Different Cell Types In the Lower Respiratory Tract of the Reindeer (*Rangifer tarandus tarandus* L.) - A Transmission Electron Microscopical Study

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Abstract: The epithelium of the trachea and distal airways of 12 healthy adult reindeer were studied with transmission electron microscopy. The ultrastructure of the reindeer respiratory tract corresponded to the findings of previous investigators studying other mammalian species. The epithelium of the trachea and bronchi, down to the level of the distal bronchioli, was composed of three main types of cell: ciliated, goblet, and basal. In the distal brochioli, non-ciliated cells similar to those known as Clara cells were predominant. Numerous electron-dense granules and the cell organelle pattern resembled the Clara cell type observed in laboratory rodents, rabbit, sheep, pig, horse, and llama. Pneumocyte 1 and pneumocyte 2 cells were readily identified in the alveoli. The pneumocyte 2 cells possessed short microvilli and granules with lamellar content. Micropinocytotic vesicles were very numerous in the alveolar wall, and a small number of alveolar macrophages occasionally seen in the alveolar lumen.

Key words: Bronchial epithelium, pulmonary epithelium, ultrastructure, Clara cell, TEM, lung.

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Introduction

Morphological investigation of histopathological specimens is based upon knowledge of normal cellular populations, upon characteristics of the normal architecture of the tissue, and upon certain changes and variation within these "normal" findings (Kayser, 1992). Since 1956, when Rhodin and Dalhamn published their study on electron microscopical findings on the respiratory tract of the rat, the ultrastructure of the respiratory epithelium has been described for a number of other animal species, with the emphasis on labotatory and common domestic animals. Special attention has been focused upon the horse as an important sports animal suffering from various respiratory disorders (Tyler *et al.*, 1971; Pirie *et al.*, 1990, Buechner-Maxwell, 1993). Among the ruminants, the ultrastructural features of the caprine (Atwal & Sweeny, 1971) and the bovine (Epling, 1964; Weekley & Veit, 1995) respiratory tract have been described. Detailed information concerning the ultrastructure of the respiratory tract of reindeer is, however, unavailable. Involving an investigation of surface characteristics and ultrastructure, the focus of the present work was the respiratory epithelium of the normal trachea and lower respiratory tract, with the main purpose being to obtain basic information for comparison in investigations of respiratory organs altered by lung-worm infection.

Material and methods

Tracheal and lung specimens came from 12 adult reindeer of both sexes slaughtered in winter in the southern part of Finnish Lapland. The animals selected for the purpose showed neither clinical signs of disease nor respiratory tract abnormalities in macroscopic and microscopic post-mortem examination.

Tissue samples came from the following sites (Fig. 1):

L1: Ventral trachea halfway between the larynx and tracheal bifurcation
L2: Tracheal bifurcation
Left caudal lobe:
L3: Large bronchus
L4: Small bronchus
L5: Terminal bronchiole
L6: Alveolar region

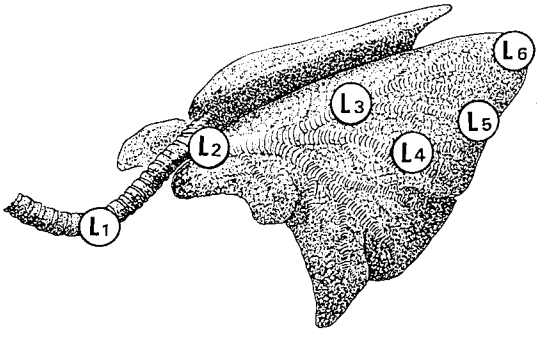

Fig.1. Sample sites L1 - L6 in the respiratory rract of the reindeer.

Samples from sites L1 and L2 were fixed in a solution containing 2.5% paraformaldehyde and 0.1% glutaraldehyde. After removal of samples L1 and L2, the left bronchus was cannulated and the lung fixed by passing a thin (Ø 1.2 mm) silicon catheter into the distal airways. The fixative was injected through this tube until the lobe was filled. Samples L3 to L6 were then taken. The samples were left immersed in the same fixative for 24 hours and then changed to a 0.1 M Sörensen phosphate buffer, pH 7.3.

The samples were trimmed into smaller pieces (approximately 1 mm^3), post-fixed in 1% osmium tetroxide, and embedded in epoxy resin. For light microscopic studies and identification of the epithelium in the blocks, sections cut at 1 µm were stained with 1% toluidine blue. Ultrathin sections were cut on the basis of light microscopical findings with a diamond knife, placed on grids, stained with utanyl acetate and lead citrate and viewed with a Jeol JEM 100 S ttansmission electron microscope.

Results

The ciliated columnar epithelial cell (Figs. 2 and 3) was clearly the ptedominant cell type in the trachea as well as in the bronchi down to the level of the terminal bronchioli (L1 - L4). Despite the tendency towards a cuboidal shape in its progression towards distal parts of the airways, only a slight ultrastructural variation appeared within these cells, and thus they were easily identified at all levels. The free surface of these cells was covered with cilia (Fig. 4) having very uniform length of about 6 µm and a width of 0.2 - 0.3 µm. Thin, short and sometimes branching microvilli were visible between the cilia. The cilia contained electron-dense longitudinally oriented tubules, and the arrangements of nine outer doublet tubules and two central single tubules of cilia could be seen in transverse sections (Fig. 5). The basal bodies of the cilia occupied the epithelial apex together with mitochondria, which were very numerous in this part of the cell. Other mitochondria-rich zones in this cell type wete the basal, subnuclear parts of the cells as well as the perinuclear zones. The oval nucleus of the cell was situated slightly basally, with the chromatin partly condensed along the nuclear membrane. The Golgi complex was usually clearly seen, closely associated with the nucleus. The cytoplasm was, throughout, loosely packed and of low electron-density, and contained scattered ribosomes sometimes arranged in little clusters. The endoplasmic reticulum seemed to be poorly developed.

The goblet cells lining the trachea were widely dispersed and usually surrounded by ciliated cells. The surface of the goblet cells was covered by microvillous projections (Fig. 6), if the cells were in a non-secretory phase. The cells showed varying amounts of membrane-bounded mucus granules, especially in the cell apex. Often the apical part of the cell was so densely packed with globular secretory granules that the upper portion bulged deeply towards the respiratory tract lumen, and no microvilli could be seen on their surface. The globules contained material that appeared finely granular, material more loosely packed at the periphery, and very dense in the central part of the globules. These

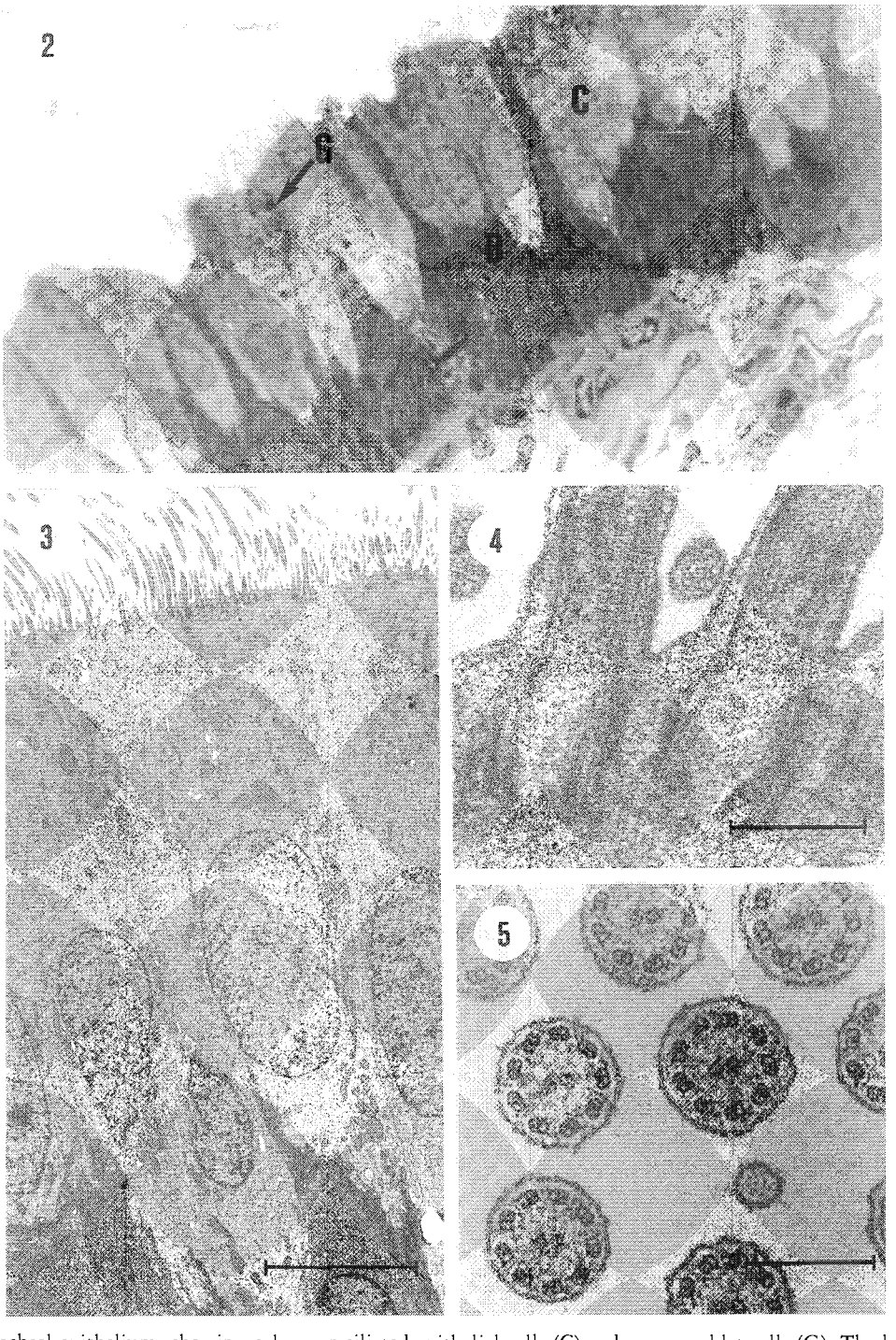

Fig. 2. Tracheal epithelium showing columnar ciliated epithelial cells (C) and sparse goblet cells (G). The basal cell layer (B) has stained more intensively. Toluidine blue, semithin section, magnification 2500x.

Fig. 3. Tracheal epithelium with ciliated epithelial cells predominant, with a large amount of mitochondria in the apical region. Beneath the ciliated cells some basal cells are visible. Bar = 3 μm.

Fig. 4. Apical region of a ciliated epithelial cell showing basal bodies and longitudinal sections of cilia. Bar = 0.3 μm.

Fig. 5. Details of ciliary ultrastructure seen in transverse section with typical arrangements of nine outer doublet tubules and two central single tubules. Bar = 0.3 μm.

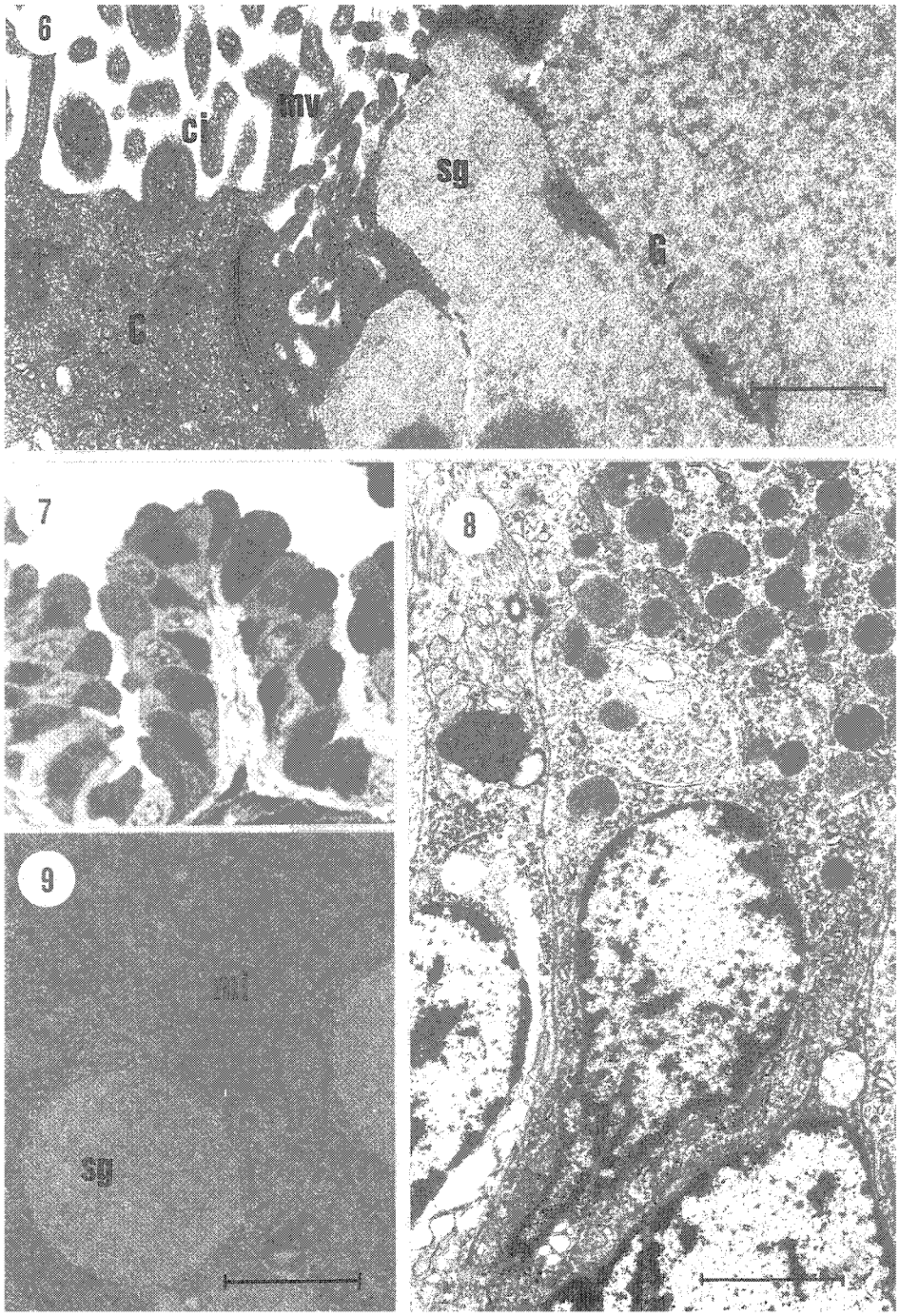

Fig. 6. Apical region of ciliated cell (C) and goblet cell (G). Numerous microvilli (mv), and cilia (ci) visible in the ciliated cell. Microvillous projections and granular appearance of secretory globules (sg) of the goblet cell also visible. Bar = 0.5 μm.

Fig. 7. Epithelium from terminal bronchiole covered by Clara cells. Toluidine blue, semi-thin section, magnification 950x.

Fig. 8. Clara cell from a terminal bronchiole showing typical homogenic secretory granules. Bar = 2 μm.

Fig. 9. Detail from a Clara cell showing secretory granules (sg), numerous mitochonria (mi), and intensively black-stained ribosomes. Bar = 0.3 μm.

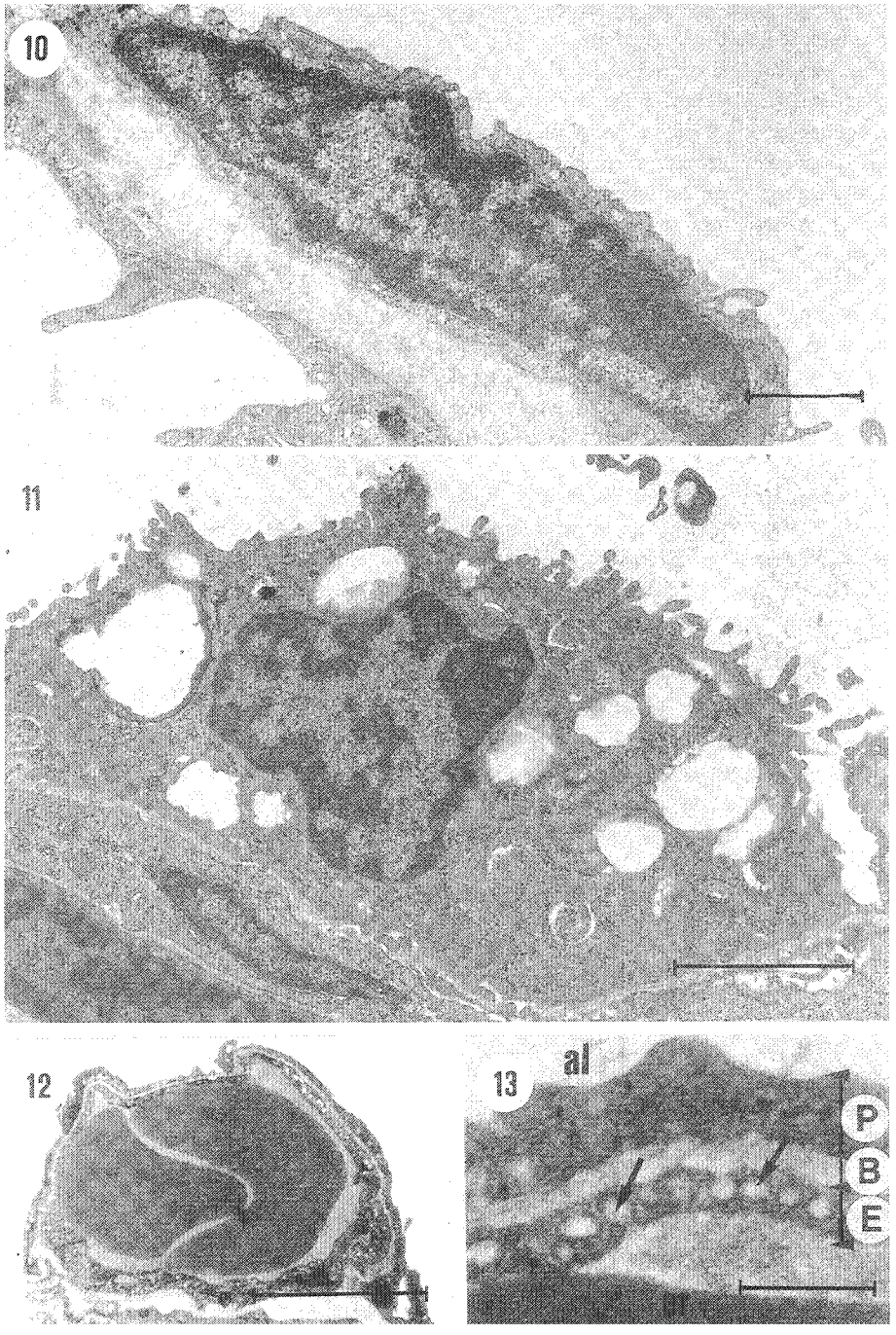

Fig. 10. Alveolar epithelial cell of type 1. Bar = 1 µm.

Fig. 11. Alveolar epithelial cell of type 2 showing numerous microvillous projections and secretory granules. Bar = 3 µm.

Fig. 12. Part of the septal wall showing the alveolar capillary complex. Two erythrocytes visible in the capillary lumen. Bar = 2 µm.

Fig. 13. Detail from an alveolar capillary complex. The upper layer consists of the cytoplasmic extension of the type 1 alveolar epithelial cell (P), the middle layer consists of the basal layer (B), and the inner layer from the endothelial cell (E). Numerous pinocytic vesicles (arrows) as well as part of an erythrocyte (er) are visible as well (al = alveolar lumen). Bar = 0.3 µm.

densities were not seen in the secretory granules that had already reached the cell surface (Fig. 6). The nucleus was situated more basally than in the ciliated cells, and also in these cells the chromatin showed a tendency to condense along the nuclear membrane. The Golgi complex was situated supranuclearly, and there was an abundant peri- and subnuclear rough endoplasmic reticulum as well as numerous mitochondria.

Beneath the ciliated and goblet cells was a line of triangular basaloid epithelial cells along the basal membrane (Figs. 2 and 3), their apices directed towards the respiratory lumen; they had an electron-dense cytoplasm. The nuclei of these cells were round or oval and filled the larger part of the cellular space.

In the terminal membranous bronchioles (level L5), another cell type became the most predominant. Although ciliated cells could also be found in this region, most of the cells represented a non-ciliated cell type (Figs. 7, 8, and 9). Each of these epithelial cells had an oval nucleus and a prominent Golgi zone. The cytoplasm of the cells was more electron dense than that of the ciliated epithelial cells. The apical part of the cytoplasm contained round, homogenous, electron-dense granules, numerous mitochondria and a rough endoplasmic reticulum. These non-ciliated cells are referred to as Clara cells.

The alveolar wall was covered by a very thin epithelial-cell component: alveolar epithelial cells of type 1 (Fig. 10). These extremely flat and elongated cells, by their cytoplamic processes covered about 90% of the alveolar surface. Their configuration meant that only a few cell organelles were visible in addition to the nucleus.

Solitary cells with microvilli protruded into the alveolar lumen (Fig. 11). These cells, alveolar epithelial cells of type 2, had large intracytoplasmic granules that contained loosely packed lamellar or amorphous material; in many of the cells these granules were empty. Ribosomes and rough endoplasmic reticulum were scattered throughout the cytoplasm. Pneumocyte 2 had a centrally located nuclei, usually indented by the intracytoplasmic granules, and the chromatin was condensed along the nuclear membrane. Comparison of the number of the nuclei detected of pneumocyte 2 type to the number of nuclei of pneumocyte 1 showed these cell types apparently to be equally numerous.

Capillaries were very numerous in the alveolar wall and were easily recognized because of electron-dense erythrocytes in the capillary lumen (Fig 12). The alveolar-capillary junction contained three distinct layers (Fig. 13), the cytoplasmic extensions of pneumocyte 1 forming the internal layer. Mitochondria, tubular and vesicular structures of the smooth endoplasmic reticulum, and small vesicular structures were the ultrastructural details detectable in this layer. The inner layer was formed by the capillary endothelium, with its the most prominent ultrastructural feature being numerous uniform vesicular structures, more numerous in the endothelium than in the alveolar epithelium. Between these two layers was a basal lamina that consisted mostly of delicate fibres. Collagen fibres were apparent in the thick portion of the alveolar septa.

Although the air spaces were usually empty, some alveoli contained round cells not connected to the alveolar walls. These cells, with their numerous microvillous projections and cytoplasmic organelles and inclusions, were considered alveolar macrophages.

Discussion

In collecting lung samples, two different fixative methods may be successful. This study used the conventional method with the lung fixed by installation of fixative into the airways. This method may, however, cause slight artefacts on the airway surface, because by this technique the extracellular components (*e.g.* alveolar macrophages) may be flushed away. To preserve the surface of the lung intact, fixation should be performed by vascular perfusion. Perfusion fixation is a suitable fixation method especially for smaller laboratory animals. Because the main interest of our study was the epithelial structures, fixation via the airways was considered satisfactory.

The tracheal surface as well as the bronchial and even bronchiolar surface was covered by a thick layer of ciliated columnar epithelial cells. Functionally the most important part of these cells is their apical portion, where numerous mitochondria, microvillous projections and particularly the cilia, thin longitudinal extensions from the free surface of a cell, are located. Similar ciliar projections have been found not only in the respiratory tract, but also in the mammalian auditory tubes, brain ventricles, spinal canal, oviducts and efferent ducts of the testes (Afzelius, 1986). The ciliary ultrastructure with

nine outer microtubule doublets surrounding two single tubules (9 + 2 pattern) is universal in the animal kingdom (Afzelius, 1986; Mehlhorn *et al*, 1988). The ciliary beat is controlled by adenosine-triphasphate (ATP), supplied by numerous apically located mitochondria. The coordinated beating of adjacent cilia moves the mucous blanket and particles trapped in it towards the pharynx (Sleigh *et al.*, 1988 ; Edwards *et al.*, 1992).

Goblet cells were interspersed among the ciliated cells. They produce a mucous secretion, which is added to the secretions of the submucosal gland and protects the epithelium by lubricating, humidifying, waterproofing, insulating, and providing an appropriate condition for normal muco-ciliary clearance. In addition, the mucus provides a barrier against certain macromolecules and micro-organisms, and serves as a suitable milieu for immunoglobulins and for enzyme activities (Buechner-Maxwell, 1993; Kaliner *et al.*, 1984). Previous studies have shown that the secretory granules of the goblet cells of the reindeer contain neutral mucopolysaccarides (Rahko *et al.*, 1992).

Basaloid epithelial cells beneath the ciliated and goblet cells are called basal cells; these cells form a reserve cell population and are capable of differentiating into either ciliated epithelial cells or goblet cells if needed (Ross & Romrell, 1989).

The distal airways before the alveolar region are dominated by Clara cells. Certain variations have been observed in the morphology of the Clara cell between different mammalian species (Ploppet, 1983), and this study revealed that the ultrastructure of the Clara cell of the reindeer with its numerous electron dense granules and the architecture of its cell organelles resembled the Clara cell type in mouse, hamster, rat, guinea pig, rabbit, sheep, pig, horse, and llama (Plopper, 1983). Typical morphological features of Clara cells of these species are apically located smooth endoplasmic reticulum and basally located rough endoplasmic reticulum. Glycogen as granular aggregates, and numerous mitochondria, present throughout the cell, are characteristic as well. The cytoplasm of the canine, feline, and bovine Clara cells is mostly filled with glycogen. In addition, endoplasmic reticulum and mitochondria are present but are significantly less abundant than in laboratory rodents, rabbit, pig, sheep, horse, and llama (Plopper, 1983). The secretion of the Clara cells is voided into the bronchiolar lumen, and it coats the surface of bronchioles. Clara cells also have P-450 monooxygenase activity and are thus capable of metabolizing xenobiotic agents; in addition, the Clata cells are consideted the progenitors for new Clara cells and ciliated epithelial cells (Plopper, 1983; Buechner-Maxwell, 1993).

Pneumocyte 1 and pneumocyte 2 were readily identified, and theit ultrastructure was in accordance with that previously described for other mammalian species. In the literature, pneumocyte 1 is given several other names, *e.g.* alveolar type I cell, squamous pulmonary epithelial cell, and small alveolar cell. Its extremely flattened cytoplasm makes the diffusion of gases between capillaries and alveolar space possible. Gas exchange is facilitated by microvesicular structures, often referred to as micropinocytotic vesicles; these vesicles were very numerous in the alveolar wall of the reindeer. The predominance of micropinocytotic vesicles in the cytoplasm of pneumocyte 1 has been described in the lung of the goat as well (Atwal *et al.*, 1971). Similarly, pneumocyte 2 is referred to by several different names: alveolar type II cell, granular pneumocyte, and large alveolar cell. The identification of this cell type is based on osmiophilic lamellar granules, granules thought to represent the precursors of pulmonary surfactant. Besides the role of secreting surfactant, pneumocyte 2 also serves as a reserve cell in case of alveolar damage, where the usual finding is hyperplasia of pneumocyte 2 (Wang, 1994).

Alveolar macrophages are considered to be derived from circulating macrophages that have migrated through the capillary wall and the alveolar epithelium into the air space (Kayser, 1992). The usual site of the macrophages in the lung is the alveolar connective tissue, but in addition, the presence of intravascular pulmonary macrophages has been demonstrated in various mammalian species, as well as in the reindeer (Staub *et al.*, 1992). Macrophages have an important tole in defending the airways at the alveolar level. These powerful cells have a capacity to phagocytize, and to produce bacteriocidal enzymes and interferon (Dungworth, 1993; Weekly & Veit, 1995).

Epithelial cells having the appearance of so-called brush cells could not be found in this study, although they have been recognized for example in the bovine lung (Allan, 1978). These cells appear to have an absorptive function in the respiratory tract (Reynolds, 1991).

These findings on the lower respiratory tract morphology of the teindeer indicate, that the cellular populations and their ultrastructural characteristics are similar to those of other mammalian species.

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