of the trachea of adult disease-free hamsters. These tracheas were removed from the animals and cultured in vitro, then infected with M. pneumoniae. After attachment of the organisms, the trachea was examined in the SEM. The attachment of M. pneumoniae was inversely correlated with the presence of extensive ciliation; the most heavily ciliated areas having the least attachment. This was explained by the author, as being due to the vigorous beating of the cilia. The SEM revealed numerous relatively large patches of unciliated cells where the mycoplasmas had attached. These were more common on the ventral luminal surface of the trachea and were quite prominent in the middle portion. According to Gabridge et al. (1977) the unciliated areas apparently contained several different types of cells. When the ventral surface of the trachea was examined in its entire length, the anterior region (nearest the larynx) and the posterior region (nearest the bifurcation) appeared to have the most extensive ciliation. The sides of the trachea were similar in that the ciliated cells outnumbered all other cell types on the surface. The dorsal surface of the trachea had a much different morphological configuration from the ventral one. The mucosa was arranged in a series of longitudinal folds heavily ciliated.

Mebus and Underdahl (1976) using the SEM, observed the effect of Mycoplasma hyopneumoniae infection on the trachea and bronchi of gnotobiotic neonatal pigs. After infections given intranasally

with M. hyopneumoniae cultures, the pigs were killed at different times and the specimens were prepared for SEM observation. The results on the control group of animals indicated that ciliated cells were the main cell in the trachea. They were intermixed with goblet cells which were obscured by the numerous long cilia present. In the infected pigs the surface of the trachea had severe changes consisting mainly of the loss of cilia, exposure of the microvilli of the epithelial cells and the presence of mucus and many spherules considered to be mycoplasms In some animals, goblet cells were noted to be protruding. Large numbers of mycoplasmas forming clusters were seen. In the early stage of the infection in the bronchi there was loss of cilia and mycoplasmas were associated with cilia. In the later stages of infection the loss of cilia was more extensive and the mycoplasmas were arranged in a layer rather than in clusters. At the end of the experiment about half of the bronchial epithelial cells had lost cilia and the presence of leucocytes was also noted. The distribution and severity of SEM bronchial changes coincided with the gross pulmonic lesions. However, the severity of the lesions was less in terminal bronchioles than in the trachea.

Williams and Gallangher (1978) studied porcine tracheal rings and lungs inoculated with a virulent strain of M. hyopneumoniae. The preparations were observed by different methods including SEM. The main objective was to characterise the primary interaction between M. hyopneumoniae and the host on the ciliated epithelial surfaces of the respiratory systems. Previous reports, (Livingston et al., 1972 and Mebus and Underdahl, 1976) had revealed that mycoplasmas appeared in close proximity to the cilia of the bronchial epithelial cells. However, it was considered necessary to conduct further investigations with the commonly occurring secondary infections such as bacteria, viruses or other mycoplasmas. An investigation of a complicated mycoplasma infection in porcine respiratory tissue cultures using SEM was considered by the authors to afford a unique high-resolution view of surface areas which could be compared with observations using other methods. The SEM methods applied were the conventional

ones. The explants of trachea and lung were infected with mycoplasmas only and with mycoplasmas plus a culture of porcine lung fibroblast (PLF). The ciliated pseudostratified columnar epithelial cells of the trachea, lobar bronchi and proximal bronchioles, were considered to play an important role in removing various inhaled particles and disease-promoting organisms from the respiratory systems. This study in vitro demonstrated that the mycoplasmas probably induced loss of cilia even when the infection was considered to be of low titre and free of other microorganisms. In this case the damage was focal and it seemed that the organisms might easily be localised either on tracheal cells or lung cells after the cilia were lost, possibly due to toxic substances of an enzyme nature produced by the mycoplasmas. When the infections were produced with mycoplasmas and PLF, the damage was greater because of the high concentration of toxic products from damaged cells caused by the M. hyopneumoniae. The authors (Williams and Gallangher, 1978) pointed out that since, during in vitro experiments, the organisms were not in contact with immune cellular responses, the complexity of a given infection in vitro could be different from what might occur in vivo.

Pneumonic calves infected naturally with Mycoplasma dispar have been studied by TEM (Allan and Pirie, 1977). The results showed that the TEM was able to demonstrate mycoplasmas in lung tissue even when attempted isolation was negative. Mycoplasmas detected by TEM were always on the bronchial epithelium and scarcely found on alveolar tissue. Usually individual organisms in close association with neutrophils and macrophages were found. The organisms were mainly seen on and between the cilia of the bronchial epithelium often in layers as judged by TEM, giving the appearance of a microcolony on the epithelial surface. They were never seen within the cytoplasm of the epithelial cells. Loss of cilia was a common feature as seen before with the SEM studies on the in vitro experiments on pig tissues (Mebus and Underdahl, 1976; Williams and Gallangher, 1978). The cellular changes such as protrusion of the apical cytoplasm of epithelial cells into the bronchial lumen were also seen.

A study using SEM on the tracheal epithelium of calves inoculated with bovine herpes virus I was described by Allan and Msolla (1980). Infecious bovine rhinotracheitis virus was experimentally inoculated onto the mucous membrane of the upper respiratory tract. The infection developed clinical signs and the virus was reisolated from the nasal passages. Damage was demonstrated on the mucous membrane of the trachea by LM. When normal calves were observed under SEM the surface of the mucous membrane appeared to be composed of ciliated epithelial cells intermixed with goblet cells. The cilia appeared to be all about the same length and quite numerous. When the infected animals were examined with SEM, loss of cilia and consequent exposure of the microvilli on the epithelial cells was detected. The injury seemed to involve large areas and was accompanied by large amounts of mucus on the luminal surface. The mucus appeared as strands of material intruding into the lumen from the ducts of submucosal glands.

Bryson et al. (1983) studied the ultrastructural features of the lower respiratory tract of calves, experimentally infected with parainfluenza Type 3 (PI3) virus. The acute stage and the repair stage of the pneumonia were investigated using TEM and SEM procedures, applied on a conventional basis. In the acute stage of the infection, viral replication was observed by TEM within the epithelial cells of the respiratory tract and within alveolar macrophages. The major bronchi were less affected that small bronchi. The nucleocapsid filaments were seen to be intracytoplasmic and occasionally intranuclear in the bronchial ciliated cells, bronchiolar ciliated cells and bronchiolar non-ciliated cells (Clara cells). Nucleocapsid was also seen in the Type I and Type II alveolar epithelial cells. Marked changes were observed in the ciliated cells of bronchi and bronchioles consisting of loss of cilia and disruption of the normal orderly-arrangement of the basal bodies within the apical cytoplasm. With the SEM, loss of cilia was observed to be more severe in bronchioles and small intrapulmonary bronchi than in large bronchi. The loss of cilia destroyed the ciliary carpet on

the luminal surface and exposed the microvilli. Proliferation of Clara cells producing zones of hyperplasia in the bronchiolar epithelium was seen with TEM confirming the features observed by LM. Within the alveoli, widespread proliferation of Type II alveolar epithelial cells led to alveolar epithelialisation. These features were not studied by SEM.

It would appear, therefore, from the literature cited that there have been few comprehensive specific studies, using SEM, on the normal or diseased bovine respiratory tract and that such an investigation could yield useful information for workers with research commitments in bovine respiratory disease.

SECTION TWO

MATERIALS AND METHODS

ANIMALS AND RESPIRATORY TRACT SAMPLES

SCANNING ELECTRON MICROSCOPY

TRANSMISSION ELECTRON MICROSCOPY

LIGHT MICROSCOPY

ANIMALS AND RESPIRATORY TRACT SAMPLES

The animals investigated during this study could be classified into three groups: (i) adult cattle (ii) week-old calves and (iii) parasite free calves three months of age.

ADULT CATTLE

The adult cattle examined, had been admitted to the Veterinary School, Clinical Department for a variety of problems. These animals had no evidence of clinical respiratory disease although minor lesions were found in the lungs post-mortem. These animals formed one of the groups in the study described in Section 3.

WEEK-OLD CALVES

These animals were purchased from a local market, at one week of age, and brought to the Veterinary School. All were the same breed, Friesian, and were male. They were kept indoors and fed with milk until the day of post-mortem examination. The calves were examined clinically and were found to be free of respiratory disease. No signifiant lesions were found post-mortem. These animals were used for the study described in Setion 3.

PARASITE FREE CALVES

These calves had been reared indoors and then brought to the Veterinary School when they were two months old. There they were left indoors until they were three months of age. These animals comprised the group of calves for the study described in Section 4.

POST-MORTEM EXAMINATION

The normal calves and the parasite free calves were anaesthetised by intravenous injections of pentobarbitone sodium solution (Euthatal - May and Baker - Dagenham, England) into the

jugular vein. To prevent the escape of stomach contents and the contamination of the airways the oesophagus was exposed and ligated before the calf was euthanasised by exanguination.

The adult cattle were stunned by shooting and killed by bleeding.

A post-mortem examination was carried out on all the animals. First of all, however, immediately after death the lower respiratory tract was removed from the carcase and dissected for sampling and fixation. This was done as quickly as possible and samples were never collected over 20 minutes after death.

After the samples for SEM had been selected the samples for light microscopy were taken.

RESPIRATORY TRACT SAMPLES

In order to make a comprehensive examination of the lower respiratory tract, five levels were selected and at these levels different sites were established for the removal of the tissues. These sampling sites in the trachea and the right lung were kept to rigorously during the two experiments described in Section 3 and Section 4 and the sites are illustrated in Fig. 2A and Fig. 2B.

Level 1 was the trachea which was sampled at three sites, cranial trachea (1a), middle trachea (1b) and caudal trachea (1c). Level 2 was the large bronchi of the right lung; two sites were sampled, one in the cranial lobe (2a) and the other in the caudal lobe (2b). Level 3 was the small bronchi of the right lung; these were also sampled at two sites, one in the cranial lobe (3a) and the other in the caudal lobe (3b). Level 4 was the bronchioles of the right lung; cranial lobe samples were (4a) and caudal lobe samples were (4b). Level 5 was alveolar tissue in the right lung; the two sites sampled were (5a) in the cranial lobe and (5b) in the caudal lobe.

Fig. 2A - The levels sampled in the trachea and right lung of the normal calves and cows described in Section 3, Level 1, Trachea (caudal, middle, cranial) Level 2, Large Bronchi (cranial and caudal lobes) Level 3, Small Bronchi (cranial and caudal lobes) Level 4, Bronchioles and Alveoli (cranial and caudal lobes).

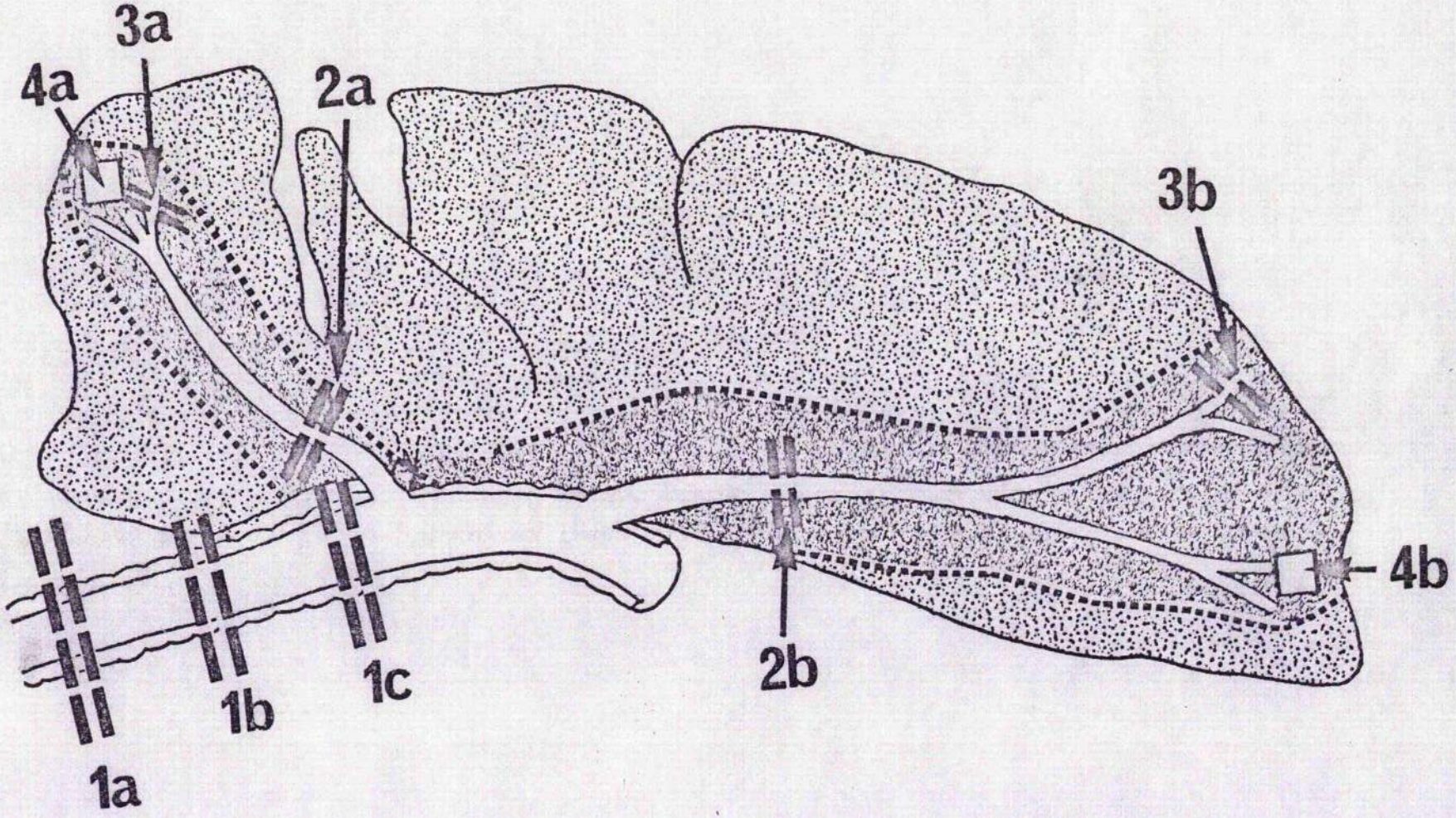
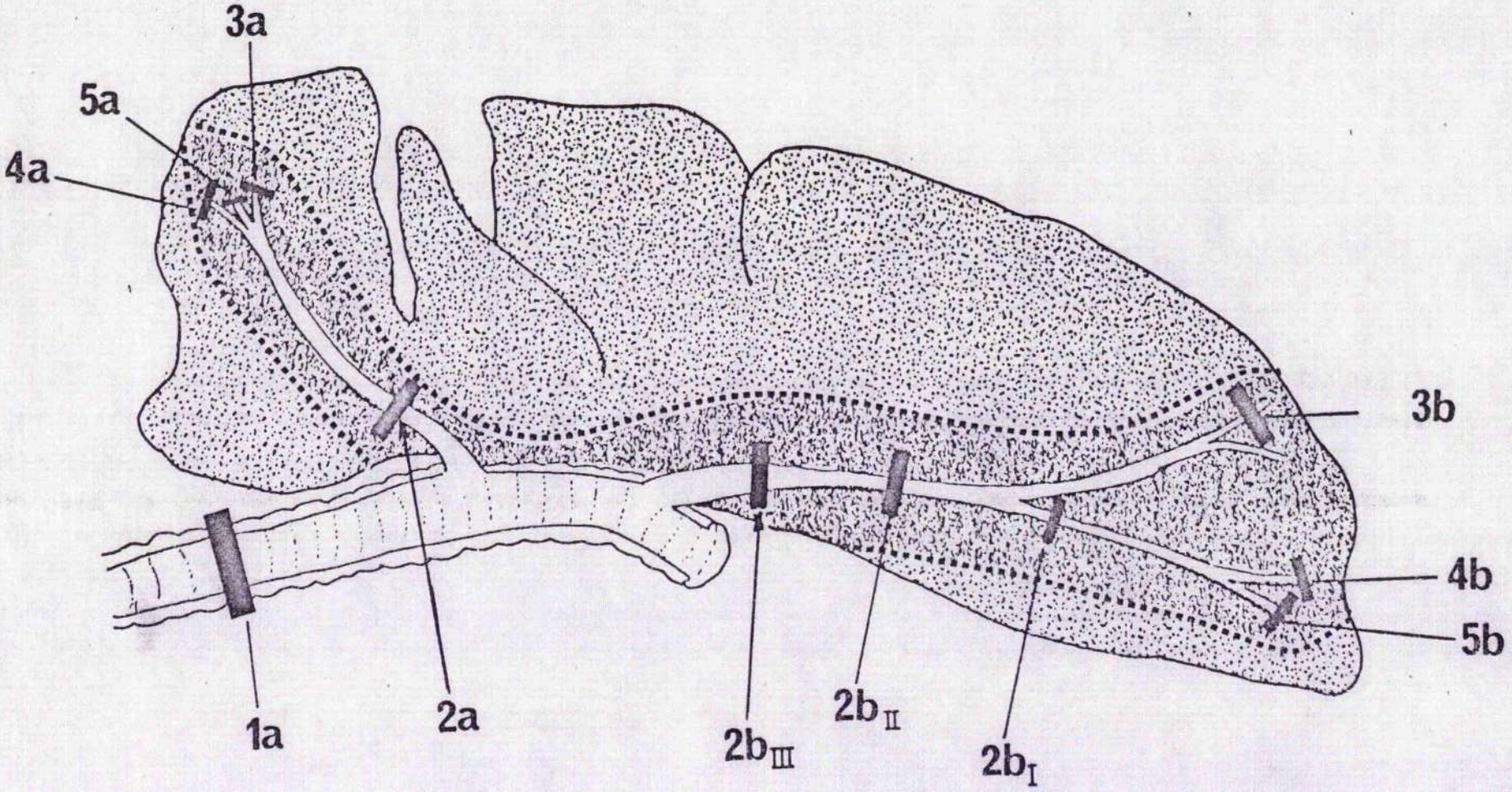

3a
4a
2a
3b
4b
1a
1b
1c
2b

Fig. 2B - Levels sampled in the trachea and right lung of the experimental animals used in the lungworm experiment, Section 4; Level 1, Trachea (Middle).
Level 2, Large Bronchi (2a in cranial lobe and 2bi, 2bii, 2biii in caudal lobe. Level 3, Small Bronchi (cranial and caudal lobes) Level 4, Bronchioles (cranial and caudal lobes) and Level 5, Alveoli (cranial and caudal lobe).


3a
5a
4a
3b
4b
5b
1a
2a
2b III
2b II
2b I

These samples were fixed by a method which involved them being either (a) non-perfused or (b) perfused).

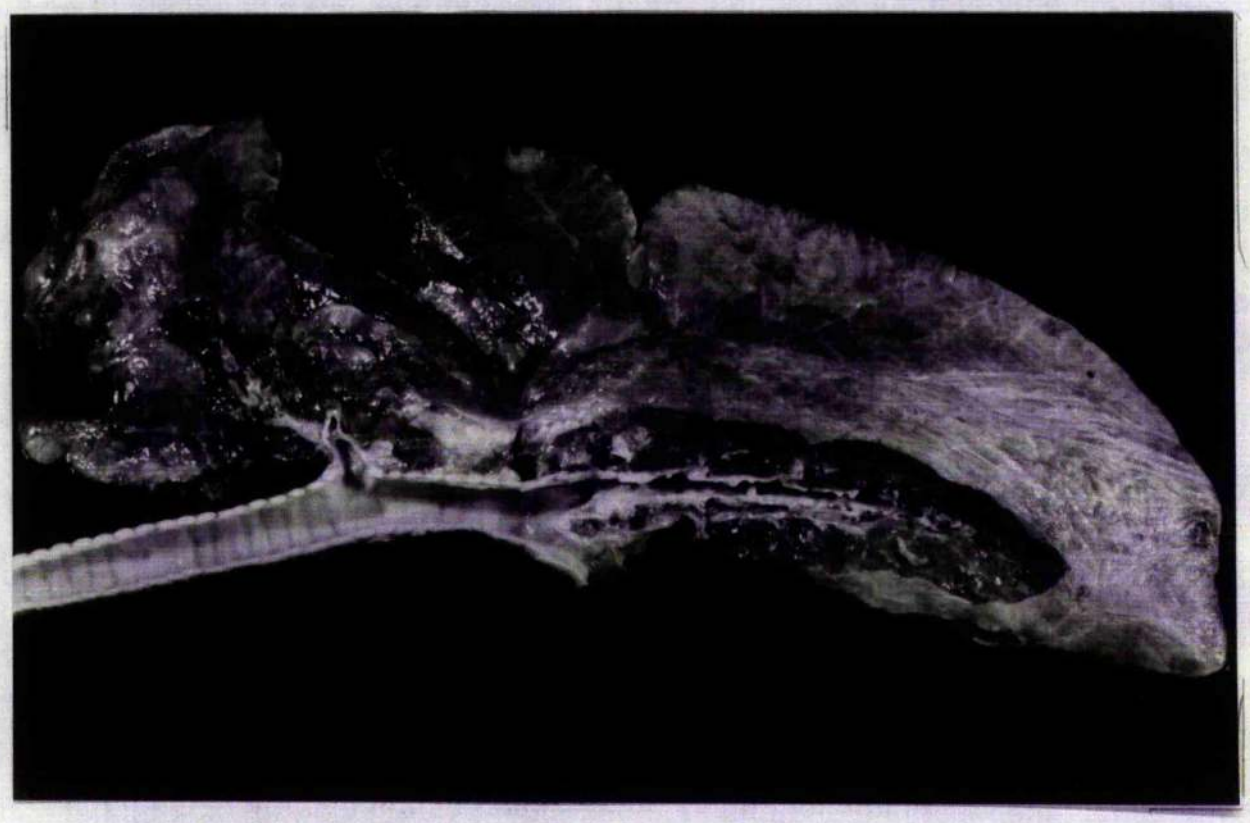
Non-perfused samples were from Level 1 and Level 2. These were complete rings of tissue, taken from each site, which thereafter were washed in a jar containing rinse solution and then transferred to a second jar to be fixed with modified Karnovsky's fixative.

Perfused samples were collected as follows: After removing the samples at Level 1 and Level 2, the remaining large bronchi in the cranial and in the caudal lobes were cannulated at the points illustrated in Fig. 3, and the lung perfused with chilled modified Karnovsky's fixative. Thereafter the perfused parenchyma was separated from the rest of the organ and kept immersed in fixative in sealed polythene boxes to complete the fixation procedures.

Slices of the perfused lung as illustrated in Fig. 4, were then examined under a dissectng microscope. Level 3, Level 4 and Level 5 were identified and appropriate samples were removed from the sites described using forceps and a scalpel blade keeping the tissues wet in rinse solution.

Fig. 3 - Levels in the cranial and caudal lobe where the points of acculations are illustrated.

Fig. 4 - Perfused lung is illustrated where Level 3, Level 4 and Level 5 are sampled.



SCANNING ELECTRON MICROSCOPY

The following methods were used on the specimens collected at post-mortem examination in order to prepare them for examination under the scanning electron microscope.

FIXATION

The tracheal rings (1a, 1b, 1c) and the bronchi (2a, 2b, 2bI, 2bII, 2bIII) were first carefully washed with a jet of cacodylate buffer 0.1M, to remove the film of mucus covering the epithelial surface, and then fixed by immersion in modified Karnovsky's fixative. The rest of the specimens (3a, 3b, 4a, 4b, 5a, 5b) were fixed by air-perfusion procedures. The route by which the fluid was injected was the remaining large bronchi of the cranial and caudal lobes. They were cannulated and the lung perfused using a 50 ml syringe filled with fixative and they attached to the cannula. The rate of perfusion was controlled by means of the syringe which could be refilled several times during the perfusion. The specimens were then immersed in the fixative and kept in cold temperature (4°C) for at least one week in airtight plastic boxes.

The following solutions were used to rinse the tissue and to prepare the Karnovsky fixative:

Rinse solution

The rinse solution used was Sodium cacodylate buffer 0.1M.

0.2M sodium cacodylate - 100 ml.
distilled water - 100 ml.

Buffer

a) 0.4M, sodium cacodylate
21.4g made up to 250 ml distilled H_2O.

b) 0.2M, sodium cacodylate buffer

50 ml 0.4M sodium cacodylate
8 ml 0.2 M HCl.
42 ml distilled water.

Control pH = 7.2

c) 0.2 M, HCl.

1.72 ml HCl in 100 ml distilled water.

Paraformaldehyde 10%:

Heat 2g of paraformaldehyde in 20 ml of distilled water to 60°C in a fume cupboard. Add a few drops of 1 NaOH to clear. Cool before used.

Modified Karnovsky's Fixative

50 ml	0.2M cacodylate buffer pH 7.2
20 ml	10% paraformaldehyde
10 ml	25% glutaraldehyde
20 ml	distilled water.

After adequate fixation the processing of the specimens for scanning electron microscopy comprised the following procedures (i) washing (ii) dehydration and critical point drying and (iii) mounting and sputter coating.

WASHING

Slices of perfused and non-perfused samples were removed from the fixative and washed in 0.1M sodium cacodylate buffer.

a) Non-perfused samples: The samples from the trachea (Level 1) and from the large bronchi (Level 2) which were in the form of rings of tissue were cut into small, flat pieces, 1 mm x 0.5 mm. A sample of this size was taken from the ventral and dorsal walls of the rings at each site and washed overnight in sodium cacodylate

buffer 0.1M. This was necessary to rinse away fixative. It was recommended to use buffer for washing to maintain the osmolarity with the tissue. When it was necessary to wash tissues for a long time the tissues in the buffer were kept in cold conditions.

b) Perfused samples: Slices of perfused lung were examined under a dissecting microscope. Small bronchi (Level 3) bronchioles (Level 4) and alveolar tissue (Level 5) were identified in slices from the cranial and the caudal lobes, keeping the tissues wet in 0.1M sodium cacodylate buffer, and appropriate samples were removed from the sites described using forceps and a scalpel blade. The selected tissues were transferred into bijou bottles containing rinse solutions and were kept there at least two hours or overnight.

DEHYDRATION AND CRITICAL POINT DRYING

Specimens from the rinse solution were passed first through graded acetone in which they were gradually dehydrated. Cold dehydration help to decrease extraction of cell components by the organic solvent.

The time in acetone had to be controlled and the tissues were kept in acetone for as short a time as possible to avoid excessive shrinkage and extraction. The dehydration schedule for acetone used in the present study is shown below:

Acetone	70%	2 hours or overnight
"	90%	2 hours
"	100%	2 hours or overnight
"	100%	2 hours

The specimens were then transferred in specimen holder baskets to the stage when the tissues were impregnated with the final substitution liquid (acetone 100%), in the liquid transfer boat. The boat was filled with the substitution liquid and the basket assembly was transferred rapidly to the boat. The boat could then

be loaded into the pressure chamber of the apparatus. When the specimen access door was closed this actuated the drain valve in the liquid transfer boat, and this resulted in a sufficiently slow draining action for liquid carbon dioxide to enter and cover the specimen before the substitution liquid level had fallen. The valve connected to the CO_2 cylinder (inlet valve) was slowly opened to let the carbon oxide fill the chamber at 20-25°C until the gauge registered 800 p.s.i. If it did not, the cause was either a leak or an exhausted CO_2 cylinder. The residual air was then briefly flushed out by slightly opening simultaneously the valve connected with the outlet and the pressure-reduction valve, while keeping the inlet open. The reduction valve was then closed and the inlet valve closed. The liquid CO_2 in the chamber was then allowed to equilibrate with the solution in the specimens. After a few minutes the CO_2-acetone mixture was flushed out for a few seconds by opening the reduction valve whilst the CO_2 was open. This was carried out several times during a period ranging from half an hour to one hour, for an average run of say ten specimens, each approximately 1 x 1 x 0.5 mm. Whether a significant quantity of acetone remained in the chamber could be crudely estimated by the smell of the outgoing CO_2 gas. Finally the chamber was isolated by closing all the valves and the whole chamber was gently heated with hot water to about 35°C. Since the pressure in this sealed system was a function of temperature it was quite sufficient to follow the pressure change alone. If the pressure did not rise appreciably on heating this could indicate that CO_2 gas, not liquid, was in the chamber, or the valve for the outlet was not properly closed.

When a pressure of 1,200 p.s.i. was reached, the liquid surface would have disappeared and the CO_2 was above its critical point. The release-outlet valve was opened and the pressume-reduction valve was slowly opened to release the now gaseous CO_2. If the pressure was allowed to drop too quickly then either the expansion of the gas might cool it below its critical point, when it would liquify or solidify or the specimen might explode due to the rapid expansion of the gas. It was important, however, that the gas was released slowly so that the chamber had

time to cool by radiation. A suitable period of time was one minute.

In the present study the Critical Point Drying Apparatus E3000 Polaron Equipment was used.

MOUNTING AND SPUTTER COATING SPECIMENS

As soon as was convenient, after critical point drying, the specimens were mounted because it was much easier to handle mounted preparations. This was specially true of small specimens because they could then be more easily prepared in batches without damaging or contaminating the faces which were to be examined.

Specimens for SEM were normally mounted by attaching them to aluminium stubs on aluminium holders using colloidal silver paint.

Glues like epoxy resin were useful for dry specimens but took a long time to set. Glue which hardened by the evaporation of a solvent was generally more convenient although some trouble could be experienced if too much glue was used because it could be slow to dry and would tend to bubble up under vacuum.

Colloidal silver paint was used as a glue but this was an expensive material for mounting large numbers of specimens. However, it was invaluable for improving electrical continuity between the specimen and the stub, and for covering an unwanted area of a specimen which would charge in the microscope.

Most biological specimens will charge unevenly in the SEM when the electron beam impinges upon their surfaces. The even charging will deflect the beam and destroy resolution by causing stigmatism and introducing brightness variations which cannot be interpreted. Charging artefacts could be reduced by reducing the primary beam energy, but this is associated with severe loss of resolution at voltages low enough (1.5 Kv) to work with an untreated specimen surface. In practice therefore the specimen's surface should

become conductive by coating with gold-palladium in an Emscope Sputter Coater.

THE SCANNING ELECTRON MICROSCOPE

The specimens studied in this thesis were examined by means of a Philips 501B Scanning Electron Microscope at a 72 to 15 Kv and 200, 500 and 1000 spot size. The SEM was in the Veterinary Anatomy Department of the University of Glasgow Veterinary School.

DISCUSSION OF SEM METHODS

Animal tissues for SEM examination generally need to be free from extraneous material in solution such as mucus, blood or tissue fluid. In the case of the natural surfaces of soft tissues, isolated cells or cell cultures the most useful approach seems to be to wash the material with a suitable isotonic medium before fixation.

Good fixation stabilises cellular organisations to such an extent that ultrastructural relations are preserved despite the subsequent drastic treatments of dehydration, embedding and exposure to an electron beam. In 1963 Sabatini et al. recommended the use of glutaraldehyde as a fixative for electron microscopy. Specimens could be stored in buffer, after having been fixed in glutaraldehyde, for considerable periods of time before further processing. Paraformaldehyde (Robertson et al., 1963) compared favourably with glutaraldehyde and had the advantage of more rapid penetration into the specimen. Subsequently various mixtures of aldehyde were tested and some of them, particularly a mixture of paraformaldehyde and glutaraldehyde (Karnovsky, 1965) were found to be very useful. Paraformaldehyde-glutaraldehyde fixatives gave better preservation of a wide variety of tissues than aldehyde alone. In consequence, they have become very widely used as primary fixatives. Formaldehyde penetrates tissues much more rapidly than glutaraldehyde and it is thought that the formaldehyde temporarily stabilises structures which are subsequently fixed more

permanently by the glutaraldehyde (Karnovsky, 1965). The fixative originally suggested by Karnovsky (1965) consisted of 4% paraformaldehyde, 5% glutaraldehyde and 0.05% calcium-chloride, in 0.08M cacodylate buffer pH 7.2 and was extremely hypertonic, consequently lower concentrations of paraformaldehyde and glutaraldehyde (0.5 to 2% and 1 to 3%) started to be used. Since and it is always advisable to try first a method that has ben used successfully with similar specimens and then make modifications as required. In this study the modified Karnovsky's fixative was used.

An ideal fixative would exactly match the natural environment of a living tissue with respect to pH, osmoliarity and ionic constitution.

The majority of fixatives were buffered with phosphate or cacodylate. Cacodylate buffers were first proposed for electron microscopy by Sabatini et al., (1963). They are easy to prepare, are stable during storage for long periods and do not support the growth of microorganisms. Their main disadvantages are that they contain arsenic which is toxic and may act as a fixative and they have an unpleasant smell.

It is clear that most biological soft tissue cannot be examined wet in the SEM but must be dried first. If this is not done the tissues' water will boil in the vacuum of the microscope and the extraction of the latent heat of evaporation will cause the remaining water to freeze. The questions of water content could be avoided by previous workers in a few instances such as examing living tissues of Tribilium sp. and Sitemia sp. These specimens were either able to conserve their free water or could tolerate its removal in the vacuum of the microscope. Usually, however, the tissue must be dehydrated prior to insertion in the SEM. The following techniques have been used: (i) air -drying from the liquid phase (ii) critical-point dryng (iii) freeze-drying from water and (iv) freeze-drying from organic liquids. Most of the materials referred to in the Review of the Literature (Section 1) were processed using either the first or second methods.

The basic limitation of the air-drying from liquid phase is drying down of tissue structure caused by the receding surface of the liquid as it evaporates, and the tendency of minute solid components to clump together. Some specimens, such as mineral and hard tissues obviously resist this. Others do to some extent. Soft tissues, however, could be seriously deformed. This deformation may be advantageous as, in the case of artificial surfaces prepared in soft tissues, by the cells shrinking in such a way as to reveal the position of cell junctions or contacts (e.g. synapses in the nervous system) and the A,I and Z bands in striated muscle fibres.

Many specimens may be prepared by allowing a very volatile solvent to dry in air, but the apparent saving of time over other methods is a delusion. The actual operator time involved in transferring the specimens through a suitable graded series of solvent mixtures certainly exceeds the operator time involved in freeze-drying the same specimens for water. However, the same considerations of time involved in dehydrating the specimen through graded solvents apply also to techniques which are at present regarded as the most promising namely, critical point drying or freeze-drying.

The aim of drying from volatile solvents should be to choose one which has both (a) low surface tension (e.g. ether 17 dynes/cm, acetone 24 dynes/cm) so that the forces tending to disturb the specimen surface will be minimal and (b) high volatility, so that the specimen-shrinkage time is minimal. The rigidity of the tissue may also be increased by the use of appropriate fixation procedures.

Boyde (1980) recommended that the specimens would be successively equilabrated, after washing in buffer to remove the fixative by processing in the following ethanol : water mixtures of 30, 50, 70, 80, 90, 96 and 100% ethanol following by diethyl-ether : ethanol mixture of 25, 50, 75 and 100% either or acetone 70, 90, 100%.

The principles of the critical-point drying method of dehydration and the apparatus used were described by Anderson (1951). The water content of the specimen was successively replaced by ethanol and amyl-acetate or acetone and liquid carbon dioxide, and the latter was then heated to a little above its critical point in an enclosed place. Above the critical point (31°C for CO_2) the liquid became a gas which could be released from the specimen. The result was that artifacts caused by the crystals and phase boundaries in frozen-dried preparations were eliminated. There was no surface-tension distortion if the drying was properly conducted. Critical point drying was quick, but suffered from the disadvantage that the organic liquids used, ethanol and amyl acetate were precipitant fixatives and fat solvents which made the tissues rather brittle. However, critical point drying was a most satisfactory way of drying specimens of soft tissues routinely because it avoided the ice-crystal artifacts associated with freeze-drying from water.

TRANSMISSION ELECTRON MICROSCOPY

Transmission electron microscopy (TEM) was used to examine tissue previusly observed with the SEM and details of these different tissues will be described in the corresponding Sections 3 and 4.

Small pieces of mucous membrane were removed from the respiratory tract as soon after death as possible and placed in drops of a chilled paraformaldehyde/gluteraldehyde fixative on blocks of dental wax. These small blocks were then transferred to glas phials containng chilled fixative and were fixed overnight in cacodylate rinsing solutions and post-fixed in osmium tetroxide for one hour.

The fixed tissue was dehydrated through an ascending series of 70%, 90% to absolute alcohol, followed by rinsing in propylene oxide. The tissues were soaked for one hour in a mixture of equal parts propylene oxide and araldite, and left overnight in an 80%

araldite mixture. Individual blocks were then embedded and the embedding resin polymerised at 57°C for 48 hours.

Sections 1µ thick were cut on an LKB Mark III ultramicrotome using glass knives; mounted on glass slides and stained with toluidine blue (Trump et al., 1961). Fields for ultramicrosopy were then selected and the original blocks trimmed accordingly.

Ultrathin sections were cut on the ultramicrotome and mounted on uncoated ATHENE 482 copper specimen grids (Agar Aids, Stanstead). Sections were stained with Urany acetate, rinsed in methanol 50% methyl alcohol in distilled water, after which the grids were dried on filter paper. They were then stained for 10 minutes with lead citrate, rinsed with 0.02N sodium hydroxide, distilled water and again air dried on filter paper.

Sections were examined using an AEI 6B electron microscope in the EM Unit of the Veterinary Pathology Department, the University of Glasgow.

1. Paraformaldehyde/Glutaraldehyde mixture

1.3% paraformaldehyde and 1.6% glutaraldehyde in cacodylate buffer pH 7.2 - 7.4.

Paraformaldehyde 2g.
Distilled water 25 ml.
IM sodium hydroxide 2-3 drops.
25% glutaraldehyde 10 ml.
* Caeodylate buffer 115 ml.
Anhydrons calcium chloride 25 mg.

*2. Cacodylate buffer: This was prepared as a 0.2M solution of sodium caeodylate in distilled water (21.4 g/l) and adjusted to pH 7.4 - 7.6 by addition of a few drops of concentrated hydrochloric acid.

Osmium tetroxide: 1% osmic acid (BOH Chemicals Ltd., Poole, Dorset) in Millonig's buffer pH 7.2 - 7.4.

Millonig's phosphate buffer was prepared as follows:

Sodium dihydrogen phosphate (2.26%)	83 ml.
Sodium hydroxide (2.52%)	17 ml.
Distilled water	10 ml.
Sucrose	0.54g.

Uranyl Acetate: A 20% solution (May and Baker, Dagenham) was made up in 100% methanol.

Lead Citrate: Lead nitrate (1.33g) and sodium citrate (1.75g) were dissolved in separate 15 ml volumes of distilled water. The solutions were then mixed, the lead citrate precipitate shaken for one minute and then allowed to stand for 30 minutes with periodic agitation. The precipitate was solubilised by the addition of 8 ml of 1.0 M sodium hydroxide. The solution was diluted to 50 ml with distilled water. The final pH was 11.9 - 12.1.

Araldite mixture: Equal parts Araldite Resin (CY212) and Araldite Hardener (HY 964) were mixed by stirring overnight and then stored at 4°C until required. Before use in embedding, 0.6 ml of accelerator (DH 064) and 2.4 ml of di-n-butyl phthalate were added to 57 ml of the aralidte mixture, and the total volume stirred for 30 minutes.

LIGHT MICROSCOPY

In all the animals studied, histological procedures were performed for light microscopy (LM) examinations. The tissues were obtained from the same levels as described for SEM samples but were selected from areas non-perfused or taken prior to the perfusion of the lung parenchyma. The samples were fixed in 10% neutral buffered formalin (NBF).

FIXATION IN 10% NEUTERED BUFFERED FORMALIN

Tissue blocks remained in fixative for a minimum of 24 hours, and then each sample was trimmed to a thickness of not more than 4 mm, with a flat surface provided to ensure accurate orientation and to facilitate sectioning. After trimming all specimens were transferred to fresh 10% NBF, and duplicate positions post-fixed in corrosive formal for a further 24 hours. All blocks were then processed as detailed below.

Fixatives were prepared as follows:

1. 10% Neutral Buffered Formalin (NBF)

Tap water	900 ml.
Concentrated formaldehyde 40%	100 ml.
Sodium dihydrogen orthophosphate	4.6 g/l.
Dipotassium hydrogen orthophosphate	8.0 g/l.

2. Corrosive Formol (Formol sublimate)

Concentrate formaldehyde 40%	100 ml.
Mercuric chloride (sat. aqueous sol.)	900 ml.

PROCESSING

Tissues were processed by dehydration through a series of alcohols, double embedded in 1% celloidin in methyl benzoate and embedded in paraffin wax, using amyl acetate as a medium. Sections were cut at a thickness of 3μ and stained using Meyer's haematoxylin and eosin; and sometimes alcian blue/periodic acid Schiff at pH 2.5.

SECTION THREE

A SCANNING ELECTRON MICROSCOPIC STUDY OF THE LOWER RESPIRATORY TRACT IN NORMAL CALVES AND ADULT CATTLE

INTRODUCTION

The entire air-tissue interface of the respiratory tract extending from the nasal cavity to the alveolar space, is covered by epithelial cells and their secretions (Breeze et al., 1976; Breeze and Wheeldon, 1977). However, little is known about this interface in the bovine respiratory tract. The SEM has been shown to be a useful instrument for studying the surface topography of tissues, particularly lung tissues. As described in Section I, the surface pattern of the ciliated and non-ciliated cells varies at different levels of the lower respiratory tract and differences related to age have been observed e.g. in the dog (Wright et al., 1983). Furthermore, when Mariassy et al. (1975) studied the lungs of young adult cattle they observed several anatomical features that apparently differed from those of other mammals.

The objectives of this study were firstly to examine the epithelial surface of the lower respiratory tract of normal young calves in order to describe the distribution of the ciliated cells forming the mucociliary apparatus, and secondly to assess differences that might be found in the surface due to age by comparing the calves with normal adult cattle.

In addition the study was intended to provide a basis for future investigations on pathological materials.

MATERIALS AND METHODS

The general procedures have been given in Section II.

ANIMALS

Two groups of animals were examined. Group A comprised six Friesian calves one week old with no evidence of respiratory disease confirmed by gross and histological examination of their lungs. Group B comprised four adult cows, two aged two years, one aged five years and one 10 years old. Macroscopically their lungs were normal and there were no major histological abnormalities. In Group A each calf was anaesthetised with pentobarbitone sodium given intravenously and the oesophagus tied before exsanguination. Animals from Group B were stunned by shooting before exsanguination. At post-mortem examination the larynx and lower respiratory tract were removed from all the animals in both groups, and sampled within 10 minutes.

SAMPLING

Samples were selected at five different levels in the lower respiratory tract as illustrated in Fig. 2A, Section 2.

In the trachea three sites were established, cranial (1a) middle (1b) and caudal (1c). At each site the dorsal and the ventral walls of the trachea were trimmed and constituted the final segment of tissue studied, cranial-dorsal-trachea (1ad), cranial ventral trachea (1av), middle-dorsal-trachea (1bd) and middle-ventral trachea (1bv). The caudal trachea (1c) was sampled at the dorsal wall (1cd) and the ventral wall (1cv).

All the other samples taken were from the right lung. The large bronchi constituted Level 2, and two sites were selected, one in the cranial (2a) and the other in the caudal lobe (2b). Two centimetres long rings of the bronchi were excised and the

large bronchi distal to the sample were cannulated as described in Section 2 in order to perfuse the lung parenchyma with fixative (Fig. 3, Section 2).

Level 3 was small bronchi within the perfused segments in the cranial and caudal lobes as described in Fig. 4, Section 2. 3a was in the cranial lobe and 3b in the caudal lobe.

Level 4, bronchioles and Level 5 alveoli were trimmed after fixation, using a dissecting microscope from the lung parenchyma as illustrated in Fig. 4, Section 2.

Samples were taken for histological examination under LM, and in some cases TEM, from adjacent sites at all levels. They were processed by standard methods as described in Section 2.

PROCESSING FOR SEM

The details of this processing have been given in Section 2. In summary the samples were treated as follows. After fixations they were trimmed to flat pieces, washed overnight in sodium cacodylatic buffer 0.1 M. and dehydrated in an ascending series of acetones and then dried in a Polaron critical point dryer. Following surface orientations the specimens were cemented on aluminium stubbs and coated with gold-palladium in an Emscope Sputter Coater for 4 minutes. The specimens were examined by means of a Philips 501B scanning electron microscope, using an accelerating voltage of 15 kV.

RESULTS

The observations made on the samples from the lower respiratory tract of normal bovine animals are described below.

TRACHEA (Level 1)

In both groups the dorsal wall of the trachea at the three sites of sampling (1a, 1b, 1c) had longitudinally orientated folds covered by a complete carpet of cilia. On the ventral walls at the three sites the mucosal surface was flat and also completely covered by cilia but it was possible to identify numerous orifices of glands (Fig. 5).

On high power observations, it was possible to identify only ciliated cells. The cilia, examined at high magnification (Fig. 6) were abundant, long, not packed together but sometimes uniformally gathered together over the surface of the cells. All the cilia in each field looked orientated in one direction.

The major part of the epithelium appeared ciliated but there was not a continuous lining on the ventral surface, because of the openings of the submucosal glands (Fig. 5 and Fig. 6).

In the adult animals, areas of the tracheal surface epithelium devoid of cilia were seen similar to areas found in the large bronchi of the adult cattle as described later.

LARGE BRONCHI (Level 2)

The large bronchi did not differ to any great extent from the trachea. Although the surface of these bronchi, in both groups of animals and in both cranial and caudal lobes, appeared to be almost completely covered by ciliated cells, non-ciliated cells were more evident. However these non-ciliated cells did not protrude into the lumen (Fig. 7). The apical surface of these cells, presumed to

be goblet cells, readily distinguished them from the ciliated cells. The surface of the goblet cells frequently had a peripheral area covered with short microvilli and a central portion devoid of surface projections. These cells were usually obscured from view because of the multitude of cilia that lay over them.

In some of the adult animals observed the non-ciliated cells were protruding and became more evident (Fig. 8). This appearance was also occasionally seen in the trachea of adult animals. It was easy to identify these mucous secretory cells because of the granules at the apices of the cells (Fig. 8 and Fig. 10).

From observations made on transverse sections of the mucous membrane it was possible to visualize that these non-ciliated cells were quite numerous even when their surface was obscured by the cilia of the surrounding ciliated cells (Fig. 9).

The mature and active mucous secretory cells seen in the adults appeared to have a swollen surface, due to accumulated mucous granules (Fig. 10). Fewer microvilli around the cell margins were seen. The secretion seemed to be extruded through pits or pores in the cell surface (Fig. 10).

The content of the mucous granules gives the mature non-ciliated secretory cells its characteristic goblet shape. No microvilli or surface projections were visible on the surface of the extruded secretion.

In Group B, the adult cows, some small irregular shaped patches of epithelium devoid of cilia were found in the bronchi as well as in the trachea (Fig. 11). These areas were mainly seen on the ventral surface of the trachea and large bronchi of the cranial lobes where they are almost extrapulmonary. These changes observed in the epithelium were considered to be due to a damage to the ciliated cells. Large numbers of these cells had lost their cilia and in the remaining cells, appeared to have less cilia which were shorter (Fig. 11 and Fig. 12). A close-up examination demonstrated

that the cilia also looked to be packed in an irregular pattern which is a sign of ciliostasis. In these areas no observations related to increased activity of the non-ciliated mucous secretory cells were made (Fig. 12).

These altered areas devoid of cilia, were not seen in the large bronchi of the cranial lobes or in the trachea of the young calves.

SMALL BRONCHI (Level 3)

In the small bronchi of animals in both groups, the non-ciliated secretory cells became much more evident. In the lower levels of the small bronchi and near the transition with the bronchioles there were approximately equal numbers of ciliated and non-ciliated cells (Fig. 13).

In a low magnification picture, a regular pattern of non-ciliated cells, more or less of the same size and at the same stage of activity was observed (Fig. 13). These non-ciliated secretory cells appeared fairly round and their apical membrane appeared wrinkled as if the apical cytoplasm had collapsed (Fig. 14). The collapsed surface could be due to an earlier discharging phase. A fringe of many microvilli was easily distinguished on the edge of these non-ciliated secretory cells (Fig. 14). These microvilli were not seen in the centre of the cells. This compares with other quiescent non-ciliated secreting cells which are covered by a dense population of short stubby microvilli (Fig. 15).

A non-ciliated cell representing a transition between those in Fig. 15 and Fig. 14 is shown in Fig. 16.

The ciliated cells surrounding the non-ciliated secretory cells looked to be well ciliated and the cilia were orientated in one direction.

BRONCHIOLES (Level 4)

In both groups of animals the proportion of non-ciliated cells increased at this level so that they became the predominant cell type (Fig. 17 and Fig. 18). The ciliated cells were still present but no longer formed a complete carpet. They were located around single non-ciliated cells or groups of non-ciliated cells and it was often noted that at this particular level they had fewer cilia than cells in upper levels (Fig. 18 and Fig. 19). The ciliated cells in the terminal bronchioles had slender cilia which were shorter than those in higher levels and therefore allowed the presence of the microvilli between cilia to be visualized more clearly (Fig. 20). The cilia were standing in a disorderly way with no definite orientation as in the bronchi (Fig. 10).

The non-ciliated bronchiolar epithelial cells, Clara cells, revealed a characteristic dome-shaped apex, rounded and smooth except for scattered microvilli or small projections at the periphery of the cell surface giving it a granular appearance (Fig. 21 and Fig. 22). The abundant non-ciliated secretory bronchiolar epithelial cell projected high above the cilia of the ciliated cells. Their luminal surfaces had an oval perimeter and appeared to have a granular surface when quiescent (Fig. 22). The central portions of the cells frequently protruded into the lumen of the bronchioles and appeared to have a wrinkled surface when in an active phase (Fig. 21). The cell boundaries were easily discernible often by virtue of the contrast provided by microvilli on the adjacent cells (Fig. 22).

The terminal bronchioles continued into the alveolar ducts and no proper respiratory bronchioles were seen (Fig. 17 and Fig. 23).

No significant differences were observed between the bronchioles in normal calves and adults.

ALVEOLI (Level 5)

The findings in Group A and Group B were similar in both cranial and caudal lobes of the right lung.

The perfused tissue had a spongy appearance due to the multitude of thin-walled air sacs or alveoli (Fig. 23 and Fig. 24). Alveoli share extremely thin partitions with other alveoli that are adjacent to them. Division of the bronchial tree were present in the sections used to study alveoli and included small bronchi, bronchioles, terminal bronchioles, alveolar ducts and alveoli (Fig. 23 and Fig. 24).

The two types of alveolar epithelial cells were readily identified (Fig. 25). Alveolar Type II cells (granular pneumocytes) could be distinguished from adjacent cells because of their shape and the presence of short microvilli (Fig. 25). The Type II alveolar epithelial cells usually had their surface protruding into the alveolar lumen and were covered by microvilli openings or pits were also seen (Fig. 26). In some adult animals the Type II alveolar epithelial cells were particularly numerous in alveoli adjacent to interlobular septa and in alveoli close to bronchioles (Fig. 26 and Fig. 27).

A second cell identified, Type I alveolar epithelial cell or membranous pneumocytes, appeared very flat and with a smooth surface (Fig. 25 and Fig. 28). Small projections could be seen scattered on the surface of these cells. Narrow linear ridges representing the intercellular junctions between the cells that formed the alveolar wall were often identified (Fig. 25).

Small opening, the interalveolar pores or pores of Kohn, were readily seen (Fig. 29). They were variable in size, round or oval and exhibited a smooth even margin (Fig. 30). Interalveolar pores were observed frequently in both groups of animals.

A third cell was the alveolar macrophage which appeared to be attached to the alveolar surface by thin tendrils (Fig. 31 and Fig. 32).

The alveolar surface also had large ridges or slopes which denoted the position of capilaries within the wall causing bulges on the surface of the alveolar epithelium (Fig. 33).

DISCUSSION

In the present study, the experiment was designed to sample the two main lobes of the lung, where respiratory diseases are most frequently established, i.e. the cranial and the caudal lobes.

The nomenclature used for the lung lobes was that recommended in the Nomina Anatomica Veterinaria (1968) by the International Committee on Veterinary Anatomical Nomenclature in Vienna from where the World Association of Veterinary Anatomist functions.

The methods for SEM were selected on the basis of using techniques that had been applied successfully by other workers with similar specimens and had been recommended by Boyd and Wood in a comprehensive review in 1969. It was observed in some references dealing with SEM methods that not much importance was paid to the fixative used, for SEM. Techniques for TEM were frequently applied and this usually meant that a second fixative was used. The second fixative however was mainly used to stain cells rather than to fix them and was invariably OsO_4. In the observations reported here, OsO_4 had to be used very carefully because the tissues became hard and brittle after the treatment resulting in artifacts causing charging effects under SEM examination, which were very difficult to correct.

Some authors presented their SEM observations on samples that had only been fixed in glutaraldehyde. Although glutaraldehyde has been demonstrated to be a very good fixative for TEM it could result in incomplete fixation of the thick sections that were often prepared for SEM.

Modified Karnovsky has been shown to be the fixative of choice for SEM since its mixture of paraformaldehyde and glutaraldehyde present all the qualities necessary for good fixation. This is particularly true for lung tissue since Karnovsky's fixative is a good agent for dissolving the mucous secretions present on the

epithelial surface of the airways.

Tyler (1983) pointed out that the information produced by investigations on the respiratory tract was sometimes inconsistent because of the differences in the methods and definitions used. The definitions adopted in this study are as follows; the airways having been defined and named by the following characteristic structure.

A bronchus was considered to have complete walls formed by irregular plates or plaques of cartilage in loose connective tissue with elastic and collagenous fibres and smooth muscle; mucous or mixed glands were present in the lamina propria or submucosa and the surface epithelium was ciliated pseudostratified columnar with mucous (goblet) cells.

A bronchiole had walls of smooth muscle and loose connective tissue with no cartilage and no glands; interalveolar septa attached to their abluminal surface and ciliated columnar to cuboidal epithelium with no mucous cells formed the luminal surfaces. Terminal bronchioles were the most distal generation of bronchioles which were not alveolarized and were considered to be the last of the conducting airways.

Respiratory bronchioles were considered to be a transition between conducting airways and respiratory air spaces with the same structure as non-respiratory bronchioles and in addition the presence of openings in the walls for alveoli. Low generations of respiratory bronchioles could be poorly alveolarized i.e. having few alveoli. Higher generation of respiratory bronchioles were typically well alveolarized i.e. having numerous alveoli.

Alveolar ducts had alveoli opening into the lumen all around the circumference and the wall contained spiral smooth muscle; Type I and II alveolar epithelial cells formed the surface epithelium of alveolar ducts.

The study established some differences in the distribution of the epithelial cells of the airways when the bovine animal was compared with other mammals.

In newborn puppies (Wright et al., 1983) the ventral wall of the trachea had areas devoid of ciliated cells which were considered to be immature tissue. In addition, ciliated cells were never observed in the bronchioles, whereas in the bovine animals ciliated cells were seen in bronchioles although they were poorly ciliated.

Contrary to the finding in the mouse (Pack et al., 1980), cells considered to be mucous secreting cells occurred mainly in the large airways of the cattle. For the mouse 50-60% of the non-ciliated cells present in the trachea and large bronchi were Clara cells. In the bovine animals Clara cells were considered to be the predominant cell type of the bronchioles. Similarities were found between cattle and sheep when the information obtained from studying the respiratory tract of lambs by SEM was considered (Tyler et al. 1971).

Although in the human, alveoli are said to develop after birth (Reid, 1972) this was not apparent in the bovine animal, since the alveoli in the one week old calves were not significantly different from those in the adult cows. Tyler et al. (1971) expressed the same opinion about the sheep.

No major differences were established when the one week old calves were compared with the adult animals, except for areas of desciliation present in the trachea and extrapulmonary bronchi. These areas were considered to be abnormal. In addition active mucous secreting cells were only identified in the adult cows and these were only present in the large airways. Some differences were found from a previous study done with SEM in the bovine animals (Mariassy et al., 1975). However their observations were obtained by examining the lung of a group of young adult cattle from a slaughterhouse where no history was recorded and where the extrapulmonary airways were not studied.

The results of this study found that the surface epithelium of the conducting portion of the lower respiratory tract of the bovine animal contained two main cell types, as judged by SEM and that in general it resembled the surface epithelium of the other mammalian species previously reported although there were some differences, referred to earlier. The two main cells, the ciliated and non-ciliated cells were present in a different pattern according to the level in the airway that was observed. The relative proportions of ciliated and non-ciliated cells varied in the trachea, the extrapulmonary bronchi, intrapulmonary large bronchi, the small bronchi and bronchioles. The ciliated cells were the predominant cells in the trachea, large bronchi and small bronchi where they represented a major component of the mucociliary escalator. The mucociliary escalator with the cough reflex and alveolar macrophages, is considered to be one of the most important factors for removing foreign particles from the respiraltory system and it is consequently an important defence mechanism. The ciliary component of this apparatus in the bovine animal appeared to be well developed in the trachea, large bronchi and small bronchi. In the bronchioles the ciliary component of this apparatus was not well developed.

Breeze and Wheeldon (1977) reviewed and gave detailed descriptions of the cell types that comprised the basic structure of the lining of the lower respiratory tract. At least 13 cell types, 11 epithelial and two mesenchymal have been recognised in the tracheobronchial epithelium, and these have been listed in Table 1 in Section 1. The majority of these cells were described by LM or TEM and less information was available on the structure of the surface of these cells as seen by SEM. The topography of the ciliated cells and non-ciliated cells of the bovine tracheo-bronchial lining has been clearly observed in the present study by the use of the SEM.

Ciliated cells, which were considered to be the most abundant of all the airway epithelial cells, were present in the epithelium at all sites of sampling from Level 1 to Level 4; they were absent

in Level 5. The ciliated epithelium appeared folded in the dorsal wall of the trachea and this was considered to be due to less firm attachment of the mucous membrane to the cartilage in the wall at this point. This folding made it difficult to identify the openings of glands in the dorsal wall compared with the ventral wall where the flat appearance made it possible to identify the opening of the glands easily. The openings of glands were not obvious in the bronchi.

The ciliated cell is known by TEM to be a columnar cell with the lower lateral cell surface forming complex connections with adjacent cells. Many mitochondria have been found in the upper part of the cell and this has been explained as giving sufficient energy to the basal body anchors of the cilia which are on the luminal face of the cell. Rootlets extend from the basal bodies into the cell.

Some authors have reported that 200 cilia are present on the luminal surface of each ciliated cell. Between the cilia, microvilli and cytoplasmic processes have been seen with TEM. These were seen with SEM more easily in the terminal bronchioles where the cilia no longer made a complete carpet capable of forming an efficient mucociliary escalator. Cilia that transport mucus are generally 5 to 7 µm long and have been reported to have a diameter of about 0.25 µm (Sleigh, 1977-1981). There did not appear to be major differences between the ciliated cells in the animals of both age groups nor in other species.

As reported by Sanderson and Sleigh in 1981 all the packed cilia in each field beat in the same direction in coordinated waves that travel short distances and spread across the surface. This could explain the harmonic pattern of all the cilia lying in one direction which was observed <u>in situ</u>. This pattern was more evident in Level 1, Level 2 and Level 3 than in Levels 4 and 5. The cilia beat, in the respiratory tract, in the periciliary fluid, while only the tip of cilia moves in the thicker viscous mucous layer. The ciliated areas appear to be continuous but they are in

fact separated by groups of non-ciliated secretory cells and the submucosal gland openings. These structures represent, among other functions, the source of the periciliary fluid and regulate its depth and composition. The abundant microvilli on the ciliated cell may play a role in the absorption or secretion of periciliary fluid. Interactions between the mucus layer and the cilia might also be critical to proper mucociliary transport (Kilburn, 1968).

The total circumference of the terminal bronchioles in the human lung is nearly 2000 times that of the trachea (Weibel, 1963) so the considerable decrease in the presence of the ciliated cells at this level in the bovine could suggest that other defence mechanisms might operate and be more important at this level. In the normal lung the mucus transport velocity appears to increase from the small to the large airways (Iravani and Melville, 1976) and some authors have explained that this progressive increase in mucus velocity is due to the increase in the proportion of ciliated cells from the bronchioles to the trachea and the increase in the average length of the cilia in the more proximal airways. The relative number of ciliated cells found at the levels observed could be sufficient evidence to allow this concept to be extended to the bovine animal. Sleigh (1977) considered greater ciliary beat frequency and increased relative numbers of ciliated cells in the larger airways as well as regional differences in the chemical composition and thickness of the mucous layer could also contribute to the accelerated mucociliary transport rates in the central airways.

The ciliated cells of large airways appear to regenerate from basal cells as judged by TEM. In the terminal bronchioles where there are no basal cells; non-ciliated Clara cells may serve as progenitors to ciliated cells. Therefore some of the ciliatd cells observed at that level could represent regenerating ciliated cells with immature ciliation. The factors that regulate the turnover of ciliated cells are not known.

One of the non-ciliated cells, the goblet cell, considered to be a mucus secreting cell, was found principally in the large airways. In the trachea they were more evident in the adult animals when the cells were mature and discharging. Immature cells or discharged goblet cells were seldom evident and this was considered to be due to the long cilia of the ciliated cell obscuring the presence of the goblet cells lying interspersed between the ciliated cells. The mature cells or active discharging cells seen in the adult animals appeared protruding into the lumen. The apical cytoplasm contained the mucous granules making this surface look granular and as described by TEM this content gives the cells a goblet shape. Immature cells or discharged cells did not seem to have this appearance when observed by SEM and their surface appeared completely covered by short microvilli.

In the calf these cells were found in a non-secretory phase, in the trachea and large bronchi while in adult cattle some areas were observed with very active goblet cells. It could be accepted that for adult animals, some abnormal pattern might be present considering that these animals could have been exposed to micro-organisms without developing major damage. Where some areas of deciliation were found, the non-ciliated cells were more evident but were either immature cells or mucous cells in a non-secretory phase.

As presented in the results, the non-ciliated cells became the predominant cells in the bronchioles and in the transitional level between the small bronchi and the bronchioles. Non-ciliated cells in this level had features similar to the non-ciliated bronchiolar Clara cells. An identical cell, in the tracheal epithelium of a rat, was considered to be an epithelium serous cell. Some authors categorized this cell as a variant of the goblet cell. Jeffery and Reid (1975) considered that the epithelial serous cells resembled the serous cell of the bronchial submucosal gland, and was probably the cell containing apical PAS-positive neutral mucosubstance granules identified in the rat tracheobronchial epithelium stained with alcian blue (pH 2.6). This observation was interesting

because one of the most frequent components of mouse airway epithelium was described as a non-ciliated cell that bore surface microvilli. When this cell was studied by TEM it was believed to be related to the Clara cells because of the similarity between the shape of its secretion granules.

However, the fact that non-ciliated cells were present sometimes as frequently as the ciliated cells in small bronchi supports the view that these are likely to be epithelial serous cells. Further studies should be conducted at this level of the small bronchus to corroborate this point of view. There is, however, increasing evidence in the literature to support the concept that the serous cells, might contribute to form the periciliary liquid layer found beneath the tracheobronchial mucus, and that these microvillous cells are involved in the transport of IgA across the epithelium. Mucous secretory cells with the characteristics of goblet cells were not seen in the bronchioles confirming the LM observations in non-pneumonic calves of Allan and Pirie (1977).

Bronchiolar brush cells, which have been identified in the large and small bronchi of bovine lung by TEM (Allan, 1978) were not detected in our study involving the SEM.

The non-ciliated bronchiolar epithelial cells resembled human Clara cells. The apical cell cap was rounded and smooth and sometimes appeared wrinkled; this was attributed to fixation artefacts. It was believed that the apical cap extruded into the bronchiole and was broken down to release the secretory product. More studies require to be done to obtain more information about these cells. Distinctive respiratory bronchioles were not seen and there was a relatively sudden transition from terminal bronchioles to alveolar ducts.

The results obtained from this study, demonstrated that Type I and Type II alveolar epithelial cells were the two cells present in the walls of the alveoli.

In adult animals Type II alveolar epithelial cells appeared forming small groups near interlobular septa, adjacent to bronchioles and in alveolar ducts and might represent progenitor cells. The protruded centre of the Type II alveolar cells appeared to have pits or orifices, through which the secretory product might have been released. This coincided with descriptions made by Nowell and Tyler (1971) who observed these pits in Type II cells in equine lung. Mariassy et al. (1975) were not able to demonstrate them in the bovine lung and they proposed that the reason for the differences could not be ascertained until more information was available on surfactant synthesis and turnover in the two species.

Pores of Kohn were consistently found in both groups of animals. The presence of these pores was considered to be normal in several species but Mariassy et al. (1975) found the pores extremely uncommon in the lungs of young adult cattle and considered that this might be typical for that age group. They attributed their presence in older animals to earlier damage to the lung. Nevertheless, Bastacky et al. (1983) considered them to be normal features. They described lung structure in the human combining different techniques such as SEM, TEM, and LM with microdissection, and examination of lung in the frozen-hydrated state. Their results suggested that alveoli varied in shape, size, and entrance width as a function of their position along the airway. They established that the transition from ciliated conducting airway eithelium to squamous respiratory epithelium was quite abrupt and the alveolar wall in the hydrated state was quite smooth, tended to be round and had surfactant occluding almost all pores of Kohn. These interalveolar pores became evident after the tissues were fixed in paraformaldehyde-glutaraldehyde. According to Boyd (1980) this destroyed selective components of the surface layer making specific surface features visible.

In contrast to the finding of Mariassy et al. (1975) alveolar macrophages were readily observed. It has been proposed that blood monocytes leave the circulation to undergo differentiation into functional alveolar macrophages. Alveolar macrophages were

detected sitting on the alveolar wall but no evidence was found related to the way they approached this place.

FIGURES

Fig. 5 - Normal Calf: Ventral wall of the trachea with a complete carpet of cilia and showing many orifices of mucosal glands.

SEM x 160

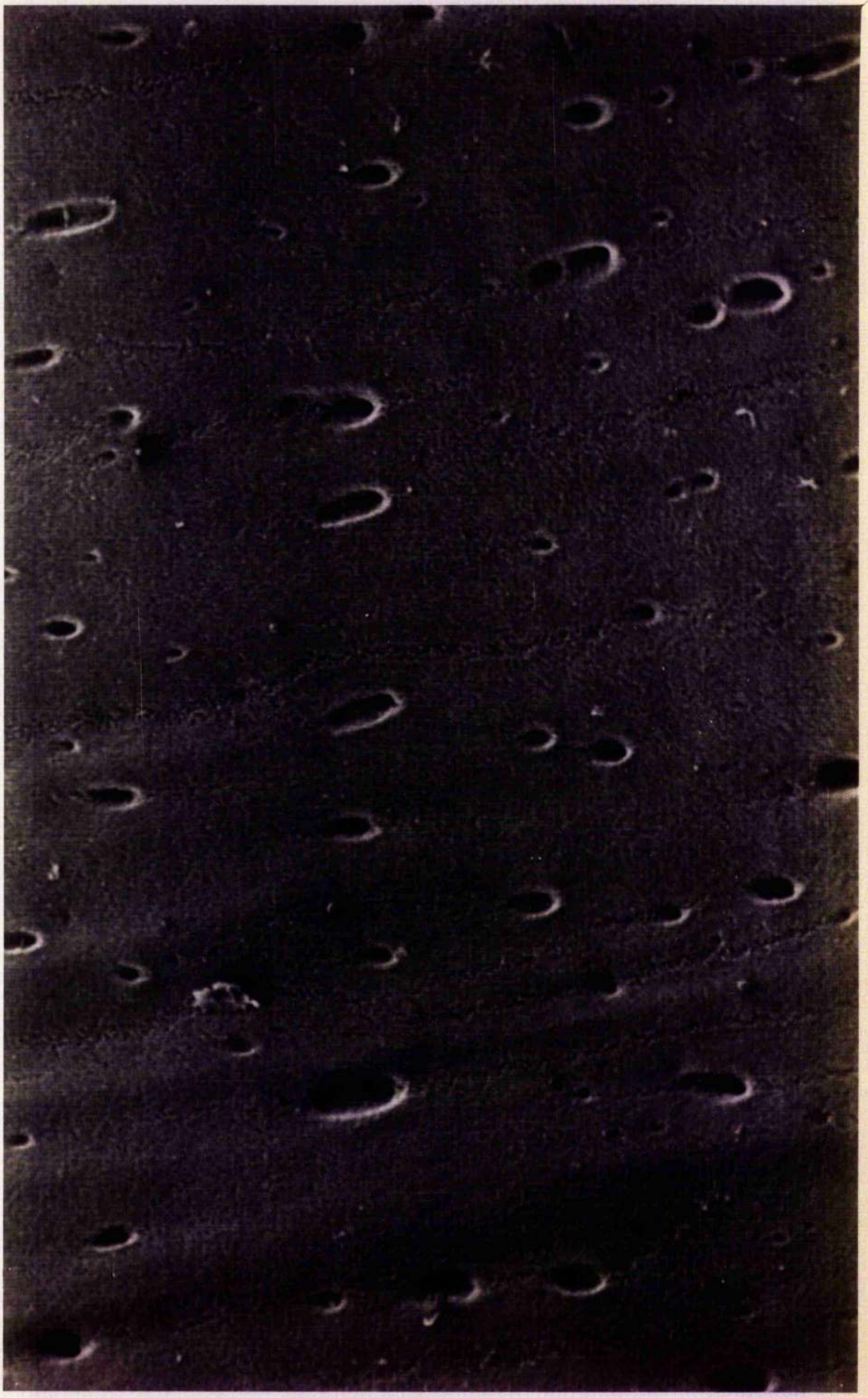


Fig. 6 - Normal Calf: Ventral tracheal wall showing the orifice of a mucosal gland and the thick uninterrupted carpet of cilia.

SEM x 640

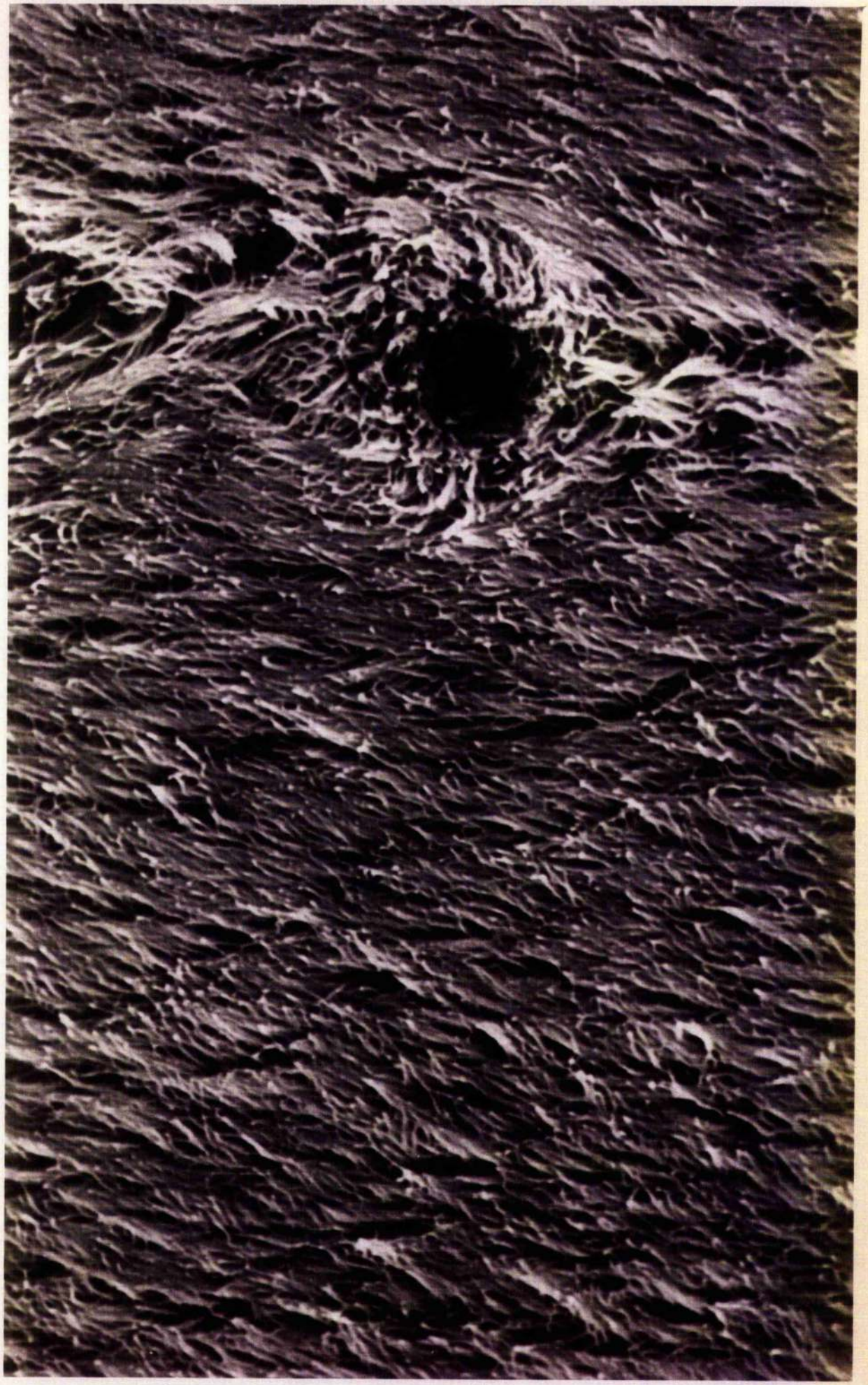


Fig. 7 - Normal Cow: Surface of a large bronchus with non-ciliated cell (*) between ciliated cells. The non-ciliated cells do not protrude into the lumen.

SEM x 2500

Fig. 8 - Normal Cow: Mucous membrane of large extrapulmonary bronchus in the cranial lobe, with non-ciliated mucous secretory cells (goblet cell arrow) with granules at the apices of the cells.

SEM x 1250

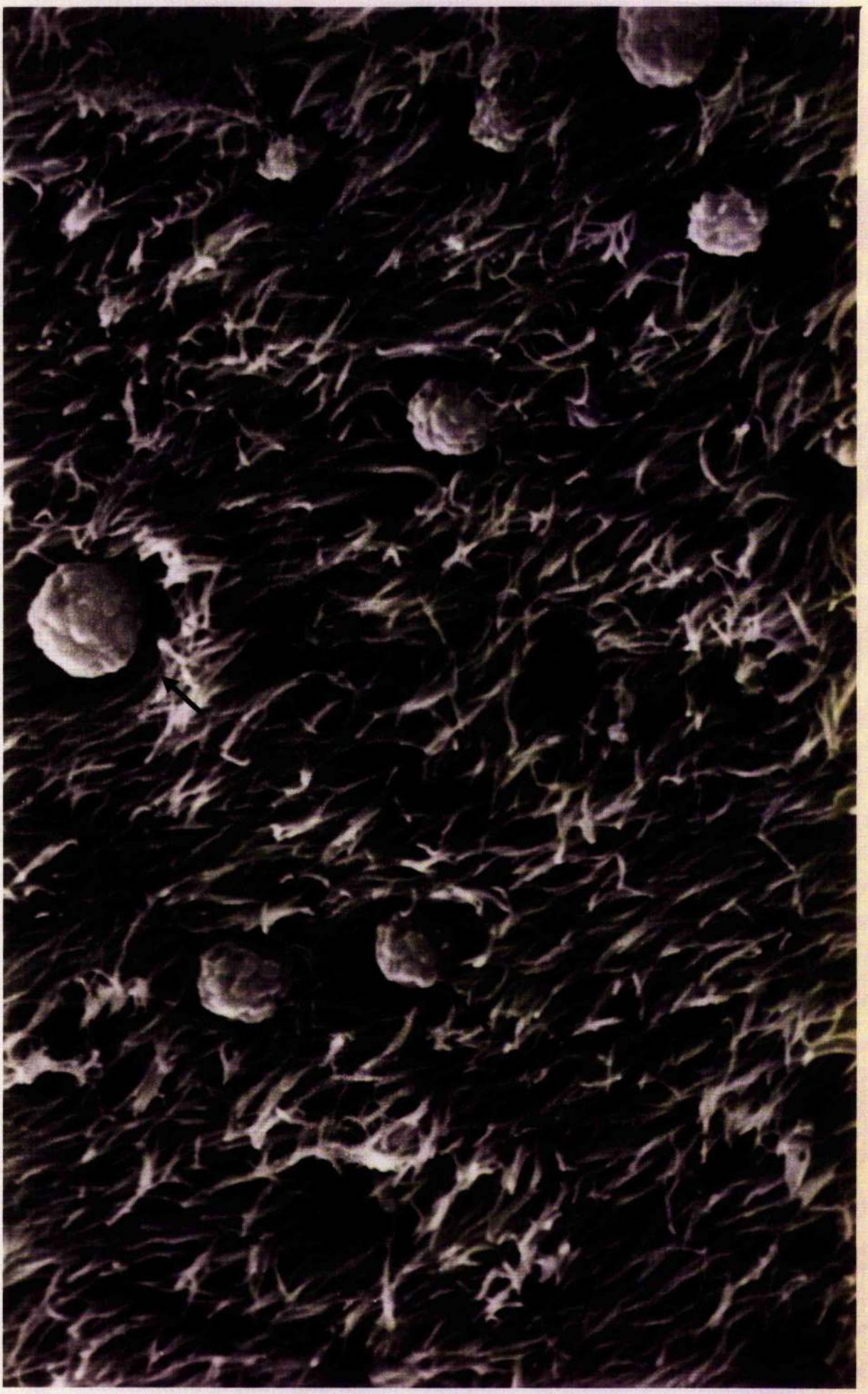


Fig. 9 - Normal Calf: Transverse section of the mucous membrane of a large bronchus. Goblet cells (G) can be seen with mucous granules as round and smooth spherules (*). There are some empty spaces left by the granules. The cilia of adjacent cells (C) seem to obscure the surface of the mucous cells.

SEM x 2500

Fig. 10 - Normal Cow: Mature and active goblet cells interspersed with ciliated cell. Observe the swollen surface (G) and a pit or pore in the apex of one cell (arrow).

SEM x 2500

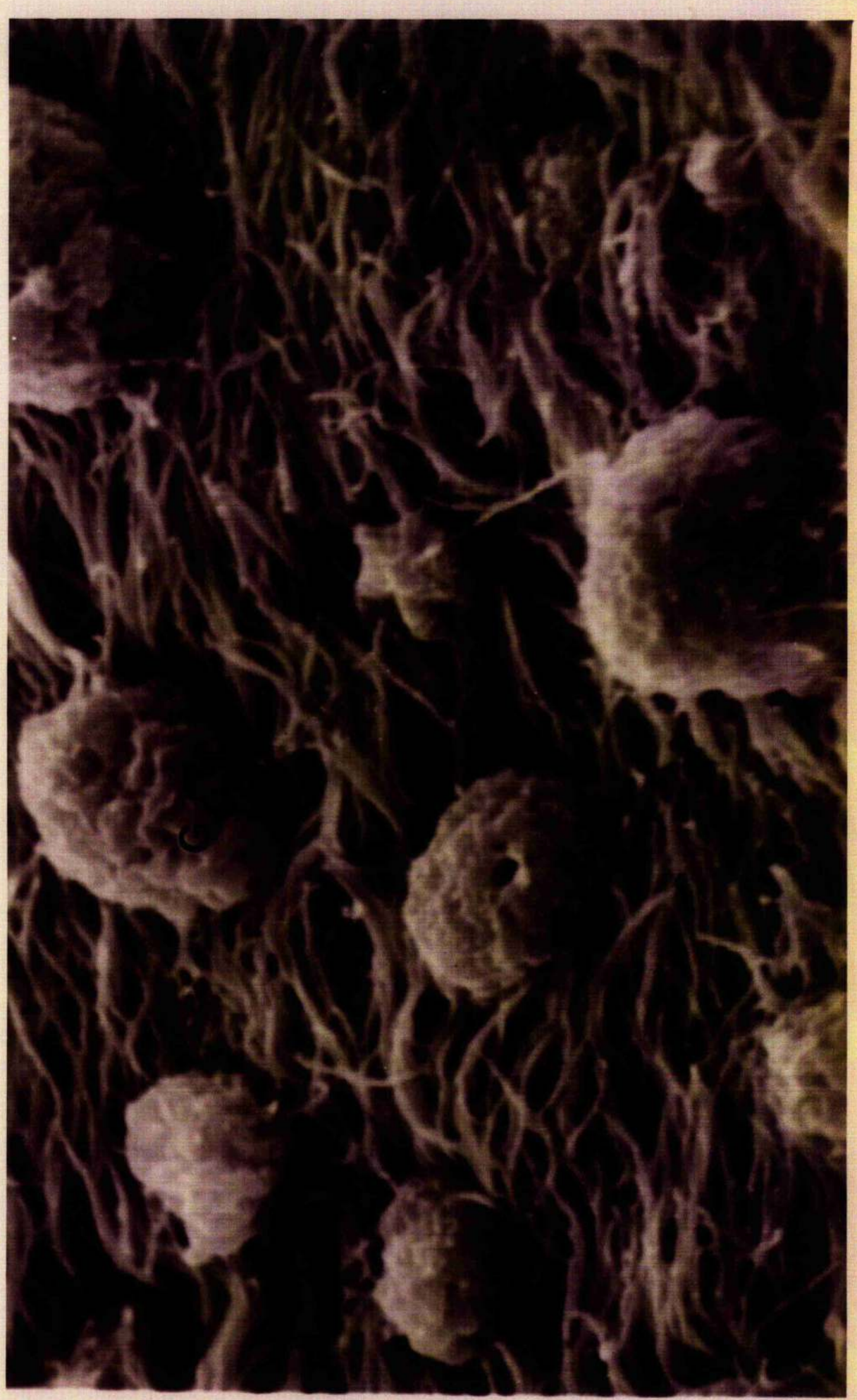


Fig. 11 - Normal Cow: Tracheal surface of an adult animal with patches of epithelium (*) devoid of cilia similar to changes seen in large bronchi.

SEM x 320

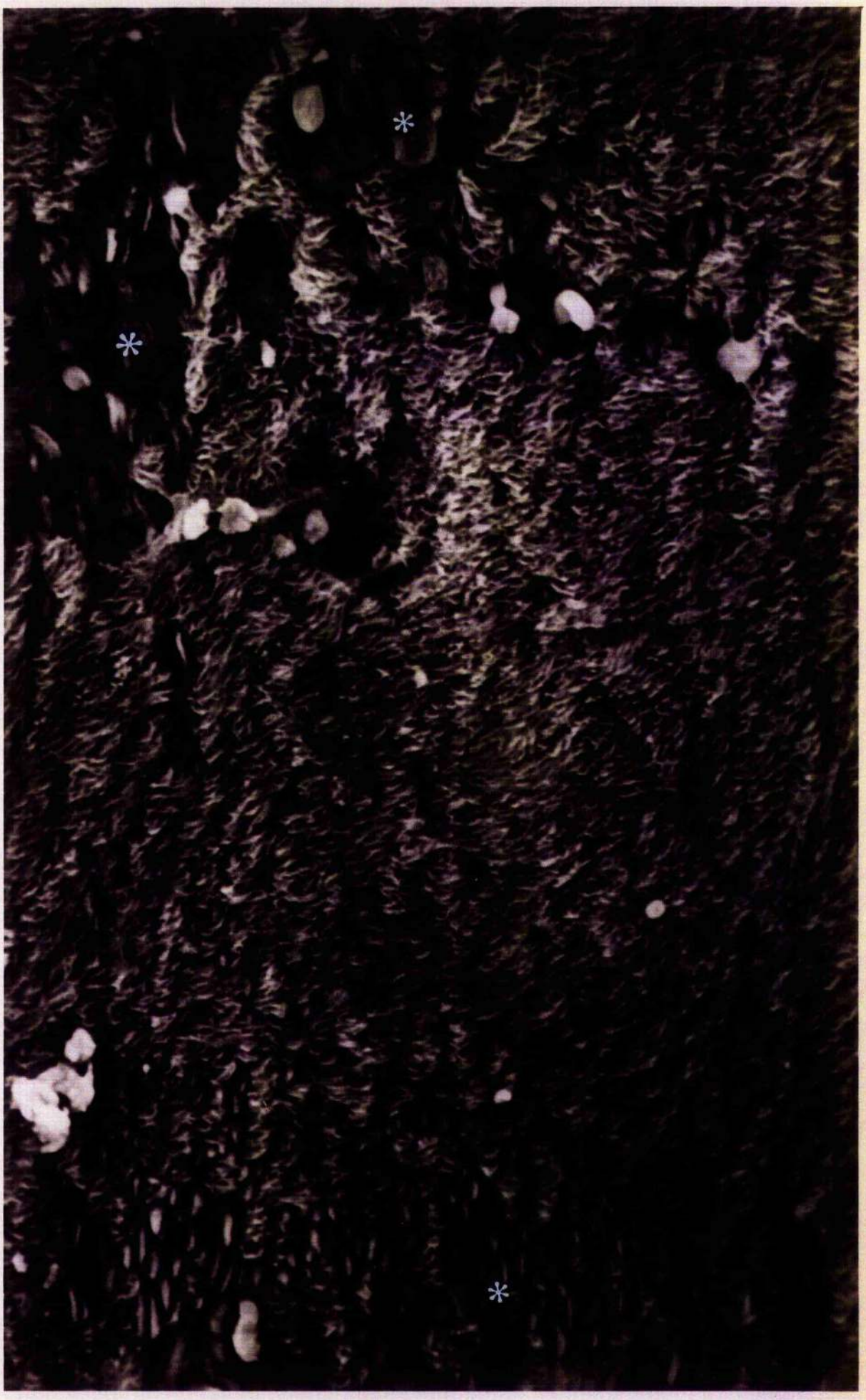


Fig. 12 - Normal Cow: High magnification of the desciliated area shown in Fig. 11. There is complete loss of cilia (*) as well as cells with sparse and poorly formed cilia. In addition the non-ciliated cells have variable surface size and shape.

SEM x 640

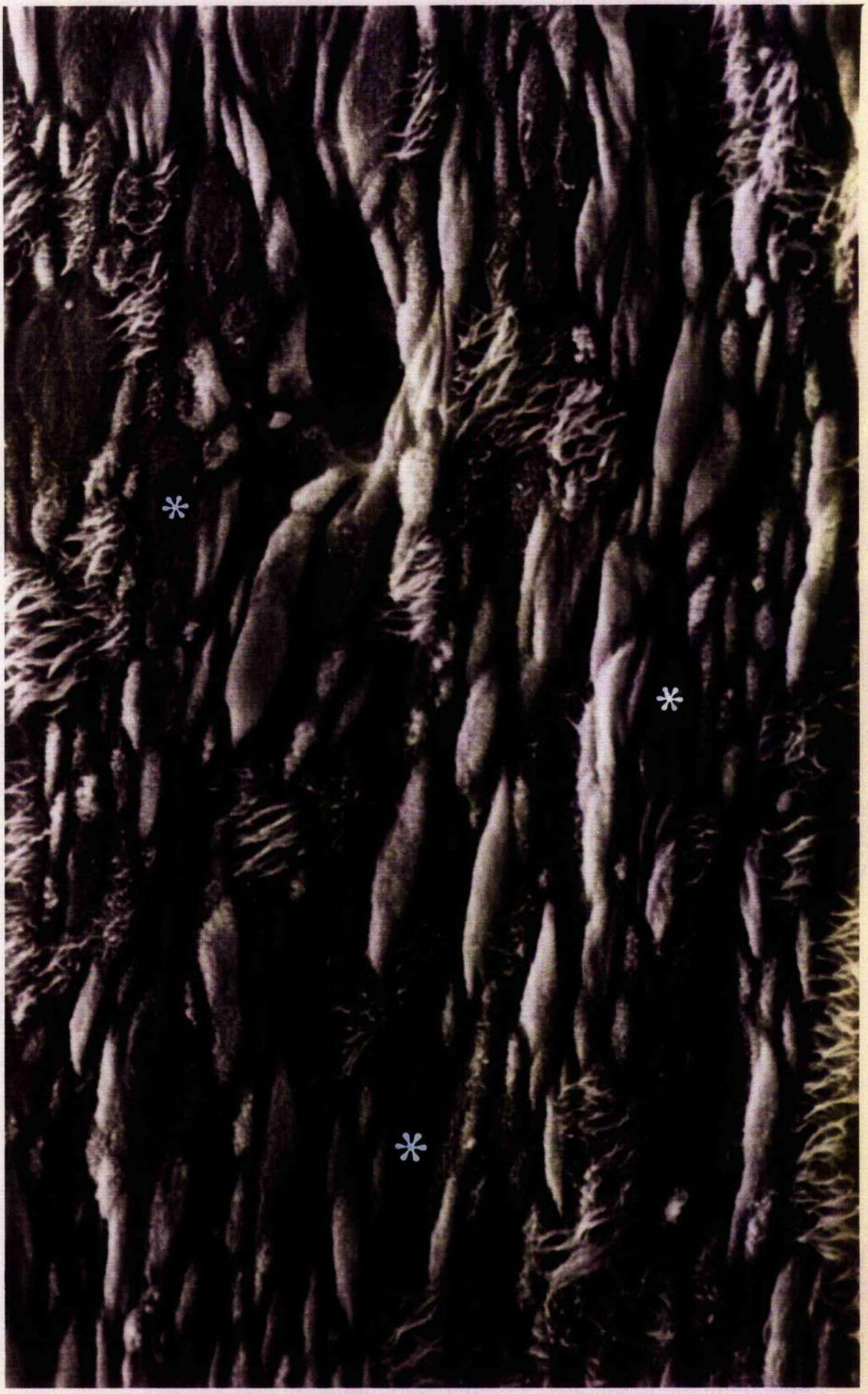


Fig. 13 - Normal Calf: The surface of a small bronchus at a transitional level with bronchioles. Observe the equal numbers of ciliated and non-ciliated cells which appear not to have mucous granules.

SEM x 160

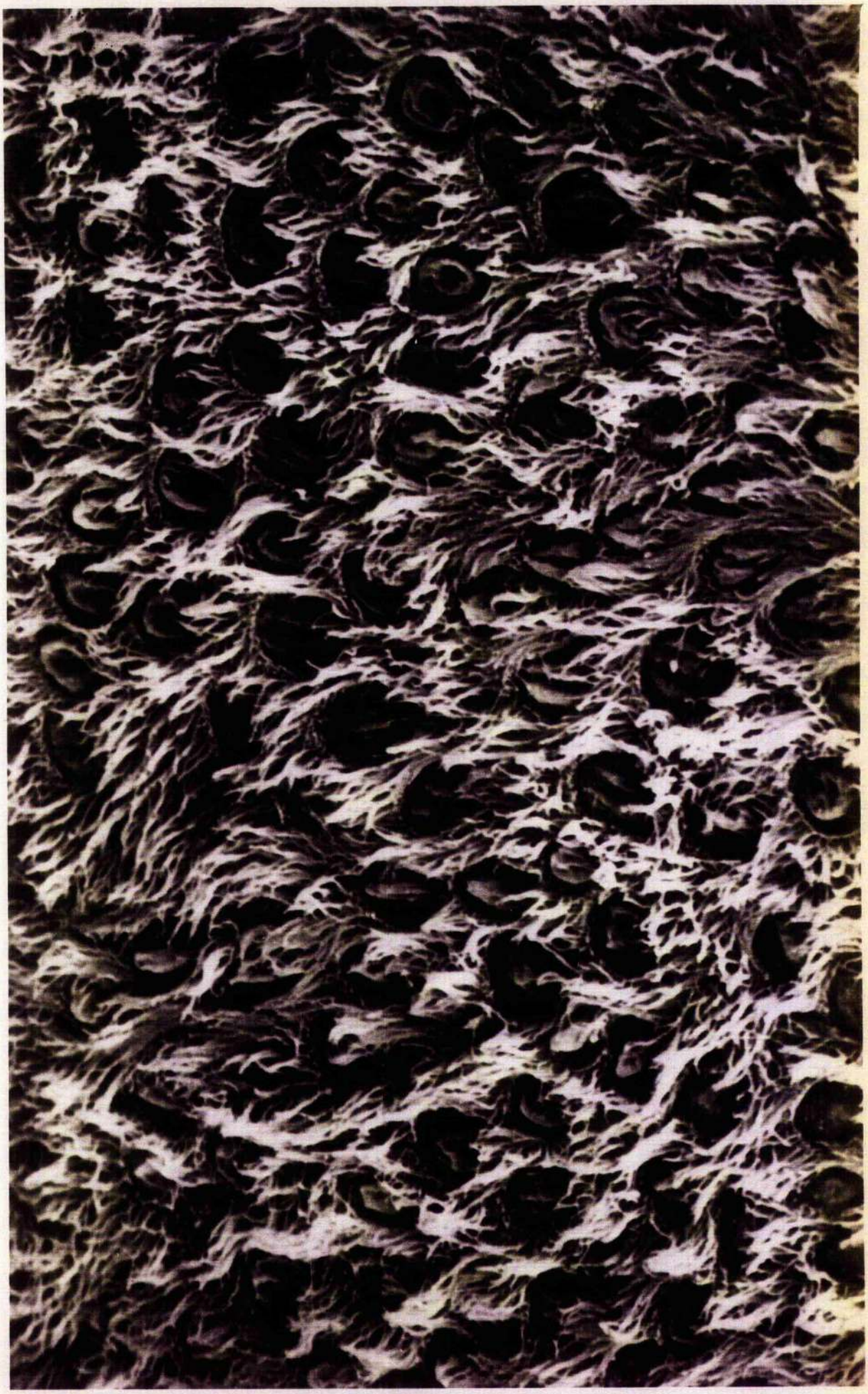


Fig. 14 - Normal Calf: The small bronchi seen in Fig. 13 with non-ciliated secretory cells appearing round with apparently collapsed apical cytoplasm (*). A fringe of many microvilli are easily distinguished on the edge of these non-ciliated secretory cells (arrow) and no projection could be seen in the centre of the cells. Cilia of the ciliated cells looks free and well orientated.

SEM x 1250

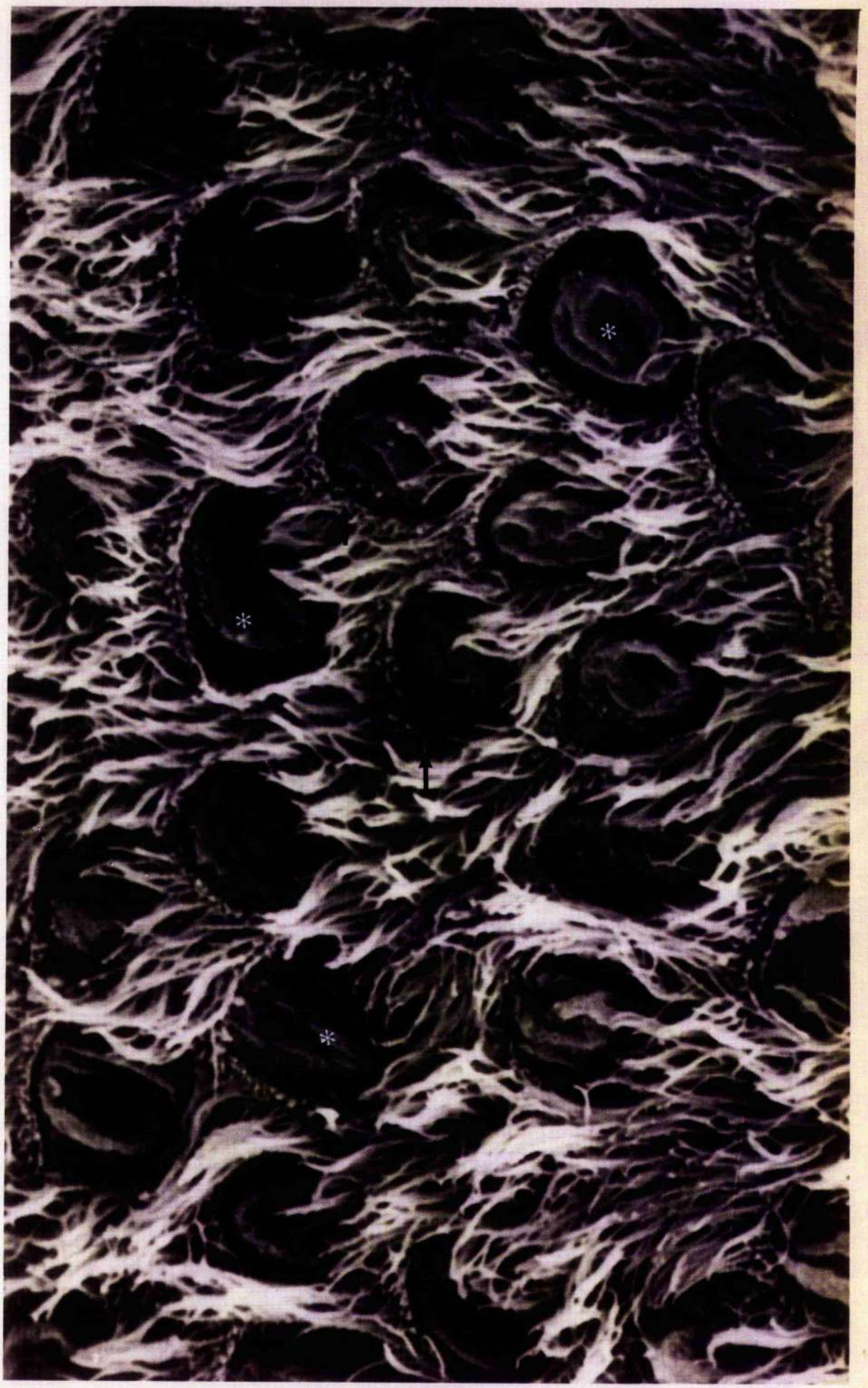


Fig. 15 - Normal Calf: A small bronchus with ciliated and non-ciliated cells. The non-ciliated cells do not protrude and its surface appears to be covered by microvilli (*)

SEM x 2500

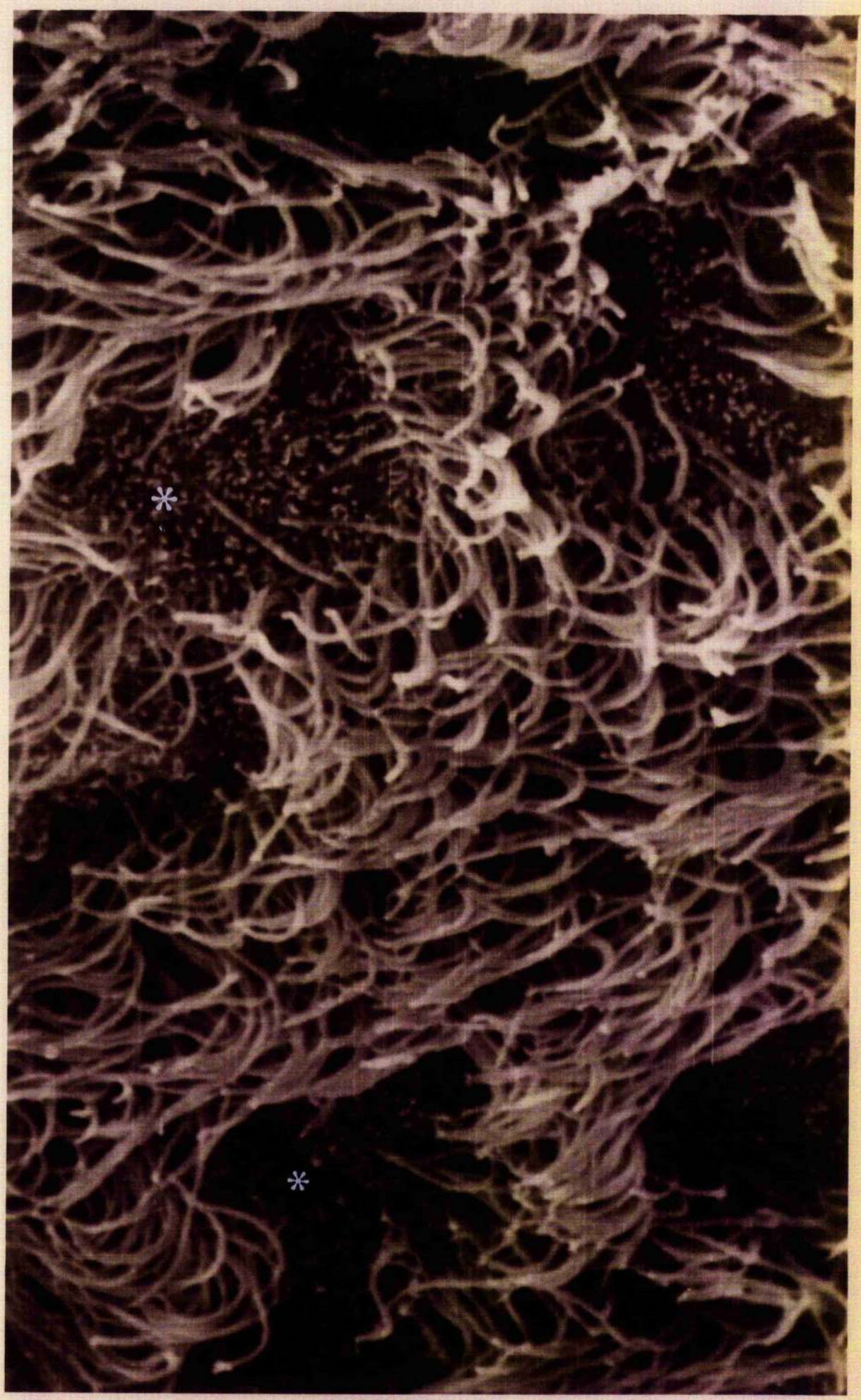


Fig. 16 -- Normal Cow: A non-ciliated cell surrounded by ciliated cells in the surface of a small bronchus. The membrane of non-ciliated cell appears to be covered by a dense population of short stubby microvilli but the central area is les densely covered and is slightly wrinkled. The cell may represent a transitional phase between the cell in Fig. 15 and Fig. 14.

SEM x 5000

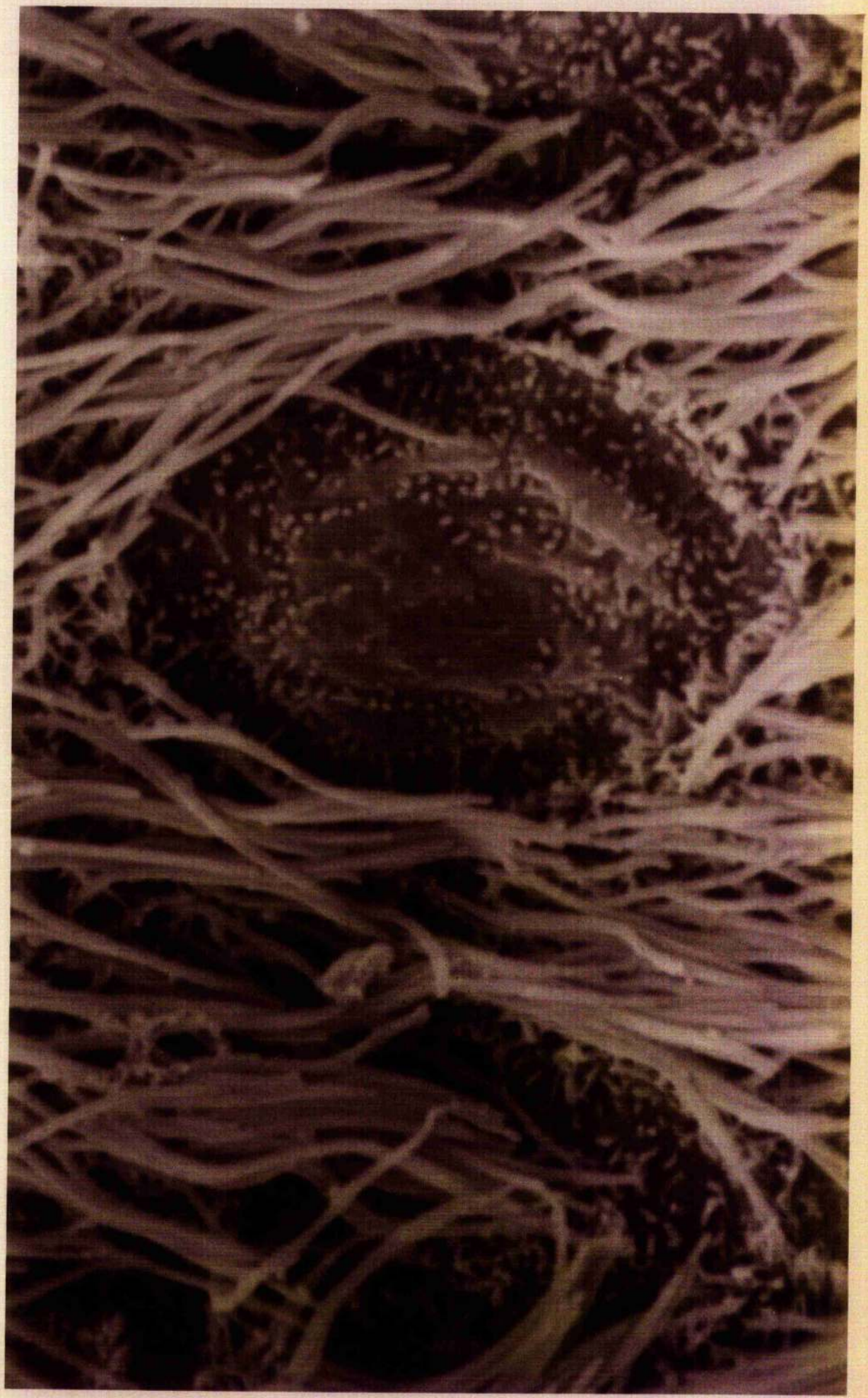


Fig. 17 - Normal Calf: A low power view of the terminal bronchiole. The surface is comprised mainly by non-ciliated epithelial cells. The terminal bronchiole continued into the alveolar ducts (arrow) no proper respiratory bronchioles could be seen.

SEM x 160

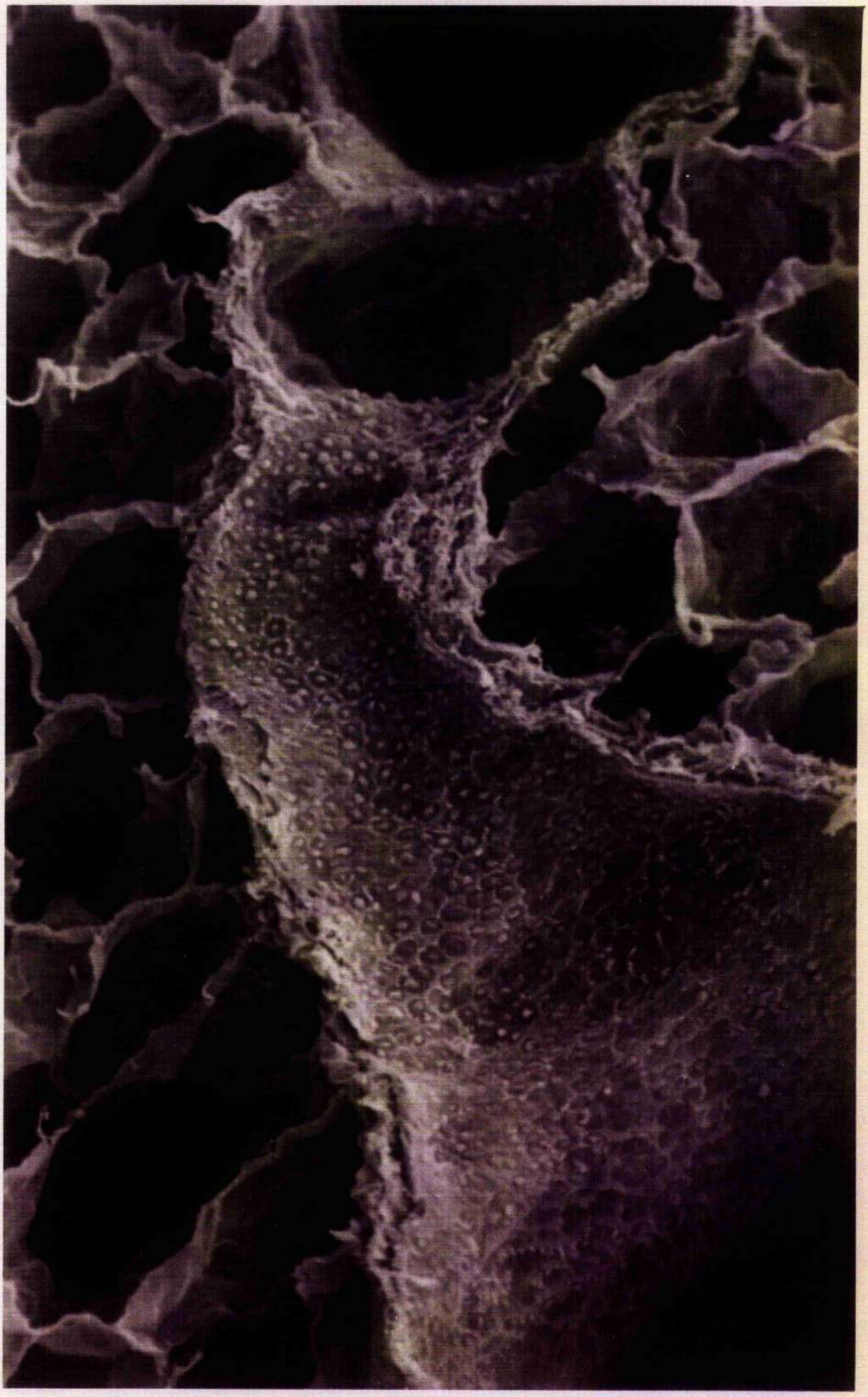


Fig. 18 - Normal Cow: Bronchiolar luminal surface illustrating the predominance of non-ciliated cells although ciliated cells are still present at this level.

SEM x 640

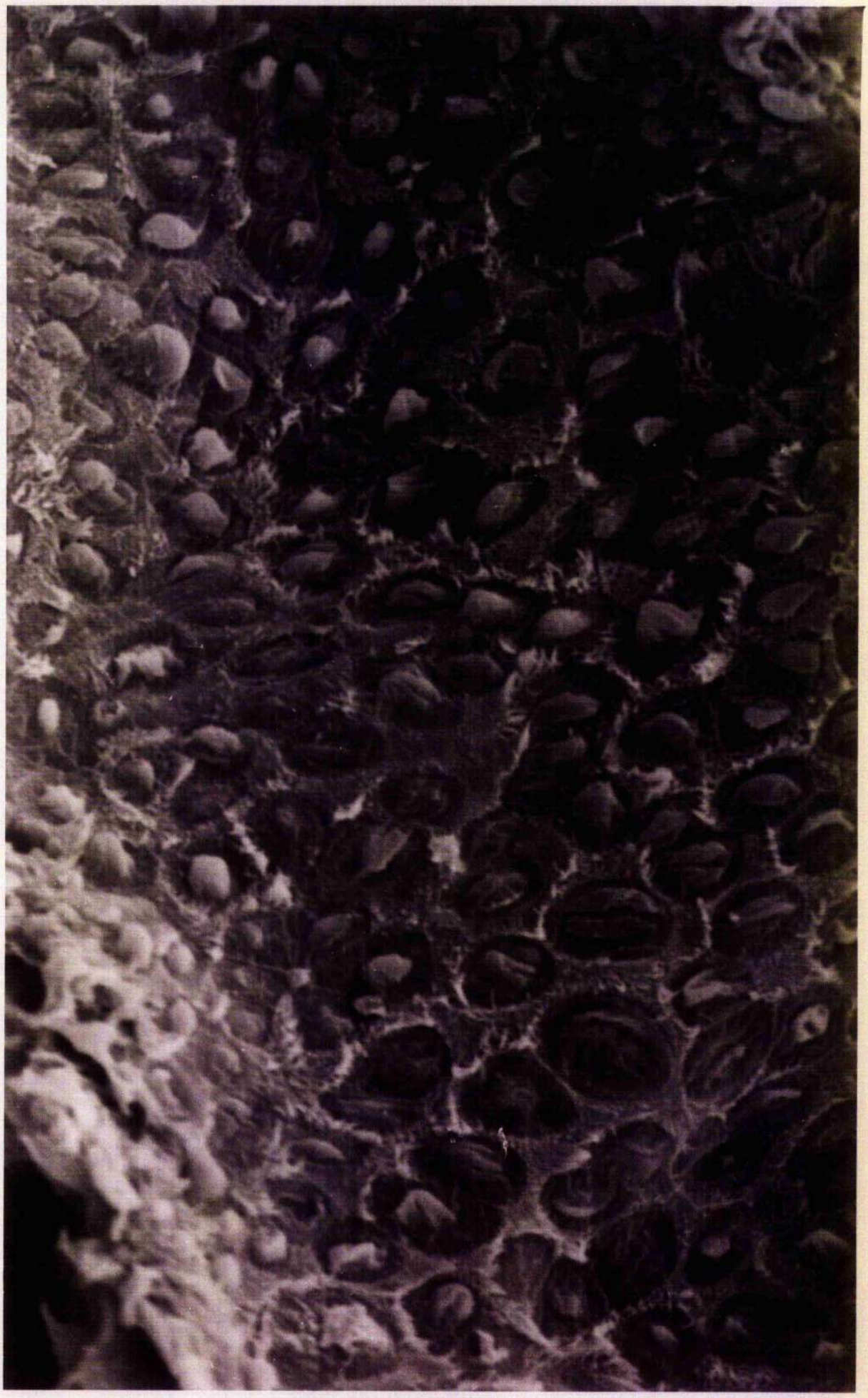


Fig. 19 - Normal Cow: The non-ciliated bronchiolar epithelial cells, Clara cells, present at this level have a characteristic dome-shaped apex, probably indicating an active phase, and protruding into the lumen. Ciliated cells appeared with short cilia and multiple microvilli.

SEM x 2500

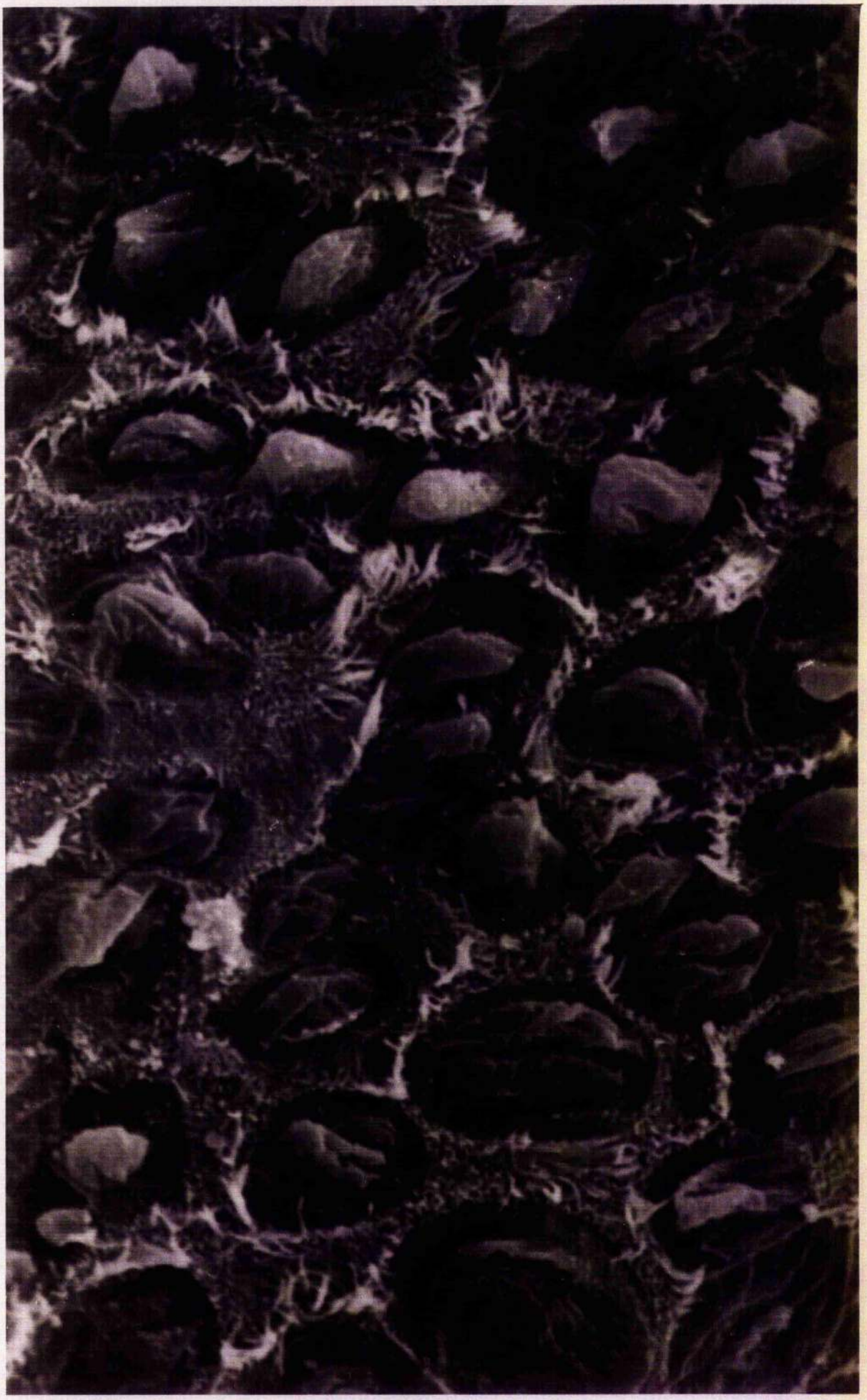


Fig. 20 - Normal Cow: The non-ciliated bronchiolar epithelial cells are abundant and their cytoplasm projects above the cilia of the ciliated cells. Their luminal apices have an oval perimeter and have a wrinkled surface. The ciliated cells appear poorly ciliated.

SEM x 1250

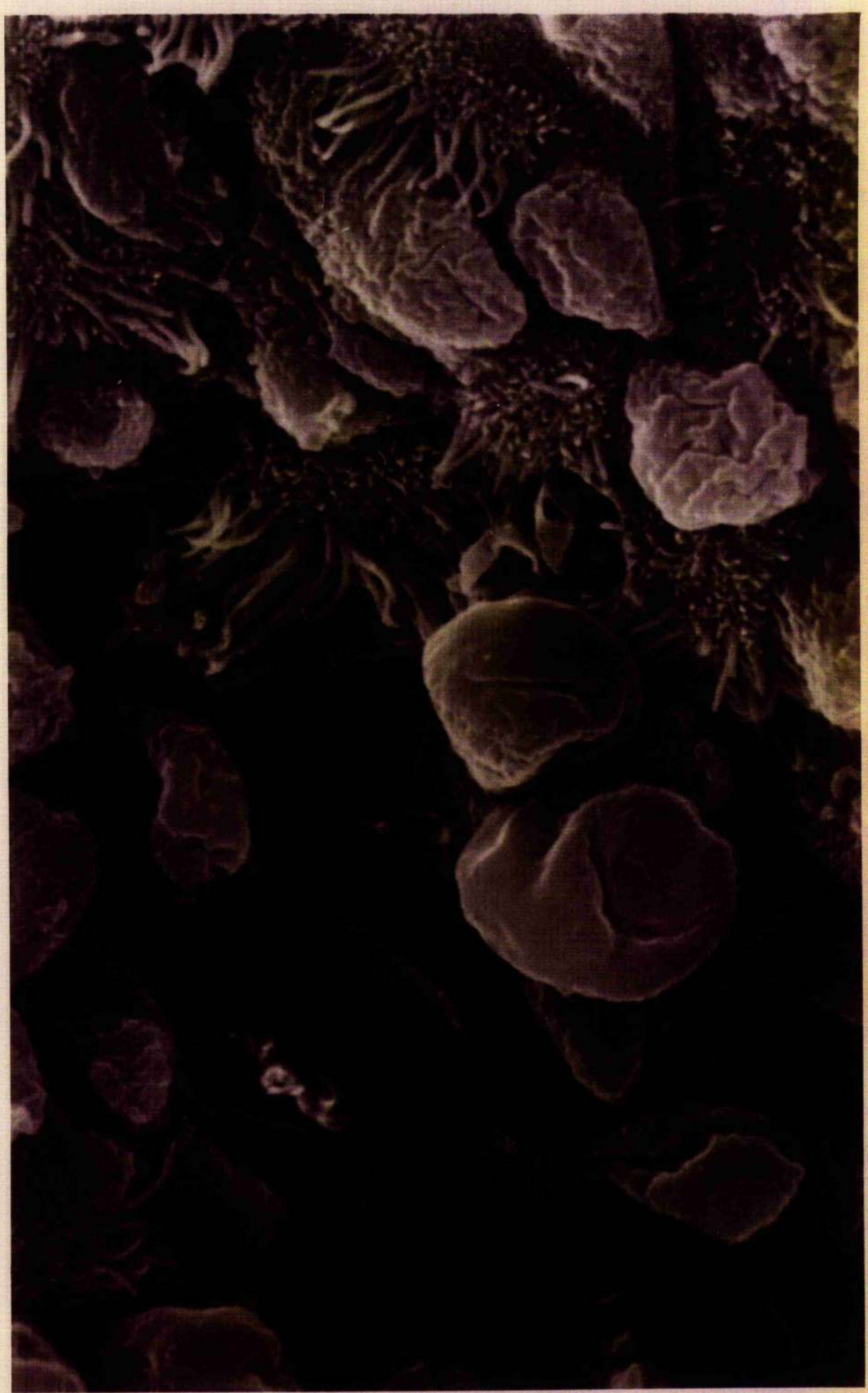


Fig. 21 - Normal Cow: Ciliated and non-ciliated bronchiolar epithelial cells. Observe the microvilli of the ciliated cells with fewer and shorter cilia (arrows).

SEM x 5000

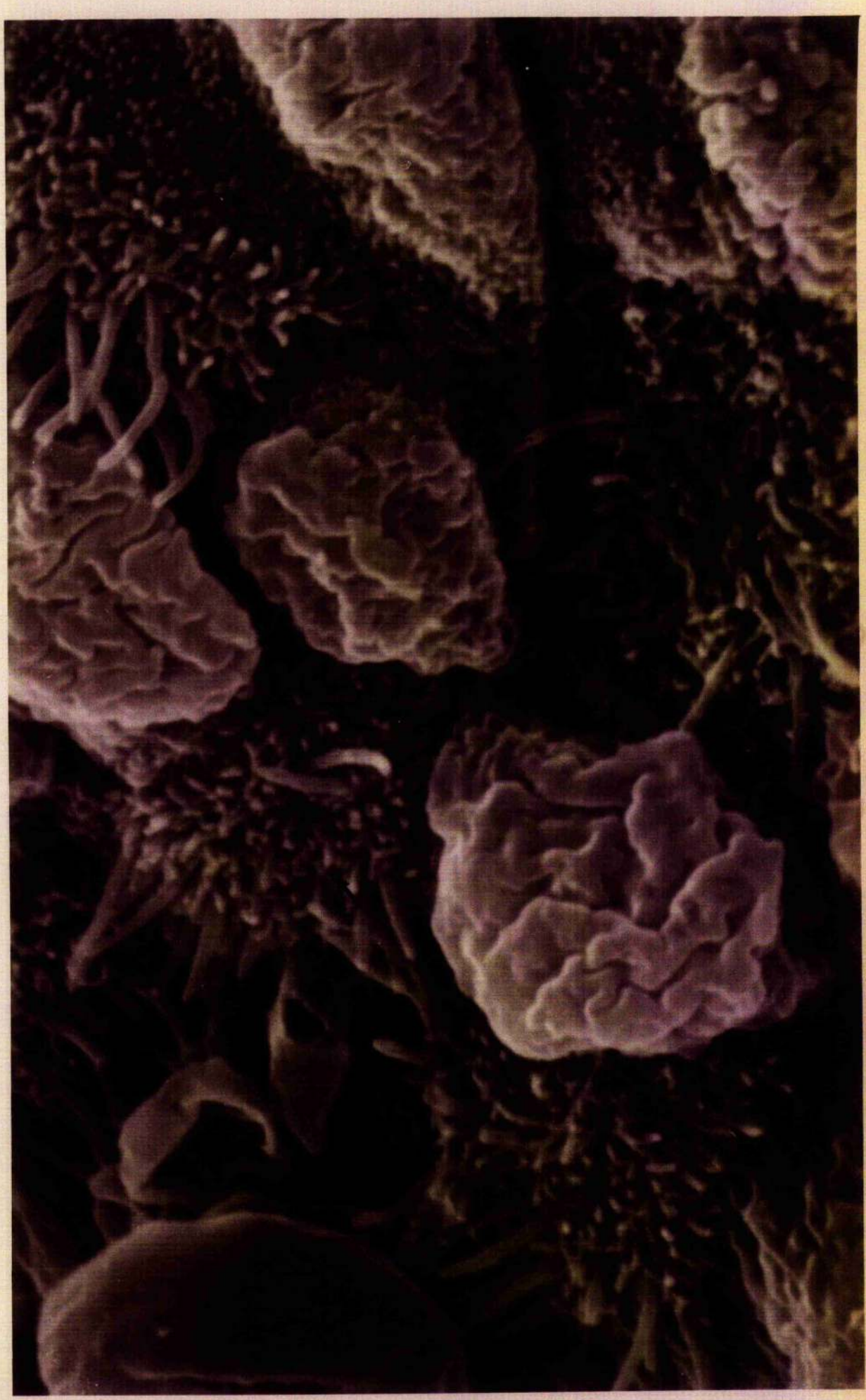


Fig. 22 - Normal Calf: Bronchiolar epithelium with Clara cells and ciliated cells with a few cilia. Cells (1-4) may represent different phases of secretory activity of Clara cells. The cell boundaries are easily discernible.

SEM x 2500

1
2
3
4

Fig. 23 - Normal Calf: Lung parenchyma. This section illustrates the entrance of a terminal bronchiole into an alveolar duct and air sacs.

SEM x 160

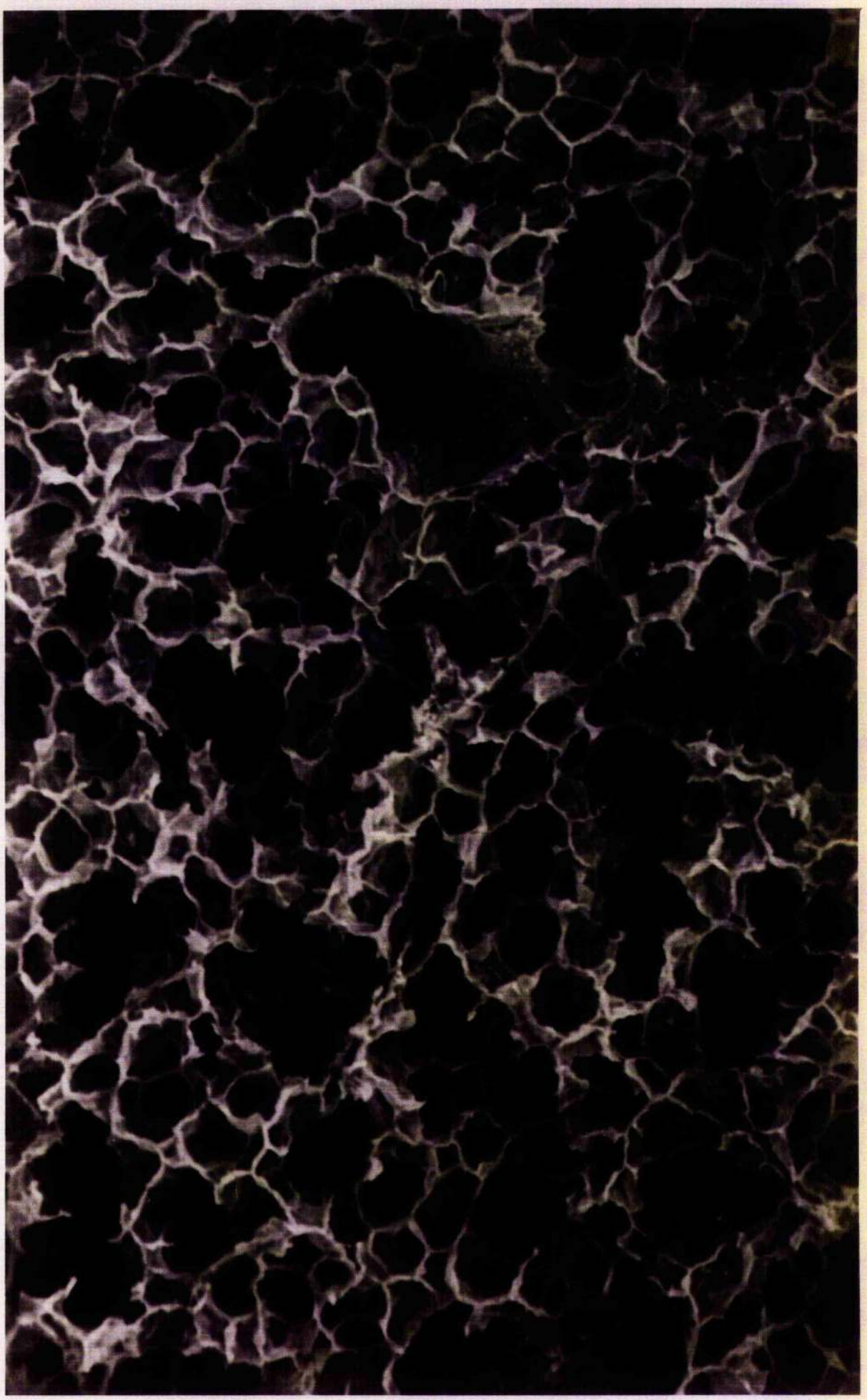


Fig. 24 - Normal Cow: Low power view of lung parenchyma with terminal divisions of the bronchial tree and many alveoli.

SEM x 80

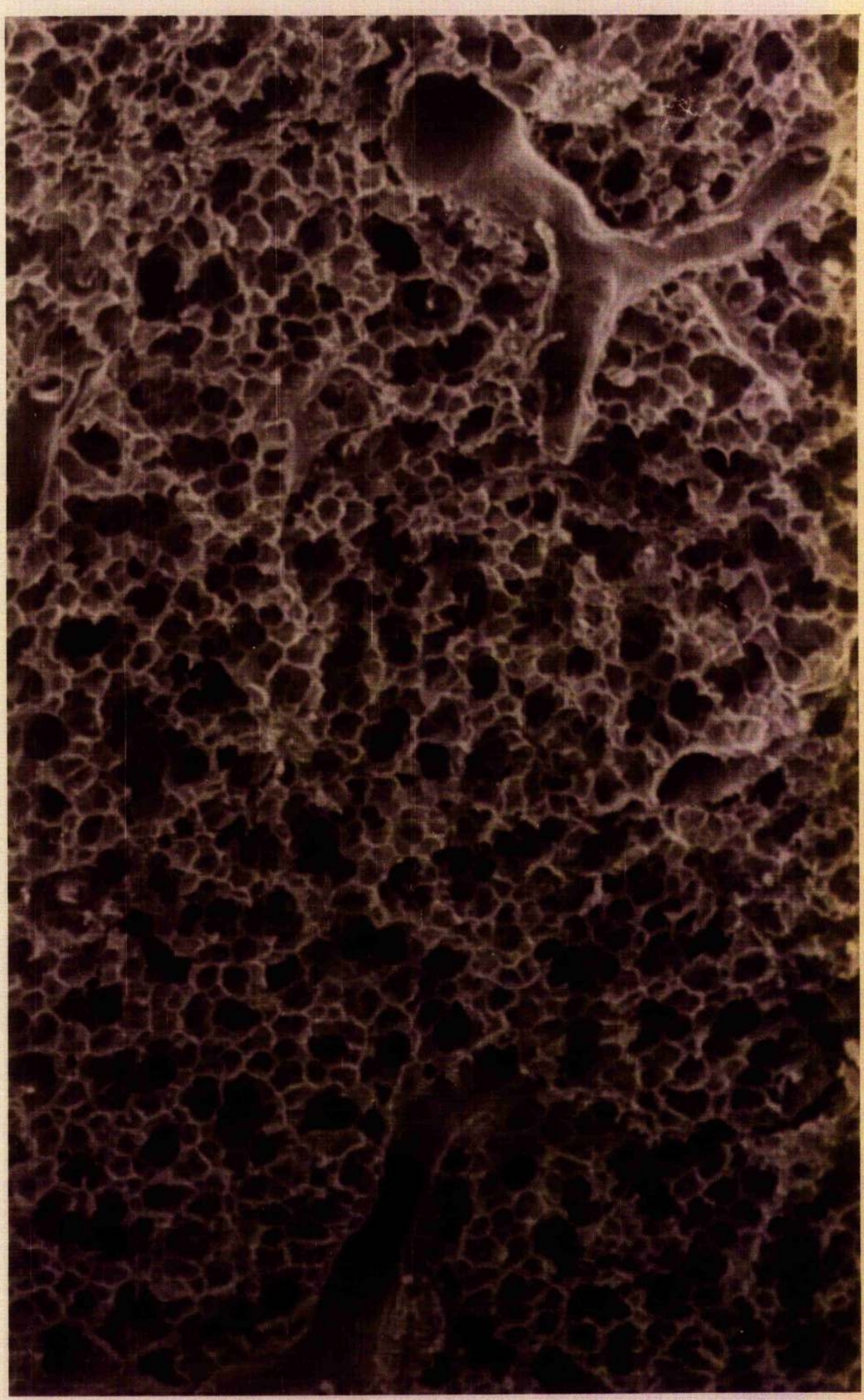


Fig. 25 - Normal Calf: Type II alveolar epithelial cells (arrows), with adjacent flat Type I alveolar epithelial cells ( ). The cell junctions are easily seen as well as a fringe of microvilli on the protruding Type II alveolar epithelial cells.

SEM x 1250

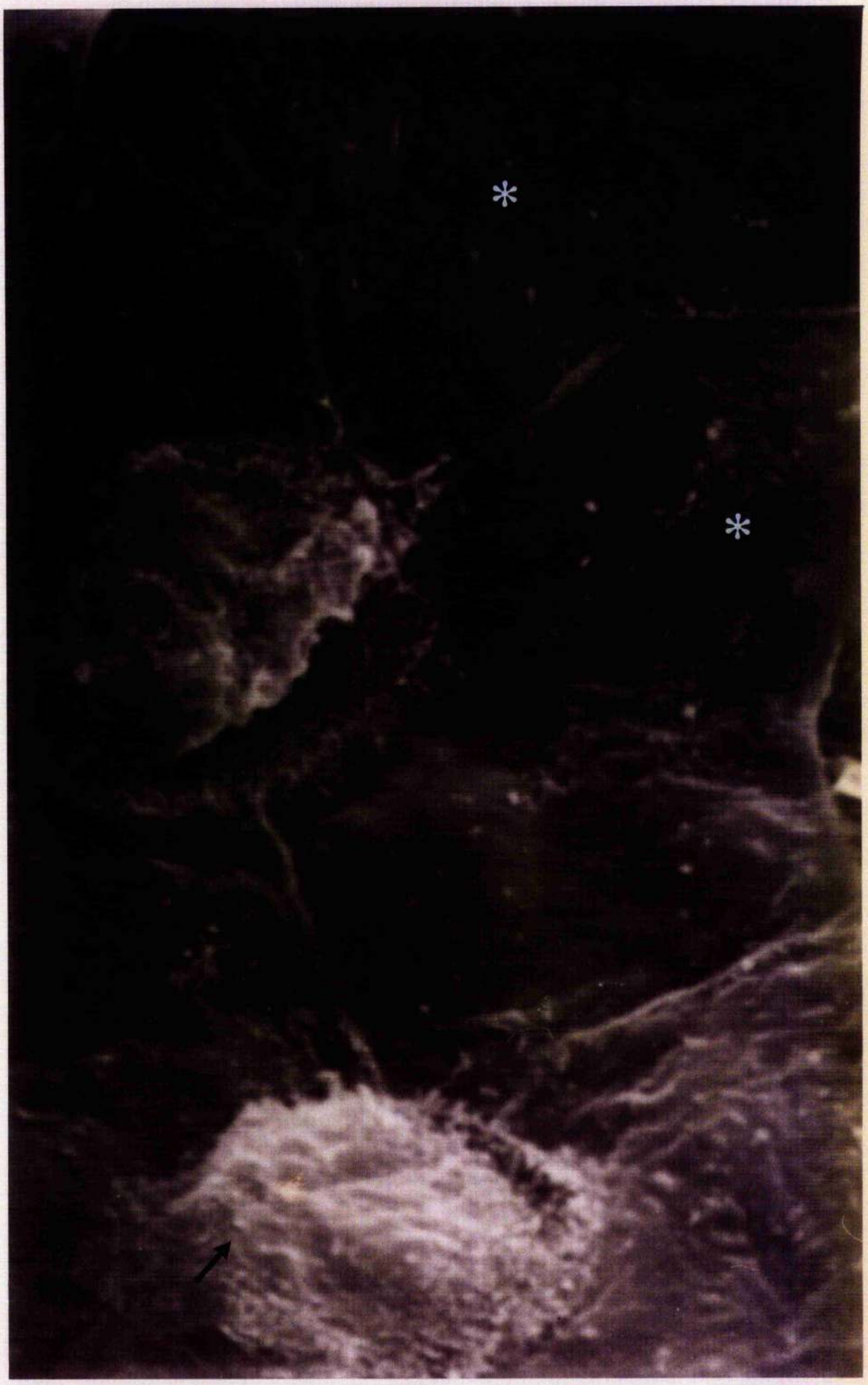


Fig. 26 - Normal Cow: Numerous Type II alveolar epithelial cells with numerous microvilli on the surface of their cytoplasm. The centre of the cells has some openings or pits (arrows) and it is protruding into the lumen.

SEM x 1250

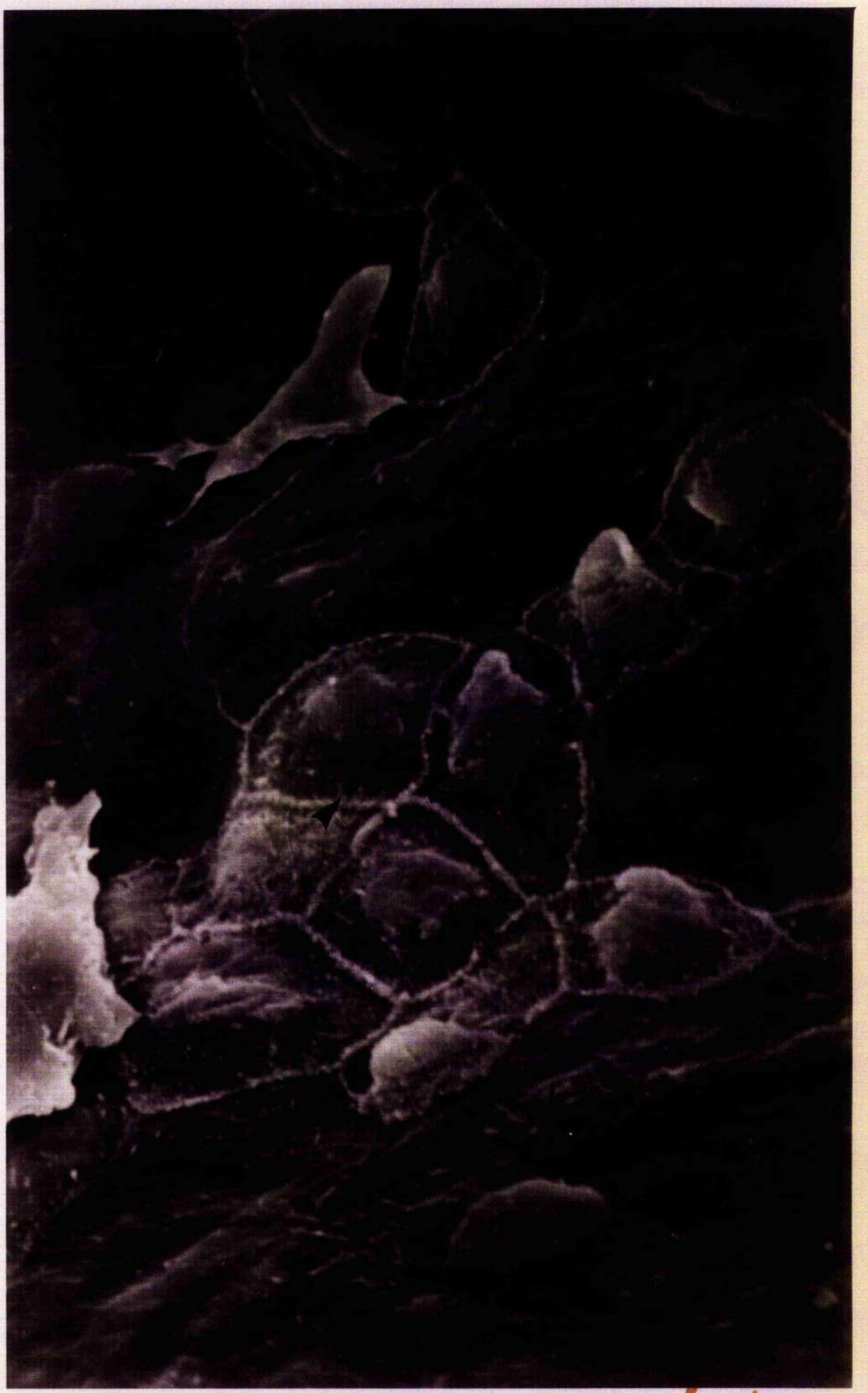


Fig. 27 - Normal Cow: This section represents alveoli with numerous Type II cells adjacent to an interlobular septa (arrows).

* Type II pneumocytes.

SEM x 640

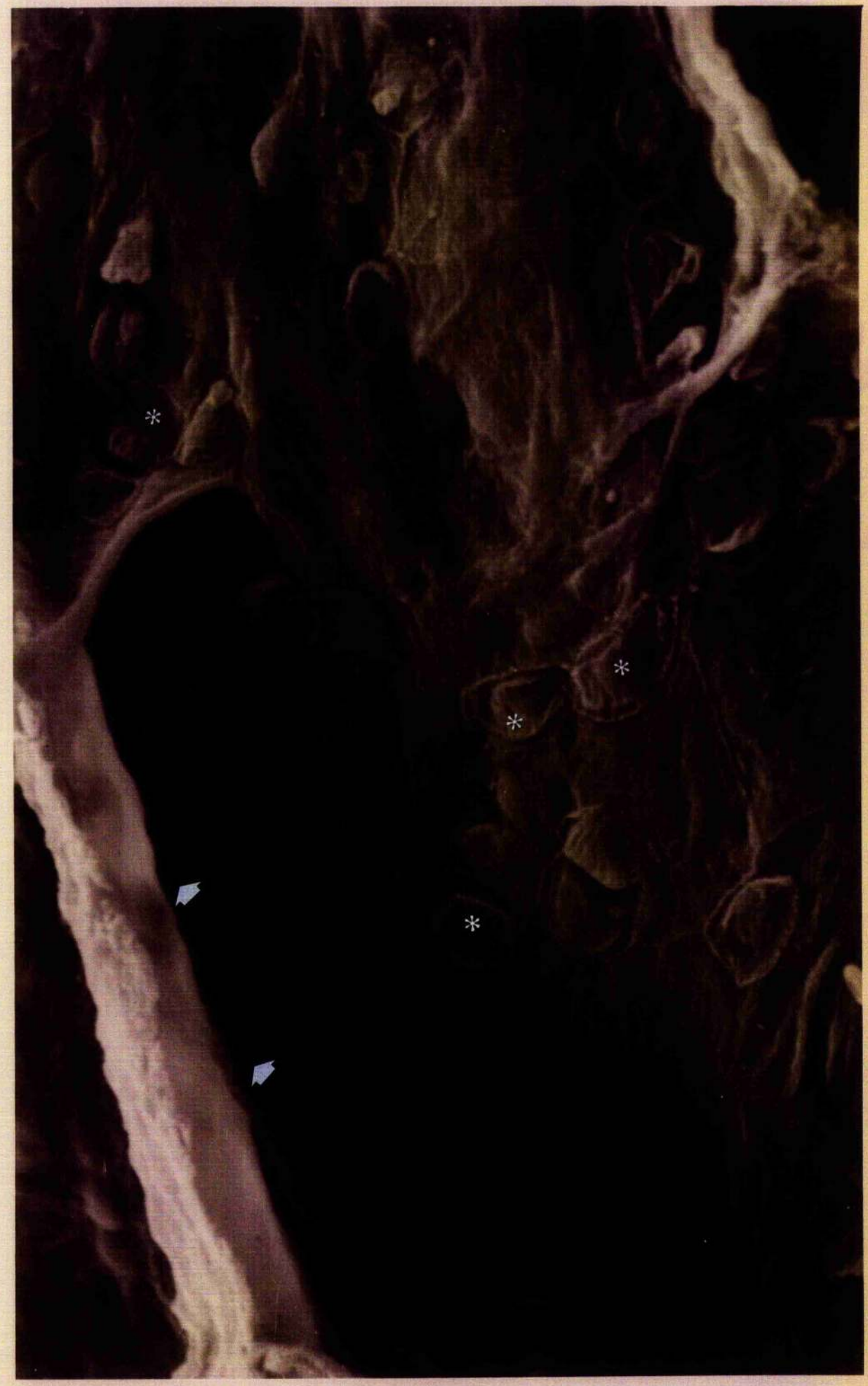


Fig. 28 - Normal Calf: Type II (arrows) and Type I alveolar epithelial (*) cells. The latter having a very smooth surface and small projections are scattered on the surface. Linear ridges of cellular junctions (*) are also visualized. A capillary (v) within the wall is bulging the alveolar surface.

SEM x 2500

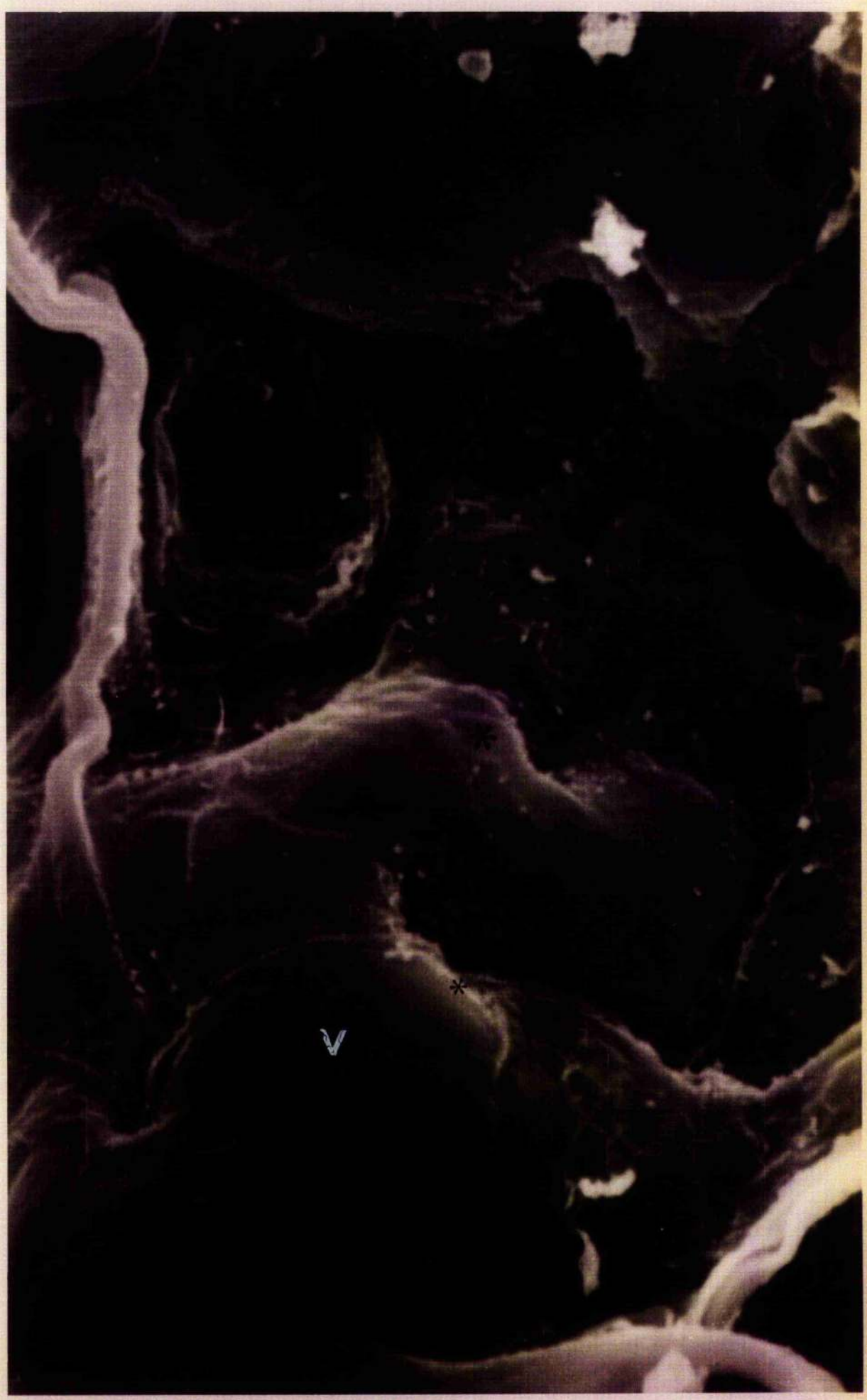


Fig. 29 - Normal Cow: A low magnification view of the lung parenchyma. Many alveoli can be seen whose walls contain interalveolar pores, the pores of Kohn.

SEM x 160

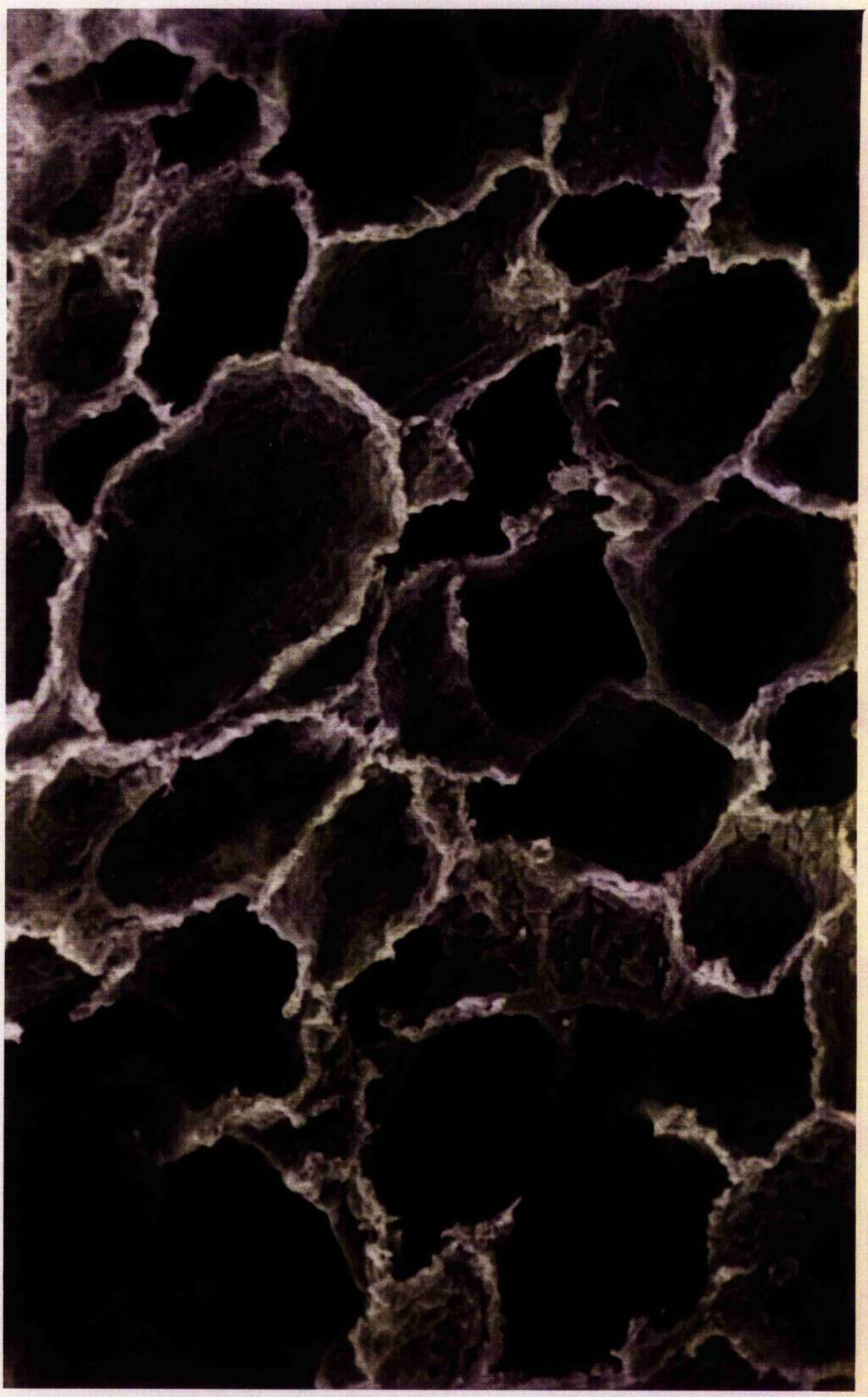


Fig. 30 - Normal Cow: A high magnification view of an alveolar wall with two pores of Kohn (arrows) which have smooth even margins.

SEM x 640

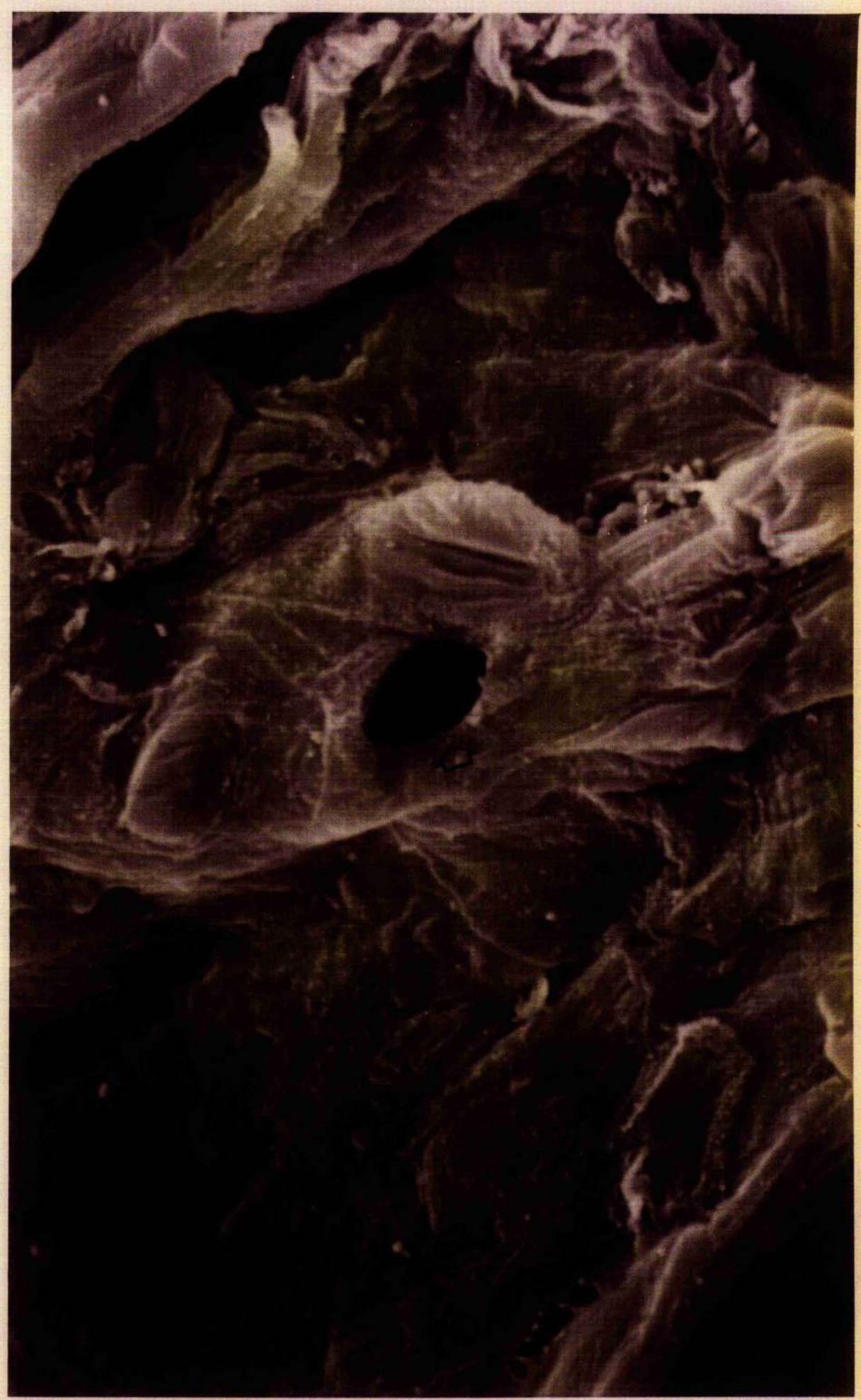


Fig. 31 - Normal Calf: Alveolar surface with alveolar macrophages (arrows) in the alveolar lumen.

SEM x 640

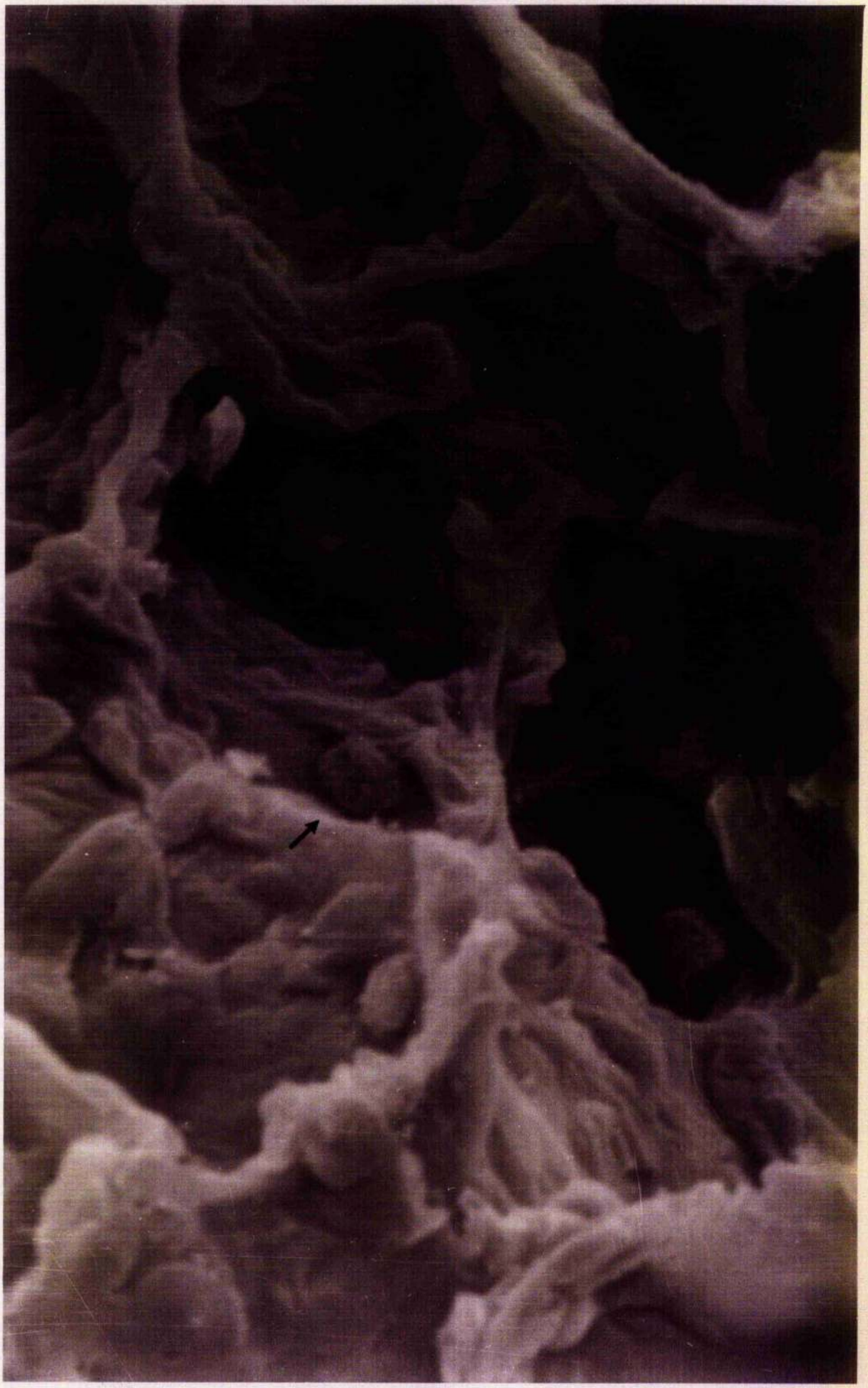


Fig. 32 - Normal Cow: The common appearance of an alveolar macrophage showing the thin cytoplasmic prolongations (arrows).

SEM x 2500

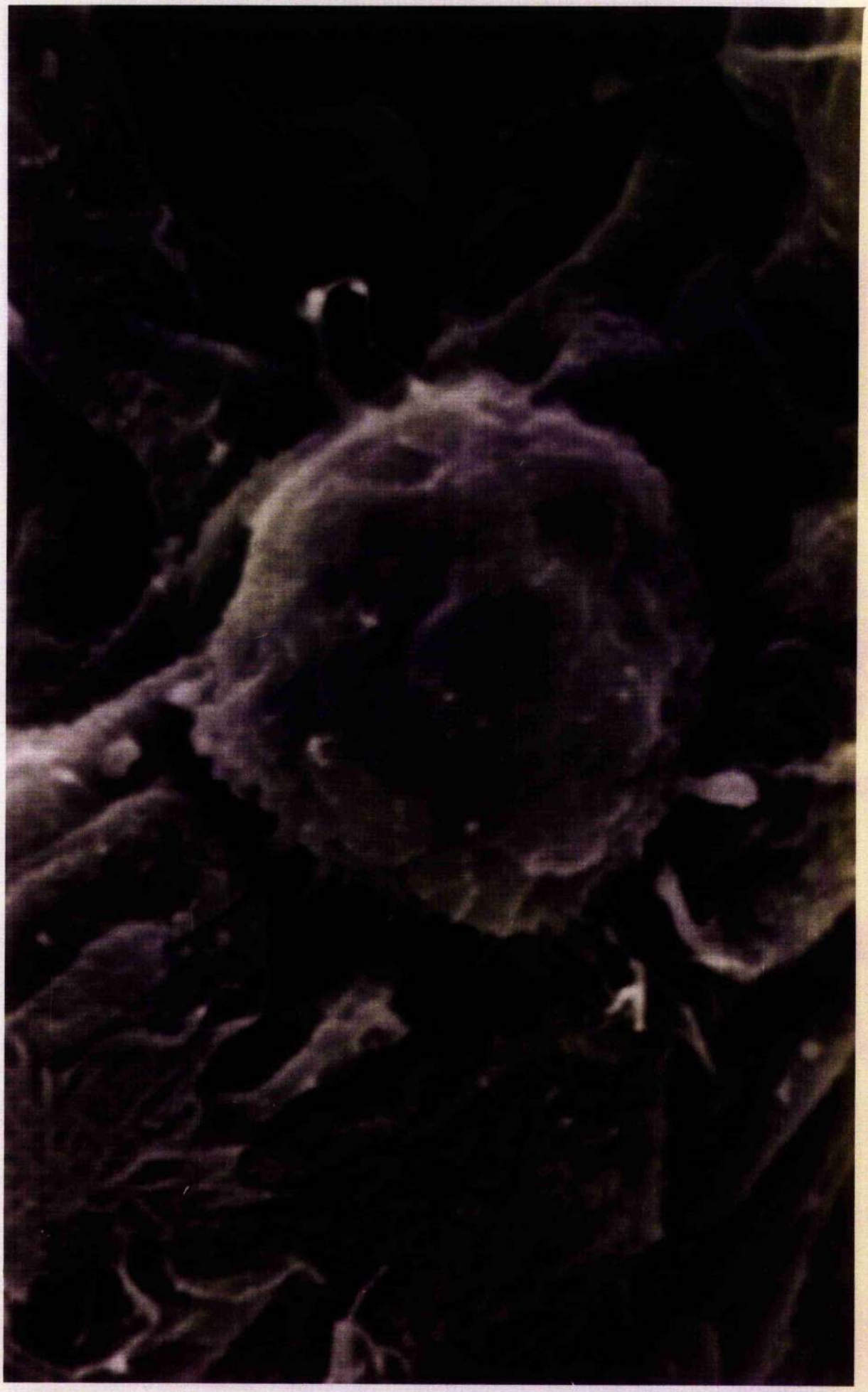


Fig. 33 - Normal Cow: An Alveolar surface with larger ridges or slopes produced by capillaries underlying the alveolar epithelium. A pore of Kohn can be seen (arrow).

SEM x 1250

SECTION FOUR

SCANNING ELECTRON MICROSCOPIC STUDY ON EXPERIMENTAL LUNGWORM INFECTION IN CATTLE

INTRODUCTION

A considerable amount of information about the pathology, parasitology and pathogenesis of lungworm infection in susceptible cattle and in immunised cattle can be found in the literature.

In 1756, Nicholls, correlated the presence of lungworm with a distinctive disease entity in cattle which occurred in an epizootic form, a parasitic bronchitis. The adult lungworms, Dictyocaulus viviparus were found in the air passages of cattle lungs.

Jarrett et al. (1957, 1960a and 1960b) stated that the pathogenesis of the disease progressed through four stages from infection 1) the penetration phase, 1-7 days after infection, 2) prepatent phase, 8-25 days after infection, 3) the patent phase, 26-60 days after infection and 4) the post-patent phase, 61-90 days after infection. The recognition of these different phases was considered to be of great importance in relation to diagnosis, prognosis and treatment.

The third stage infective larvae (L3) when ingested by a susceptible bovine animal penetrate the alimentary tract and from there pass through the lymphatic and blood circulation to reach the pulmonary capillaries from which the larvae reaches the alveoli. During the prepatent phase an intense eosinophilic exudate was described into the lung that resulted in blockage of small bronchi and bronchioles. In this period, 8-25 days, several complications due to secondary bacterial or viral infection were described by Jarrett (1960).

During the third phase (26-50 days) the adult reproducing nematodes, present in the lung, produced a severe reaction and a parasitic pneumonia was established which Jarrett et al. (1960b) stated was the reason for the clinical signs observed at this time.

The post-patent phase was described as a gradual recovery stage in which the respiratory signs decreased and the lesions resolved. However Jarrett et al. (1957) also reported that in this last stage there was risk of death, in apparently 25% of infected animals due to alveolar "epithelialization".

The great advance in the field of parasitic bronchitis was the discovery and development of a vaccine. Jarrett et al. (1957) developed a method of vaccination, using living X-irradiated L3 of D. viviparus, which conferred a very high degree of protection against husk and was used successfully as a major way of controlling the disease in the field.

Pulmonary lymphoid nodules developed in immune calves following challenge and were a good criterion of an immune response to vaccination (Pirie et al., 1969).

Although a considerable amount is known about the cellular and structural changes in bovine lungs during lungworm infection there is no information about the damage to the surface of the respiratory portion and conducting portion of the lung that can be detected using SEM.

The present study was prepared to investigate using SEM, the surface damage produced by D. viviparus in lung tissue and in addition to assess the differences that occurred in these changes related to immunity.

MATERIALS and METHODS

In the experimental design parasite free calves were divided into three groups which received different treatments (Table 3).

ANIMALS:

Ten Friesian cross calves, 10 weeks old and reared indoors were divided into three groups. Group 1 comprised two calves which were vaccinated with Dictol as recommended by the manufacturer i.e. two doses given orally one month apart; the calves were not challenged. Group 2 comprised four calves which were infected orally with a dose of third stage infective larvae of D. viviparus, at 30 L3/Kg bodyweight; these calves had not been vaccinated. Group 3 were four calves vaccinated with Dictol at the same time as Group 1, then challenged one month later with an oral dose of L_3 D. viviparus, 30 L3/Kg of bodyweight at the same time as calves in Group 2.

Animals	Time after First Vaccination		
	0	1 month	2 months
Group 1	V	V	-
Group 2	-	-	C
Group 3	V	V	C

TABLE 3: The timing of vaccination and challenge applied to the animals in the lungworm experiment.
V = vaccination, Dictol, Glaxovet:
C = challenge, 30 larvae/Kg bodyweight, approximately 5,000 larvae per calf.

VACCINE

Dictol (Glaxovet. Ware, Hertfordshire) was a living oral vaccine against D. viviparus produced with L3 D. viviparus attenuated by exposure to X-irradiation of 40Kr. The optimum numbered of X-irradiated larvae in one immunising dose was established as 1000, according to the manufacturer.

CHALLENGE LARVAE

Normal infective D. viviparus L3 were supplied by Glaxovet - Ware, Hertfordshire, and were used to challenge the vaccinated calves of Group 3 and to infect the non-vaccinated challenge control calves in Group 2.

The larvae were suspended to approximately 1200 L3/ml. In order to provide an accurate concentration of L3/ml, 20 aliquots of 0.025 ml were counted, then challenge doses were made up to provide 30 L3/Kg bodyweight, approximately 5000 L3 per calf.

The infection and challenge was performed by oral administration of the above established dose.

POST-MORTEM EXAMINATIONS

Tissue samples for SEM were collected from calves examined post mortem on the days after challenge shown in Table 4.

At post-mortem examination the tissues were sampled for histological and SEM procedures as described in Section 2. Parasitological samples were also collected after gross-pathological examinations were performed.

Animals	Days after Challenge			
	15	25	35	45
Group 1	A	-	-	-
Group 2	A	A	A	A
Group 3	A	A	A	A

TABLE 4: Days of post-mortem examination after challenge with infective D. viviparus larvae of the calves in the lungworm experiment.
A = one animal examined.

PARASITOLOGICAL PROCEDURES

For recovery of lungworms, the left lung was obtained immediately after death and the large bronchi were opened, with scissors, down to the smallest bronchi. These surfaces were washed and the solution recovered was suspended in warm water overnight. The washing was incubated at 37°C then sieved and then the sieved material obtained was immersed in formalin. The numbers of worms present were then counted and the figure recorded was multiplied by two, to give the approximate infection rate for both lungs.

PATHOLOGICAL PROCEDURES

The lung parenchyma was examined carefully for the purpose of recording the number of lymphoid nodules, 2-3 mm diameter present on the surface of the lungs. This examination was done by scanning the pleural surface of both, right and left lungs.

SCANNING SAMPLING PROCEDURES

Tissues were selected from the trachea and the cranial and caudal lobes of the right lungs, as illustrated in Fig. 2B, Section 2.

Five levels were established according to previous studies on the normal animals, presented in Section 3. However three samples were taken at level 2b in this study compared with that on the normal animals because the adult lungworms were likely to be present at this position. After fixations the samples were processed according to methods described in Section 2.

RESULTS

The parasitological results, detailed in Table 5 demonstrated that the non-vaccinated animals of Group 2 were infected when challenge with D. viviparus. At the different days of examination after challenge the calves in Group 2 had a considerable worm count which demonstrated that a moderately heavy infection had been established. One calf in Group 1 died of intercurrent infection, so only one animal was available for study and it was examined on Day 15.

The worm counts in Group 3, vaccinated and challenge calves were very low indicating that a good state of immunity had developed. The calves of Group 1 were only vaccinated and the calf examined at day 15 had no worms.

The gross pathological changes in the calves of Group 2 were typical of primary lungworm infection. Similar but more extensive lesions were found in Group 3. Apart from the smaller number of worms in the calves in Group 3 the most striking difference pathologically between Group 3 and Group 2 was the presence of many lymphatic nodueles 2-4 mm in diameter, on the pleural surface of the vaccinated animals (Table 6). These nodules were present in all the calves of Group 1 and Group 3 but the number found was greater in the vaccinated and challenged calves in Group 3. The number of these nodules present decreased with time. The macroscopic appearance of the nodules was slightly varied in size and consistency. Some could be described as large and dense while others were small and translucent. They were present throughout the surface in all lobes. The majority were 2.0 to 4.0 mm in diameter, grey or pinkish-grey in colour and bulged slightly. They were counted at post-mortem and the results presented in Table 6, show a consistent number present in Group 3, the vaccinated and challenged calves, demonstrating that an immune state was established in these animals. No nodules were recorded in Group 2, the unvaccinated animals.

Animals	Days of Examination After Challenge			
	15	25	35	45
Group 1	0	-	-	-
Group 2	644	428	2190	38
Group 3	8	NI	18	4

Table 5. Parasitological results: The number of lungworm present in the calves in the Scanning Electron Micropic Study on Lungworm Infection.
NI = No immunity.

Animals	Days of Examination after Challenge			
	15	25	35	45
Group 1	14	-	-	-
Group 2	0	0	0	0
Group 3	47	30	24	5

Table 6. Pathological results: The number of lymphocytic nodules found in the lungs of the calves in the Scanning Electron Microscopic Study on Lungworm Infection.

Scanning Electron Microscopic Results

The results after examing the different levels are presented in the following order: first trachea and large bronchi, then bronchioles and finally alveoli; these results summarise the most representative feature observed in each calf examined. Group 1 is described first, then each day in Group 2 and finally each day in Group 3.

Group 1

One calf died of intercurrent infection, so only one was available for study. A fairly normal pattern was observed in this calf. The calf examined at this time did not have many changes in the surface of the respiratory airways at the different levels examined. The ciliated epithelium of the large airways appeared normal (Fig. 34). The cilia appeared to cover the whole surface interspersed with small round spaces which according to previous results on normal tissue probably represented sites occupied by non-ciliated secretory cells, in a quiescent state.

The bronchioles had the typical pattern with non-ciliated cells predominating and no brush cells being observed. No larvae, worm-eggs or abnormal secretions were found. The lung parenchyma did not have signs of abnormal structure (Fig. 35). At this level the clean alveolar area exhibited a normal pattern of Type I and Type II alveolar epithelial cells and the pores of Kohn and junction of Type I alveolar cells were clearly seen.

Group 2

The most striking changes were found in Group 2, the infected calves which had not been vaccinated.

Day 15

At this time after challenge small bronchi were seen, which appeared to be occluded by plugs of mucus (Fig. 36). The same infected calf had an abnormal lung parenchyma. These changes were represented by a slight thickening of the wall of the bronchioles (Fig. 37) and changes due to increased cellular activity present in their epithelial cells. The alveoli still had a regular pattern although a cellular infiltration was beginning to be evident.

Some observations were made during the experiment in this calf and in others of this group which were not attributed to the parasitic infection. When the trachea and large bronchi of the infected calf were examined changes were observed especially on the cilia of the ciliated cells (Fig. 38). These areas were fairly extensive, intermixed with areas of normal tissue. The features comprised ciliated cells which appeared to be covered by small rounded particles sitting on and between the cilia. Based on the size, approximately 0.5 µm diameter and the morphological appearance of the surface of these bodies, the elements were considered to be microorganisms. These organisms were seen in the large bronchi in other calves in Group 2, through the duration of the experiment. In Group 3 they were only seen at the beginning of the experiment and they were seen only in the trachea. In Group 2, in the trachea and large bronchi areas of epithelium appeared with abnormal patches of different sizes. In these areas the ciliated cells seemed to be devoid of their cilia and some of the remaining cells had very short cilia as if they were regenerating after injury (Fig. 39). The non-ciliated cells were easily distinguished but there were no major signs of secretory activity (Fig. 39). Other damaged areas of epithelium again in the trachea and large bronchi contained some columnar cells with cilia, which appeared to be extruding from the epithelium, as shown in Fig. 40. The extruding cells were surrounded by ciliated cells which appeared poorly ciliated and some appeared to have lost their cilia and were considered to be dying cells. At lower levels, small bronchi and bromchioles did not have this altered epithelium at any time.

Those patches of deciliation were seen at the same levels, the large airways, on other infected calves in Group 2.

Day 25

In the large bronchi from the caudal lobe, represented in Fig. 41, worm eggs were seen sitting on the ciliated surface. The ciliated epithelium did not seem to be seriously damaged, although some secretions and debris and red blood cells contaminated the surface. Lower in these airways damaged epithelium associated with worms and eggs were found. Other areas at this level 2 in the caudal lobe (Fig. 42) were seen, where the mucous secreting cells appeared to be very active and protruded between the ciliated cells. The granules of mucus in the apices of these cells were easily identified. At this time after the infection, lungworms were seen, occupying the lumen of small bronchi (Fig. 43). When the epithelium of this area in particular, was observed at high magnification (Fig. 44) it appeared completely altered and worm eggs were often seen. Some alterations could be described as cracks or depressions in the epithelium with, in addition, impressions or marks as if made by worm eggs. The surface of the eggs which were still occupying the area looked slightly wrinkled with small imprints on their shells.

When the bronchial epithelial surface was examined at high magnification it had the characteristic of a dysplastic tissue with complete loss of its regular pattern (Fig. 45). It was impossible to identify either ciliated cells or secretory cells.

Lungworm eggs were seen in alveoli where they occupied the whole lumen (Fig. 46).

Day 35

Some areas of the dysplastic epithelium were still present at this time as well as adult lungworms of D. viviparus and parasitic eggs on the surface of the bronchi and bronchioles. The

non-ciliated bronchiolar secretory cells appeared very active and the ciliated cells possessed cilia which were much longer than those occurring in normal tissue (Fig. 47).

The major striking features at this stage of the disease was represented by a severe inflammatory infiltration of the lung parenchyma. At a low magnification (Fig. 48) examining level 5, it was possible to appreciate the regular organisation of the alveolar tissue although some alveoli had thick walls and the alveolar lumena were frequently occupied by clusters of infiltrating cells. It was also possible to note larvae obstruting the lumena of the alveoli (Fig. 48). A high magnification view of these areas (Fig. 49) sometimes showed a cluster of cells surrounding the body of a larva, possibly derived from aspirated eggs, in the lumen of an alveolus. The infiltrated cells were identified as inflammatory cells but it was not possible to characterize the type present. Some of these cells (Fig. 50 and Fig. 51) in other alveoli appeared to have a ruffled surface, while others were more simply spherical. They were of different sizes, large, medium or small, exhibiting prolongations or cytoplasmic process. The smaller ones appeared to have a granular surface rather than a surface with proper prolongations of the cell membrane (Fig. 50).

Several alveoli (Fig. 52 and Fig. 53) were examined and found to illustrate different inflammatory cells which were thought to be macrophages, eosinophils, or lymphocytic cells. Type II cells could be identified in alveoli which were usually free of large numbers of infiltrated cells (Fig. 53) and these Type II alveolar epithelial cells often appeared to be in an active stage. These cells had the centre of the cell bulging into the lumen of the alveolus and numerous, easily seen, microvilli surrounding the protruding centre (Fig. 54).

Day 45

At 45 days after challenge the features observed had no major changes compared with the previous stages. Several of those most often found are illustrated in Figs. 55, 56, 57 and 58.

Group 3

Group 3, the vaccinated and challenged animals, had minor changes considting of cellular infiltration of the alveolar tissue (Fig. 59) or patches of epithelium with modified ciliation (Fig. 59 and Fig. 60). These were not considered to be significant when compared with the striking changes observed in the infected Group 2. The lesion characteristic of Group 3 was the lymphocytic nodules.

These nodules, when observed on a cut section (Fig. 61) were fairly smooth and seemed to be localized in the lung parenchyma sometimes in close relation to bronchioles. The structue was comprised mainly of rounded small and fairly smooth cells considered to be lymphocytes (Fig. 62).

DISCUSSION

Susceptible animals exposed to lungworm infection seemed to be severely affected by the damage produced by the parasite and the inflammatory processes that take place in the respiratory tract. The present study compared the changes that occurred in the surface epithelium of the airways at different levels. The findings obtained with SEM appeared to corroborate the features described by other methods particularly those using LM. Scanning electron microscopy however has illustrated these changes in a more striking fashion and has illustrated changes on the surface of the epithelium, particularly in the bronchi and bronchioles that were not recognised before. In addition the obstructive effects of the parasites and inflammatory cells within the bronchi, bronchioles and alveoli has been more effectively demonstrated. No information was available in the literature on the changes produced in bovine lungs by lungworms as seen by SEM.

This study included observations on calves vaccinated against *D. viviparus* and experimentally challenged with this lungworm as well as calves vaccinated but not challenged. The vaccinated calves without being challenged appeared to be relatively normal since no major signs of altered lung tissue structure were observed by SEM although a few pulmonary lymphocytic nodules were found.

After the vaccinated calves had been challenged some features were detected associated with the tissue reaction to antigens present in the lungs. These were inflammatory cells infiltrating the lung parenchyma and lymphocytic nodules which were found in significant numbers. These nodules which develop around dead larvae were similar to those described previously by Jarrett *et al.*, 1957 and Pirie *et al.*, 1969. The changes found in the surface epithelium of the vaccinated and challenged calves were essentially similar qualitatively to those in the susceptible challenged calves but they were much less extensive and were accompanied by the lymphocytic nodules.

In the infected calves the lungs had evidence of damage to the surface epithelium as well as cellular infiltrates 45 days after challenge. To investigate the regenerating and healing phase more fully would require and investigation over 50-90 days post challenge.

Although SEM gives a useful impression of the space occupying effects of exudates and inflammatory cells in the lungs and also demonstrates their surface topography very well identification of cells can be difficult. This is particularly true for inflammatory cells and the size of the cell is one of the most useful criteria especially when trying to differentiate neutrophils and eosinophils from macrophages. Increased numbers of pseudopodia or ruffles or small vesicular-like protrusions from the cell membrane were taken to reflect increased metabolic activity.

Eggs and larvae aspirated into alveoli during the patent phase of the disease were described as a major cause of pneumonia leading to clinical signs (Jarrett, 1960). This reaction is readily detected by SEM. In the alveoli eggs were sometimes seen with no reaction around them. These were thought to have been either recently aspirated from the position in the bronchi where they were produced or to have been artefacts resulting from eggs washed into alveoli by the perfusion fixation technique. Larvae possibly with the remains of sheaths or egg shells and surrounded by inflammatory cells were also seen. In the bronchi eggs near adults were on badly damaged dysplastic epithelium almost completely devoid of cilia. This contrasted with eggs seen further up the bronchi lying on normal ciliated epithelium. These eggs would be moved out of the lung by the mucociliary escalator and coughing to be swallowed and complete the life-cycle of the parasite.

It was pointed out that other changes were seen in patches in the trachea and the bronchi particularly of the cranial lobes in Group 2. These were surface bacteria, patches of deciliated cells and exfoliating single ciliated cells. Since these changes occurred as early as 15 days in an area which the parasite would

not have reached at that time they were attributed to intercurrent infection. When lungworms had become established in Group 2 bacteria were more frequently seen than in Group 3. Jarrett (1960) pointed out that intercurrent infection could occur.

It was interesting that increased activity of Type II alveolar epithelial cells was seen later in the infection as well as increased activity of non-ciliated bronchiolar cells, Clara cells. Whether or not this represented a minor form of the striking hyperplasia of alveolar Type II cells that occur in a proportion of animals during the post-patent phase was not clear. No obvious groups or sheets of these cells were seen which might have resulted from hyperplasia.

FIGURES

Fig. 34 - Vaccinated calf: The epithelial surface of a large bronchi with the normal appearance of a ciliated carpet.

SEM x 640

Fig. 35 - Vaccinated calf: The lung tissue structure appears normal one month after vaccination. Type I and Type II epithelial cells are present (black arrows) and there is a visible pore of Kohn (white arrow)

SEM x 1250

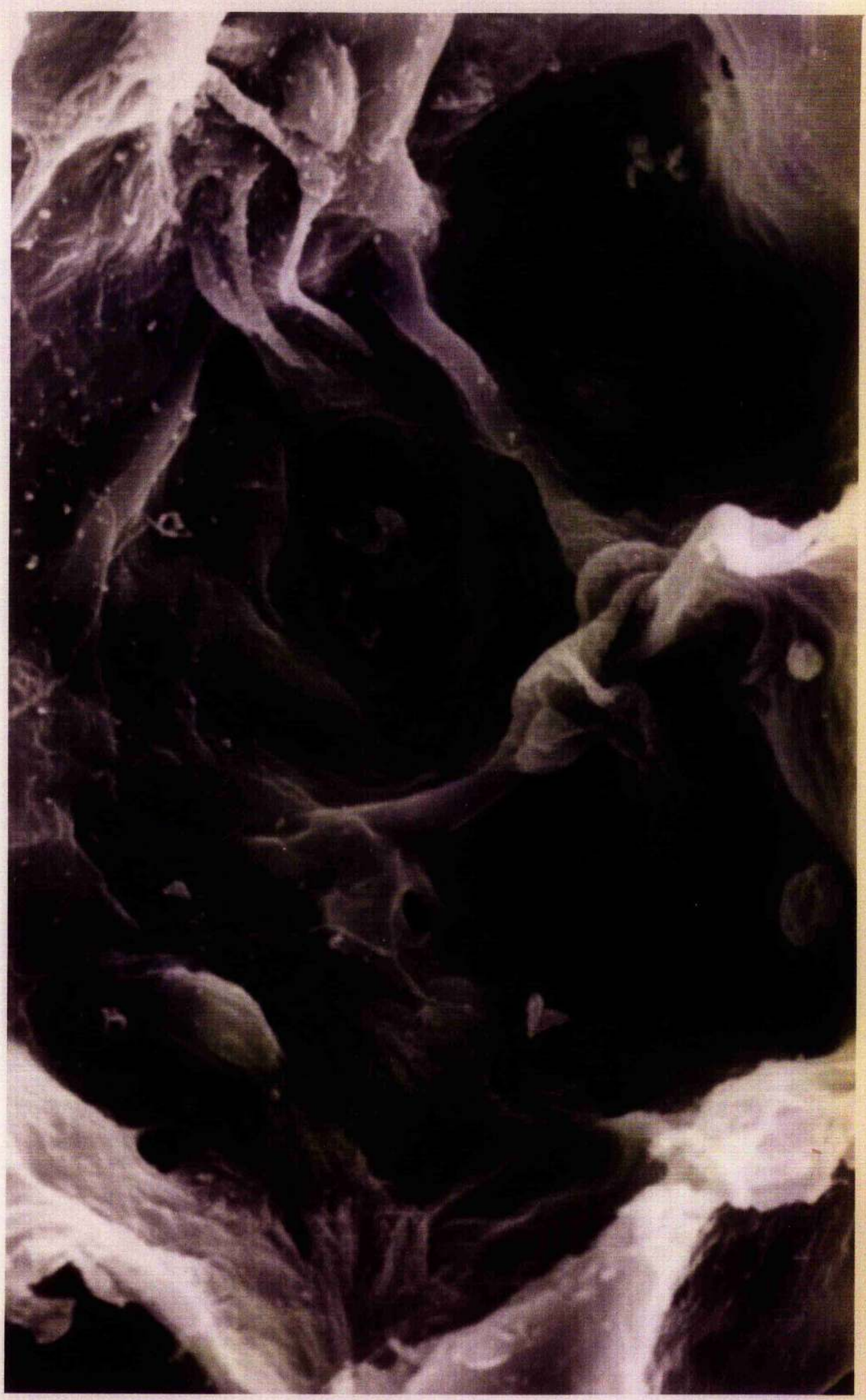


Fig. 36 - Infected calf: 15 days after challenge a small bronchus appears blocked by a plug of mucus (arrow).

SEM x 80

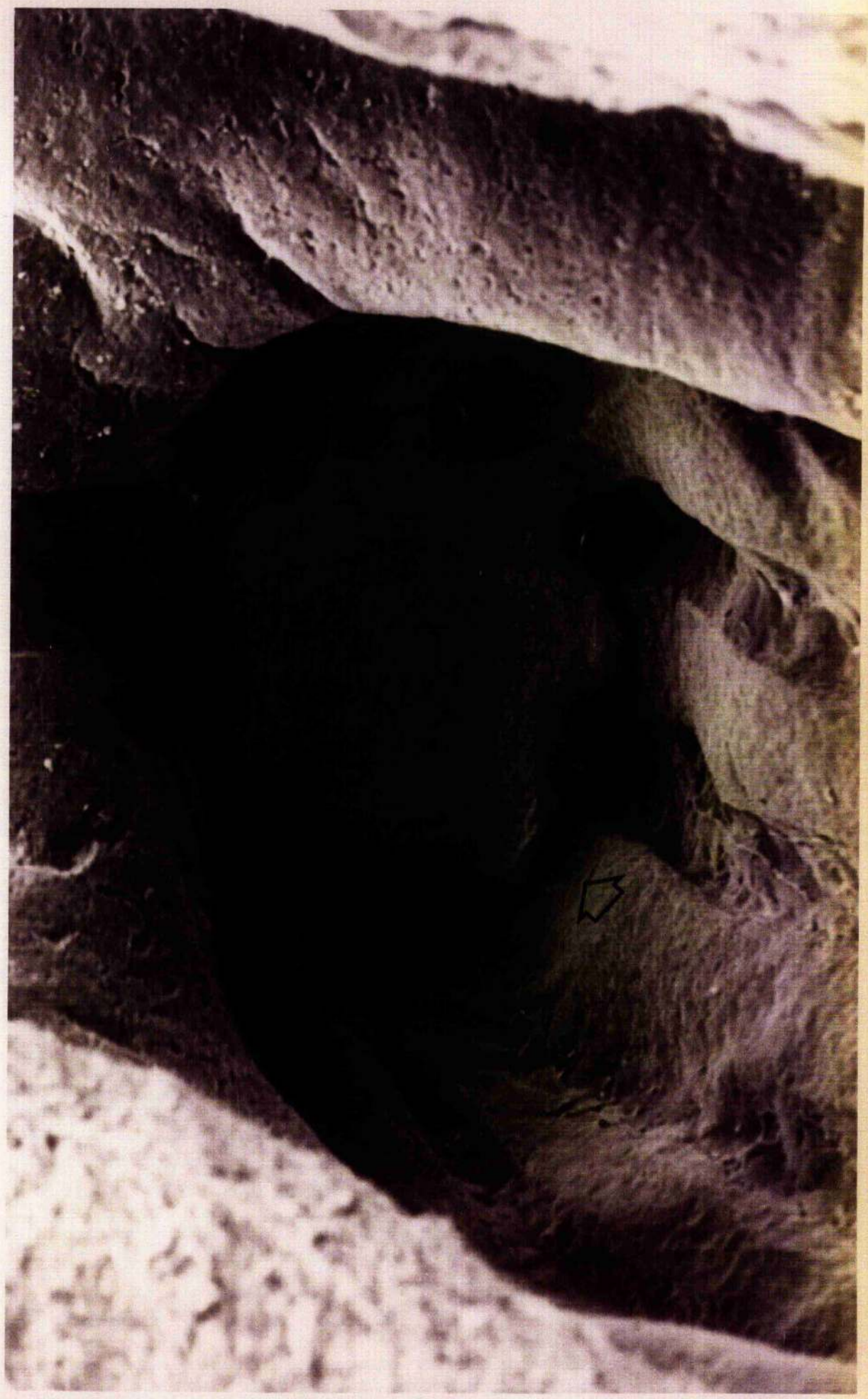


Fig. 37 - Infected calf: Low magnification of lung tissue illustrating a bronchiole with hyperplastic epithelium (arrow) and cellular infiltration in alvoli.

SEM x 160

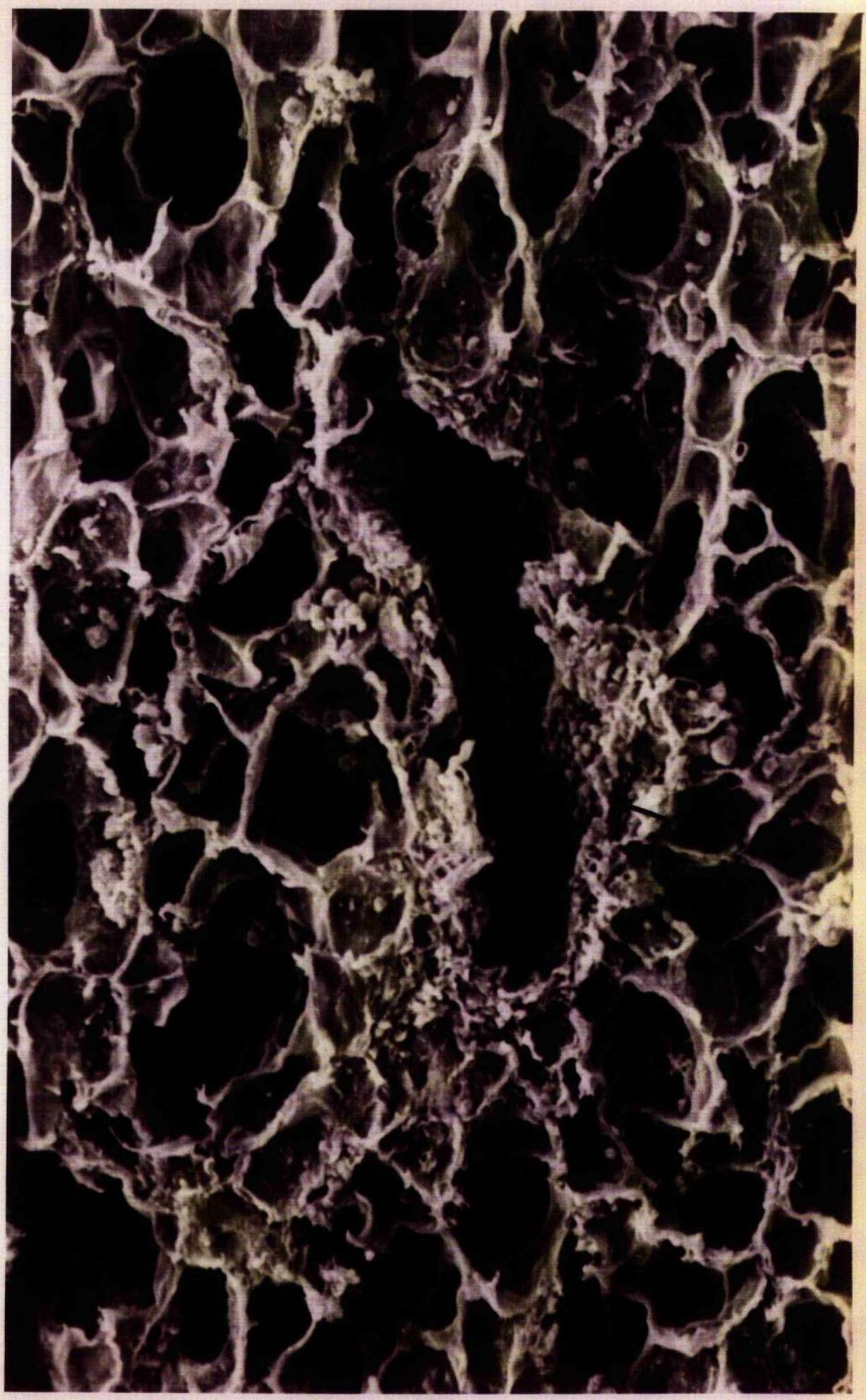


Fig. 38 - Infected calf: Microorganism colonising the tip of the cilia in a large airway.

SEM x 5000

Fig. 39 - Infected calf: A large bronchus of the caudal lobe with patches devoid of cilia. The ciliated cells appear disorganised (arrow). No secretory active cell can be seen (*).

SEM x 640

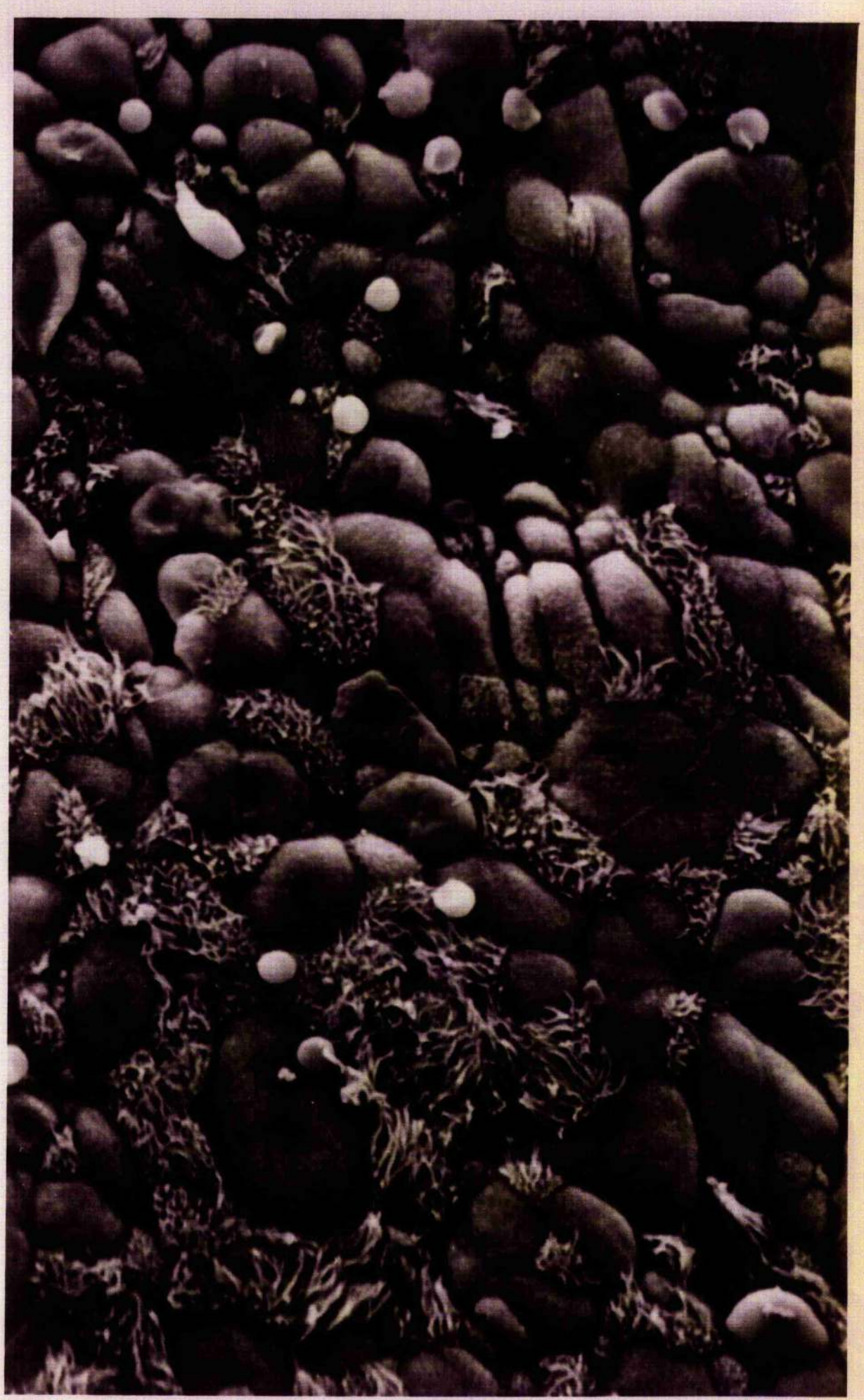


Fig. 40 - Infected calves: A large bronchus in the cranial lobe with a columnar ciliated cell, being shed from the epithelium.

SEM 1250

Fig. 41 - Infected calf: A calf 25 days after challenge with worm eggs on the ciliated surface of a large bronchus (arrow).

SEM x 320

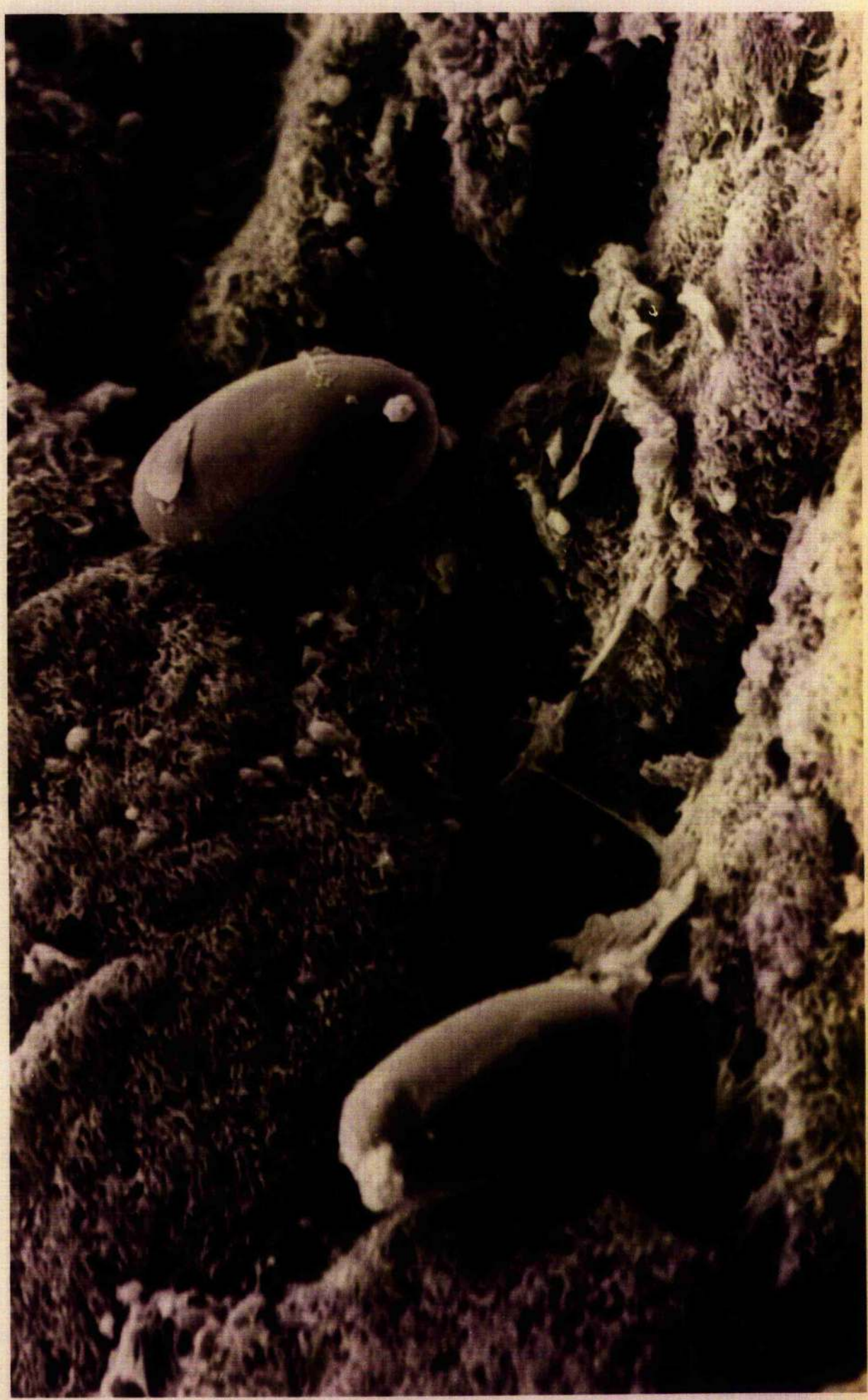


Fig. 42 - Infected calf: A large bronchus in a cranial lobe, 25 days after infection showing active mucous secretory cells (arrow) interspersed with ciliated cells.

SEM 1250

Fig. 43 - Infected calf: A small bronchus with an adult lungworm occupying the lumen of its airway.

SEM x 20

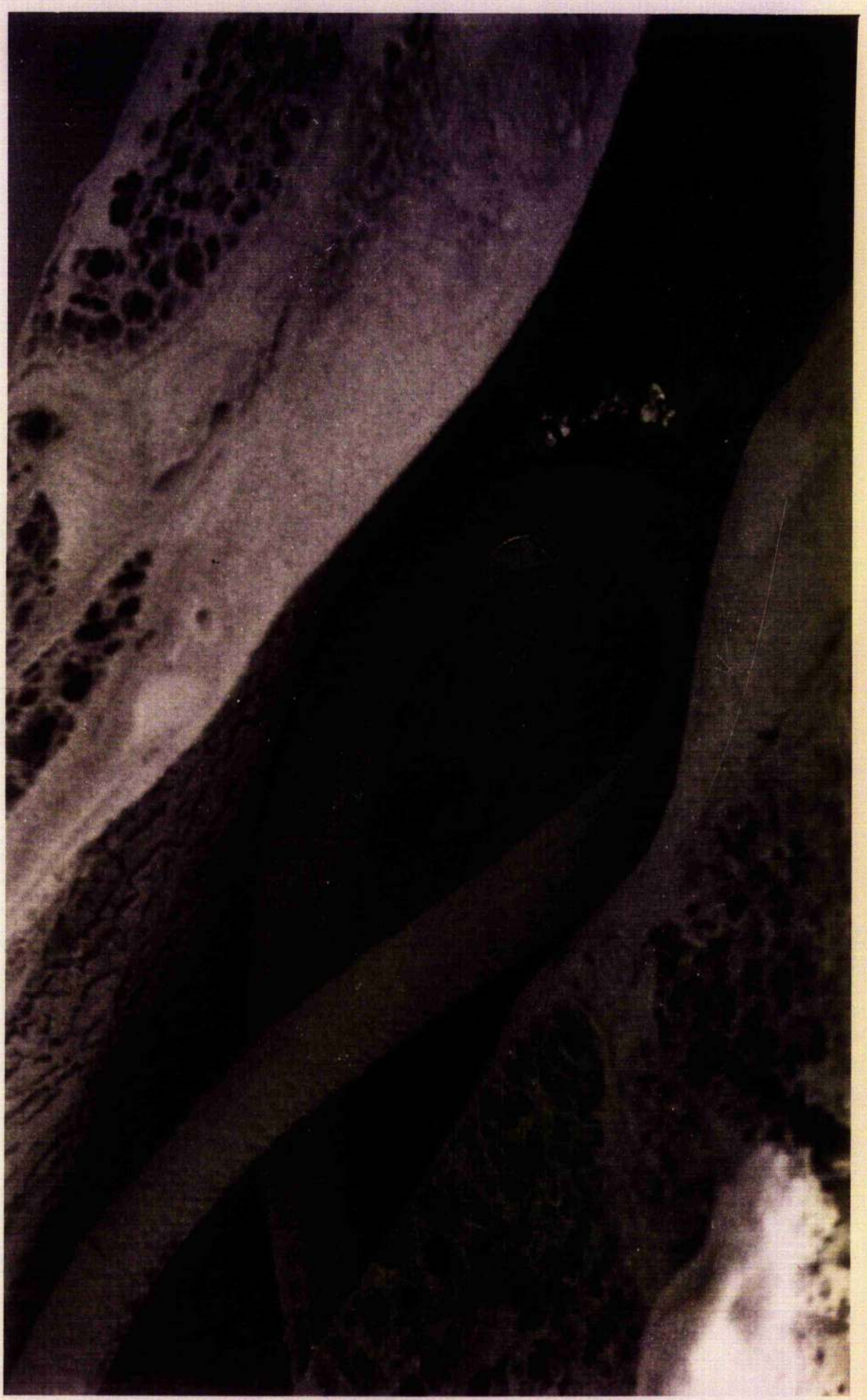


Fig. 44 - Infected calf: Damaged bronchial epithelium 25 days after challenge. Worm eggs can be seen with slightly wrinkled surface (arrow) and marks(✲) on the epithelial surface of the small bronchi possibly made by eggs.

SEM x 160

Fig. 45 - Infected calf: Undifferentiated cells forming patches without cilia on the bronchial epithelial surface.

SEM x 640

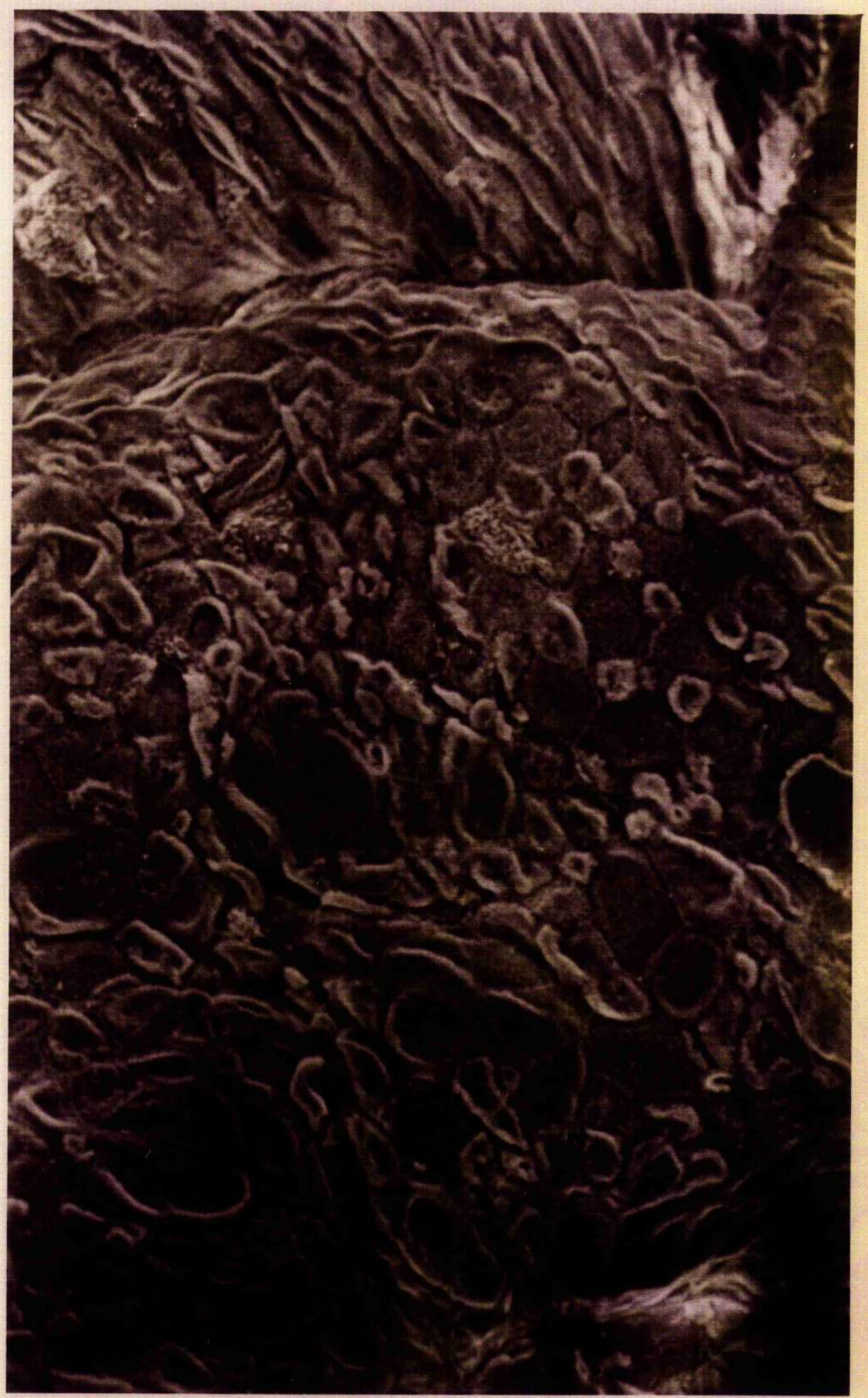


Fig. 46 - Infected calf: Worm eggs seen 25 days after infection in the lumen of an alveolus, possibly due to aspiration.

SEM x 640

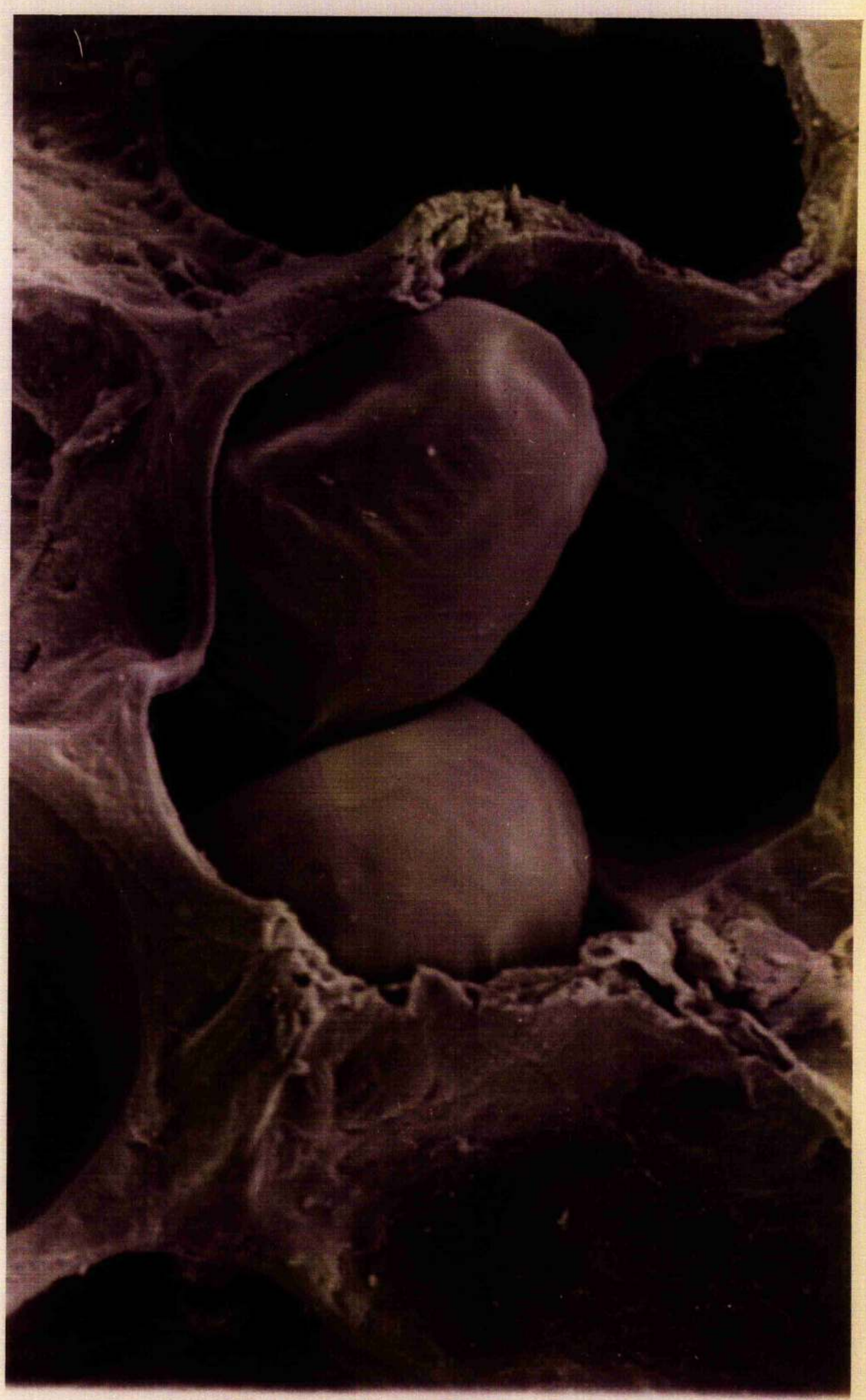


Fig. 47 - Infected calf: The non-ciliated bronchiolar epithelial cells (Clara cells) appear very active 35 days after infection. They have a granular centre protruding into the lumen (arrow). The cilia of the surrounding ciliated cells appear excessively elongated.

SEM x 2500

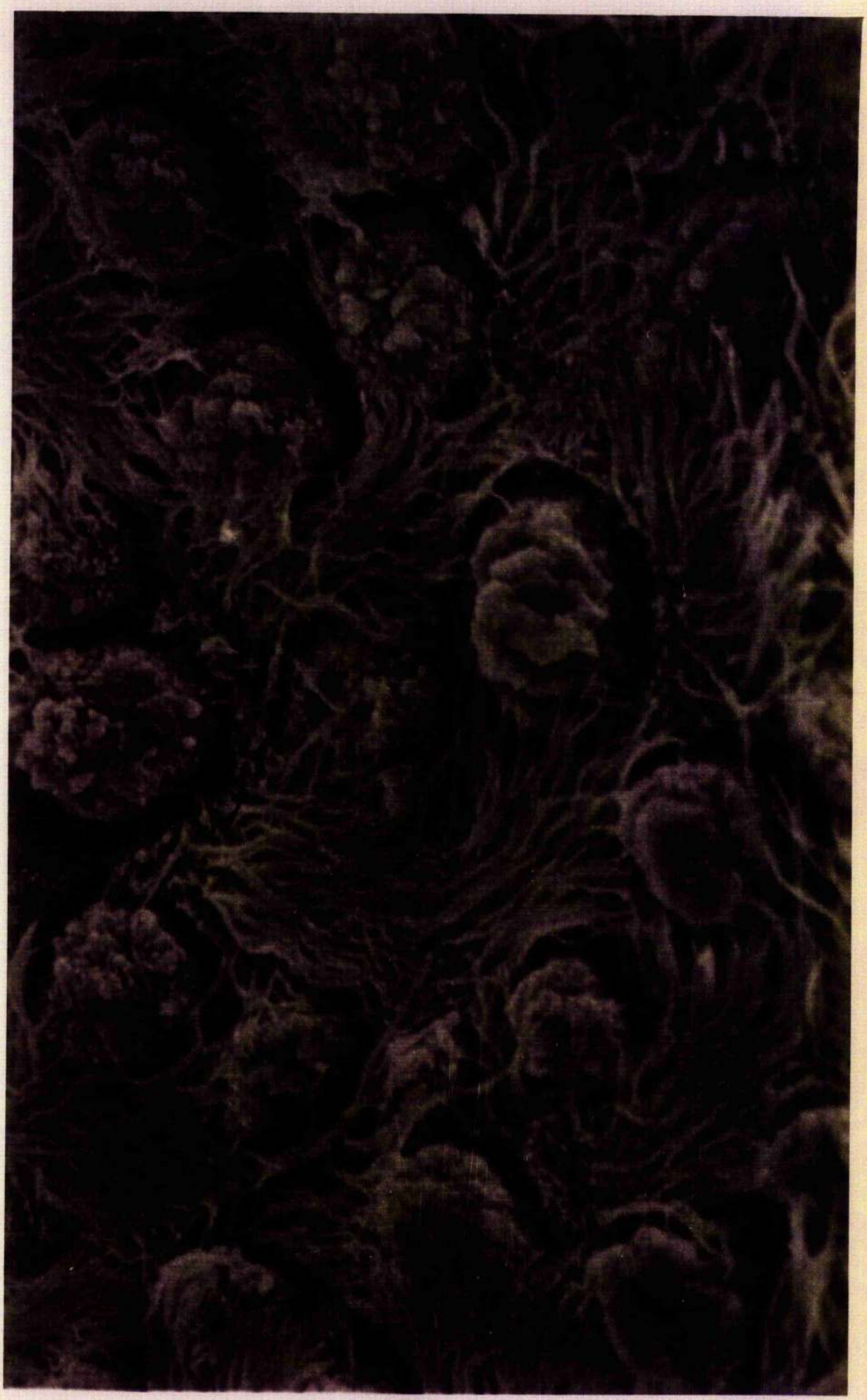


Fig. 48 - Infected calf: A low magnification view of the lung parenchyma where a severe cellular infiltration is present occupying the alveolar tissue. Larvae of D. viviparus can be seen surrounded by inflammatory cells (arrows).

SEM x 160

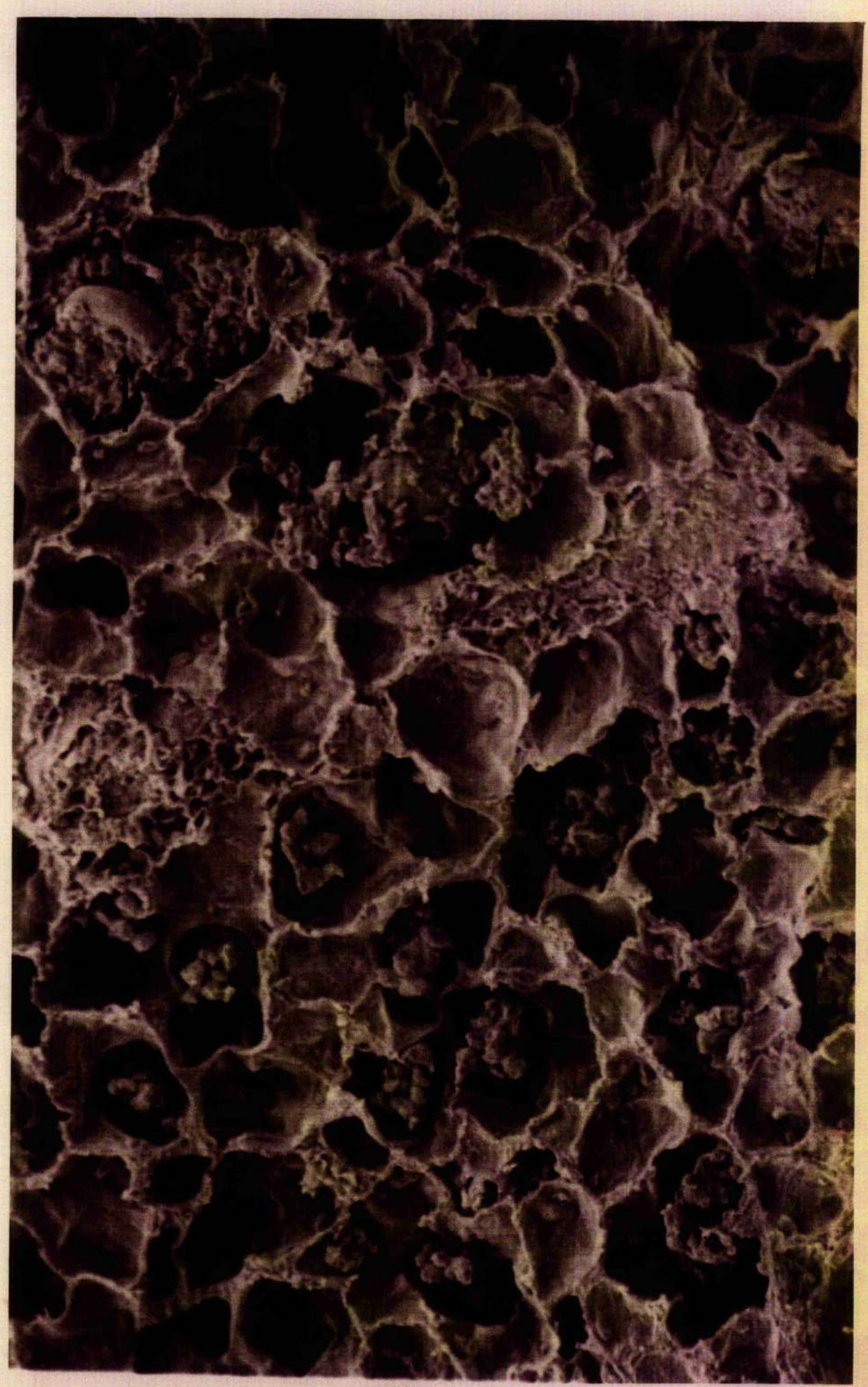


Fig. 49 - Infected calf: A high power picture demonstrating the presence of a lungworm larvae (arrow) surrounded by a cluster of inflammatory cells, 35 days after challenge.

SEM x 640

Fig. 50 - Infected calf: A high power picture of a cluster of different inflammatory cells in an alveolus of a calf infected with lungworms.

SEM x 1250

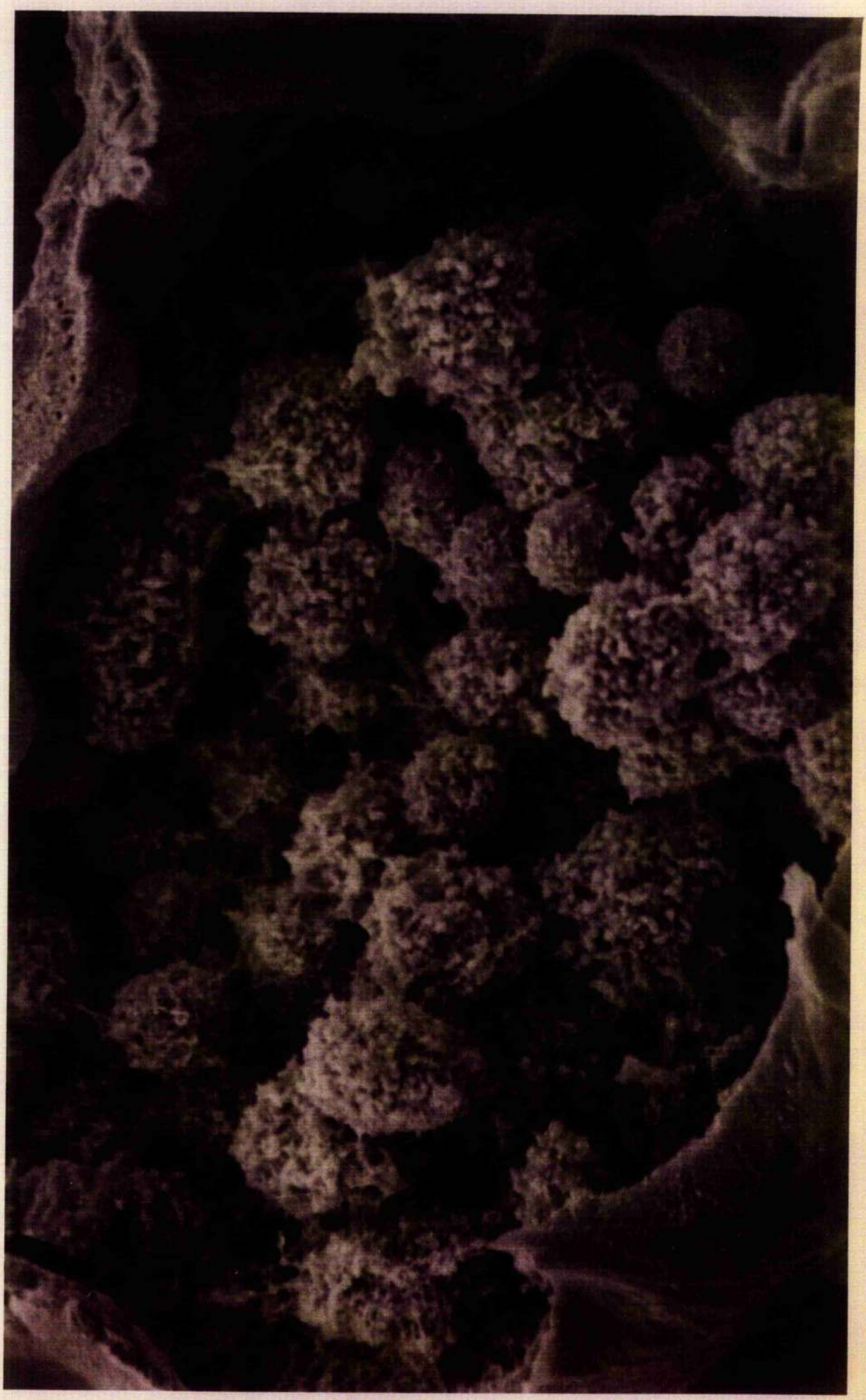


Fig. 51 - Infected calf: The alveolar surface occupied by three different types of inflammatory cell. Lymphocyte (white arrow) macrophages (black arrows) and possibly an eosinophilic cell (red arrow).

SEM x 1250

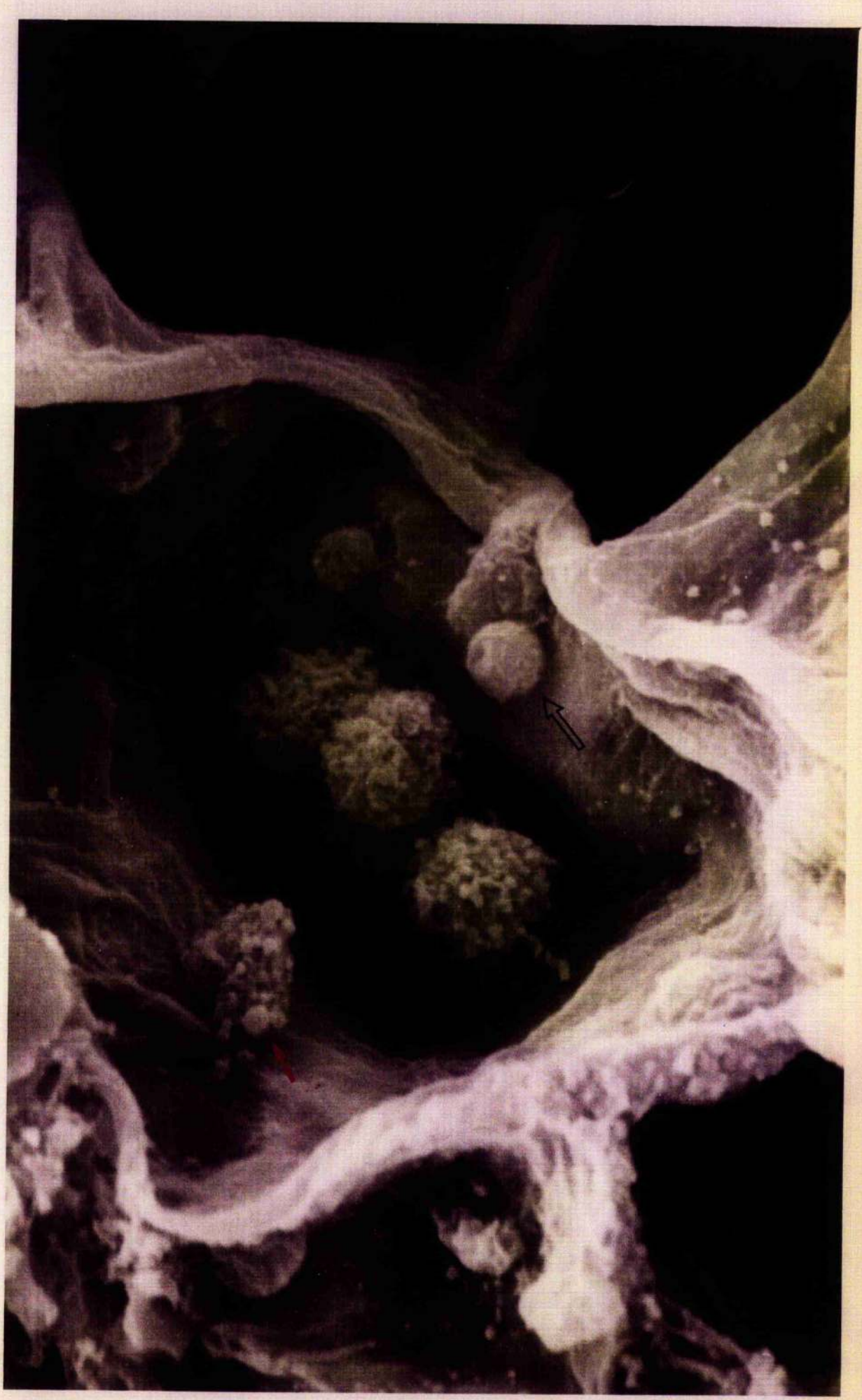


Fig. 52 - Infected calf: Lung tissue with cluster of inflammatory cells at 35 days after challenge. Note two different types of cells based on size. The small cells are probably lymphocytes and the large cells macrophages.

SEM 1250

Fig. 53 - Infected calf: Alveolar macrophages with its typical ruffled cytoplasmic appearance (arrow). A Type II alveolar cell is also identified in this picture (red arrow).

SEM x 2500

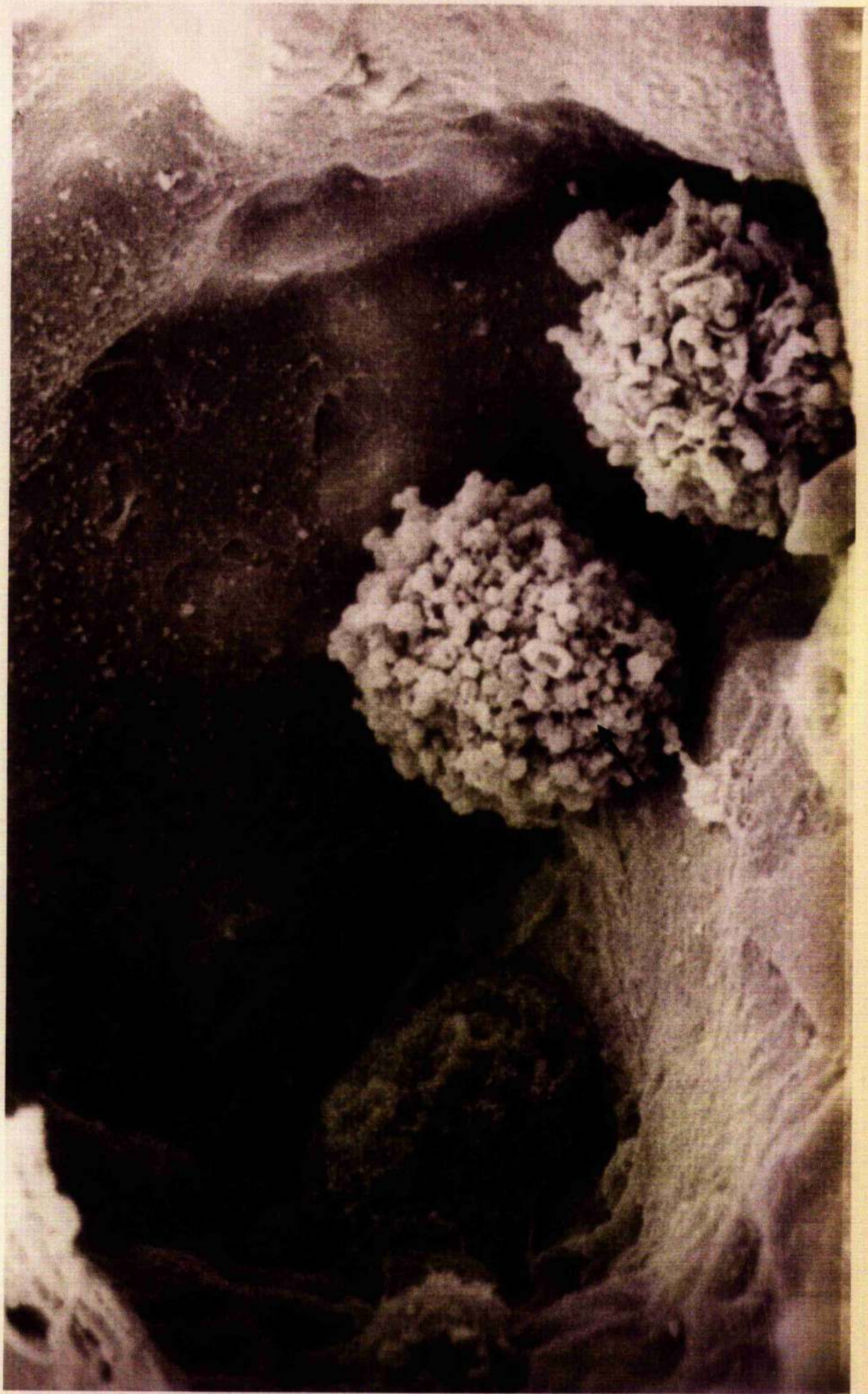


Fig. 54 - Infected calf: Type II alveolar epithelial cells in an active stage. Many microvilli (arrow) surrounding the protruding centre.

SEM x 2500

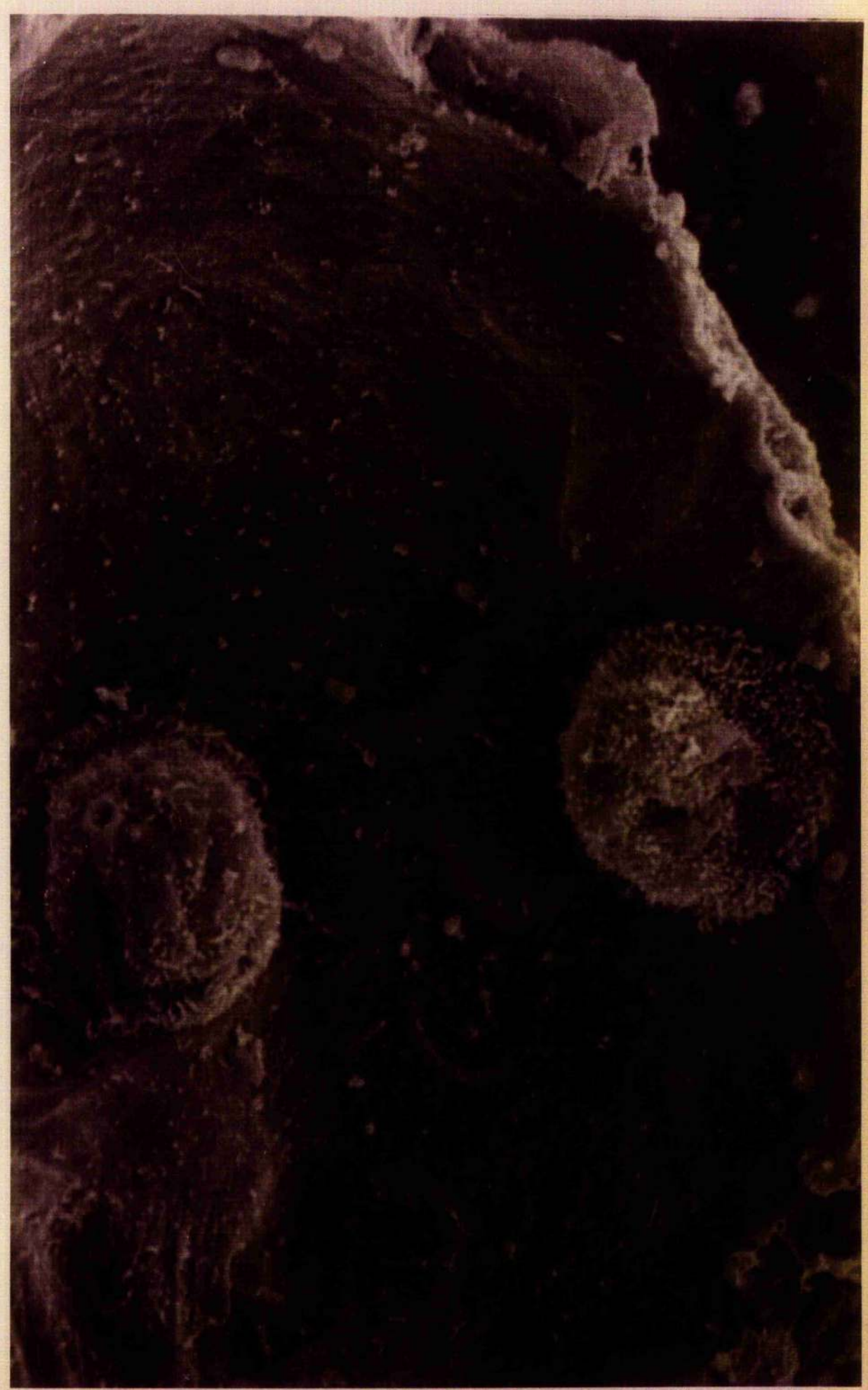


Fig. 55 - Infected calf: The surface of the trachea appears with a modified ciliated epithelium with fewer cilia (arrow).

SEM x 2500

Fig. 56 - Infected calf: A low magnification picture illustrating the non-ciliated bronchiolar epithelial cells.

SEM 640

Fig. 57 - Infected calf: A small bronchus occupied by an adult parasite of D. viviparus.

SEM x 20

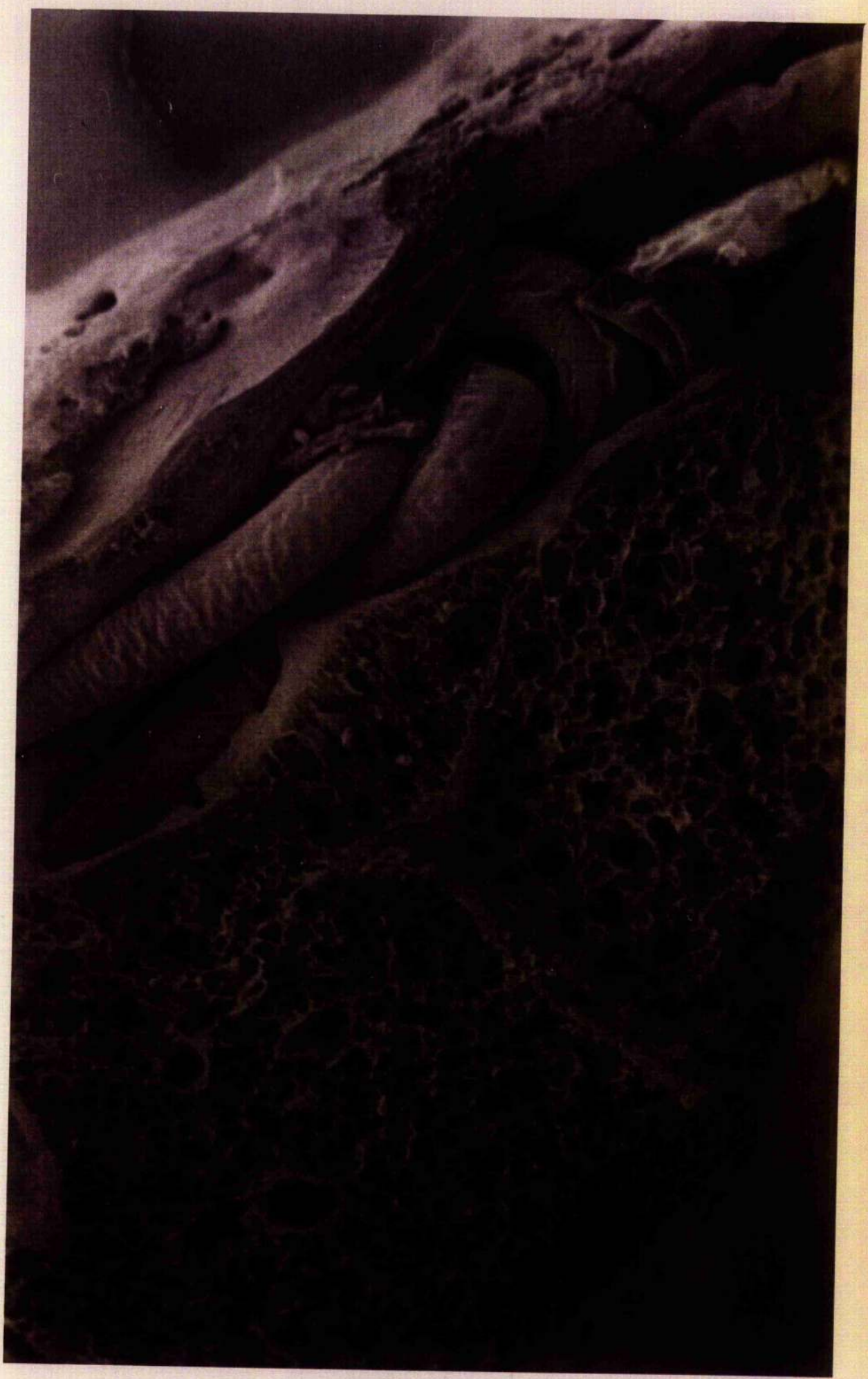


Fig. 58 - Infected calf: An aspirated worm egg occupying the alveolar lumen

SEM x 320

Fig. 59 - Vaccinated and challenged calf: The epithelium of a bronchiolar surface appears covered by active non-ciliated cells and with slight cellular infiltration of the lung parenchyma.

SEM x 160

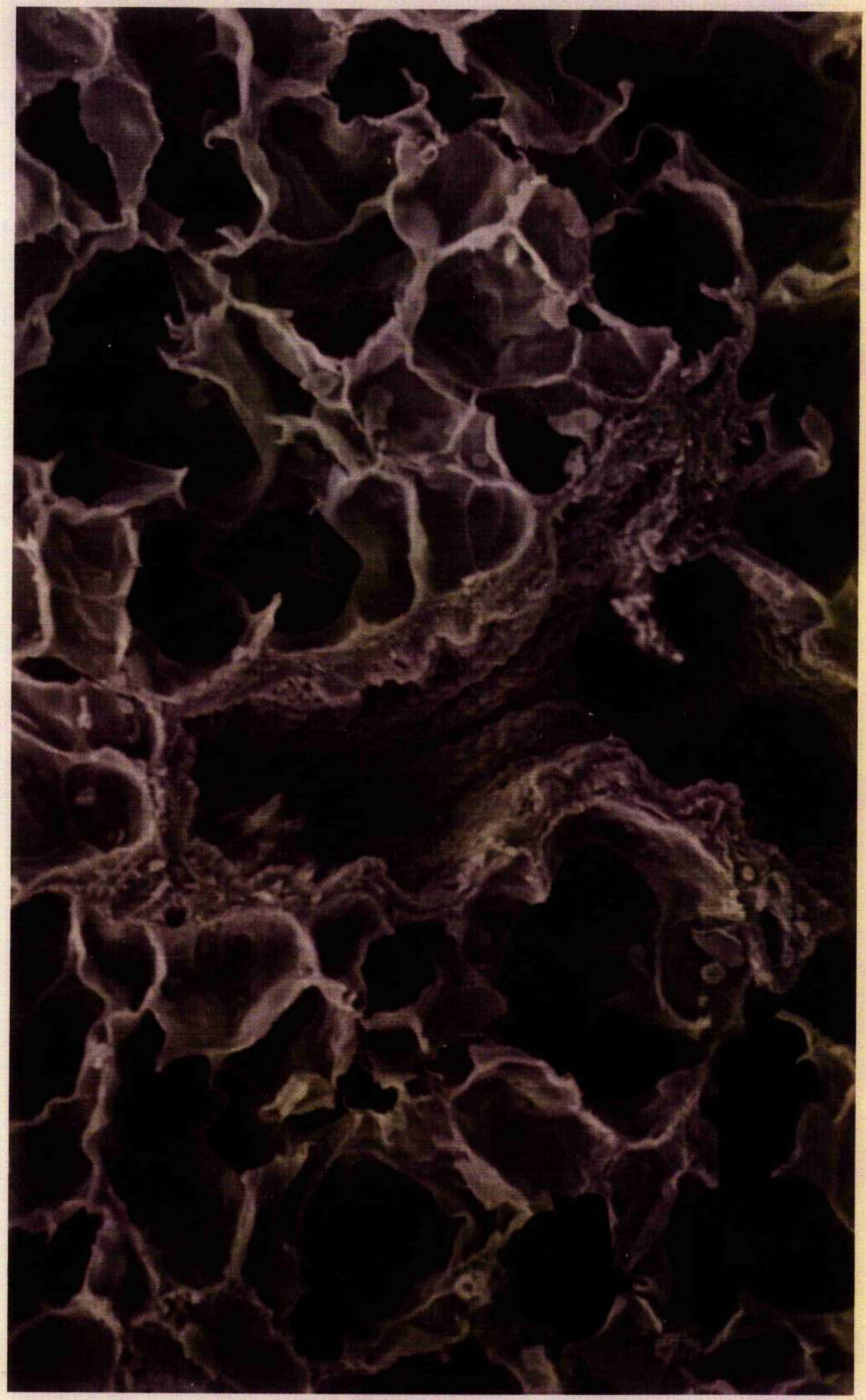


Fig. 60 - Vaccinated and challenged calf: The epithelium of a large bronchus appears with patches of ciliated cells devoid of cilia (arrow).

SEM x 1250

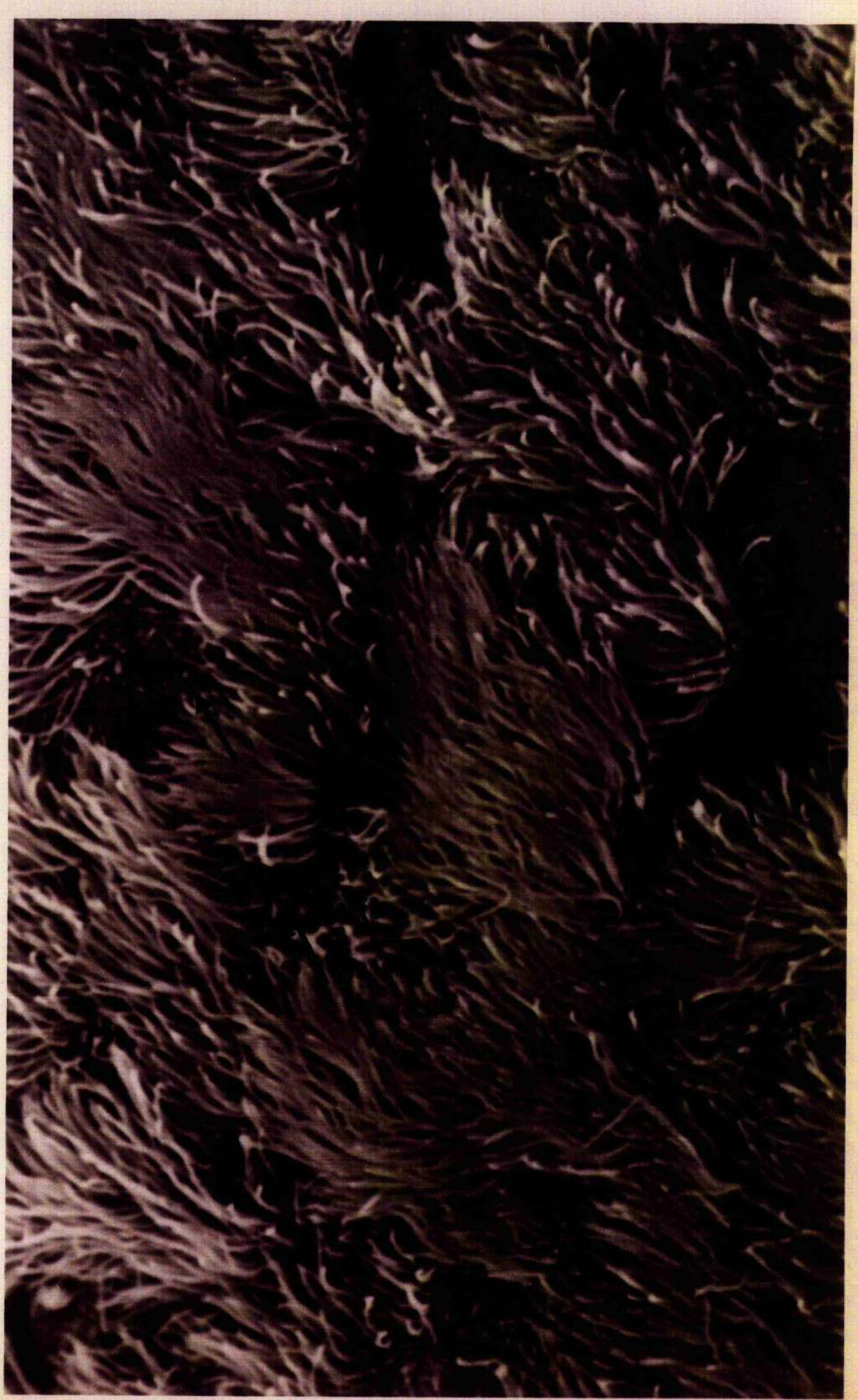


Fig. 61 - Vaccinated and challenged calf: A lymphocytic nodule (arrow) included in the lung tissue.

SEM x 40

Fig. 62 - Vaccinated and challenged calf: A high magnification view of the previous picture. Note the cells that comprise this lymphocytic nodule (arrow).

SEM x 320

SECTION FIVE

CONCLUSIONS

CONCLUSIONS

Scanning electron microscopy can be used effectively and profitably to study the bovine respiratory system in health and disease. The outstanding characteristic of the image obtained by SEM is the remarkable three-dimensional quality, whether the image represented a picture of the tracheal wall, a ciliated cell or a close-up of clusters of inflammatory cells, it conveyed a sense of reality normally lacking in micrographs made by other means such as TEM and LM.

Biological specimens such as the respiratory tract were easily prepared to be inspected by SEM no matter the kind of tissue e.g. trachea or lung parenchyma nor its size, as long as it fitted the column.

Another striking attribute of the image presented by SEM was the amount of information available about the surface topography of the cells. However, the complete interpretation of the information collected by SEM would not be possible ignoring the facts obtained by other methods of investigation e.g. LM or information of tissue such as physiology, anatomy and pathology. These provide the necessary background to interpret fully and usefully details of ultrastructure.

The SEM has proved to be an excellent instrument for studying the respiratory system, since the structure of the lungs and trachea offered a great variety of surface morphology. In the present study, the normal pattern of this tissue was assessed for young and adult cattle and the information achieved was compared with modified patterns observed on the same type of tissue when affected by a pathogenic agent. The normal bovine lower respiratory tract was found to be generally similar to that of other mammals although there were some differences.

Having in mind that a disease is like a cinematographic process it is necessary to take into account the fact that only one picture of the film was recorded. Therefore further investigations will have to be designed to obtain a sufficient amount of evidence to completely characterize the entity studied i.e. lungworm infections. Nevertheless this study has provided a good stimulus to continue the investigation of bovine respiratory diseases by SEM in the hope that a better understanding of these diseases will be found.

SECTION SIX

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