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Original Research Article

LIGHT AND SCANNING MICROSCOPIC STUDIES ON THE TRACHEOBRONCHIAL EPITHELIUM OF THE ONE-HUMPED CAMEL (*CAMELUS DROMEDARIUS*)

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Abstract:

This study aims to elucidate the light and ultrastructure morphology of the epithelial lining of the tracheobronchial tree of camel. Using both light and scanning electron microscope. The basic histology of the air-conducting portions of the camel's respiratory tract was similar to that of other mammalian species. Thesurface morphology of the trachea, extra- and intrapulmonary bronchi were the same. They were lined by respiratory epithelium. The tracheal epithelium exihibited some features along its course. There was a general cranio-caudal decrease in the numbers of goblet cells along the trachea and bronchi. In parallel with this, the number of the subepithelial glands was also decreased with decreasing airway calibre. Histochemically, the goblet cells were described to be predominantly acidic in character as they revealed strong alcianophilia.Neutral mucosubstances were rarely observed in these cells. The subepithelial adenomeres produced predominantly mixed mucosubstances.Intrapulmonary bronchi were formed of pseudostratified columnar ciliated with goblet cells.The ciliated and basal cells were the most numerous cell types. As the caliber of the airway diminished, the columnar cells became shorter with sparse goblet cells. The brush cells were seen more often in the proximal bronchi. With Scanning electron microscope (SEM), the mucosa of the bronchi had regular longitudinal folds with transverse furrows in-between. The majority of the cells appeared as ciliated columnar carrying abundant long cilia. Goblet cells were seen bulging into the lumen, each with dome-shaped apical end.All bronchioles had the same basic structure. The mucosawasformed mainly of columnar ciliated cells.The cilia were found to decrease in length, thickness and number distally.Both the basal and the goblet cells were also scarce or absent in the bronchiolar epithelium.The brush cells were rarely found between the bronchiolar epithelium.TheClara cells were more frequently encountered in the lining of thebronchiolar tree. With SEM, the bronchioles were easily identified since they had a wall uninterrupted by alveoli.The mucosashowed ascalloped appearance. The epithelium of large bronchioles, was usually ciliated low columnar with few non-ciliated Clara cells.In smaller bronchioles, the

ciliated cells were arranged in small patches between the non-ciliated cells.The terminal bronchioles were lined by both cuboidal ciliated and Clara cells. With SEM,the ciliated cells predominated in their proximal portions. More distally, the ciliated cells were replaced by the Clara cells. They were characterized by their dome-shaped apical protrusions. The initial portions of respiratory bronchiole showed reduced ciliated and Clara cells. Towards the alveolar ducts, the lining cells were replaced by flat epithelium and the Clara cells disappeared.

Keywords: epithelium, light microscope, scanning microscope, tracheobronchial and *Camelus dromedaries*.

Introduction

Camels are well known for their positive desert characteristics. Their economical importance has urged many scientists and researchers to study the biology of this organism in details. The respiratory tract of camel has attracted considerable interest. A feature of their nostrils is that a large amount of water vapor in their exhalations is trapped and returned to the camels body fluids, thereby reducing the amount of water lost through respiration. In an attempt for better understanding of the structure-function relationships of the respiratory tract of the one-humped camel and its accommodation with the surrounding hard environment of the desert. Our objectives were to study the ultrastructure morphology of the epithelial lining of the different parts of the tracheobronchial tissue, using both light microscope (LM) and scanning electron microscope (SEM).

Raji and Naserpour (2007) mentioned that, the trachea of camel was lined by a pseudostratified ciliated columnar epithelium with numerous goblet and basal cells. The goblet cells of trachea in camel produce exclusive amounts of acidic and neutral mucosubstances. Numerous submucosal glands (branched, coiled and tubuloalveolar) were observed in the trachea, with mucous (acidic and neutral) secretions. The luminal surface of the trachea in camel was completely covered by cilia, which is similar to cattle, goat and neonatal kids. The primary structure of the viscoelastic layer consists of glycoprotein and mucin, synthesized by surface goblet cell and mucous cell of the submucosal glands. **Raji (2006)** studied the light microscopy of the lung parenchyma of the one-humped camel. The intrapulmonary bronchi were lined by a respiratory epithelium composed of ciliated, secretory cells (goblet cells) and basal cells. The bronchiolar mucosa of camel was simple cubiodal or columnar and devoid of goblet cell. He mentioned that the respiratory bronchioles were absent in camel. They were poorly developed in horses and humans. **Zaghloul (2004)** investigated the ultrastructure of the camel`s lung. The bronchial epithelium was pseudo- stratified columnar ciliated with goblet cells. Three types of cells in the bronchial epithelium, ciliated, goblet and basal cells, in addition to some migratory cells. Moreover, she identified two types of ciliated in the bronchiolar epithelium; light and dark cells, in addition to a non-ciliated Clara cells. Few goblet cells were seen in large bronchioles. The Clara cells were bulged into the lumen with dome- shaped apical end and carry very few short microvilli. Their cytoplasm characterized by the presence of abundant oval shaped lamellate bodies and round to oval electron- dense membrane bounded granules. This epithelium decreased suddenly in height towards the alveolar duct. The alveolar ducts were relatively long and

were interrupted by numerous openings of several alveoli. They were lined by simple squamous. Also in the camel, **Sayed (2002)** described the morphologic alteration concerning the disintegrating cells observed at the principal bronchi in early fetal stages. **Whitsett et al. (2002)** commented on the structure and functions of the airway of the lung change dramatically along their lengths. According to **Reynolds et al. (2000)** the Clara cells represent the most abundant secretory cell type of distal airways in the human lung, and of both proximal and distal airways in the lungs of rodents and rabbits. In addition to their secretary function, they serve as the principal source of progenitors cells for repopulation of airways after injury. **Abdel-Rahman (1999)** mentioned that, the tracheal epithelium of the camel was a typical example of respiratory epithelium composed of four main cell types: ciliated, goblet, basal and intermediate cells in addition to some migratory cells. The respiratory epithelium of the trachea of the camel contained neither brush cells nor Clara cells. The ciliated cells were the most numerous cell – type encountered within the tracheal epithelium of the camel, structurally similar to those of the other mammalian respiratory epithelial cells. Histochemically, the goblet cells revealed strong alcinophilia and moderate PAS reaction. **Kahwa and purton (1996)** described the histological and histochemical study of epithelial lining of the respiratory tract in adult goats. The trachea was lined by a pseudostratified ciliated epithelium. Individual surface mucus- producing cells were relatively few in number and predominantly acidic in character. Submucosal glands were numerous and produced predominantly acidic mucosubstances with only a few producing a mixed reaction, neutral mucosubstances were rarely observed. The number of individual mucus-producing cells was seen to increase with a decrease in airway diameter. **Bouljihad and Leipold (1994)** examined the ultrastructure observations of the pulmonary bronchiolar and alveolar epithelium in sheep. Three distinct cells, basal, ciliated and non ciliated (Clara) cells were lining the primary and secondary bronchioles. Whereas, the terminal and respiratory bronchioles were lined by ciliated and non- ciliated (Clara) cells only. In **1994, Baldwin** investigated the morphology and distribution of basal cells within human bronchial epithelium. The morphometric analysis of human lung was studied by **Baldwin et al. (`1991).** The precise thickness of bronchial epithelium, the sizes of its different cells, and the distances for their nuclei from potentially harmful influences at the mucociliary surface had established. **Pirie et al. (1990)** described the surface features of the lower respiratory tract of the equine using SEM. They were populated mainly by non-ciliated epithelial cells and showed an abrupt Junction with alveolar ducts. **Reznik (1990)** studied the anatomical characters of the upper respiratory tract of various experimental animals and man. A pseudostratified respiratory epithelium composed of ciliated, goblet and basal cells lines the upper parts of the rat airway from the trachea to the segmental bronchi. The mucous cells are the goblet cell type in the trachea and main bronchi. Brush cells and APUD cells are extremely rare. **Matsumura and Setoguti (1989)** have identified three morphological types of tight junction's in-between bronchial epithelial cells in human using electron micrograph of a freeze fractured cells replica. **Plopper et al.(1989)** in rhesus monkey, concluded that the characteristics of the epithelial lining of the mammalian tracheobronchial airway tree were very species specific. **Moussa (1987)**

described the light and ultrastructure of the terminal bronchioles of the camel's lung. They were lined by ciliated and non ciliated cells or Clara cells. The former cells appeared cuboidal and carry long cilia. The other cells were tall, dome-shaped with their apical portions bulging and contained numerous dense granules and smooth endoplasmic reticulum. Both cells were firmly attached by tight junctions. **Souma (1987)** studied distribution of the epithelial cells of the rat airways continuously from the trachea to terminal bronchioles by SEM. From their surface structures, the epithelial cells could be classified into ciliated and non–ciliated, the latter including brush cells, Clara cells and other non – ciliated (secretory) cells. Ciliated cells have longer, thicker and more numerous cilia in the trachea, they decrease in length, thickness and number toward the periphery. Brush cells possess thin microvilli in the trachea and extra- pulmonary bronchi, with a rounded end. Other non – ciliated (secretory) cells, their apical cytoplasm seems to contain secretory granules. They often gather in groups in the trachea and extrapulmonary bronchi. **Chang, Mercer and Crapo (1986)** studied the differential distribution of brush cells in the rat lung using electron microscopic morphometry. The brush cells have a distinct spatial location in the lung, being in high concentration in the trachea and in areas where first generation alveolar ducts bifurcate. The brush cells made up 10% of the volume of epithelium covering the first alveolar duct, 2% of the proximal alveolar epithelium, 1.4% of the terminal bronchiolar epithelium, and 3% of the tracheal epithelium. No brush cells were found in the lobar bronchi or in the distal alveolar walls. The brush cells exhibit stubby microvilli on their apical borders protruding into the air space. **Tyler and Plopper (1985)** studied the organization and nature of epithelial populations in the distal airways of the rhesus monkey. Using SEM the epithelial populations of non alveolarized terminal conducting airways was pseudostratified columnar, consisting of ciliated, mucous and basal cells. The respiratory bronchiole found immediately distal to the terminal conducting airways had two clearly demarcated zones of distinctly different epithelial populations. The remainder of the respiratory bronchiole, was simple non ciliated cuboidal with a few squamous cells. **Mariassy & plopper (1984)** described the ultrastructural and morphometric analysis of the epithelial secretary cell types within tracheobronchial epithelium of the sheep. They could distinguish six distinct granule-containing secretary cells: four type of mucous cells, serous cells, and Clara cells in the epithelium of conducting airways depended up on cell height and width, nuclear dimensions, and granule electron density. **In 1983 Mariassy and Plopper** investigated the distribution pattern of cell populations of the tracheobronchial tree in sheep lung. The total mucous cell population in proximal airways was relatively constant. The basal cells were found in the epithelium of airways without cartilage or glands. **Spicer et al. (1982)** investigated the structure of rat respiratory glands. The glands exhibited a tubulo-acinar organization. Individual secretory units were composed of serous tubules or serous demilunes and mucous tubules. The secretory product of these gland cells varied depending up on the location of glands in the airway. Tracheal glands were composed of serous tubules, a few mucous tubules and prominent mucous ducts. **Pack, Al-Ugaily and Morris (1981)** investigated that three principal cell types in the tracheobronchial epithelium of the mouse: basal, ciliated and non- ciliated (secretory) cells. All these cells

were decreased in number and reduced in thickness in the more distal airways. The majority of the mucus-containing cells. Stained with Alcian blue–PAS were AB–positive. This indicates that they contained acid monosaccharide. Non-ciliated or Clara cells at all airway epithelium by their characteristic apical projection into the lumen. As with the ciliated cells the luminal surface was delineated by an alcianophilic band. Occasionally, PAS-positive granules were seen in the cytoplasm of the Clara cell. In rats, the bronchiolar Clara cells contained filamentary rod- shaped granules. They appeared to be most numerous in the small bronchioles. **(Baert and Vandenberghe, 1981).Spicer et al.(1980)** studied a complex carbohydrate of rat tracheobronchial surface epithelium using Alcian blue-PAS method. They related the biologic significance of this mucous to such function as protection from infection, maintenance of hydration, ion content, muco-ciliary flow and possibly other functions.

Material and Methods:

The airways used in this study were obtained from 13 apparently normal and clinically healthy adult one-humped camels of both sexes. They were collected from *"El-Warak Abattoir" - Giza.* The samples were taken from different areas of the trachea and extra-pulmonary bronchi, intra- pulmonary bronchi, bronchioles, terminal and respiratory bronchioles.

For light microscopy:

Small strips of selected tissue were fixed in 10% buffered formalin and in Zenker's formal for 24 hours. They were then dehydrated in ascending series of ethanol, cleared in benzene and were embedded in paraffin. Sections, 4 – 6 µm, thick were cut and stained by the following stains:

1. Harris haematoxylin and eosin as a general staining method.
2. Crossmon's trichrome stain for identification of collagen fibers, smooth muscle fibers, fuchsinophilic and orangophilic substances.
3. Toluidin blue stain for the semi thin sections.
4. Alcian blue technique (PH 1.0) for demonstration of the sulphated mucins and (PH 2.5) for the detection of acid mucins.
5. Periodic acid Schiff's (PAS) technique for localization of neutral mucopolysacchrides.
6. PAS-alcian blue combination for differentiation of neutral and acidic mucopolysacchrides.

The aforementioned staining methods were used as outline by **Bancroft and Stevens (1990).**

For Scanning Electron Microscopy (SEM):

The collected samples were immediately immersed in the fixative 4F1G (2% formaldehyde, 1.25% glutaraldehyde in 0.1 M sodium cacodylate, pH 7.2) and stored at 4^0 C. The fixed samples were washed in 0.1 M sodium cacodylate containing 5% sucrose, processed through tannic acid, dehydrated in ascending ethanol series (50, 70, 80, 90, 95 and 100%) for 15 minutes in each. Then critical point dried from carbon dioxide, attached to stubs with colloidal carbon, and coated with gold palladium in a sputtering device.

Specimens were examined and photographed with Joel scanning electron microscope operating at 25 KV at the Regional Center for Mycology and Biotechnology (RCMB) at Al-Azhar University.

Results

Light microscopy

Thesurface morphology of the trachea, extra- and intrapulmonary bronchi were the same and will be described together. They were lined by respiratory epithelium.

The trachea was lined by a pseudostratified epithelium in which ciliated and mucous cells predominate **(Figs.1a&b).**In addition, basal short cells stand out because their nuclei form a row close to the basement lamina to give the epithelium its apparently stratified appearance. These cells do not extend to the free surface and evidently serve as a reserve population for the epithelium.

The subepithelial loose areolar connective tissue housing numerous seromucoid glands **(Fig.2)** of which the majority were serous in nature.

The tracheal epithelium exihibited some features along its course. In the anterior third, the abundant ciliated cells were mostly crowded and squeezing the other cell types **(Fig.1).** In the middle third these lining cells appeared less crowded and the goblet cells became less numerous **(Fig.3).** Towards the lung, the decrease in the number of goblet cells became distinct **(Fig.4).** Therefore, in the examined sections, there was a general cranio-caudal decrease in the numbers of goblet cells along the trachea **(Fig.5)** and bronchi **(Fig.6).** In parallel with this the number of the subepithelial glands was also decreased with decreasing airway calibre.

Histochemically, the goblet cells were described to be predominantly acidic in character as they revealed strong alcianophilia **(Fig.7).**Neutral mucosubstances were rarely observed in these cells **.**The subepithelial adenomeres produced predominantly mixed mucosubstances **(Fig.8)** with only a few showing a neutral reaction **(Fig.9).**Like the other conducting portions, the trachea conditions the air as it passes to the lungs and provides protection from dust and airborne infection.

Intrapulmonary airways

The sequential intrapulmonary orders of airways, include intrapulmonarybronchi, bronchioles, terminal bronchioles and respiratory bronchioles **(Fig.10**). The later were connected to the alveolar sacs by alveolar ducts.

Bronchi*:*

The type of epithelium that lined the bronchial tree was similar to that observed in the trachea, with only a reduction in the height of the epithelial lining being apparent.Four cell types were recognized in the lining of the intrapulmonary bronchi. These were ciliated columnar cells, goblet cells, brush cells and basal cell **(Fig.11)**. These cells of the pseudostratified mucosa rested on the basal lamina, but only the first three cells were exposed to the lumen.

The ciliated cells appeared tall columnar and having large number of long cilia **(Fig.12)**. The cytoplasm was somewhat densely stained. The nucleus appeared large oval to ovoid in shape and were mostly located near the apical half due to crowding of cells. The chromatin was condensed on the inner surface of the nuclear envelope.

The goblet cells: They were found only in the large airways. Theyoccurred singly or in groups of 2 or 3 cells between the columnar cells **(Figs.12&13)**. Their apical cytoplasm was faint and finely granular than that of the surrounding cells. The nucleus was often found in the narrow basal portion.

The brush cells were seen more often in the proximal bronchus. They constituted less than 20% of the total cell population of the bronchi. Brush cells were distributed sparsely but rather uniformly, and apt to be grouped in two or more cells. Each cell appeared narrow columnar in shape and lacking cilia **(Fig.12)**. They were immediately recognized by their apical vacuolated dense cytoplasm **(Fig.13)**. Their nuclei were elongated or ovoid and denser than those of the neighboring cells. They

were occupying a middle position in the cell. In H&E stained sections, their cytoplasm appeared densely stained **(Fig.12)**.

The basal cells were smaller in diameter and were seen resting on the basement membrane, but not approaching the luminal border. They had different shapes with large oval or spherical centrally located nuclei and unstained cytoplasm **(Figs.12&13)**. Some of these cells attained a larger size and became insinuated in between the other lining cells to reach the luminal surface **(Fig.14)**. This view could support their role to replace dying (apoptotic) columnar, goblet and brush cells. Many ***migratory cells*** **(Fig.11)** e.g. lymphocytes, eosinophils, plasma cells and mast cells were observed in the lamina propria.

Bronchioles:

The last branching of the intrapulmonary bronchi resulted in the formation of the bronchioles, which are usually branched and formed several generations before ending as terminal bronchioles. Light microscopy showed that all bronchioles had the same basic structure **(Fig.15)**. Each bronchiole consisting of mucosal lining, smooth muscles and fibrous tissue.Unlike the rigid bronchi, they had no glands and cartilage support and had a diameter less than 1mm.

The mucosa was mainly formed of the usual ***ciliated columnar*** and ***basal cells***. ***Brush cells***were rarely found in the bronchiolar epithelium**(Fig.16)**. The ***goblet cells*** were scarce or absent and were replaced by the ***Clara cells*** which were frequently encountered in the epithelial lining of the bronchiolar tree **(Fig.17).** They appeared as tall non-ciliated cells with apical dome-shaped protrusion. The apex of the Clara cells was commonly at a higher level than the tips of neighboring epithelial cells. After H&E stain, their cytoplasm was vacuolated, housing a basal nucleus which appeared oval or elongated in shape. This cell type was being identified by its positive staining reaction to AB / PAS **(Fig.18).**

The bronchioles branched into several smaller ***terminal bronchioles*** that form the last component of the conducting portion of the respiratory system (**Fig.19**). These bronchioles were lined by both ciliated and non-ciliated Clara cells. The former type was cuboidal or low columnar and born cilia. The second type was the predominant cell type. Both types rested on a thin fibrous lamina propria rich in elastic fibers.

Each terminal bronchiole undergoes a final subdivision to form ***respiratory bronchioles***. In terms of its structure, the respiratory bronchiole can be thought of as a continuation of a terminal bronchiole. With the continued decrease in the height of the cell lining, the cells became more cuboidal especially in the smaller bronchioles. Each respiratory bronchiole was lined by numerous Clara cells with few low ciliated cells scattered in-between. This lining became flattened and decreased gradually in height towards the alveolar duct where the Clara cells disappeared **(Fig. 20).**

With Scanning Electron Microscopy:

Trachea & Pulmonary bronchus

The luminal surface of the trachea was thrown longitudinally into mucosal folds separated by shallow grooves **(Fig.21)**. At higher magnification, the mucosal surface was formed mainly of ciliated and goblet cells **(Figs. 21&22)**. The ciliated cells were the most numerous and abundant cell type encountered within the tracheal epithelium of the camel. Their cilia were thin, long and protruded above the level of the adjacent goblet cells.

The goblet cells were numerous among the abundant ciliated cells **(Fig.23)**, with some surface mucus was always present. These cells might bulge out to varying

degrees from the surface or appeared relatively flat. Many of them were provided with apical few and short microvilli**(Fig.24)**. Occasionally some of the goblet cells were also observed singly with their apical protrusion was ruptured exposing their contents of mucigenous granules **(Figs.25&26).**

Intrapulmonary airways

Bronchi *:*

The mucosa of the bronchi, as in the trachea, had regular longitudinal folds with transverse furrows in between. The majority of the cells appeared as ciliated columnar carrying abundant long cilia **(Fig.27).** Goblet cells were seen bulged into the lumen of the bronchus. Each with dome-shaped apical end carrying few short microvilli **(Figs. 28a&b).**The bronchial epithelium was resting on a basal lamina supported by fine collagen fibers. The basal cells appeared large oval or ovoid in shape and were arranged on the basal lamina **(Fig. 29).**

Bronchioles

The bronchioles were easily identified with the SEM since these airways have a wall uninterrupted by alveoli and lacking cartilage and submucosal glands **(Fig. 30)**. They appeared with lesser diameter. Bronchioles split up into terminal bronchioles and then respiratory bronchioles that were connected to the alveolar sacs by alveolar ducts. The continuity between a terminal bronchiole, respiratory bronchiole, and alveolar duct was denoted**.** Air is conducted to the alveoli via these intrapulmonary airways. Exchange of gases occurred between air within the alveoli and blood in the surrounding extensive capillary networks.

The mucosa of the examined bronchioles showed as calloped appear-ance due to contraction of the smooth muscles surrounding the bronchiolar wall **(Fig. 31).** The mucosa in most of the examined bronchioles was thrown into folds**.** These folds were clearly distinguished from one another by deep furrows ((**Fig. 32).**The epithelial lining and the underlying lamina propria were clearly identified **(Fig. 33)**.The bronchiolar epithelium was seen to vary at different levels of the bronchiolar tree. However, the type of epithelium that lined the bronchial tree was similar to that observed in the bronchi, with only a reduction in the height of the lining cells being apparent. In large bronchioles, the epithelium was usually ciliated columnar with few non-ciliated Clara cells **(Fig. 34)**. Lateral and surface views of the epithelium were shown in **(Fig. 35).**The columnar cells resided basally on a basement membrane, and cilia extended from the apical surface of the cells. The length of the cilia appeared uniform. whereas the **smaller bronchioles** were lined with ciliated low columnar or cuboidal cells and Clara cells. A small amount of connective tissue belonging to the lamina propria was present. The rounded apical surfaces of a number of Clara cells appeared among the cilia **(Fig. 36)**. The secretory materials appeared inside the cells as small-granules present within the apical cytoplasm of the secretory cells. In small bronchioles, the ciliated cells arranged in groups interspersed with non-ciliated cells throughout the epithelial surface. The length of the cilia was uniform, approximately 4.5 to 5 µm. Approximately less than one-half of the cells in the bronchioles were ciliated. The cilia were typically shorter than those on the taller columnar cells lining the bronchi. These cilia were found to decrease in length, thickness and number distally.

Terminal bronchioles were lined by both ciliated and non-ciliated cells **(Figs.37a&b)**. In their proximal portions the mucosal structure was identical to that found lining the bronchiole where the ciliated cells

predominate. More distally, however, variation in mucosal structure was observed, and the pattern of variation differed. The ciliated cells became less numerous in the distal bronchiolar areas. They were replaced, in some areas, by the non-ciliated cells of Clara. The dome-shaped apical protrusions of these non-ciliated cells were covered on their luminal surfaces by few scattered stubby microvilli. Down in the bronchiole and towards the respiratory bronchioles, the epithelium continued to be composed of both ciliated cells and non-ciliated Clara cells. However, the former cells became appreciably reduced in number and height. A bronchiole was branched into several smaller **terminal bronchioles**, which were the last component of the conducting airways. Each terminal bronchiole undergoes a final subdivision to form **respiratory bronchioles**. The latter were lined by simple cuboidal epithelium and each was interrupted by alveoli.At the level of the initial portions of **respiratory bronchioles** a transition took place between ciliated and nonciliated epithelium **(Figs. 38a&b)**. Only past this transition point, the nonciliated bronchiolar cells did appear. This transition was usually gradual in camel's lung. More distally in respiratory bronchioles, the wall became much more highly alveolarized, and the lining cells were partially replaced by flat epithelium. The ciliated cells were no longer present except for occasionally isolated and irregular small patches of ciliated epithelium were seen interrupting the dome-shaped protrusions of the non-ciliated cells **(Fig.39).**

Discussion

Light microscopy supported by SEM was used to study the structural morphology of the respiratory airways surfaces in camel, from the trachea to the respiratory bronchiole.

The examined epithelial lining of the camel's conducting airways (trachea and bronchi) was a typical example of respiratory epithelium. It was formed of ciliated, goblet, basal and intermediate cells together with some migratory cells. This result was confirmed in trachea of camel (**Abdel-Rahman, 1999**), and in bronchi of camel (**Zaghloul , 2004**).

The epithelia of the upper respiratory tract of camel showed neither brush cells nor Clara cells. The former cells were described in the trachea of rat (**Rhodin and Dalhamn, 1956**) and of man(**Rhodin, 1966**). Clara cells were observed in rat trachea by **Hansell and Moretti (1969).**

Both light and scanning EM investigations revealed that the columnar ciliated cells were the most numerous cell type encountered within the examined airways. This finding resemble that described in bronchi of dog (**Fresco et al., 1968**) and trachea of rat (**Rhodin and Dalhamn, 1956**) and mouse (**Greenwood and Holland, 1972**).

Numerous goblet cells were observed within the respiratory epithelium of the camel similar to that mentioned by **Rhodin** and **Dalhamn (1956)** in rat trachea, **Frasca et al. (1968)** and **Baskerville (1970)** in bronchi of the dog and pig. However, **Karrer (1956)**and **Hanseli and Moretti (1969)**observed that the respiratory epithelium of the trachea and bronchi of mouse respectively are virtually devoid of goblet cells. The structural features and distribution of the goblet cells in the camel's airways resembled those described by **Amal et al. (2005)** and **Gewaily (2009)** in the respiratory epithelia of camel. Histochemically, the goblet cells of the examined material contained mainly acidic mucins. **Pack et al. (1980)** and **Newman et al. (1996)** suggested that the goblet cell granules were serous and mucous granules,

respectively. In agreement with **Newman et al. (1996)** in the guinea pig trachea, the secretory granules of the goblet cells of the present study were discharged either by simple exocytosis or apocrine-like mode of secretion.

The intermediate cells have been observed in the camel's respiratory epithelium.This cell usually reaches the airway lumen, while in the other species it often does not. This cell is thought to be an undifferentiated cell capable of differentiation into either a ciliated or a goblet cell **(Rhodin, 1966).**In the rat trachea, the accumulation of fibrogranular material within the cell cytoplasm, suggesting ciliogenesis (**Jeffery and Reid, 1975**). However, their differentiation into ciliated cells could not be observed in the present study.

The structure of the basal cell of the camel's respiratory epithelium was similar to that described by **Amal et al. (2005)** and **Gewaily (2009)** in camel. **Abdel-Rahman (1999)**suggested that, the basal cells represent the layer from which the other cells differentiate, particularly in the extrapulmonary airway**. Holliday (1971)** stated that, some of the basal cells contained immature mucous granules, which indicate their future development into goblet cells. In contrast, the intrapulmonary airways of the camel, and more especially the distal bronchioles, have effectively no basal cells. In this view, **Jeffery and Reid (1975**) described the single lining layer being concerned both with division and maturation. **Erjefalt et al. (1997)** mentioned that the basal cells play an important role in airway defense against sever insults as a barrier structure, where they promptly flattened out to cover the basement membrane at loss of neighbour columnar cells.

Smith et al. (1979) and **Plopper et al. (1980**) demonstrated in the rat bronchioles that the Clara cells projected their entire apical surface high above the surrounding ciliated cells. On the other hand, **Andrews (1974)** described the Clara cells in rat bronchioles as knobby-surfaced. The present study, however, showed both surface structures of Clara cells in the camel bronchioles. The various surface structures may possibly reflect differences in active secretory phases or cell maturation. Moreover, the distribution of these cells varies. **Jeffery and Reid (1975)** briefly noted that the cells were located proximally as far as the hilum of the lung. The present study, however, demonstrated that the Clara cells occurred distal to the furcations of the bronchi into bronchioles.

Clara (1937) suggested that the presence of this cell type was characteristic of the terminal bronchioles. In the present study Clara cells were found in airways as far proximally as the hilum. In other species cells with features of the Clara cell have been described as far centrally as the trachea **(Hansell and Moretti, 1969).** Whether it is the Clara cell or the type-II alveolar cell which contributes most to the surfactant lining of the lung is not yet established **(Meyrick and Reid, 1973).** If the Clara cell does contribute, it could do so at several airway levels.

The brush cell has not previously been described in the airways of domestic animals **(Pirie et al., 1990).** The brush cells found in the present study resemble those previously described in rat and pig airways **(Rhodin and Dalhamn, 1956; Luciano et al.,1968 ; Baskerville, 1970)** and in the alveoli of the rat **(Meyrick and Reid, 1968).** In agreement with the findings of **(Meyrick and Reid , 1968)**, the brush cell is a rare cell type in the lung as a whole. But in some locations it represents as much as 10% of epithelial cell

volume, and in others it covers up to 2% of the airway surface. Although the role of the respiratory brush cell is not understood, the presence of many pinocytotic vesicles at its luminal edge suggests an absorptive function. The densities of brush cells in the trachea and the bronchi found by the current study agree with previous observations**(Jeffery and Reid, 1975**).**Luciano et al., (1968)** suggested a chemoreceptor function for brush cells based on the observation of synaptic junctions between brush cells and afferent nerves.

The camel's respiratory epithelium showed also inter-epithelial migratory cells as lymphocytes, plasma cells and polymorphonuclear leukocytes. In addition to these cells, macrophages were observed within the respiratory epithelium of the human nose (**Busuttil et al., 1977**). On the other hand, only lymphocytes were seen in the respiratory epithelium of the larynx of donkey, goat and dog (**Abdel-Rahman, 1990**). **Busuttil et al. (1977)**stated that these cells may play an essential role in the formation of local IGA, IGE and in cell mediated immunological response, where the respiratory mucosa is of such importance in allergic and hypersensitive states, they may be the cells which trigger such immunological reactions.

The tracheobronchial system of the camel presented a basic histological morphology similar to that of other mammalian species. The intrapulmonary bronchi were densely carpeted with ciliated cells. Mucus secreting cells protruded between the cilia and was pronounced in the camel's bronchi. The same features were also observed in cattle (**Lovannitti et al., 1985)** and in non-human primates (**Wilson et al., 1984 ; Maina, 1988**). These surface mucus-secreting cells produce mixed mucosubstances. In the bronchi, they were relatively fewer in comparison to those seen in the upper respiratory tract as they showed a cranio-caudal decrease in the numbers. Similar observation in goat **(Kahwa and Purton, 1996),**in sheep (**Goco et al., 1963; Mariassay and Plopper, 1983**) and in cat (**Gallagher et al., 1975**) supported the present findings.

Although the ciliated cells were the most numerous cell types in the small bronchi, goblet cells became also evident. The latter were either flat or bulged into the lumen; others were discharging mucus and appeared similar to the mucus secreting cells described in the bronchi of rat (**Andrews, 1979 and1974)** and hamster (**Becci et al., 1978)**.

In the camel's bronchial tree, the mucosubstances produced by the surface mucus goblet cells exhibited an equal proportion of both acidic and neutral mucosubstances. Therefore the present finding is in agreement with observations made in ox (**Allan et al., 1977**), sheep (**Mariassay et al., 1988**), goat (**Kahwa and Purton, 1996)**, Rhesus monkey (**Plopper et al., 1984**) and in man (**Spicer et al., 1983**). The histochemistry of muco-substances in the tracheobronchial tree of the camel differs from that observed in other mammals such as sheep (**Mariassay et al., 1988**), pig (**Jones et al., 1975**) and ox (**Allan et al., 1977**) in which neutral mucosubstances were seen to predominate.

The histology of the camel's bronchioles, proximal to the terminal bronchiole, appear to be similar to that seen in mammals, including pig (**Baskerville, 1970**), horse (**Pirie, 1990**), dog (**Majid, 1986**) and man (**Ten Have-Opbroek et al., 1991**). In the bronchioles, ciliated cells gradually became less numerous and the non-ciliated cells became the predominant cell type in the terminal bronchioles. This is

in accordance with the findings of **Plopper (1983)** who stated that the ciliated cells form up to 75 per cent of the epithelial population of bronchioles in the horse. However, **Jeffery and Reid (1975)** reported 35%ciliated cells in the main bronchi in rat. This finding seems to correspond to the report by **(Andrews, 1979)** who mentioned that areas populated with few ciliated cells are occupied by goblet cells.

Although respiratory bronchioles are present in most mammalian species, they have been shown to be rudimentary in rat (**Massaro et al., 1984**) and in rabbit (**Plopper et al., 1983**), poorly developed in ruminants (**Getty, 1975**) including the ox (**Lovannitti et al., 1985**) and absent in horse (**Pirie, 1990**). The present study has shown that the respiratory bronchioles are prominent and well-developed. In camel, the terminal bronchioles were lined by ciliated low columnar or cuboidal cells and Clara cells. The respiratory bronchioles can be thought of as a continuation of a terminal bronchiole. Their lining cells became more cuboidal and the ciliated cells did become appreciably reduced in number especially in the smaller bronchioles.The presence of ciliated cells in the respiratory bronchioles has also been reported in pig (**Baskerville, 1970**) and in guinea pig (**Lechner and Banchero, 1982**), but not in dog (**Majid, 1986**), as well as in rhesus monkey (**Plopper et al., 1989**) and in goat (**Kahwa and Purton, 1996).**Submucosal glands were not observed in the bronchiolar tree of the camel. Very few goblet or surface mucus-producing cells were observed proximal to the terminal bronchioles. The secretory functional cell being the Clara cell which was in abundance at this level. The absence of goblet cells distal to the terminal bronchioles has previously been observed in ox (**Lovannitti et al., 1985**) in dog (**Majid, 1986**) and in horse (**Pirie, 1990**). But these cells have been seen in the Rhesus monkey (**Plopper et al., 1989**) and in human (**Ten Have-Opbroek et al., 1991**) where individual mucus-producing cells, producing acidic to neutral mucosubstances have been observed within the epithelial lining of the distal airways.

Conclusion

The basic histology of the tracheobronchial treeof the camel's respiratory tract was similar to that of other mammalian species. The mucosa of the trachea,extra & intrapulmonary bronchi consisted of pseudostratified columnar ciliated with goblet cells, a basement membrane, and a lamina propria. Several kinds of migratory cells were observed in the bronchial epithelium, e.g. lymphocytes, mast cell, eosinophil and plasma cell. The type of epithelium that lined the bronchiwas similar to that observed in the trachea, with only a reduction in the height of the epithelial lining being apparent.Four cell types were recognized. These were ciliated columnar cells, goblet cells, brush cells and basal cell. These cells of the pseudostratified mucosa rested on the basal lamina, but only the first three cells were exposed to the lumen.The structural features of these cells were described. The ciliated and basal cells were the most numerous cell types. The brush cells were seen more often in the proximal bronchi. Each cell appeared narrow columnar and lacking cilia. They had vacuolated dense cytoplasmand elongated or oval nucleus. As the caliber of the airway diminished, thelining columnar cells of thebronchi became shorter with the goblet cells tended to be sparse. The Clara cells within the epithelial lining became obvious and numerous. Histochemically, the goblet cells were described to be predominantly acidic in character as they

revealed strong alcianophilia. Neutral mucosubstances were rarely observed in these cells. The subepithelial glands were decreased with decreasing airway calibre. They produced predominantly mixed mucosubstances and were similar to that reported for other domestic mammals.With SEM, the mucosa of the trachea & bronchi had regular longitudinal folds with transverse furrows in between. The majority of the cells appeared as ciliated columnar carrying abundant long cilia. Goblet cells were seen bulging into the lumen, each with dome-shaped apical end.

All bronchioles had the same basic structure. The mucosa showed ascalloped appearance due to contraction of the smooth muscles that surrounding the bronchiolar wall. It was lined mainly by the usual ciliated columnar and basal cells. Brush cells were rarely found in the bronchiolar epithelium. The goblet cells were scarce or absent and were replaced by the Clara cells which were frequently encountered in the epithelial lining of the bronchiolar tree. as tall non-ciliated cells with apical dome-shaped protrusion. The larger bronchioles were lined with ciliated columnar cells, Clara cells and very rarely scattered brush cells. Whereas the smaller bronchioles were lined with ciliated low columnar or cuboidal cells and Clara cells. The cilia were found to decreasing in length, thickness and number distally. The brush cells were rarely found between the bronchiolar epithelium. Both the basal and the goblet cells were also scarce or absent in the bronchiolar epithelium. The terminal and respiratory bronchioles were lined by both ciliated and non-ciliated Clara cells. With SEM, the ciliated cells predominated in their proximal portions. More distally, the ciliated cells became less numerous and were replaced, in some areas, by the non-ciliated Clara cells. Towards the alveolar ducts, the lining cells were replaced by flat epithelium and the Clara cells disappeared. The bronchioles were easily identified with SEM, since they had a wall uninterrupted by alveoli.

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Annexure:

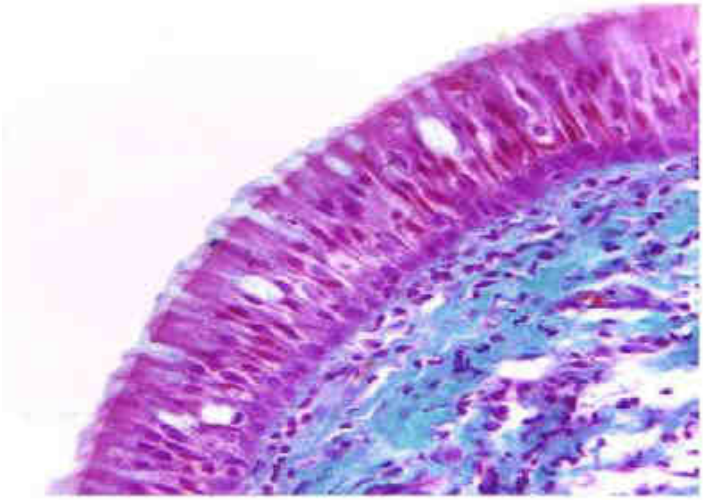

Fig.1a : The trachea is lined by a pseudostratified epithelium in which the ciliated and mucous cells predominate, Crossmon's trichrome stain, X 320.

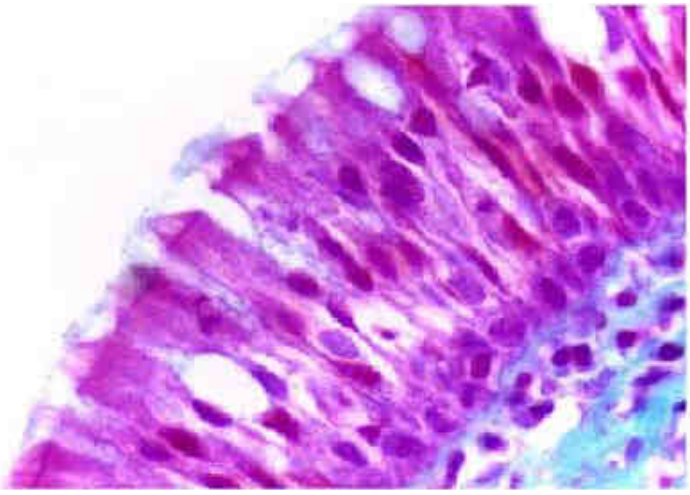

Fig.1b : High power view of (Fig.1a) Crossmon's trichrome stain, X 800

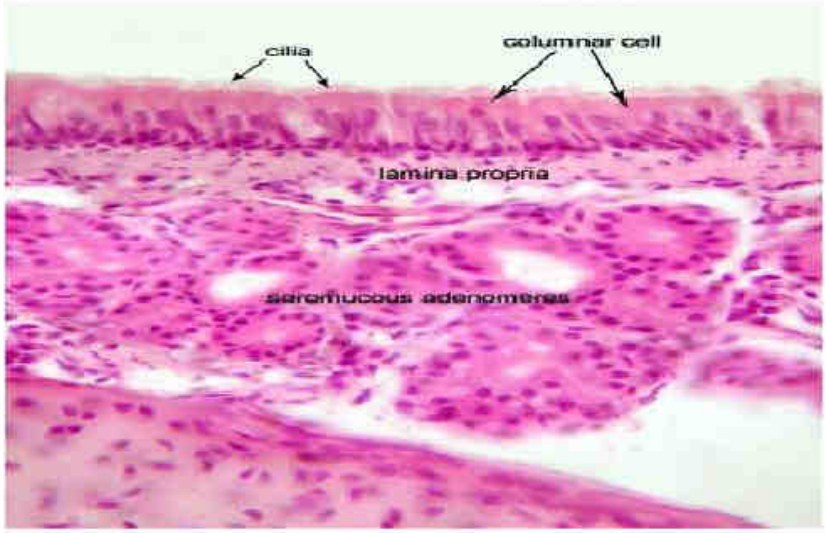

Fig.2 : The subepithelial loose areolar tissue housing numerous seromucoid glands of mostly serous nature, H&E stain, X320.

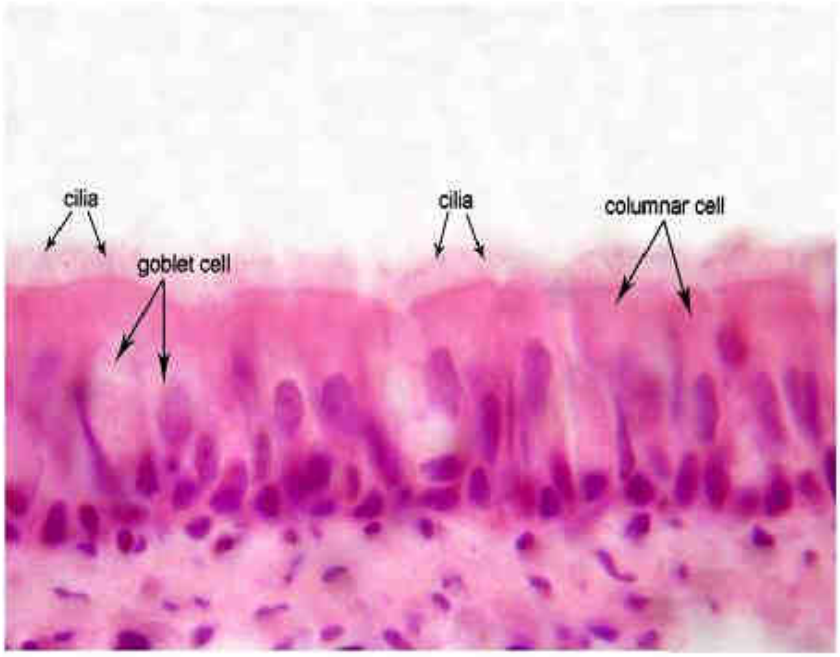

Fig.3 : The tracheal epithelium exihibits abundant ciliated cells. The goblet cells become less numerous particularly in the middle third the trachea. H&E stain, X 800

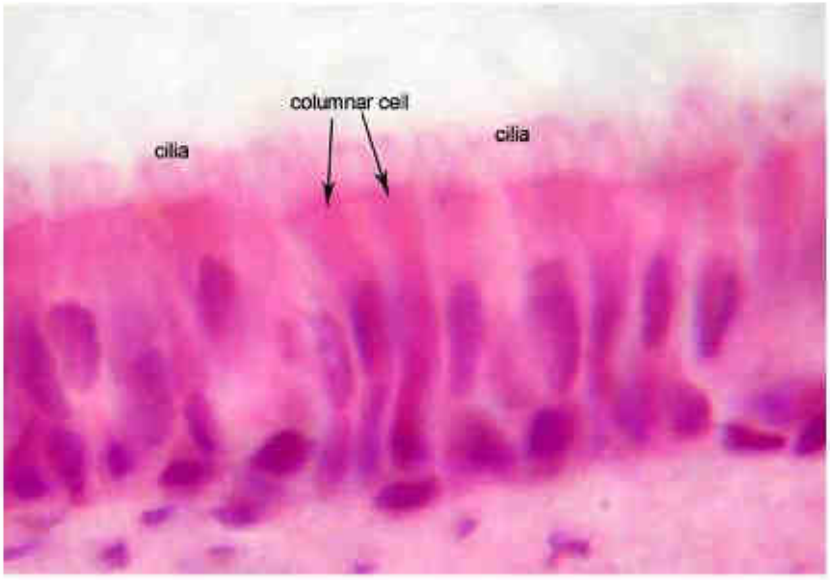

Fig.4 : The tracheal epithelium showing marked decrease in the number of the goblet cells, H&E stain, X 800.

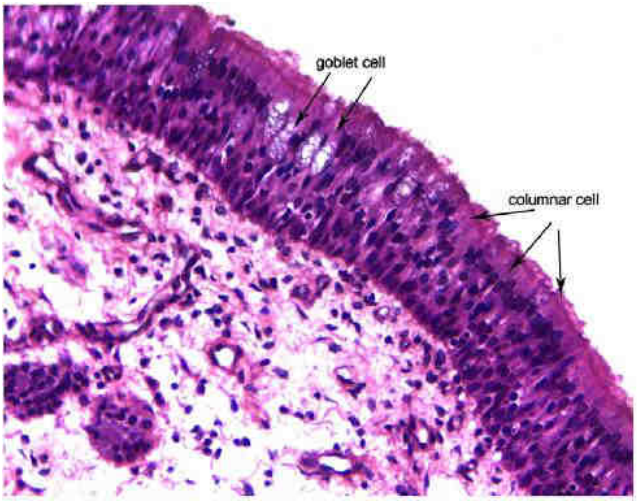

Fig.5 : Towards the lung, the tracheal epithelium showing very few goblet cells, H&E stain, X 800.

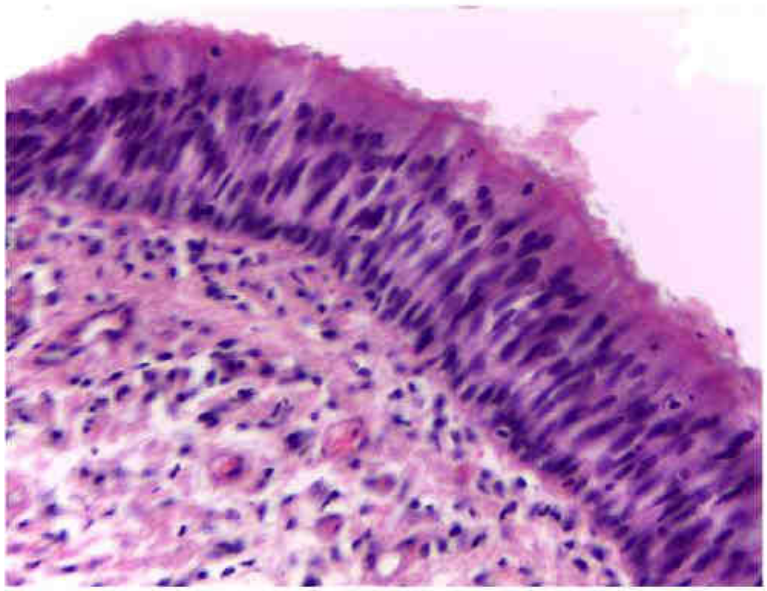

Fig.6 : The goblet cells are very few or absent in the bronchial epithelium. H&E stain, X 800

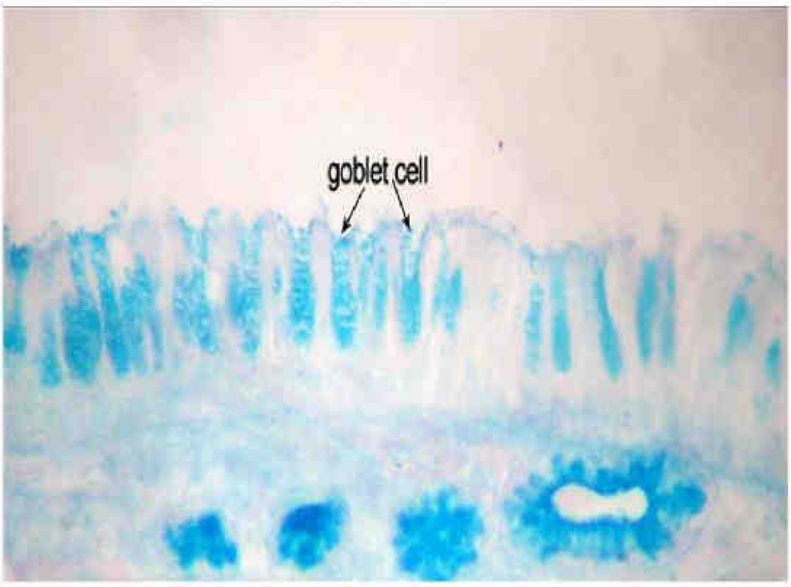

Fig.7 : Abundant goblet cells in the lining epithelium of the trachea. They react positively with the alcian blue. Alcian blue stain, X 320.

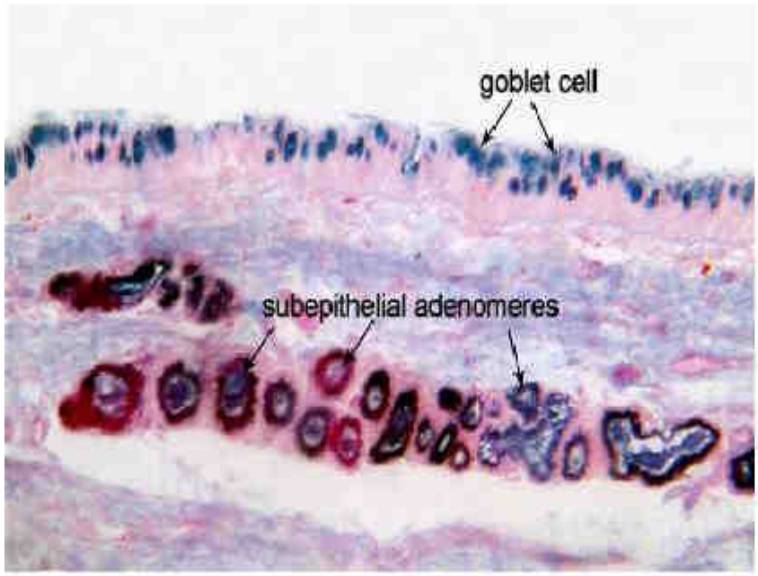

Fig.8 : Subepithelial seromucous adenomeres in the lamina propria of the trachea reacting positively with both alcian blue-PAS indicating their mixed contents of mucosubstance. The goblet cells react positively only with the alcian blue. Alcian blue-PAS combination, X 200

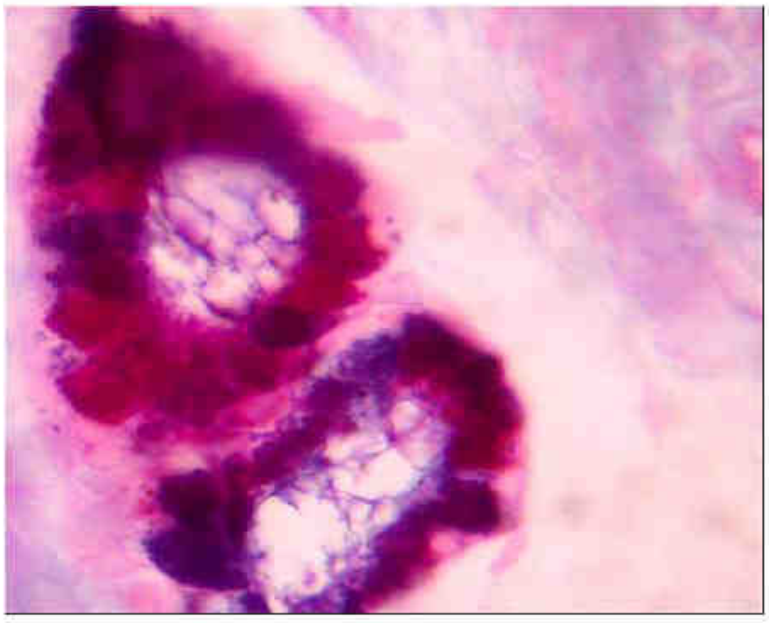

Fig.9 : High power view of (Fig. 8). Alcian blue-PAS combination, X 800

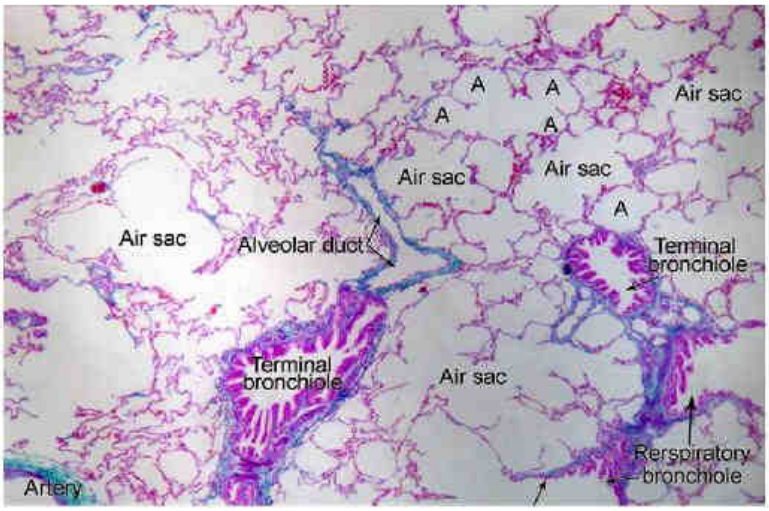

Fig.10 : The sequential intrapulmonary orders of airways, including terminal bronchioles and respiratory bronchioles. A: alveolus. Crossmon's trichrome stain, X 50

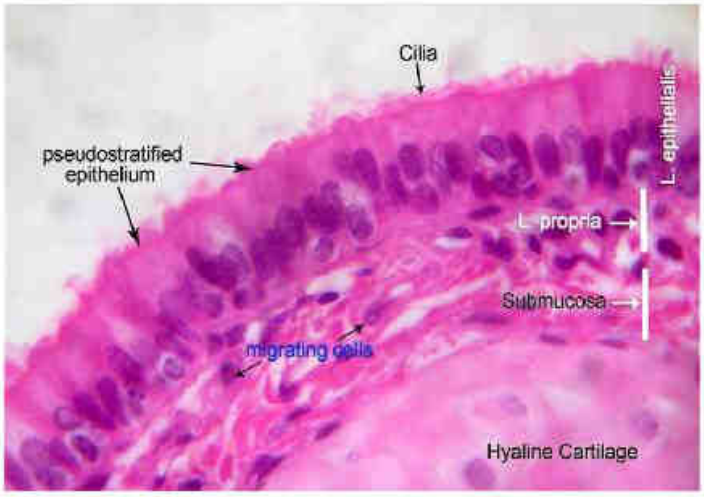

Fig.11 : The epithelial lining the bronchial tree is pseudostratified resting on a basal lamina. It is similar to that of the trachea with only a reduction in the height of the epithelium. H&E stain, X800

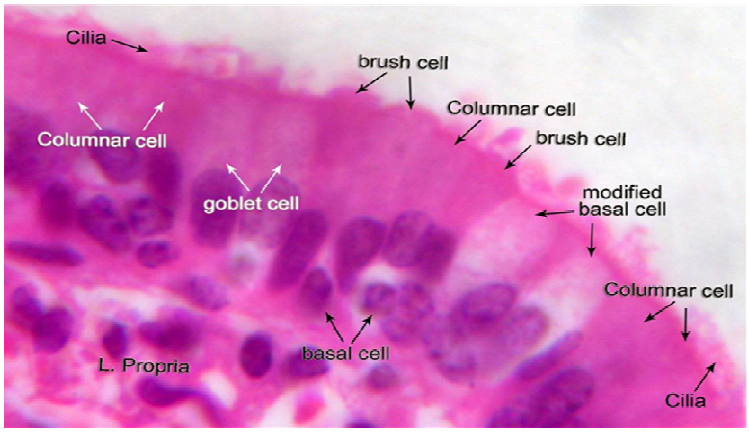

Fig.12 : The cytoplasm of the ciliated cells is somewhat densely stained. The nucleus appears large oval to ovoid in shape. Brush cells are distributed sparsely but rather uniformly. Each cell appeared narrow columnar in shape and lacking cilia. H&E stain, X800

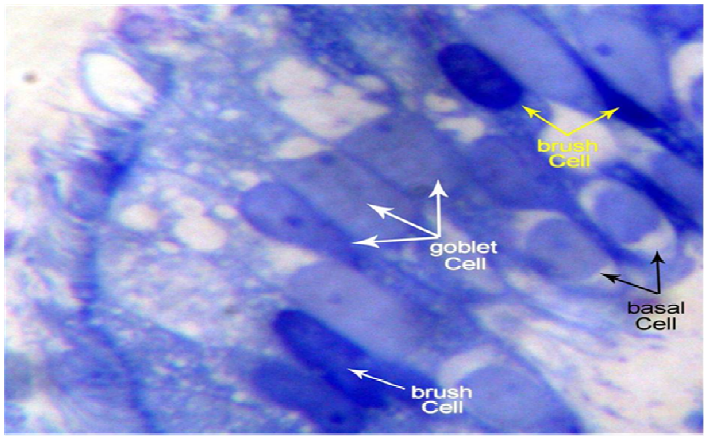

Fig.13: The goblet cells occur singly or in groups of 2 or 3 cells between the columnar cells. Their apical cytoplasm is faint and finely granular than that of the surrounding cells. Toluidin blue stain, X800

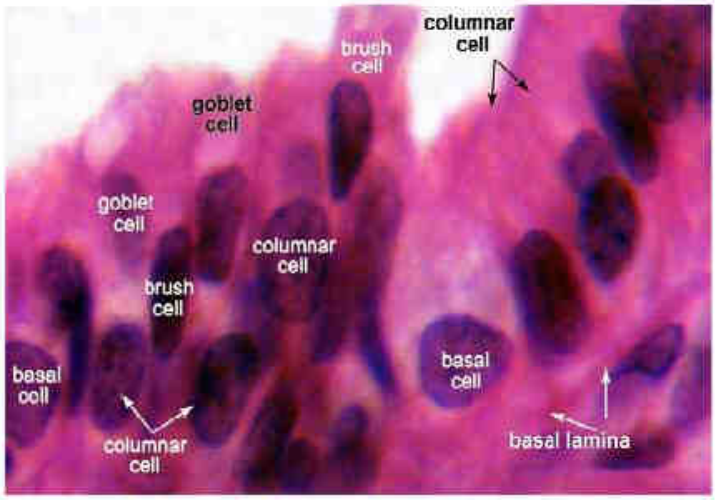

Fig.14: Some of the basal cells attains a larger size and become insinuated in-between the other lining cells to reach the luminal surface. Notice the brush and goblet cells. H&E stain, X800

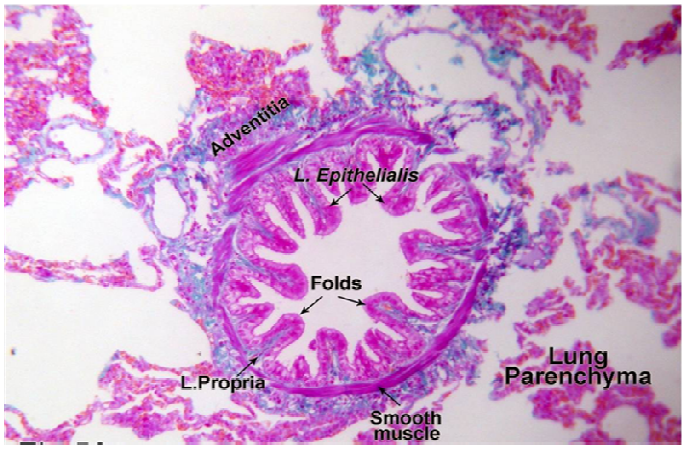

Fig.15 : Each bronchiole consists of mucosal lining, smooth muscles and fibrous tissue. They has no glands and cartilage support. Each has a diameter less than 1mm. Crossmon's trichrome stain, X 50

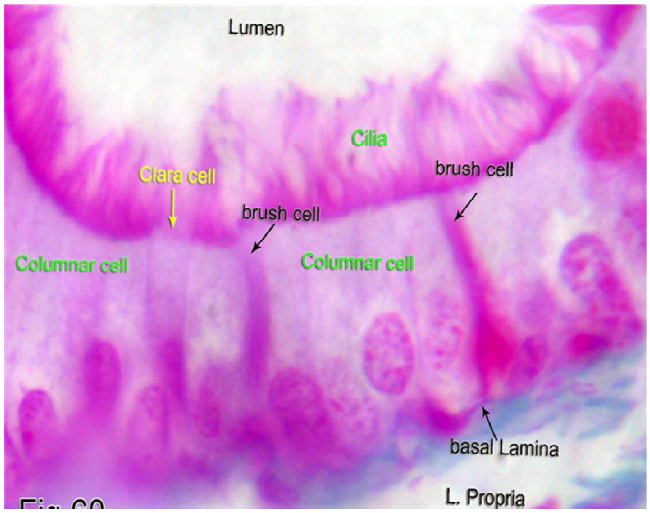

Fig.16 : The mucosa of the bronchiole is mainly formed of the usual ciliated columnar and basal cells. Brush cells are rarely found in the bronchiolar epithelium. Crossmon's trichrome stain, X800

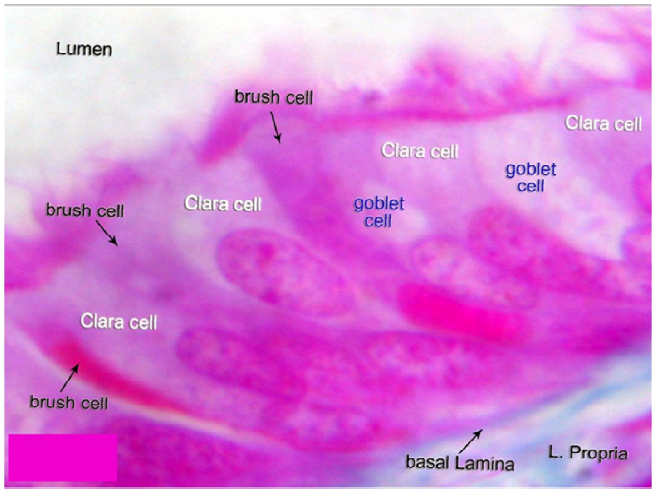

Fig.17 : Clara cells are frequently seen in the epithelial lining of the bronchiolar tree. Goblet and brush cells become rare.Crossmon's trichrome stain, X800

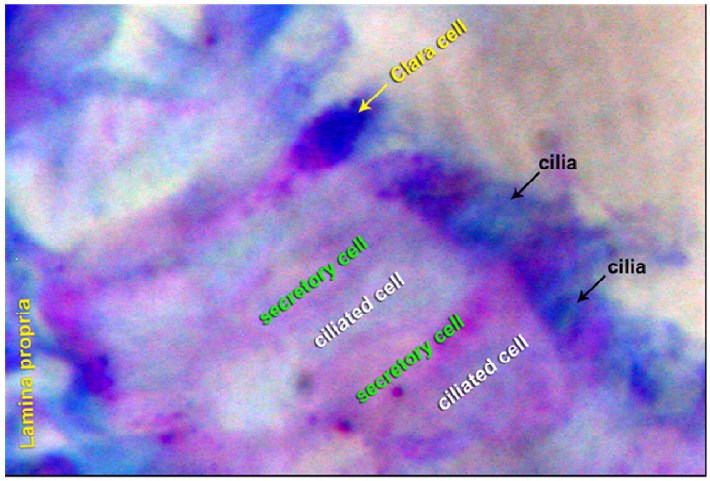

Fig.18 : Clara cells is characterized by its PAS-alcian blue positive staining. PAS-alcian blue combination, X800

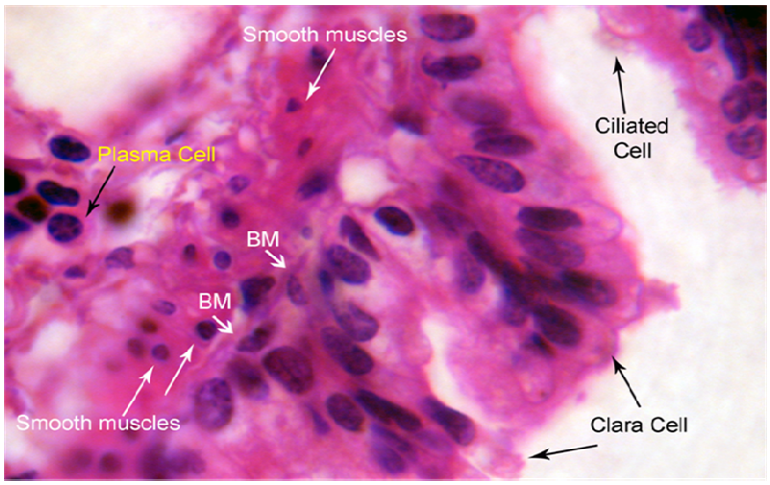

Fig.19 : Terminal bronchiole lined by both ciliated and non-ciliated Clara cells. The Clara cells are the predominant cell type. Both cells rest on a thin fibrous lamina Propria. BM : Basement Membrane. H&E stain. X800

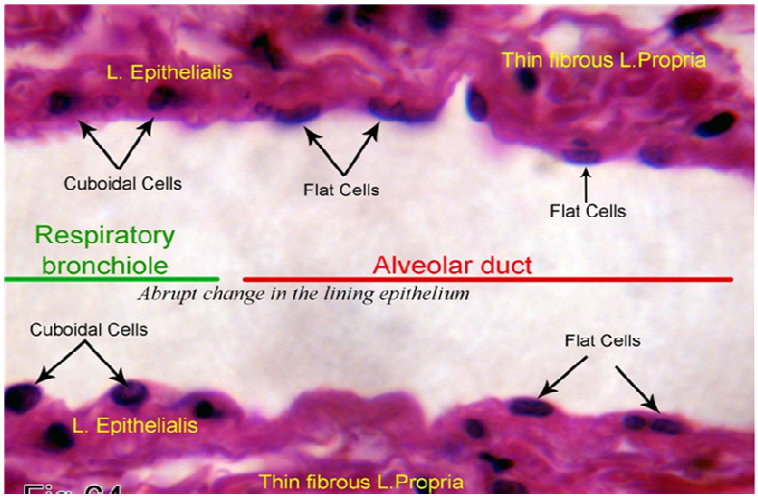

Fig.20 : Respiratory bronchiole lined by cuboidal cells which gradually become flattened towards the alveolar duct where the Clara cells disappeared. H&E stain, X800

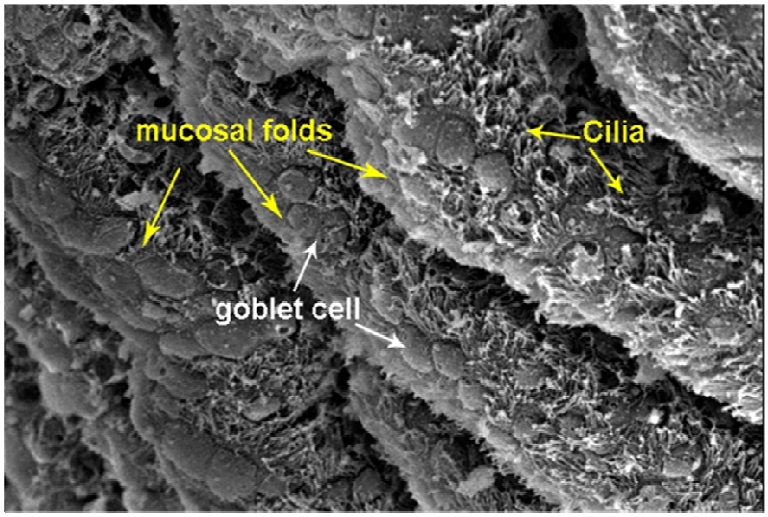

Fig. 21: The luminal surface of the trachea is thrown longitudinally into mucosal folds separated by shallow grooves. X 1.100

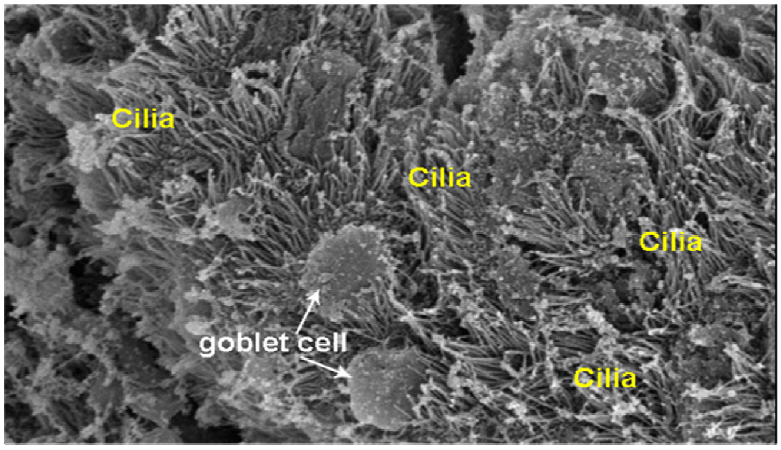

Fig. 22: At higher magnification, the tracheal mucosal surface is formed mainly of ciliated and goblet cells. X 2.300

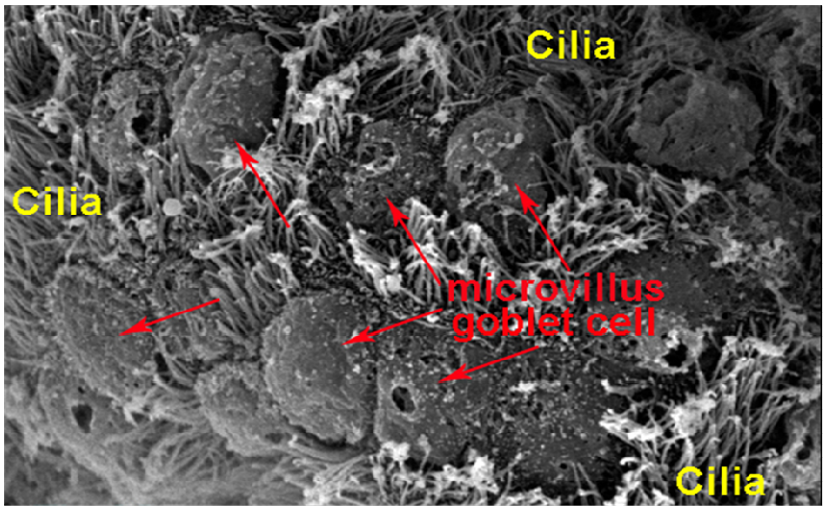

Fig. 23: Higher magnification of Fig.(90 and 91) to show ciliated and goblet cells forming the tracheal mucosal suface. X 3000

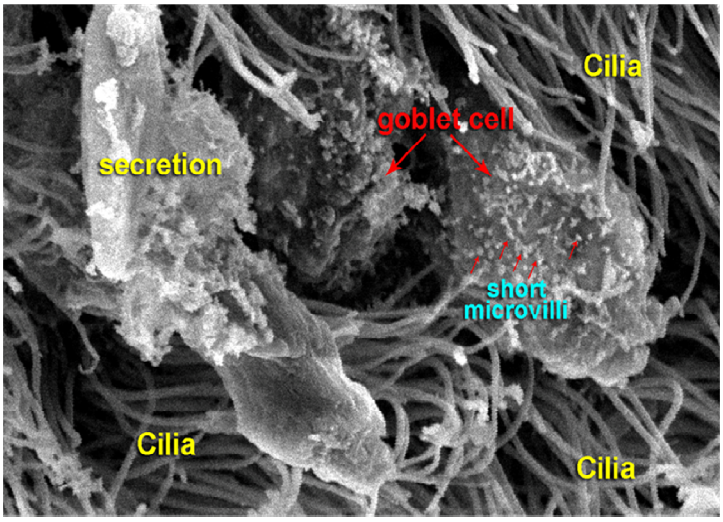

Fig. 24: Tracheal mucosal surface showing microvilli goblet cells among the ciliated cells. X 9.000

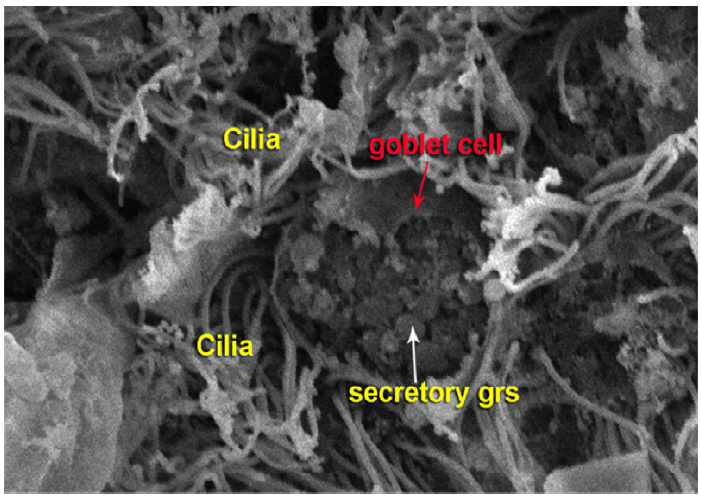

Fig. 25: Tracheal mucosal surface showing one of the goblet cells exposing their content of mucigenous granules. X 8.000

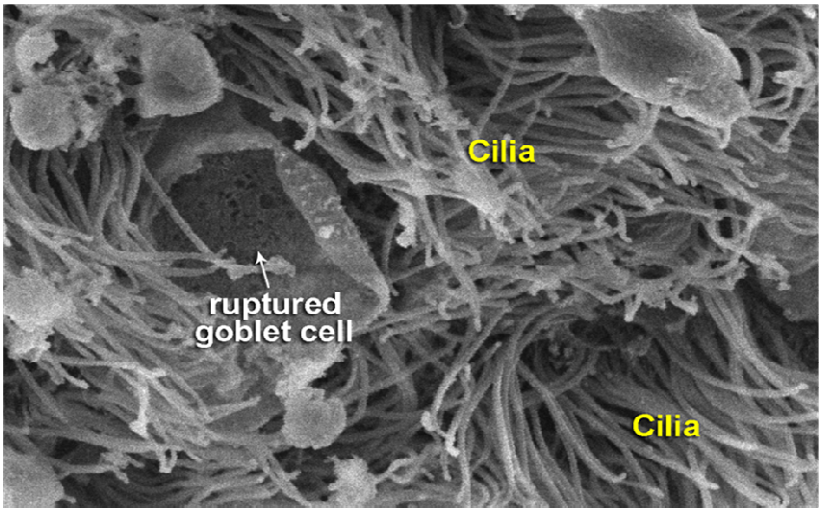

Fig. 26: Higher magnification of the tracheal surface showing single goblet cell with ruptured apical portion exposing its content of mucigenous granules. X7.500

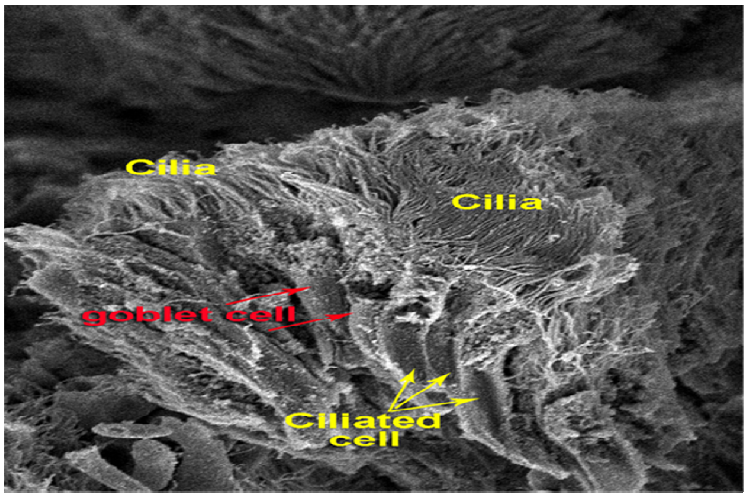

Fig. 27: Bronchial mucosa lined with numerous ciliated columnar cells carrying abundant long cilia. Goblet cells are also found. X 1.800

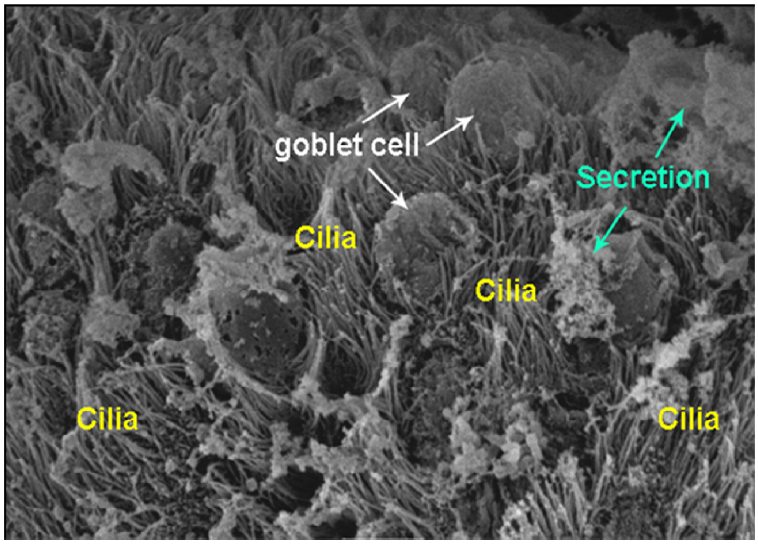

Fig. 28a : Surface view of the bronchiolar epithelium showing both ciliated and goblet cells. X 2.700

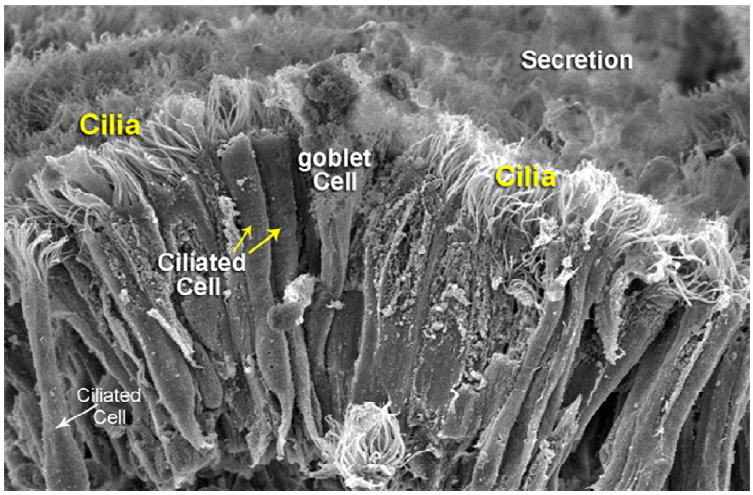

Fig. 28b: Side view of a bronchiolar epithelium. It is formed of flask-shaped goblet cells and tall ciliated cells. X 1.700

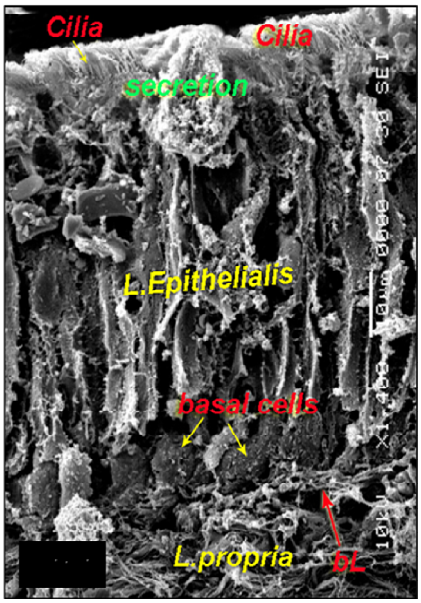

Fig. 29: The ciliated bronchial epithelium is resting on a basement membrane supported by fine collagen fibers. Notice the basal cells (arrows).

bl :basal lamina , X 1.400.

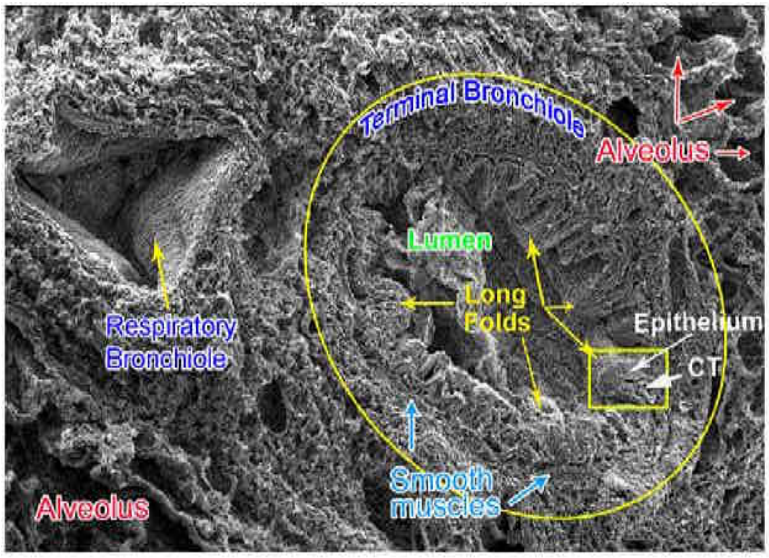

Fig. 30: Terminal bronchiole showing folded mucosa surrounded by smooth muscles. It is uninterrupted by alveoli and lacking cartilage and submucosal glands. X 140

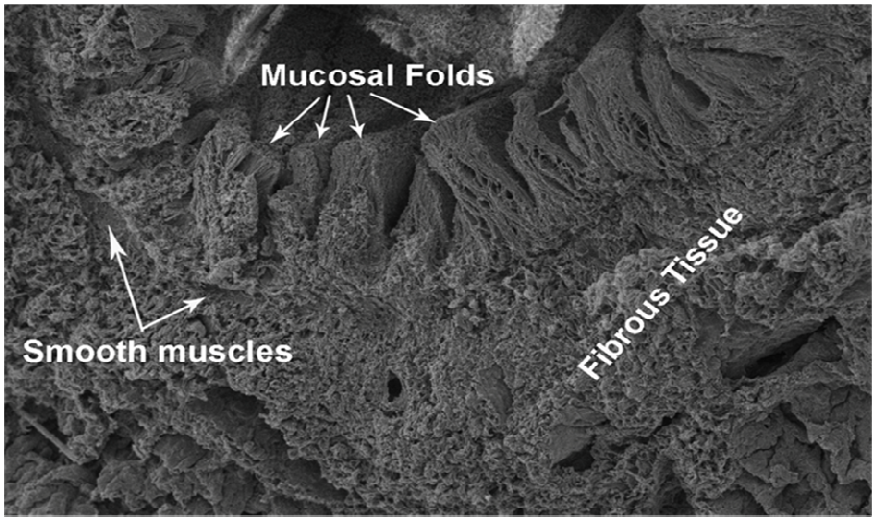

Fig. 31: Terminal bronchiole showing folded mucosa surrounded by smooth muscles and fibrous tissue. X 160

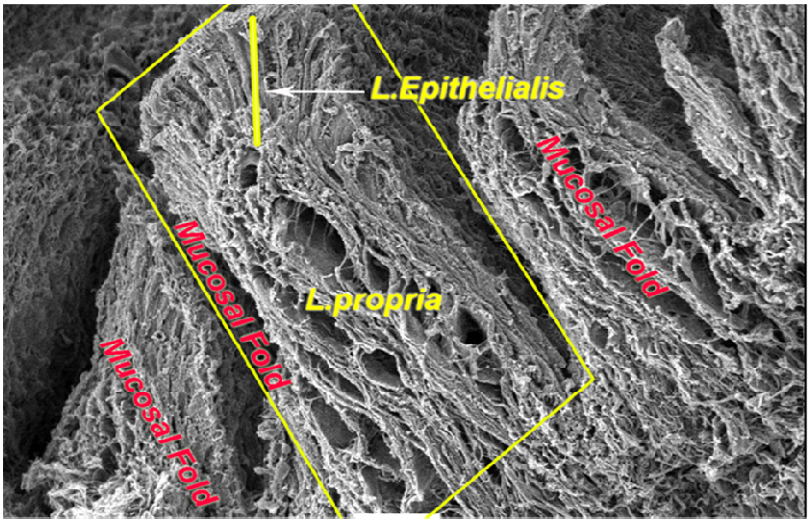

Fig. 32: Terminal bronchiole showing mucosal folds. Each fold consists of lamina epithelialis resting on a clear lamina propria. X 650

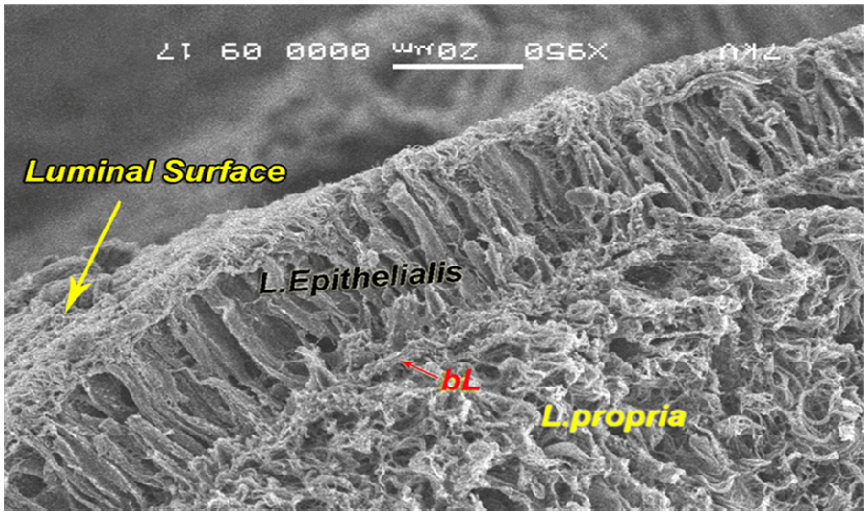

Fig. 33 : Bronchiolar mucosa formed of lamina epithelialis resting on a clear lamina propria. Basal lamina (bL) is present at the junction between the two laminae. The luminal surfaces carries numerous cilia. X 950

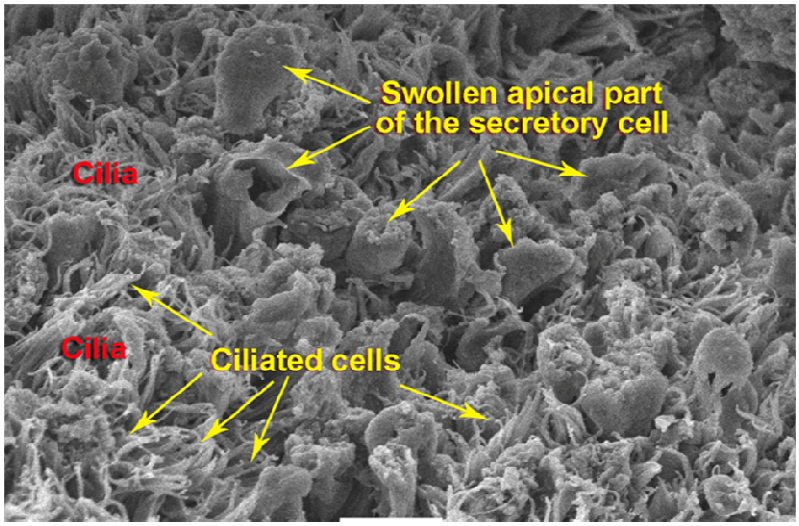

Fig. 34 : Bronchiolar epithelium showing swollen apical part of the secretory cells together with ciliated cells bearing cilia. X 3.300

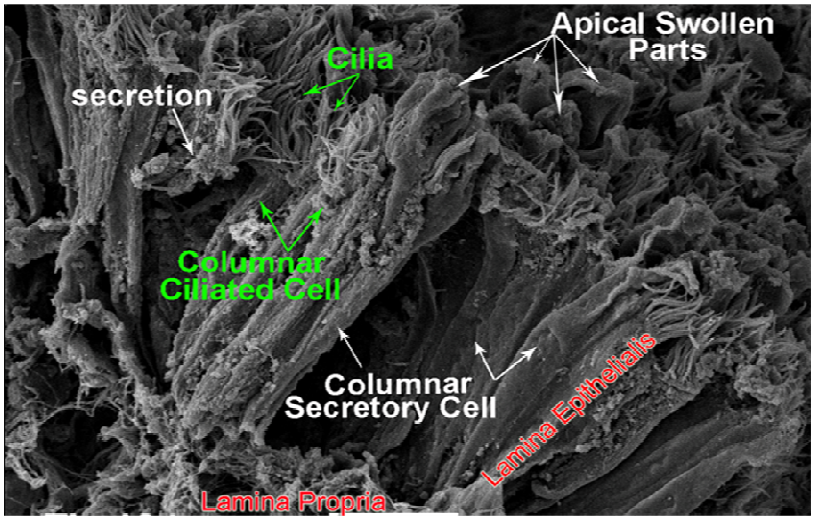

Fig. 35 : Bronchiolar epithelium showing lamina epithelialis resting on a clear lamina propria. The epithelium is formed of secretory cells and columnar ciliated cells carrying numerous cilia. X 2.000

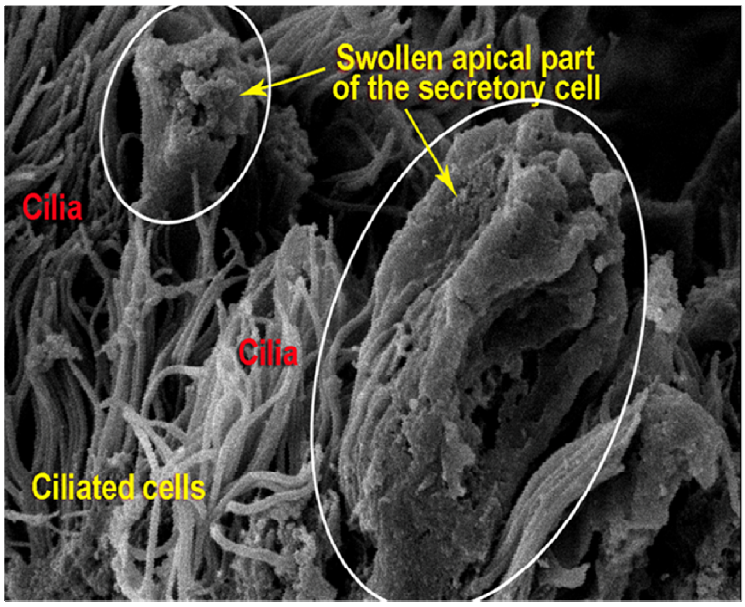

Fig.36 : Bronchiolar epithelium showing swollen apical part of the secretory cells. The ciliated cells showing long and uniform cilia. X 7.500

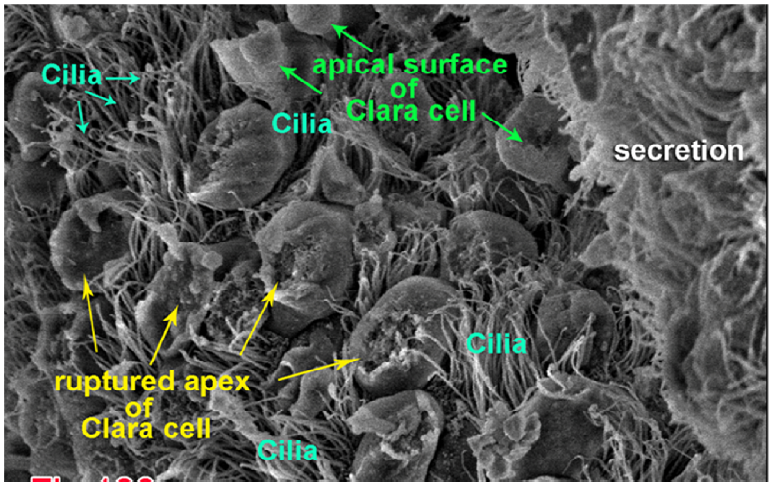

Fig. 37a : Apical surface of the terminal bronchiole epithelium showing swollen apical part of the Clara cells interspersed between the ciliated cells. Notice the secretion covering a portion of the surface. X 3.000

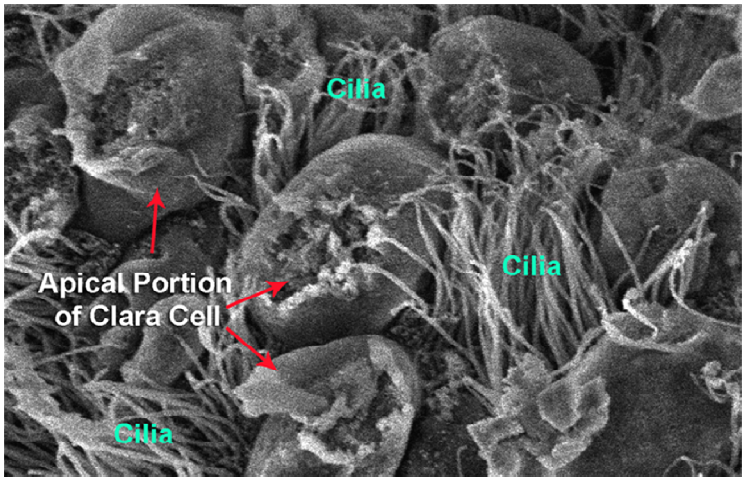

Fig. 37b : Apical surface of the terminal bronchiole showing that the number of Clara cells are nearly equal to that of the ciliated cells. X 6.000

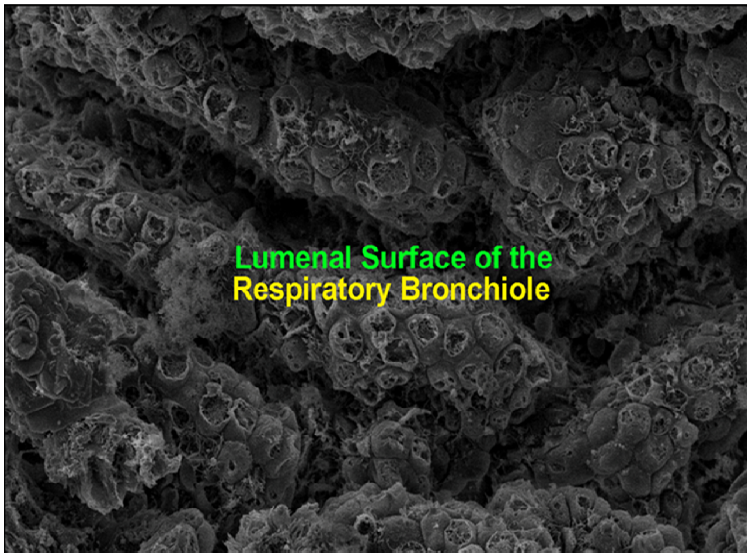

Fig. 38a : The luminal surface of the respiratory bronchiole. It appears to be mostly lined by the Clara cells. X 1.100

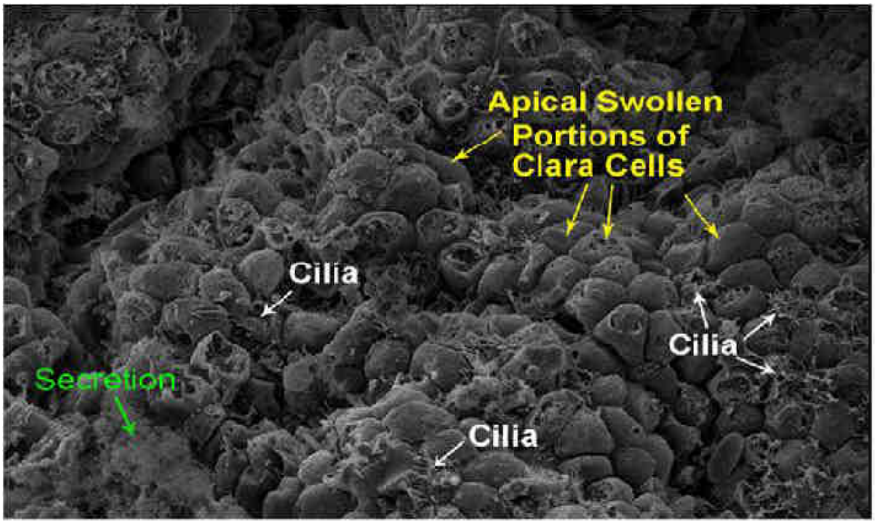

Fig. 38b : Apical surface of the respiratory bronchiole showing swollen apical part of the Clara cells. Only a few scattered ciliated cells are present in between. X 1.500

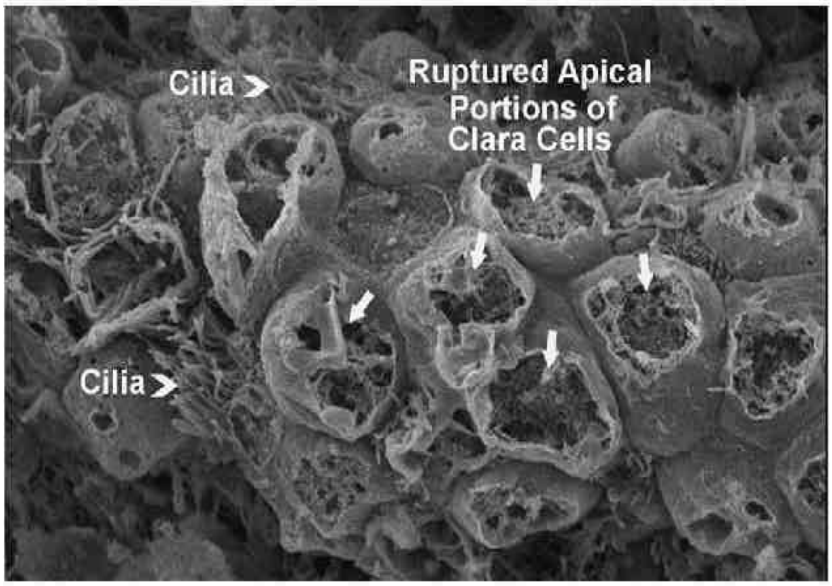

Fig. 39 : Apical surface of the respiratory bronchiole showing ruptured swollen apical parts of the Clara cells. Notice the few scattered ciliated cells in between. X 4.000