To the Graduate Council:

I am submitting herewith a dissertation written by Mohammed S. Al-Tikriti entitled "Pre- and Post-Partum Growth of Feline Lung: Light, Scanning and Transmission Electron Microscopic and Morphometric Studies." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

Robert W. Henry, Major Professor

We have read this dissertation
and recommend its acceptance:

Accepted for the Council:

Vice Provost
and Dean of The Graduate School

PRE- AND POST-PARTUM GROWTH OF FELINE LUNG: LIGHT, SCANNING AND TRANSMISSION ELECTRON MICROSCOPIC AND MORPHOMETRIC STUDIES

A Dissertation

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ABSTRACT

Development of the lung in prenatal, postnatal, and adult cats was observed. After fixation by intratracheal instillation of 3% glutaraldehyde in phosphate buffer at a pressure of 20 cm H_2O, tissue samples were processed for light, SEM and TEM. The epithelial lining of the tracheobronchial tree (TBT) was pseudostratified ciliated with goblet cells. Ciliated, goblet, microvillus-rich, intermediate, nonciliated bronchiolar (NBC), and basal cells were present in growing cats. The serous cell was observed only in the adult. Epithelial thickness increased significantly with age (r^2=0.518, P<0.0025) but not linearly (r^2=0.353, P<0.0196) with body weight. However, regressing with body weight, the epithelial thickness was correlated when all ages were included (r^2=0.644, P<0.0001). The submucosa between and over cartilage was correlated with age (r^2 = 0.655, P<0.0003; 0.534, P<0.0020). Submucosal glands were present in the bronchi of all ages. However, in the adult, they extended into the medium bronchioles. Bronchial-associated lymphoid tissue extended into the terminal bronchiole (TB) and adventitia of pulmonary vessels only in the adult. The adult ciliated cell was covered with microvilli which often branched near or away from the cell's surface. Ciliated cells undergoing ciliogenesis were observed in TBT of growing cats and adult bronchi. The goblet cell was covered with microvilli in the early stages of their secretory cycle, but lacked microvilli late in the cycle. NBCs lining the (TBs) had

apical protrusions covered with microvilli, except in prenatal kittens. NBCs contained abundant mitochondria and a and b glycogen inclusions. Serous cells were observed near the basal cell in the trachea and near the luminal surface of adult bronchi. Lung volume and weight increased proportionately with the body weight and age. Surface density increased from 648 cm^2/cm^3 in prenatal to 1218 cm^2/cm^3 in 21-day-old kittens. The harmonic mean thickness reduced significantly (from 0.54μm to 0.31μm) with age. Prenatal saccules were surrounded by thick primary septa, lined by type II pneumocytes (TII), divided by secondary septa with double capillaries. Eosinophils were observed in the septum, intraepithelium, and in alveolar space of growing cats. Saccular division was not completed by 21 days of age. Elastic fibers accumulated at the tip of the septum and play a role in alveolar formation. Microvilli of TII increased with age. TII were located at the base of the secondary septum, giving stability to the septum. Type I pneumocytes formed by transformation from TII pneumocytes. Pores of Kohn were observed only in the adult cats.

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I. REVIEW OF THE LITERATURE CONCERNING THE ANATOMY, DEVELOPMENT AND MORPHOMETRY OF THE FELINE LUNG

MACROSCOPIC ANATOMY OF THE LUNG

The lungs are located in the thoracic cavity, where they are covered by pulmonary pleura. The lungs are divided by fissures of varying depths into lobes. The right lung has four lobes: cranial, middle, caudal, and accessory. The left lung has two lobes: cranial and caudal. The left cranial lobe is subdivided into cranial and caudal portions by a deep fissure.

The respiratory system provides gas exchange which takes place at the alveolar level. However, there are a number of conductive tubes of different sizes that the air routinely passes through to get to the alveolus. These passages warm and moisten the air before it reaches the alveolus.

The proximal conducting passage is the trachea which is non-collapsible tube consisting of 38-43 cartilages in the cat (Nickel et al., 1979). The tracheal cartilages are "C" shaped and their ends do not meet dorsally. Here the trachealis muscle bridges the ends of the cartilage. The trachea is continued with the other respiratory segments through its bifurcation into two principle bronchi.

The bronchial tree consists of six lobar bronchi. On the left side, the cranial lobar bronchus divides into cranial and caudal segmental bronchi which serve the cranial and caudal parts of the cranial lobe. In the caudal lobe, the caudal lobar bronchus gives off dorsal and ventral segmental bronchi. On the right-side, the

cranial lobar bronchus supplies the undivided cranial lobe by means of dorsal and ventral segmental bronchi. The middle lobar bronchus supplies the middle lobe, and the caudal lobar bronchus enters the caudal lobe and gives off dorsal and ventral segmental bronchi. The accessory lobar bronchus arises from the right caudal lobar bronchus and supplies the accessory lobe.

The segmental bronchi divide and subdivide into varying numbers of generations,followed by division into bronchioles, respiratory bronchioles, and alveolar ducts. The generations of the bronchial tree are best described by Weibel (1963) who numbered successive generations of air passages from the trachea (generation 0) down to alveolar sacs (generation 23).

MICROSCOPIC ANATOMY OF THE LUNG

The lung is the only organ that has intimate contact with the environment and plays a key role in supplying the organism with oxygen. It has to filter 12,000 liters of air per day and perfuse more than 12,000 liters of blood in order to convey the oxygen to the site of consumption (Burri, 1985).

The alveoli are connected to the outside by a highly organized airway tree. In man about $300x10^6$ alveoli provide a gas exchange surface of $150m^2$ (Gehr et al., 1978). These alveoli are separated by thin tissue plates, the interalveolar septa. The septum is design to minimize the thickness of the air-blood barrier. Despite its delicacy, the lung parenchyma has an appropriate mechanical stability

achieved by the formation of surfactant and the presence of a fibrous skeleton and dense capillary network in the alveolar wall. The air-blood barrier is a three-layered structure composed of a superficial epithelial layer, intermediate interstitial connective tissue sheet, and an inner endothelial lining.

The most important cell type is the interstitial fibroblast, also called myofibroblast. Kapanci and coworkers (1974) reported these cells possessed intracytoplasmic contractile filaments. Weibel and Bachofen (1979) observed that the cytoplasmic processes of these fibroblasts frequently spanned the entire width of the septum. These authors suggest that the interstitial myofibroblast could control the width of the septum and hence adjust the compliance of the interstitial space. By doing so, the myofibroblasts may prevent the accumulation of fluid which normally leak from the septal capillaries. Other cells of the alveolar interstitium include: smooth muscle cells located in the proximal alveolar entrance ring, pericytes that adhere to the capillary, and free cells which relatively rare, the most frequent being the mast cell.

The type I pneumocyte is an epithelial cell that covers between 92 and 97% of the alveolar surface area (Burri, 1985) as a continuous sheet. Type I cells have very thin cytoplasmic plates that can extend up to 50 μm from the nuclear region. These cells have tight junctions with a 1nm gap (De Fouw, 1983). The tightness of these junctions prevents the escape of large molecules into the alveoli, thus preserving the oncotic pressure gradient which is essential to

avoid pulmonary edema. However, these junctions permit the free passage of macrophages. Type I pneumocytes are unable to divide (Kauffman et al., 1974; Evans et al., 1975) and cannot be grown in tissue culture (Weibel, 1984) indicating a lack of self-reproduction capability.

Type II pneumocytes are cuboidal with no lateral cytoplasmic extensions. Its characteristic cytoplasm is rich in organelles: mitochondria, rough and smooth endoplasmic reticulum, Golgi apparatus, multivesicular bodies, and lamellar bodies. Its apical surface is covered by microvilli and protrudes into the alveolar lumen.

DEVELOPMENT OF THE LUNG

Postnatal growth of mammalian lungs has been the subject of controversy for many years. Much of the work on the development of the lung was conducted in small animals (Boyden and Tompsett, 1961; Burri, 1974; Amy et al., 1977; Winkler and Cheville, 1985). In these studies most of the attention was concentrated on alveolar development.

During the last two decades, there has been great debate over the development of the acinar structures, especially, those experiments conducted on laboratory animals, and particularly the rat (Loosli and Potter, 1959; Vaccaro and Brody, 1978; Brody and Vaccaro, 1979; Antonia and Ten Have-Opbroek, 1981). From their work, one can explain two possible modes of lung growth. First the animal could be

born with a full complement of alveoli, and further increases in the lung would occur solely by increase in the volume of its components. In agreement with this hypothesis, conflicting reports have been published regarding the development of mammalian lungs (Hislop and Reid, 1974; Kauffman et al., 1974; Lechner and Banchero, 1982; Thurlbeck, 1982; Burri, 1984). Yet, they all agree there is a certain degree of postnatal development and differentiation in the lung. Hislop and Reid (1974) and Burri (1984) reported that alveoli of human lungs do not appear until after birth. During the first one to three years of postnatal life Lechner and Banchero (1982) reported that neonatal guinea pigs are born having mature air-blood barriers. Thereafter, double capillary layers were rarely seen in septal walls.

The above notion is an excellent test to justify Engel's hypothesis (1953) that pulmonary development parallels the overall level of development at birth. For example mammals with longer gestation periods have more developed lungs at birth.

The second mode of lung growth consists of acinar unit duplication. In rats, for instance, there is rapid cell multiplication in postnatal life as shown by a sharp increase in DNA content of the lung (Kauffman et al., 1974). Histological examination of newborn animal indicates that there are numerous air saccules which differ from adult alveoli in that they are larger and of different shape (Burri, 1974; Thurlbeck, 1982).

Many investigators agree that the development of the lung can be divided into distinguished periods of respiratory tract development:

pseudoglandular, canalicular, terminal sac or saccular, and alveolar or postnatal periods. This model of lung development has gained support from extensive morphological and morphometrical analysis of postnatal lung growth in the rat (Burri et al., 1974; Lechner and Banchero, 1980 a; b; Lechner et al., 1982). The new born rat possesses alveolar septa whose appearance and dimensions are indistinguishable from those of the adult (Burri, 1974; Amy et al., 1977; Vaccaro and Brody, 1978; Hilfer, 1983). However, other investigators suggest that mammals such as dog, pig, sheep, as well as man, are born with lungs containing primitive, thick-walled saccules or terminal sacs. Postnatally, saccules undergo rapid septal elaboration and structural rearrangement into true alveoli (Loosli and Potter, 1959; Boyden and Tompsett, 1961; Hislop and Reid, 1974; Baskerville, 1976; Brody and Vaccaro, 1979; Alcorn et al., 1981; Thurlbeck, 1982; Lechner and Brody, 1982; Fukuda et al., 1983).

The postnatal development of the rat lung has been most extensively studied. Burri et al. (1974) showed an increase in gas exchange surface area starting after day four. In addition, rapid increase of pulmonary parenchyma mass was due to the increase in size of existing cells rather than the formation of new alveoli. Many investigators (Burri et al., 1974; Burri, 1974; Amy et al., 1977) have demonstrated that the new born rat has no true alveoli. The respiratory exchange of these animals is carried out in large, smooth-walled sacs (primary saccules). Primary saccules have thick

walls called primary septa with a cellular interstitium (Amy et al., 1977; Vaccaro and Brody, 1978).

The primary saccule differs from an alveolus by its large size and lack of complexity. The primary septa differs from mature interalveolar septa by the presence of a double capillary system in the wall of the former (Amy et al., 1977). The wall of the primary saccule is modified by the elevation of low secondary crests, which appear as narrow ridges. The secondary crest consists of outer alveolar epithelial cells, whose cytoplasm is separated by a compound basal lamina from the interstitial layer (Amy et al., 1977).

Much of the surface of the air space is lined by type II pneumocytes with relatively few type I cells. These are progenitors of type I cells which form the major part of the lining surface of adult alveoli (Dunnill, 1982). Type I pneumocytes in the rat do not form until after birth on day 19 or 20 (Hilfer, 1983). The proliferation rate of different septal cells vary both in quantity and with time (Kauffman et al., 1974).

The alveoli may be formed by an out pouching in the walls of terminal bronchioles (Burri, 1974). Antonia and Ten Have-Opbroek (1981) disagreed with this hypothesis, since the cuboidal epithelium appear in the pseudoglandular and following period. Therefore, alveolar appearance may not be restricted to one of the periods, based on studies using fluorescent antibodies to investigate the occurrence of the lung specific antigen present in type II pneumocytes.

Boyden and Tompsett (1965) and Emery (1969) have described two patterns of lung alveolization. One involves out pouching of gas exchange units from the walls of terminal bronchioles, thus transforming these conducting air ways into respiratory airways. The second involves the adding of new septa from saccular walls of the immature lung. Burri (1974) and Kauffman and co-workers (1974) reported that there was a coincidence between the formation of new septa and the proliferation of the interstitial fibroblast. They also noted that elastin frequently appeared at the tip of newly formed alveolar septa. Elastin and collagen fibers present between the capillaries in the growing alveolar septa was found to play a key role in the alveolar development (Amy et al., 1977). Similar studies in early developing sheep lung have shown that elastic fibers were found exclusively in the primordia of the alveolar septa (Fukuda et al., 1983). In contrast to these findings, Lechner and Banchero, (1982) found elastic fibers in both bronchiolar and duct walls in the fetal pig.

Vaccaro and Brody (1978) described two types of interstitial fibroblasts present at the tips of newly formed septa. One which has the characteristic of a myofibroblast and appears to be engaged in the synthesis and secretion of elastin. The other which appears at the base of new septa, is filled with lipid, and contains few cytoplasmic organelles. They also described the process of postnatal alveolization of lung parenchyma involving differentiation of interstitial fibroblasts and elastogenesis.

Lung volume, alveolar surface area, total volume of alveolar wall, and the number of pores were found to increase with age in the mouse lung (Kawakami et al., 1984). The major increase in the number of interalveolar pores occurred by 68 days, but there was a significant increase from 68 to 270 days of age (Kawakami et al., 1984).

Short (1950) considered lung growth to be determined by purely physical factors, and thought that simple distension of the new born rabbit lungs resulted in a structure resembling that of our adult lung. The lungs of new born animals are quantitatively different from those of adults of the same species, in that, alveoli are few or absent at birth. Therefore, a considerable differentiation occurs postnatally as has been described in several species including man (Loosli and Potter, 1959; Leeson and Lesson, 1964; Krause and Leeson 1973; 1975).

Boyden and Tompsett (1961) described reduction in the number of non-respiratory branches of the axial bronchus and of non-respiratory peripheral bronchioles in the postnatal life of the dog. He also stated that the respiratory bronchioles previously lined by cuboidal epithelium were converted into respiratory units. Following inflation of the bronchioles by inspired air, the cuboidal epithelium becomes drawn out into segmented clumps of cells between which alveoli are present. In addition, new terminal alveoli are formed by ingrowth of new septa.

Alcorn and co-workers (1981) described the formation of alveoli in the sheep by subdivision of the large terminal spaces by alveolar crests. This subdivision was followed by differentiation of alveolar lining epithelium during the canalicular and alveolar periods of development. Unlike the rat, new born pigs have shown no signs of postnatal tissue proliferation (Baskerville, 1976; Lechner and Banchero, 1982). The thickness of the air-blood barrier decreases with additional age. As a matter of fact, these animals were born with highly advanced stages of lung development (Winkler and Cheville, 1985). A stage that could make them live independently and without mother care (Wagner and Manning, 1976).

Burri (1984) divided the development of human lungs into five distinct phases or periods; embryonic, pseudoglandular, canalicular, saccular, and alveolar. The embryonic period, during which the lung primordium is laid down as a diverticulum of the foregut, lasts for about seven weeks. The pseudoglandular period (in which the lung looks like a tubulo-acinar gland) starts as early as the fifth week and extends to the 17th week. During this period an epithelial tube is sprouting and branching into the surrounding mesenchyme. The canalicular phase (17-26th week) is characterized by widening of the peripheral tubules, differentiation of cuboidal epithelium into type I and type II cells, formation of the first thin air-blood barriers, and surfactant production. The saccular phase follows the canalicular phase until birth. It shows growth of pulmonary parenchyma, thinning of connective tissue between the air spaces, and

further maturation of the surfactant system. At birth, although the lung is functioning, it is still morphologically immature (there are no alveoli which resemble adult alveoli). During the first 1-3 years of postnatal life, alveoli are formed through a septation process which greatly increases the gas exchange surface area (Burri, 1984). Throughout childhood, the primitive septa with their capillaries undergo complete remodeling, gaining the mature slender morphology found in the adult lung.

In humans, conflicting reports have been published regarding the time of alveolar multiplication. Dunnill (1962b) reported that alveolar multiplication slowed after the age of four years and stopped at eight years. Hislop and Reid (1974) suggested the increase in alveolar number occurred in the first seven years of life. However, recent investigations have shown there is rapid alveolar multiplication during the first two years of life, and alveolar dimension and number per unit area and volume do not change much during this period (Thurlbeck, 1982). However, little or no increase in the total number of alveoli occurs after the age of two years. Furthermore, the same study showed that sexual differences occurred not in the number of alveoli per unit area and volume, but in the total number of alveoli and alveolar surface area. This is greater in males when compared to females.

MORPHOMETRY OF THE LUNG

Ethical and procedural considerations limit the study of pulmonary development in the human fetus. Many investigators perform experimental studies on laboratory animals, including sub-human primates, which have more similarity to man than other mammalian species (Hislop et al., 1984). Cats are less expensive than primates, and their lungs have some morphological similarities to those of man. Therefore, investigators have used the cat as a model in their studies (Tsunoda et al., 1974; Plopper et al., 1980, 1983a, b).

Most lung studies are done on lung preparations fixed by instillation of glutaraldehyde into the airways (Weibel, 1970; Gehr et al., 1981; Bachofen et al., 1983). This method preserves the blood content of capillaries and causes the alveolar-capillary tissue barrier to unfold on the alveolar side. The value obtained on such preparations reflects maximal capacity of the gas exchange apparatus (O_2 between alveolar air and capillary blood by passive diffusion) (Weibel et al., 1973; 1981).

Many investigators agree that the development of the lung can be divided into distinguishable periods: pseudoglandular, canalicular, terminal or saccular, and alveolar or postnatal periods (Hislop and Reid, 1974; Ten Have-Opbroek, 1981; Hilfer, 1983; Burri, 1984). This model of lung development has gained credibility from extensive morphological and morphometrical analysis of postnatal lung growth in the rat (Burri et al., 1974; Lechner and Banchero, 1980a,b; Lechner

et al., 1982). The histological phases of normal fetal lung development have been identified in several species (Meyrick and Reid, 1977; Collins et al., 1986). During the early phases of lung growth, development of the conducting airways is prominent. Whereas, later in gestation, the distal air spaces undergo extensive alteration and enlargement in preparation for gas exchange.

A few studies have selectively appeared in recent years which studied the terminal bronchiolus and/or proximal alveolar region. This indicates an increased awareness of this region being a site of toxic injury, and the necessity for development of appropriate morphometric techniques to study this area (Lum et al., 1978; Boorman et al., 1980; Barry et al., 1982). Systematic morphometric examination of human (Dunnill, 1962a,b; Thurlbeck, 1982) and rat (Weibel, 1967; Burri et al., 1974; Vidic and Burri, 1983) pulmonary development have demonstrated remarkable lung immaturity at birth. Pigs have a thicker air blood barrier than other mammalian species, but the barrier composition is similar (Meban, 1980). It is known that alveoli are formed postnatally through the process of septation which greatly increases the gas exchange surface area (Burri, 1984). However, in the pig these septa appear thickened in cross section (Winkler and Cheville, 1984). Similar findings were also described in rodents (Burri, 1979; Amy et al., 1977) and man (Hislop and Reid, 1974).

Morphometry is a sensitive quantitative tool which can be of great value in evaluating changes in lung tissue. It uses two

dimensional information derived from a specimen to derive three dimensional information concerning that specimen (Barry and Crapo, 1985). Weibel (1963) was the first to extensively use morphometric methods to study the three dimensional architecture of the normal human lung. This work, which combined the use of large macroscopic sections through the entire lung and paraffin and plastic sections of lung tissue, presented quantitative methods to quantitate the alveolar portion of the human lung and to describe the branching patterns of the airways.

In the lung, two of the main factors affecting gas exchange are alveolar surface area and alveolar capillary surface area. These morphometric properties are essential determinants of the gas exchange function of the lung. Their estimation requires precise methods of measurement to correlate structure with function. Lechner and Banchero (1982) studied the morphometry of the guinea pig lung from the 56th day of gestation to 16 days postpartum. They reported that lung volume and surface area increase exponentially with body weight in pre and post partum animals. In contrast, data from the canalicular phase until birth shows good linear correlation between lung volume and body weight (Collins et al., 1986). Total lung volume increases linearly with gestational age in the rhesus monkey (Bunton and Plopper, 1984; Hislop et al., 1984; Collins et al., 1986) and in man an exponential relationship exists (Langston et al., 1984).

The lungs of new born animals are quantitatively different from those of adults, in that, alveoli are few or absent at birth. Therefore, extensive differentiation occurs postnatally in the human, rat, and opossum, (Loosli and Potter, 1959; Leeson and Leeson, 1964; Krause and Lesson, 1973; 1975). Collins et al. (1986) showed that the guinea pig lung gains increased surface area by increasing alveolar numbers. Guinea pigs possess 3 - 15 times as many alveoli as humans at birth (Dunnill, 1962a,b; Davies and Reid, 1970; Thurlbeck and Angus, 1975; Langston et al., 1984). In humans it is suggested that alveolar numbers increase throughout the first seven years of life. The number of alveoli in the guinea pig increases, but alveolar size decreases [$1cc^3$ lung volume in the guinea pig contains 60 times as many alveoli as the same volume of human lungs (Davies and Reid, 1970)]. Tenney and Rommer (1963) have shown that the high metabolic rate and oxygen demand of small animals is accommodated by increased alveolar partitioning, resulting in decreased alveolar size. These findings were recently supported by Collins et al. (1986) who worked with guinea pigs.

OBJECTIVES

1. Record the normal development of the feline lung during late uterine life (60 post-conception) and the changes which follow the onset of breathing.
2. Record the structures involved in these changes through data obtained by light microscopy and transmission and scanning electron microscopy.
3. Define the age when alveolar epithelial cells differentiate into type I and type II pneumocytes and into the various other respiratory elements.
4. Record the first appearance in the alveolar epithelium of the lamellated osmiophilic inclusion bodies, which are considered to represent pulmonary surfactant.
5. Quantitate alveolar surface area, volume density, and air-blood barrier thickness.

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II. PRE- AND POST-NATAL GROWTH OF FELINE TRACHEAL EPITHELIUM WITH SPECIAL EMPHASIS ON CILIATED AND GOBLET CELLS: A QUANTITATIVE LIGHT AND SCANNING ELECTRON MICROSCOPIC STUDY

ABSTRACT

The growth of the trachea was investigated in prenatal (60 days post-conception), postnatal (1-, 7-, 14-, and 21-day-old), and adult cats by light and scanning electron microscopy. The epithelial lining of the trachea was similar in all cats examined. Four cell types were identified in the growing cat; these are ciliated and nonciliated cells possessing microvilli, goblet, and basal cells. In the adult cats, a fifth cell, a serous cell, was also observed. The ciliated cells had a convex luminal surface with few cilia. Postnatally, the cell surface became flattened and the number of cilia was increased. Goblet cells were present in prenatal kittens. Postnatally, their number was not increased significantly. However, the amount of secretion increased and thus the cells became more obvious. The goblet cells possessed neither microvilli nor cilia, but instead had a smooth dome-like apical surface. Quantitative results showed an increase in the thickness of epithelium and submucosa between and over cartilage, which were highly correlated (r^2 = 0.518, 0.655, 0.534) with age in the growing cat. Only epithelial thickness was strongly correlated (r^2 = 0.644) with body weight.

INTRODUCTION

There have been many light microscopic descriptions of the airway epithelial lining (Lucas, 1932; Von Hayek, 1960; Azzopardi and Thurlbeck, 1969). These studies concluded that the epithelium of large airways has several cell layers and contains goblet cells. Rhodin (1966) described the respiratory epithelium as pseudostratified ciliated columnar, resting on a basement membrane composed of a dense network of fine collagenous and reticular fibrils. Recently, Kawamata and Fujita (1983) identified three types of epithelial cells (ciliated, nonciliated and basal cells) by light microscopy. The nonciliated cells are usually more numerous than the ciliated cells. In addition, the ciliated cells have less electron-dense cytoplasm.

Considerable data on ciliary length, especially for the trachea and nasal passages, have been reported (Pavelka et al., 1976; Serafini and Michaelson, 1977). Pavelka et al. (1976) reported the length of cilia in the cat, but not for early postnatal life.

Frasca et al. (1968), using scanning electron microscopy, demonstrated that smoking has a remarkable effect on the surface epithelium of the respiratory tract. After short exposure to smoke the epithelium in dogs has fewer cilia and goblet cells. Long exposure of the epithelium to smoke increased numbers of ciliated and goblet cells. Similar observations were reported for humans by Ebert and Terracio (1975).

It is essential to understand the normal airway morphology of an animal in order to use it as a respiratory model or to evaluate pathological changes. Although the cat has been used as a model to study the tracheobronchial tree (Gallagher et al., 1975; Lamb, 1975; Pavelka et al., 1976; Jeffery, 1977, 1983; Das et al., 1979; Tandler et al., 1981; 1983a, b), no investigations report the normal morphology of the developing cat trachea. Therefore, the objective of this study was to evaluate the morphological aspects of differentiation and development of the cat tracheal epithelium from prenatal to 21 days postnatally.

MATERIALS AND METHODS

Thirty-nine domestic cats (Felis catus) representing the following six age groups: prenatal (60 days post-conception), postnatal (1-, 7-, 14-, and 21-day-old), and adult were used in this study, and body weights were recorded (Table 2-1). The cats were anesthetized by intramuscular injection of ketamine (5 mg/kg) and xylazine (0.4 mg/kg). The trachea was cannulated, and the lungs were fixed by infusion of 3% glutaraldehyde in phosphate buffer (pH 7.4, 0.1M) at a pressure of 20 cm H_2O. After fixation *in situ* for 30 minutes, a midline incision through the skin and subcutaneous tissue from the larynx to the xiphoid region was made to expose the trachea and the sternum. The thorax was opened and the lungs and trachea were carefully dissected free and removed. Tissue samples were taken from the mid-portion of the trachea, cut into small pieces and

Table 2-1: Mean and standard deviation of cellular density and thickness of mucosa and submucosa of feline trachea. Number of cats (N), Body weight (BW), Length of cilia (LC), Epithelial thickness (ET), Submucosa over cartilage (SOC), Submucosa between cartilage (SBC), Number of ciliated cells (NCC), Number of goblet cells (NGC).

	PREPARTUM	POSTPARTUM				ADULT
	60 DAYS	1 DAY	7 DAYS	14 DAYS	21 DAYS	2-4 YEARS
N	8	9	5	6	7	4
BW (gm)	80.94 ±3.8	89.29 ±6.5	172.42 ±44.1	264.9 ±20.1	360.0 ±44.3	3317.00 ±792.3
LC (μm)	5.10 ±0.7	5.20 ±0.3	5.22 ±0.7	5.33 ±0.3	5.63 ±0.6	5.82 ±0.5
ET (μm)	23.71 ±1.1	28.40 ±0.7	29.16 ±5.4	30.29 ±2.0	32.25 ±2.2	40.10 ±1.4
SOC (μm)	34.85 ±8.2	45.60 ±22.5	51.62 ±1.7	63.59 ±4.5	67.21 ±15.9	69.57 ±3.8
SBC (μm)	114.50 ±51.8	115.30 ±27.3	120.40 ±38.8	200.90 ±39.2	243.00 ±12.4	291.30 ±37.3
NCC/100μm	20.11 ±2.8	22.00 ±6.9	18.67 ±2.4	21.67 ±2.3	21.67 ±1.7	20.78 ±1.4
NGC/100μm	14.33 ±1.2	13.00 ±1.0	16.11 ±3.0	13.00 ±1.0	11.00 ±2.0	12.11 ±1.3

immersed into the fixative for an additional four hours. The samples were then processed for both light and scanning electron microscopy (SEM).

For light microscopy, glutaraldehyde fixed tissue was washed three times in phosphate buffer (pH 7.4, 0.1M). Subsequently, the samples were dehydrated in a graded series of ethanol (50%, 70%, 95%; two changes at 10 minutes each). Infiltration of the samples was done at room temperature using standard JB-4 methods (Polysciences Inc., Warrington, PA). The samples were embedded and precisely oriented in the mold to yield longitudinal sections through the trachea. Sections, 3μm thick, were taken at 25μm intervals from each block with glass knives using a Reichert Jung Autocut model 1140 microtome and stained with hematoxylin and eosin and periodic acid-Schiff reagent. Some of the samples were embedded in paraffin using standard procedures, 6μm sections were cut and then stained with Weighert's stain to identify elastic fibers.

Glutaraldehyde fixed samples for scanning electron microscopy were postfixed for 90 minutes in 1% osmium tetroxide in phosphate buffer (pH 7.4, 0.2M). They were washed three times in phosphate buffer for 10 minutes each, then dehydrated in a graded series of acetone solutions (70%, 80%, 95%, with three changes in 100% acetone). The samples were dried in a critical point drying apparatus with liquid CO_2. Tissues were then mounted on aluminum stubs, coated with a 15nm gold-palladium layer, and examined with an ETEC Scanning Electron Microscope operating at 25 kV. Data were

collected from three samples of the trachea for each group observed by SEM. However, only the epithelial lining over the cartilaginous part was observed by SEM.

For morphometric evaluation of the trachea, tissue was randomly selected from three cats in each group. Six blocks were then selected from each animal, yielding 18 blocks per age group. Three micron longitudinal sections were stained with periodic acid-Schiff reagent and examined with a light microscope at a magnification of 500X.

Length of cilia was measured from the terminal bar to the luminal free tip, whereas the height of epithelial cells was measured from the basement membrane to the terminal bars. The thickness of the submucosa over the cartilage was measured from the epithelial basement membrane to the perichondrium, where the cartilage surface was nearly parallel to the surface epithelium. The thickness of the submucosa between cartilages was measured from the epithelial basement membrane through the total depth of the tracheal glands. This also included profiles that were located at the ends of the cartilage plates, where the edge turns away from the surface (Fig. 2-1). These measurements were made using a computerized digitizer (Zeiss Videoplan Image Analysis System).

The numbers of goblet and ciliated cells were tabulated with a calibrated 50μm ocular reticule. Only cells which extended from the lumen to the basement membrane were included.

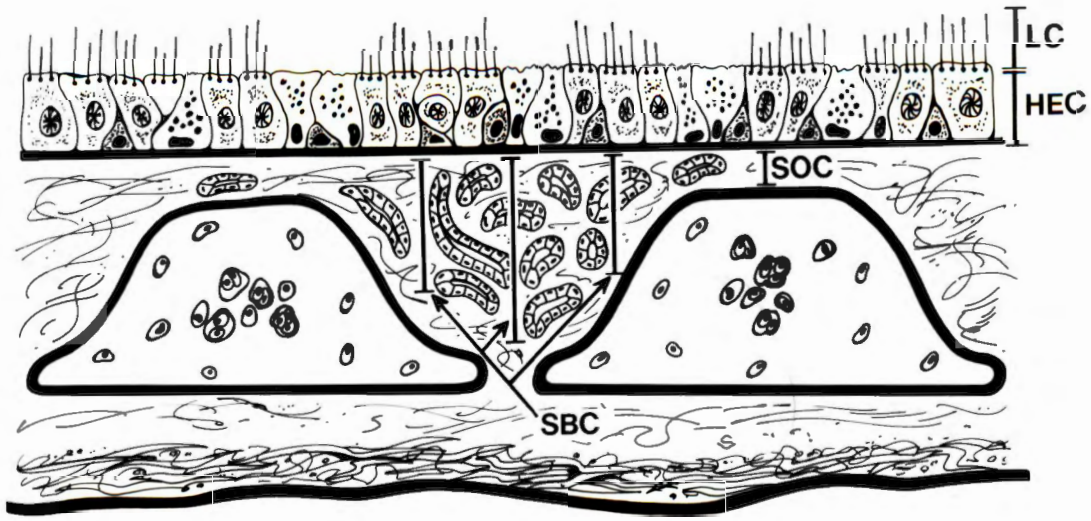


Fig. 2-1: Schematic drawing of the trachea (longitudinal section) illustrating measurements points. Length of Cilia (LC), Height of epithelial cells (HEC), Submucosa over cartilage (SOC), Submucosa between cartilages (SBC).

Statistical Methods

The measurements in this study were tabulated from eighteen cats of random age and sex (Table 2-1). Numerical data were analyzed from three cats per group, using General Linear Model (GLM) procedures contained in Statistics Analysis System (SAS) software. The functional relationship between body weight and age were described by simple regression equations. Since this functional relationship changes with age, and since the ages of the adult cats were not precisely known, regression analysis was performed with and without inclusion of the adult age group. A difference of less than 5% statistical probability of being due to chance ($P<0.05$) is considered as the level of significance. The coefficient of determination (r^2) was used to characterize the closeness of fit. A value of $r^2 = 1$ indicates a perfect correlation, whereas an $r^2 = 0$ indicates no correlation (Snedecor and Cochran, 1980). In this study, an r^2 greater than 0.500 was considered to be a significant correlation. In addition, the root mean square error for each relationship was determined.

RESULTS

Morphometry

Length of cilia, thickness of epithelium, thickness of submucosa over cartilage, and thickness between cartilage are summarized in Table 2-1. Mean epithelial thickness was increased 200% from before birth to adulthood. The thickness of submucosa both over (35 - 70μm) and between (115 - 291μm) cartilages increased in prenatal kittens to

adult cats. Cilia length increased slightly (5.10 - 5.82μm), number of ciliated cells per 100μm remained constant, and number of goblet cells per 100μm decreased slightly (14 - 12) in prenatal kittens to adult cats.

Regression analyses of cilia length, epithelial thickness, thickness of submucosa over and between cartilages, number of ciliated cells, and number of goblet cells versus body weight are shown in Table 2-2. Only epithelial thickness was correlated with body weight (r^2 = 0.644) (Table 2-2). In addition to epithelial thickness ($P<0.0001$), submucosal thickness between cartilages ($P<0.0021$) was a significant predictor of body weight. Regression analysis of data for developing cats result in epithelial thickness ($P>0.0196$) , submucosal thickness over and between ($P>0.0002$) cartilages being significant descriptors of body weight. The thickness between cartilages is also strongly correlated (r^2 = 0.662) with body weight (Table 2-3). Neither with nor without the adult cat did the length of cilia, number of ciliated cells, or number of goblet cells correlate with body weight (Table 2-3).

The epithelial thickness (r^2 = 0.518) and thickness of the submucosa both over (r^2 = 0.534) and between (r^2 = 0.655) cartilage, were correlated with age, among growing cats (Table 2-4). Yet the length of cilia, number of ciliated cells, and number of goblet cells were not correlated with age. By regressing the average of the dependant variables used in this study as a function of age, they were significantly and strongly correlated (Table 2-5).

Table 2-2: Correlation of tracheal parameters as a function of body weight including the adult. Length of cilia (LC), Epithelial thickness (ET), Submucosa over cartilage (SOC), Submucosa between cartilage (SBC), Number of ciliated cells (NCC), Number of goblet cells (NGC). Significant and/or linear (*).

Dependent Variables	n	Intercept	Slope	r^2	s	P<F
LC (μm)	18	5.271	0.000	0.138	0.502	0.1295
ET (μm)	18	28.064	0.004	0.644*	3.421	0.0001*
SOC (μm)	18	51.189	0.006	0.198	15.098	0.0646
SBC (μm)	18	150.494	0.043	0.456	59.049	0.0021*
NCC/100μm	18	20.808	0.000	0.000	1.411	0.9824
NGC/100μm	18	13.621	-0.000	0.133	1.853	0.4775

Table 2-3: Correlation of tracheal parameters of growing cats as a function of body weight. Length of cilia (LC), Epithelial thickness (ET), Submucosa over cartilage (SOC), Submucosa between cartilage (SBC), Number of ciliated cells (NCC), Number of goblet cells (NGC). Significant and/or linear (*).

Dependent Variables	n	Intercept	Slope	r^2	s	P<F
LC (μm)	15	4.968	0.002	0.149	0.480	0.1558
ET (μm)	15	24.966	0.019	0.353	3.152	0.0196*
SOC (μm)	15	34.309	0.094	0.429	12.906	0.0080*
SBC (μm)	15	71.844	0.447	0.662*	38.082	0.0002*
NCC/100μm	15	20.539	0.002	0.009	2.360	0.7319
NGC/100μm	15	16.190	-0.010	0.136	2.928	0.1763

Table 2-4: Correlation of tracheal parameters of growing cats as a function of age. Half of these variables present a significant linear relationship (*). Length of cilia (LC), Epithelial thickness (ET), Submucosa over cartilage (SOC), Submucosa between cartilage (SBC), Number of ciliated cells (NCC), Number of goblet cells (NGC).

Dependent Variables	n	Intercept	Slope	r^2	s	P<F
LC (μm)	15	3.973	0.018	0.124	0.487	0.1975
ET (μm)	15	8.444	0.281	0.518*	2.721	0.0025*
SOC (μm)	15	-37.437	1.243	0.534*	11.661	0.0020*
SBC (μm)	15	-223.515	5.281	0.655*	38.460	0.0003*
NCC/100μm	15	19.135	0.025	0.011	2.358	0.7121
NGC/100μm	15	20.571	-0.087	0.076	3.028	0.3187

Table 2-5: Correlation of mean tracheal parameters of growing cats as a function of age. Significant linear relationships (*), Length of cilia (LC), Epithelial thickness (ET), Submucosa over cartilage (SOC), Submucosa between cartilage (SBC), Number of ciliated cells (NCC), Number of goblet cells (NGC).

Dependent Variables	n	Intercept	Slope	r^2	s	P<F
LC (μm)	5	3.971	0.018	0.870*	0.085	0.0208*
ET (μm)	5	8.445	0.281	0.854*	1.401	0.0248*
SOC (μm)	5	-37.432	1.243	0.966*	2.807	0.0027*
SBC (μm)	5	-223.514	5.281	0.860*	25.764	0.0233*
NCC/100μm	5	17.826	0.041	0.094	1.551	0.6156
NGC/100μm	5	20.929	-0.103	0.324	1.792	0.3165

Light Microscopic Findings

Tunica mucosa respiratoria: The mucosa lining the trachea was characterized by an absence of folds on the cartilaginous portion, while the mucosa of the membranous portion was often thrown into longitudinal folds. The mucosa lining the trachea was similar in all ages, and was composed of pseudostratified ciliated columnar cells (Epithelium pseudostratificatum columnare ciliatum). This epithelium consisted principally of three cell types: ciliated (Epitheliocytus ciliatus), goblet (Exocrinocytus calciformis) and basal (Epitheliocytus basalis) (Fig. 2-2).

The ciliated cells were columnar, occurred singly or in groups of 2 to 4, and rested on a relatively thick basement membrane (Fig. 2-2). The nuclei, forming the upper layer of surface epithelium, were large, oval, and centrally located with prominent nucleoli. The cytoplasm contained vesicles concentrated above the nucleus, giving a lightly stained foamy appearance (Fig. 2-3). Near the apical border, the cytoplasm also contained dark pleomorphic granules (Fig. 2-2). The apical surface was covered with cilia and microvilli projecting into the lumen. In *prenatal kittens*, ciliated cells had only a few short cilia which tended to cluster, yielding a patchy appearance (Fig. 2-2). By *day 21*, these cells became large and had dense cilia (Fig. 2-4). In addition, the ciliary border was irregular in the growing cat and flattened by adulthood (Fig. 2-4).

PLATE 2-1

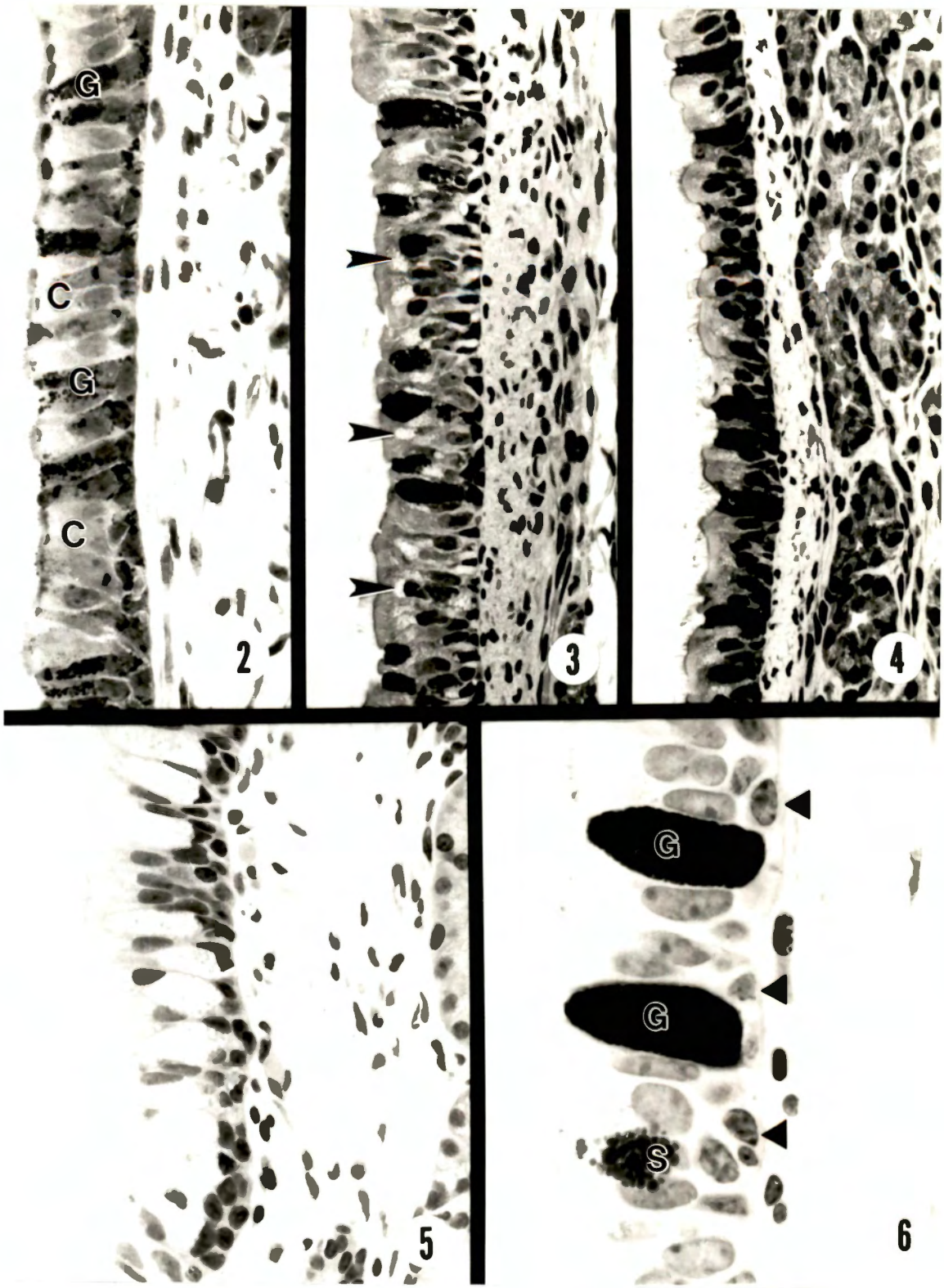
Fig. 2-2: Light micrograph of prenatal tracheal epithelium. Ciliated cells (C) have apical electron dense granules. Goblet cells (G). Plastic section, PAS stain. X460.

Fig. 2-3: Light micrograph of tracheal epithelium of a 1-day-old kitten. Ciliated cells have apical vesicles (arrowheads) which give a foamy appearance to the cytoplasm. Plastic section, H&E stain. X460.

Fig. 2-4: Light micrograph of tracheal epithelium of a 21-day-old kitten. Note the density of cilia and the irregularity of the ciliary border. Plastic section, H&E stain. X230.

Fig. 2-5: Light micrograph of the tracheal epithelium of an adult cat. Note many goblet cells had characteristic morphology. Plastic section, H&E stain. X460.

Fig. 2-6: Light micrograph of the tracheal epithelium of an adult cat. Goblet cells (G), Epithelial serous cell (S), Basal cells (arrow heads). Plastic section, PAS stain. X1150


G
C
G
C
2
3
4
5
G
G
S
6

The goblet cells occupied the middle layer of the epithelium and were identified by their chalice-shape (Figs. 2-3, 2-4). These cells were usually found in groups of 2 to 4 and situated between ciliated cells or ciliated and basal cells. They varied in shape depending upon their secretory status, and ranged from columnar to ovoid in shape (Figs. 2-2 to 2-6). The cytoplasm was filled with mucous granules of various shapes and sizes. The luminal surface was small and was either cup-shaped or flat. Their nuclei were elongated, dark, and located near the basal border. In *prenatal kittens*, this cell was columnar and contained few mucous granules in its cytoplasm (Fig. 2-2). However, soon after birth it became chalice-shaped and filled with mucus granules (Figs. 2-3, 2-6).

The basal cells were similar in all cats examined. Structurally, they were triangular and wedged between other cells. They formed the lower layer of the epithelium and never reached the epithelial surface (Figs. 2-5, 2-6). They also were frequently clustered, had centrally located nuclei and little cytoplasm.

A fourth cell type, the serous cell, was found only in *adult cats*. They were few in number and of variable location in the epithelium. They were characterized by a few spherical granules of varying size and electron density (Fig. 2-6). In addition, migratory cells were found lodged between the epithelial cells. These cells were neutrophils, lymphocytes, and globular leukocytes.

The lamina propria mucosae consisted of loose subepithelial connective tissue composed of elastic and collagenous fibers.

Adjacent to this layer was a layer of longitudinally oriented elastic fibers which was confined mainly to the upper region of submucosa. In *prenatal kittens*, this layer consisted of two layers (Fig. 2-7) which gradually increased in thickness postnatally (Fig. 2-8). In the *adult*, it was prominent and consisted of at least four layers condensed into a single lamina (Lamina fibrarum elasticarum) (Fig. 2-9). Elastic fibers were distributed randomly among the collagenous fibers and extended occasionally below this lamina (Fig. 2-8).

Tela submucosa: Collagenous fibers were randomly distributed throughout the submucosa. The tela became denser postnatally and was continuous with the collagen fibers that formed the perichondrium of the cartilage. Among these fibers, numerous small clusters of simple coiled branched tubuloacinar mixed glands extended into the depths of the submucosa both between and overlying the cartilage (Figs. 2-10, 2-11).

Scanning Electron Microscopic Findings

Scanning electron microscopy confirmed the light microscopic findings of the surface epithelium as having at least two cell types which reach the surface, ciliated and goblet cells (Figs. 2-12, 2-15). In addition, since SEM permits the visualization of large areas of the surface epithelium, a third type of cell was identified (Epitheliocytus microvillosus) which was overlooked by light microscopy. These cells were characterized by their microvilli which evenly covered their surface (Figs. 2-12, 2-13).

PLATE 2-2

Fig. 2-7: Tracheal epithelium of a prenatal kitten showing transverse sections of dark stained bundles of elastic fibers. Lamina elastica (LE), Surface epithelium (SE). Paraffin section, Weighert's stain. X1150.

Fig. 2-8: Tracheal epithelium of a 21-day-old kitten. Well-developed elastic fibers are observed in the lamina elastica (LE). Surface epithelium (SE). Paraffin section, Weighert's stain. X 1150.

Fig. 2-9: Tracheal epithelium of an adult cat. Notice the large bundles of elastic fibers forming the lamina elastica (LE). Paraffin section, Weighert's stain. X 1150.

Fig. 2-10: Longitudinal section of the trachea of a prenatal kitten. Tracheal glands (G) are located over and between the cartilage (Ca). Plastic section, H&E stain. X 115.

Fig. 2-11: Tracheal epithelium of a 14-day-old kitten. Note the tracheal glands (G) extending into the submucosa. Plastic section, H&E stain. X 460.

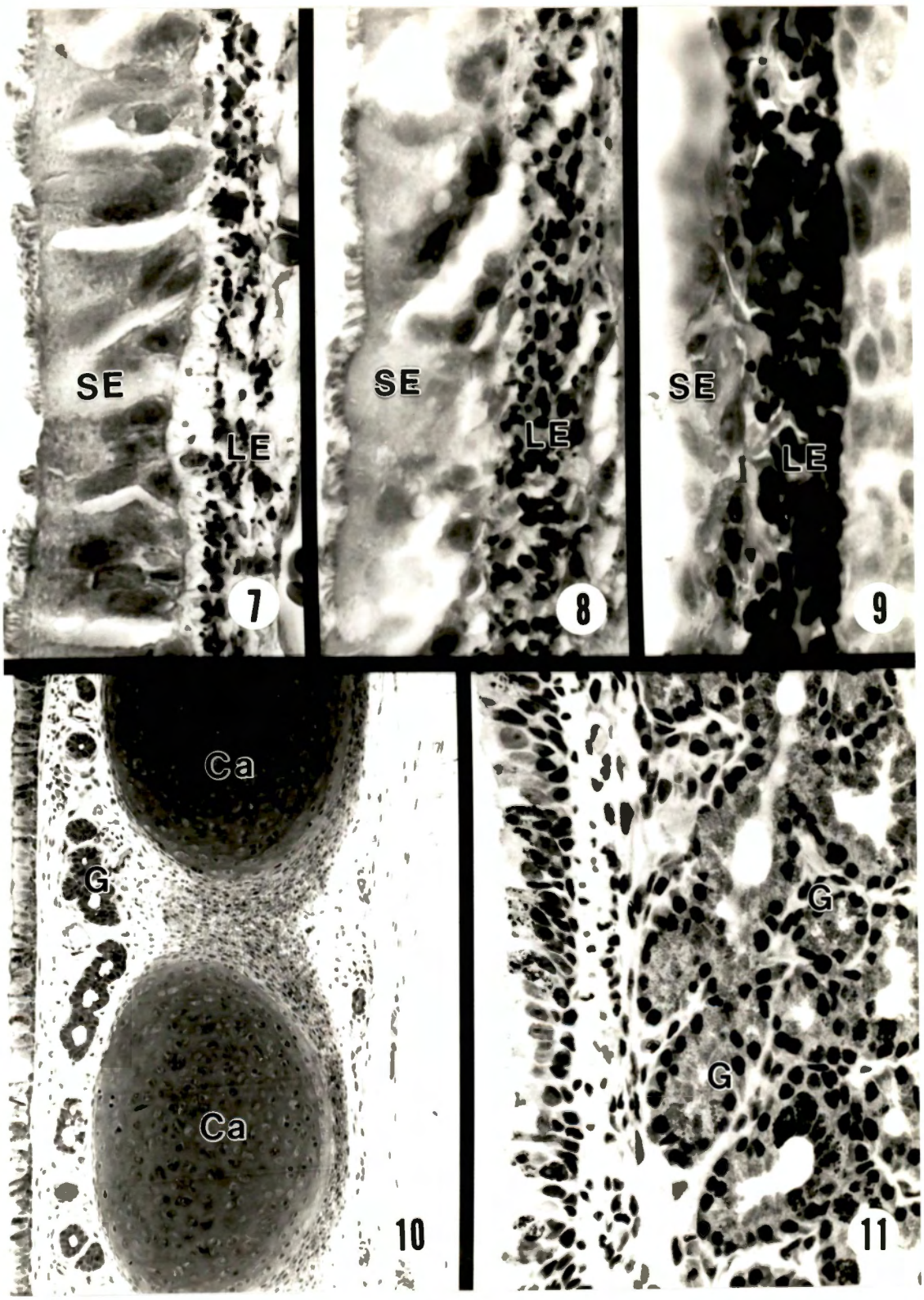

SE
LE
7
SE
LE
8
SE
LE
9
Ca
G
Ca
10
G
G
11

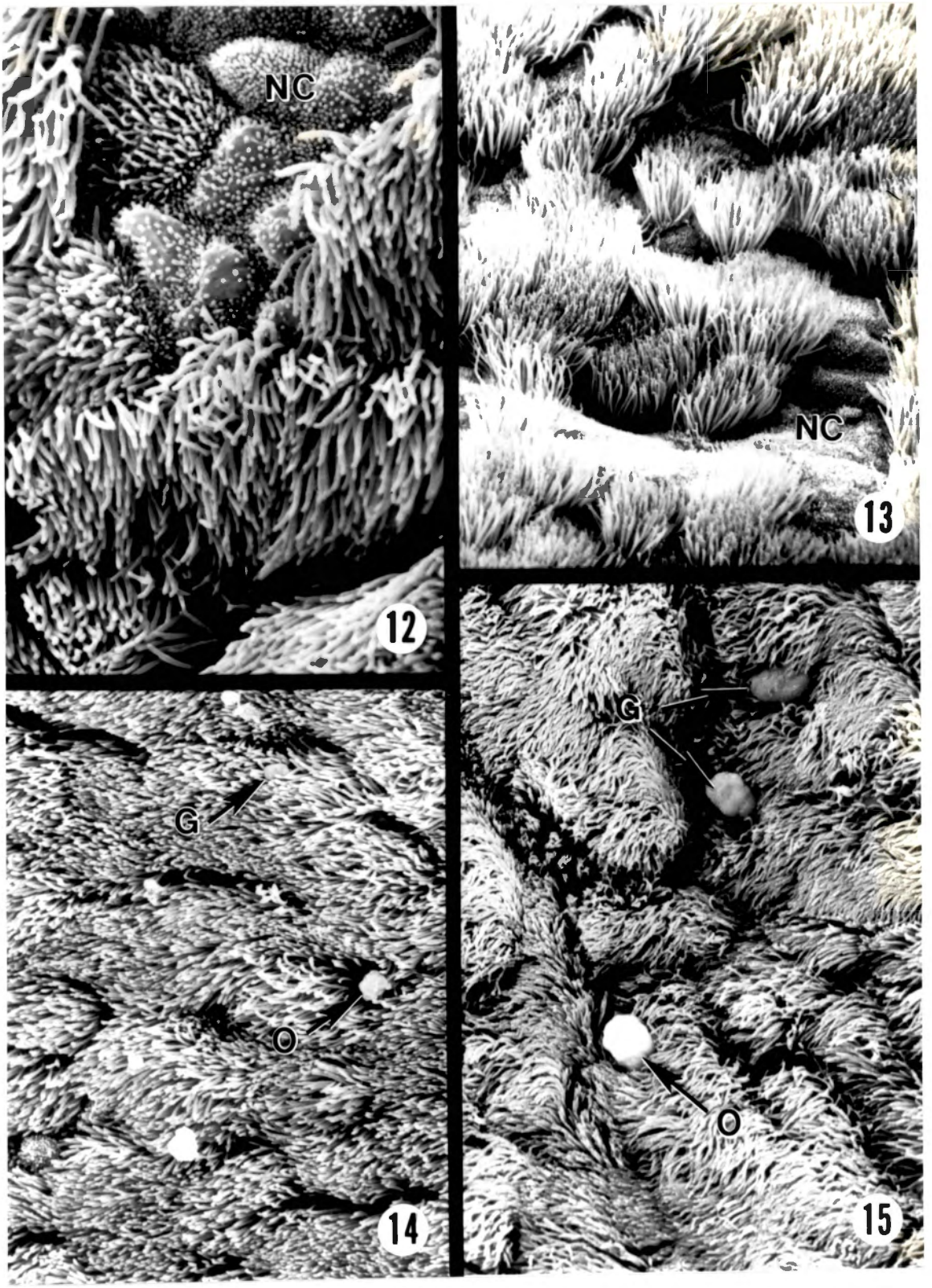
PLATE 2-3

Fig. 2-12: Scanning electron micrograph of the tracheal epithelium of a prenatal kitten. Ciliated and nonciliated (NC) cells are observed. X4200

Fig. 2-13: Scanning electron micrograph of the tracheal epithelium of a 14-day-old kitten. Notice patches of non-ciliated cells (NC) among the ciliated cells. X1950.

Fig. 2-14: Scanning electron micrograph of the tracheal epithelium of a 21-day-old kitten. Numerous cilia cover the surface and have a uniform direction. Goblet cells (G), Ostia of submucosal gland (O). X2250

Fig. 2-15: Scanning electron micrograph of the tracheal epithelium of an adult cat. Goblet cells (G), Ostia of submucosal gland (O). X2100.


NC
12
NC
13
G
14
G
O
15
O

Generally, the surface epithelium of *prenatal kittens* exhibited patches of ciliated cells, but gradually the cilia became denser by 3 weeks of age and similar to those of the adult cat (Fig. 2-14). In prenatal kittens, the surface epithelium had numerous cells with ciliary tufts interrupted by occasional non-ciliated cells. The non-ciliated cells were clearly delineated, polygonal in shape, and evenly covered by microvilli. The cilia on the majority of epithelial cells were of uniform length, but cilia orientation was random. In addition, cells with shorter cilia were occasionally observed (Fig. 2-12).

The surface epithelium of *1- and 7-day-old kittens* was similar except for the orientation of the cilia. However, by day 14, more of the surface was covered by ciliated cells and among them were a few nonciliated cells (Fig. 2-13). By *day 21*, the surface epithelium was similar to that of the adult with a carpet of ciliary tufts interrupted by goblet cells were observed (Fig. 2-14). The cilia were increased in density, which decreased cell delineation. Precipitated mucus, which marked the opening of submucosal tracheal glands, was seen only in 21-day-old kittens and adults (Figs. 2-14, 2-15). In the adult cats, goblet cells were increased in size (Fig. 2-15) and had smooth, dome-like apical surfaces. In addition, many ostia of submucosal tracheal glands were observed.

DISCUSSION

Since early light microscopic studies, it has been known that the respiratory tract mucosa has ciliated and non-ciliated cells. However, the cellular orientation, relative distribution of cell types, and surface topography have been difficult to define by either light or transmission electron microscopy. The present findings indicate that surface structural data are best obtained using SEM. In addition, SEM can be used to describe normal postnatal growth and differentiation of surface morphology of the tracheal lining epithelium.

Quantitative studies show that the height of the epithelium, and the thickness of submucosa over and between the cartilage increased gradually toward adulthood. Pavelka and co-workers (1976) suggest that the thickness of epithelium increases with the size of the animal. In this study, to prove this hypothesis, the above parameters were regressed with two independent variables: the body weight of and the age of the cat. Regression as a function of body weight was significant but not correlated. Thus an increase in body weight does not necessarily produce an increase in the thickness of the lining epithelium.

Only the thickness of the submucosa between tracheal cartilages was strongly correlated with body weight. This was probably due to an increase in the number of acini of the tracheal submucosal glands. However, when regressed as a function of age a significant correlation was observed. These analyses reveal that age was a major

contributor to an increase in the thickness of submucosa and not body weight "size" as described by Pavelka et al. (1976). Although the cats were held in a similar environment, variations among them were found when the means of all parameters were regressed with age. These analyses show strong linear relationships between age and mucosal and submucosal components but not all such relationships were significant. This suggested that other factors including hereditary, played a key role in these variations.

In all ages, the lining epithelium was pseudostratified ciliated columnar with goblet cells. Four cell types were identified at the light microscopic level: ciliated, goblet, basal and serous cells. The serous cell was only observed in the adult cat and was similar to that described in the rat (Jeffery and Reid, 1975; Spicer et al., 1980; Pack et al., 1981). With SEM, a fifth cell type, a nonciliated cell with microvilli was observed. It is possible that this cell is an intermediate stage of differentiation of the ciliated cells. In the rat (Andrews, 1974) and in man (Rhodin, 1966) tracheal cells have surface microvilli prior to the appearance of cilia.

The subepithelial connective tissue was a mixture of collagenous and elastic fibers. The lamina elastica was present in prenatal kittens, but did not become well-developed until adulthood. No folds in the surface epithelium were noticed on the cartilaginous part of the surface epithelium, because it lacked substantial amounts of connective tissue in the submucosa. However, folds were observed in the membranous part, similar to the earlier findings by Tandler et

al., (1983b) in the cat. These folds caused corrugation of the epithelium, however, their functional significance remains unknown. Monkhouse and Whimster (1976) suggest that the corrugation in surface epithelium equalizes tension in the tracheobronchial tree during inspiration and provides elastic recoil during expiration.

Similarities and differences in pulmonary airway structure were noted between the cat and other mammals (Castleman et al., 1975; Becci et al., 1978). In mice (Greenwood and Holland, 1972), scanning observations of the trachea allowed characterization of two predominant cell types: ciliated cells and nonciliated cells covered with microvilli.

The distribution of ciliated cells in the trachea varied with age. Ciliated cells did not increase in number through adulthood. They occurred singly or in groups; but they have a convex luminal surface with few cilia in prenatal kittens and flattened surface in adults. Unlike puppies (Wright et al., 1983), cats were born with almost all of the epithelium ciliated, suggesting that the cilia were functional before birth. A similar finding was described in mice by Kawamata and Fujita (1983) where ciliated cells appeared in the trachea before term. The early appearance of cilia would make the newborn kitten better equipped to resist respiratory disease. No change in average cilia length (5μm) in any age was observed, which is similar to findings by Pavelka et al. (1976) in the adult cat. Occasional ciliated cells had shortened cilia, suggesting that these cells were immature. In the mouse, a shortening of the cilia toward

the lung periphery has been demonstrated by SEM (Greenwood and Holland, 1972). The relatively longer cilia can achieve higher tip velocities than those of more peripheral airways and thereby compensate for the lesser number of ciliated cells found proximally (Greenwood and Holland, 1972; Jeffery and Reid, 1975). In prenatal kittens, the sweep of motion of cilia was directed randomly, but soon after birth, the orientation was uniform as respiration combs the cilia in one direction.

The ciliated epithelial lining of the cartilaginous portions shows a smooth contour in the adult cat which is similar to the findings of Tandler et al. (1983a). In the other age groups, the ciliated cells were either observed in patches or individually, delineated by the microvilli-covered nonciliated cells. Tandler, et al. (1983 a, b) observed regional differences in the morphology between the membranous and cartilaginous portion of the tracheal epithelium of the cat. No mucosal folds were observed over the cartilaginous portion of the trachea due to the lack of substantial submucosal connective tissue.

The number of goblet cell per 100μm of epithelial length declined in developing cats. Pavelka and co-workers (1976) report that the cat has the highest relative number of goblet cells among mammals. However, in this study, no significant change in goblet cell number was observed, which suggests that cats are either born with a fixed number of goblet cells or that goblet cells have a longer life span than other epithelial cells, causing newly formed

cells to be overlooked. The only noticeable change was in their morphology, being columnar and containing fewer mucin granules in prenatal kittens. Soon after birth, the quantity of mucin increased and the cell exhibited the traditional chalice-shape. Microvilli have been observed on the luminal surface of goblet cells of man and the rat (Rhodin, 1966; Andrews, 1974); however, they decrease in number when goblet cells prepare to secrete mucus (Rhodin and Dalhamn, 1956). In this study, using scanning electron micrographs, goblet cells had smooth, dome-like apical membranes with no observed microvilli. Precipitated mucus was observed, suggesting that the submucosal glands were functional.

In conclusion, the kittens were born with their tracheal epithelial linings similar to those of adult cats. The ciliated cells of the prenatal kitten had convex luminal surfaces with few cilia, but soon after birth, they gradually became flattened and covered with numerous cilia. Goblet cells were present in prenatal kittens, and the quantity of mucin granules increased with age. Quantitatively, the number of ciliated and goblet cells was not significantly increased with age. However, the thickness of the epithelium and submucosa was strongly correlated with age, but not with body weight. Only the submucosa between cartilages was strongly correlated with body weight. Variation among growing cats was predicted and hereditary factors were considered a key factor. Future experiments should include ultrastructural studies to identify

changes at the subcellular level in the tracheal epithelium of growing cats.

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III. FINE STRUCTURAL ASPECTS OF THE DEVELOPING FELINE TRACHEAL EPITHELIUM

ABSTRACT

Regardless of age, the lining epithelium of developing cats is pseudostratified ciliated columnar with goblet cells. Seven cell types were identified by their morphological characteristics: light ciliated, dark ciliated, goblet, microvillus-rich, intermediate, serous, and basal cells. Light ciliated cells were observed only in growing cats and were considered an intermediate cell type, destined to become ciliated cells. Light ciliated cells undergoing ciliogenesis had an electron-lucent cytoplasm and few mitochondria. They lacked lateral interdigitations in prenatal kittens. Dark ciliated cells were the predominant cell type and consistently present in all ages. Goblet cells had a few mucous granules in prenatal kittens, but mucous granules became more abundant soon after birth. Microvillus-rich cells lacked cilia, were covered by microvilli, and had a few scattered mitochondria. Intermediate cells were observed in growing cats and were presumed to be progenitors for goblet cells. Serous cells were observed only in the adult and had large characteristic electron-dense cytoplasmic granules. The basal cell was joined to the underlying basal lamina by hemidesmosomes and to the superficial cell layer by desmosomes. Minimal postnatal change was observed in the basal cell, suggesting their early formation.

INTRODUCTION

The epithelial lined airways are responsible for clearing inhaled particulate and gaseous pollutants. The mucosa produces mucus in which pollutants become trapped and hence moved to the pharynx by the action of cilia. The predominant epithelial cells are ciliated and goblet cells. The later are more abundant in the cat (Pavelka et al., 1976) and contain mucin, with a predominance of sulfate esters similar to the dog and man (Jeffery, 1977).

Three cell types contribute to the glycoprotein secretion of the trachea: mucous and serous cells of the submucosal glands and goblet cells of the overlying epithelium (Rhodin, 1966; Gallagher et al., 1975; Lamb, 1975; Jeffery, 1977). Taira and Shibaski (1978) noted that subepithelial glands were usually absent in mice and mucus-containing cells were rare in their trachea. These observations were confirmed by Al-Ugaily et al. (1980) and Pack et al. (1981). Rhodin and Dalhamn (1956) identified four cell types of tracheal epithelium in the rat: ciliated, goblet, brush, and basal cells. Their classification of cells has been adopted for the rat (Jeffery and Reid, 1975) and for other animals (Breeze and Wheeldon, 1977).

With the advent of the transmission electron microscope, eight types of epithelial cells have been identified in animals, as well as, two migratory cell types (Jeffery and Reid, 1975; Jeffery, 1983). Cat airway epithelium has a complete representation of cell types (Jeffery, 1977). Ciliated, mucous, Clara, and basal cells are consistently identified in all mammals (Jeffery 1977, 1983). In the

ferret, Robinson and co-workers (1986) described four cell types: ciliated, basal, goblet, and nonciliated dark cytoplasmic cells. The nonciliated dark cytoplasmic cells are characterized by their dark electron-dense cytoplasm and smaller diameter when compared with ciliated cells. The purpose of this study was to evaluate the morphologic aspects of differentiation and development of the tracheal epithelial cells at the ultrastructural level.

MATERIALS AND METHODS

Thirty-nine domestic cats (Felis catus) representing the six following age groups: prenatal (60 days post-conception), postnatal (1-, 7-, 14-, and 21-day-old), and adult were used in this study. The cats were anesthetized by the intramuscular injection of ketamine (5 mg/kg) and xylazine (0.4 mg/kg). The trachea was cannulated and the lungs were fixed by infusion of 3% glutaraldehyde in phosphate buffer (pH 7.4, 0.1M) at a pressure of 20 cm H_2O. After fixation *in situ* for 30 minutes, a midline incision through the skin and subcutaneous tissue from the larynx to the xiphoid region was performed to expose the trachea and the sternum. The thorax was opened and the lungs with the trachea were carefully dissected free and removed. Tissue samples were taken from the mid-portion of the trachea, cut into small pieces and immersed into the fixative for an additional four hours. The samples were then processed for transmission electron microscopy.

For transmission electron microscopy the samples were washed three times in phosphate buffer (pH 7.4, 0.1M), postfixed in 1% osmium tetroxide in 0.1M phosphate buffer (pH 7.4) for 90 minutes, dehydrated in a graded series of ethanol and acetone, and embedded in an Epon-Araldite mixture. Thin sections were stained with 2% (w/v) aqueous uranyl acetate, contrasted with lead citrate, and viewed by Phillips electron microscope Model 201 working at 60 kV.

RESULTS

In *all ages*, the surface epithelium was pseudostratified with both ciliated and goblet cells lying on a common basement membrane. The ciliated cells had an extensive basal surface. Whereas, goblet cells reached the basement membrane through a narrow cytoplasmic extension. Basal cells were interposed between these cells and the basement membrane.

The ciliated cells of the *prenatal kittens* were columnar (Fig. 3-1) and their apices were convex with a few cilia surrounded by short microvilli (Fig. 3-2). Their lateral surfaces interdigitated with adjacent cells, leaving an expanded intercellular space near the basal cells. Two types of ciliated cells were identified by the electron density of their cytoplasm: dark ciliated cells (DC) and light ciliated cells (LC).

The Dc cells were characterized by an electron-dense cytoplasm, basally located nucleus with an irregular outline, and a prominent nucleolus (Figs. 3-1, 3-2). The apical cytoplasm contained abundant

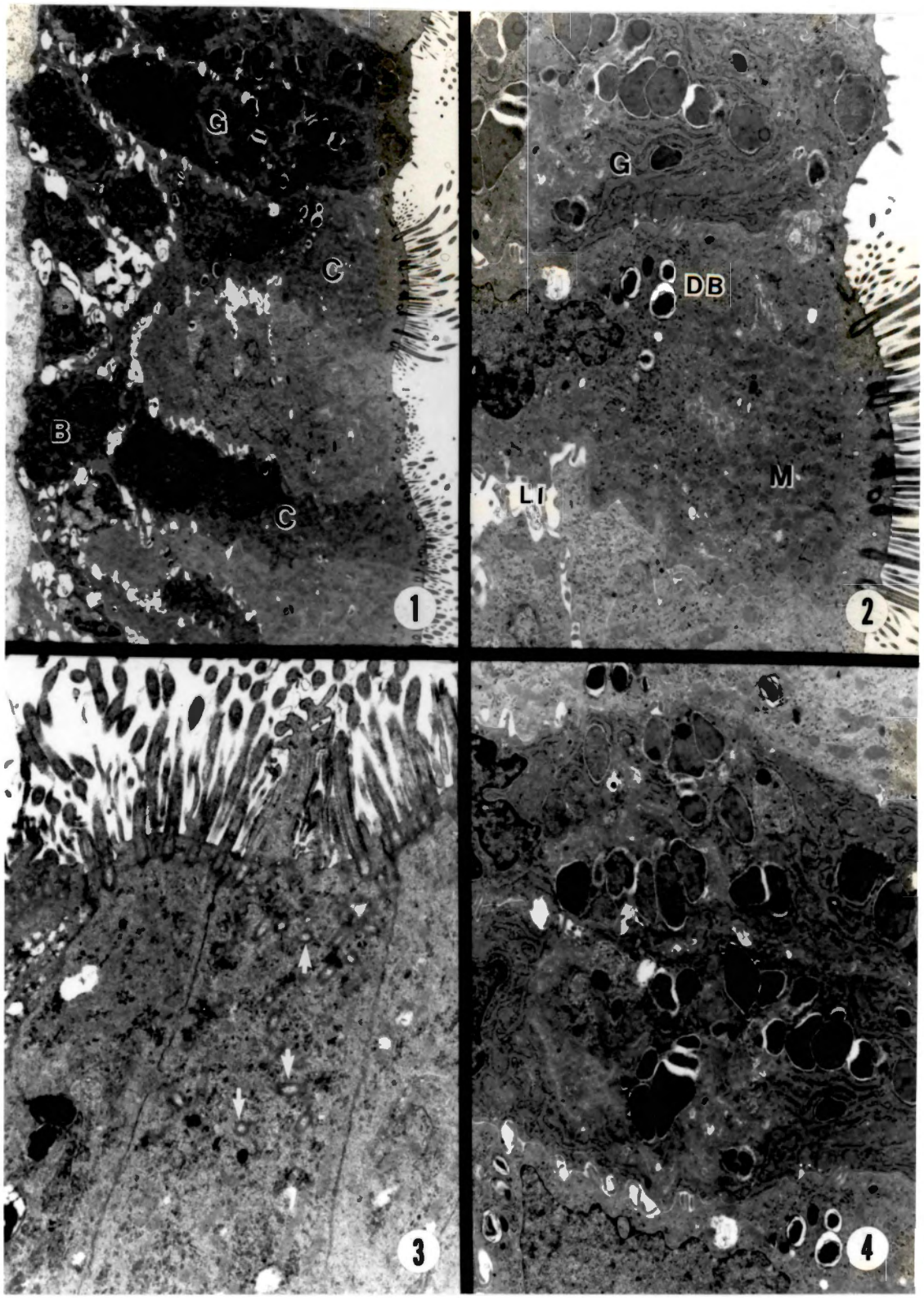
PLATE 3-1

Fig. 3-1: Transmission electron micrograph of prenatal tracheal epithelium. Two ciliated cells (C) surround a cell undergoing ciliogenesis. Goblet cell (G), Basal cell (B). X3,000

Fig. 3-2: Transmission electron micrograph of a ciliated cell of prenatal tracheal epithelium with apical mitochondria (M) and supranuclear dense-bodies (BD). Lateral interdigitations (LI), Goblet cell (G). X10,500

Fig. 3-3: Transmission electron micrograph of a ciliated cell of prenatal tracheal epithelium with apical cytoplasmic extensions and undergoing ciliogenesis. Note the large number of basal bodies deep in the cytoplasm (Arrows). The cell lacks lateral interdigitations. X15,000

Fig. 3-4: Transmission electron micrograph of two goblet cells of prenatal tracheal epithelium containing mucous granules which vary in size and shape. Some granules exhibit electron-dense granules. Rough endoplasmic reticulum (rER). X10,500


G
C
B
C
1
G
DB
M
LI
2
3
4

mitochondria of varying size and shape and a well-developed Golgi apparatus. Dense bodies of varying sizes were observed above and below the nucleus, being more abundant in the apical cytoplasm (Fig. 3-2). Near the luminal surface numerous basal bodies were arranged linearly with cilia projecting from them into the lumen.

The LC cells had a lighter cytoplasm with similar morphology to the Dc cells. However, the LC cells contained fewer mitochondria and dense-bodies. LC cells were observed with fewer basal bodies and no cilia (Fig. 3-1), while others had undergone ciliogenesis with their basal bodies scattered in the apical cytoplasm and thus having fewer cilia. Cytoplasmic extensions were observed on these cells and often they lacked lateral interdigitations (Fig. 3-3).

Goblet cells were columnar and were observed singly or in pairs. They varied in size and electron density. Their electron-dense cytoplasm contained large numbers of membrane bound mucous granules in the apical cytoplasm, while some contained small eccentrically-located electron-dense granules (Fig. 3-4). Most of organelles were confined to the apical cytoplasm. Elongated mitochondria with an electron-dense matrix and few cristae were observed in the apical cytoplasm (Figs. 3-2, 3-4). Long profiles of rER were found in the perinuclear region and were scattered among the mucous granules in the apical and lateral cytoplasm (Fig. 3-4).

The basally-located electron-dense nucleus had an irregular outline (Fig. 3-1). The apical surface was convex and displayed microvilli (Fig. 3-2). Goblet cells interdigitated with adjacent

cells. They were joined by tight junctions near the luminal surface and joined by desmosomes to the basal cells (Fig. 3-5). The basal one half of the cell was separated by a prominent intercellular space (Figs. 3-1, 3-4).

The basal cells were either ovoid or triangular, occurred either singly or in groups of three, and were wedged between adjacent epithelial cells. They were characterized by an electron-dense cytoplasm and a large, irregular, centrally-located electron-dense nucleus. The cells were separated by a pronounced intercellular space, but were joined by desmosomes to adjacent cells and hemidesmosomes to the underlying basement membrane (Fig. 3-5).

In *1-day-old kittens*, DC cells and LC cells were observed. The DC cells had similar morphology to prenatal cells, but their luminal surface was covered by more cilia. In their cytoplasm, increased numbers of electron-dense bodies were located apically and lateral to the nucleus. The nucleus of the DC cell was indented, elongate and irregular in outline. Whereas, the nucleus of the LC cell was small and rounded, and its cytoplasm contained a few small electron-dense granules (Fig. 3-6).

Seven days later, the luminal surface of most ciliated cells was irregular. In addition to dense cilia, cytoplasmic extensions with small microvilli were observed. The LC cell contained increased numbers of dense-bodies which varied in size and shape. Large dense-bodies were located either immediately above the nucleus or in the central cytoplasm, whereas small dense-bodies were concentrated near

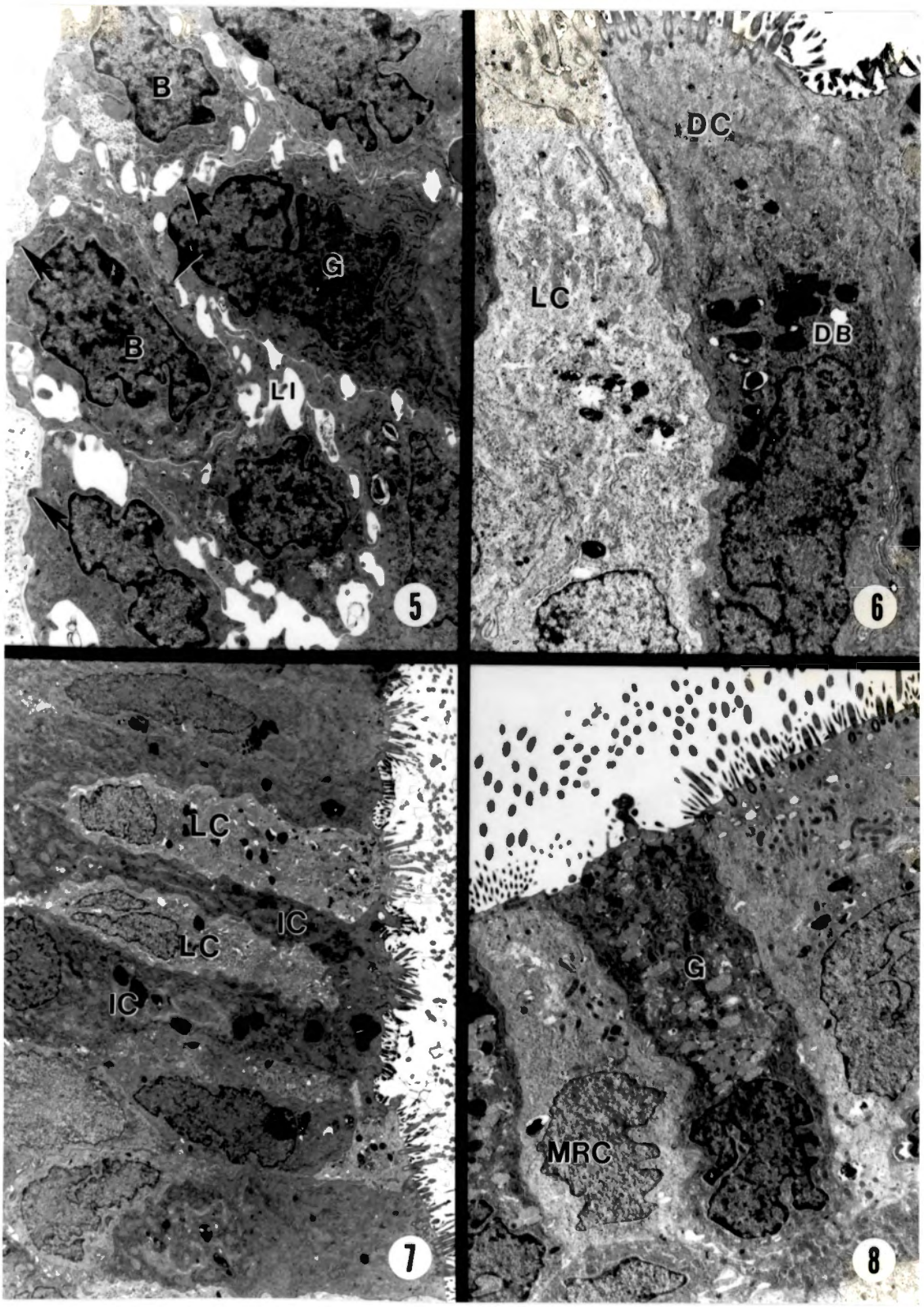
PLATE 3-2

Fig. 3-5: Transmission electron micrograph of basal cells (B) of prenatal tracheal epithelium resting on a thin basement membrane and joined to it by hemidesmosomes (arrows). The basal cell is joined to a goblet cell (G) by desmosomes (arrowhead). Note the large intercellular space interrupted by lateral interdigitations (LI). X10,500

Fig. 3-6: Transmission electron micrograph of tracheal epithelium of 1-day-old kitten with dark ciliated (DC) and light ciliated (LC) cells. Dense-bodies (DB). X10,500

Fig. 3-7: Transmission electron micrograph of tracheal epithelium of 7-day-old kitten with two light ciliated (LC) and intermediate cells (IC). X4,500

Fig. 3-8: Transmission electron micrograph of tracheal epithelium of 7-day-old kitten. Note the right cell contains cilia and an abundance of mitochondria, vesicles and dense-bodies; and the microvillus-rich cell (MRC) with an abundance mucous granules. X15,000.


B
G
B
LI
5
DC
LC
DB
6
LC
IC
LC
IC
7
G
MRC
8

the apical surface (Fig. 3-7). In addition, vesicles of varying size were observed near the luminal surface of the LC cell (Fig. 3-8). The LC cell nuclei was sometimes indented.

Microvillus-rich cells (MRCs) were characterized by the presence of microvilli covering their luminal surface. Mitochondria were scattered throughout the cytoplasm and occasional dense-bodies were aggregated in their apical cytoplasm. In addition, profiles of rER and Golgi apparatus were observed (Fig. 3-8).

Intermediate cells (IC) were columnar with a basally-located, indented nucleus. The characteristic dark cytoplasm contained scattered electron-dense granules whose granular contents partially coalesced. The IC luminal surface was covered by long slender microvilli. The electron-dense nucleus of the IC had clumps of heterochromatin attached to the inner nuclear membrane (Fig. 3-7). Goblet cells were well-developed with a dark,indented, irregular nucleus. Its apical cytoplasm was filled with mucous granules of low electron density which pushed the nucleus basally (Fig. 3-8).

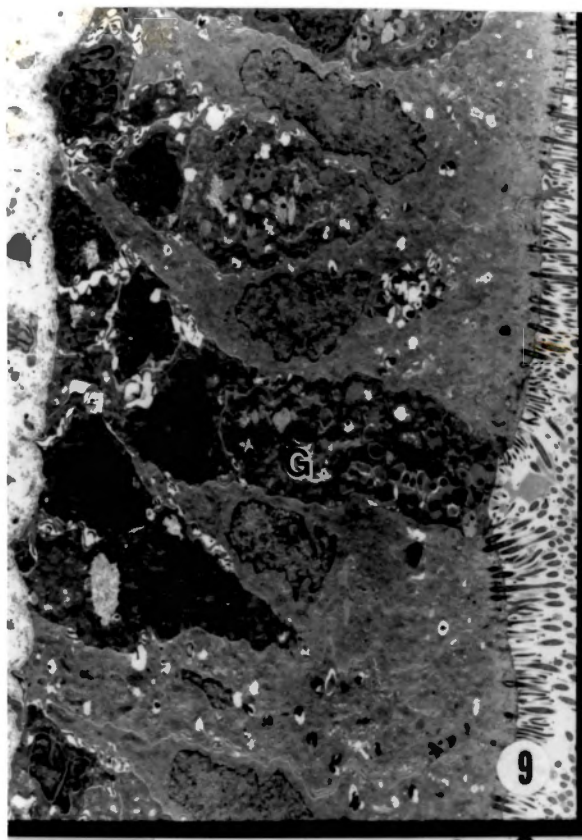
At *14 days of age*, the luminal surface of the ciliated cells was flattened and covered with numerous microvilli which sometimes branched near their base. Dense-bodies were observed either grouped immediately above the nucleus or scattered throughout the cytoplasm. Ribosomes filled the cytoplasm and a few mitochondria were concentrated in the apical cytoplasm. Goblet cells had large numbers of mucous granules of different sizes which contained a pale

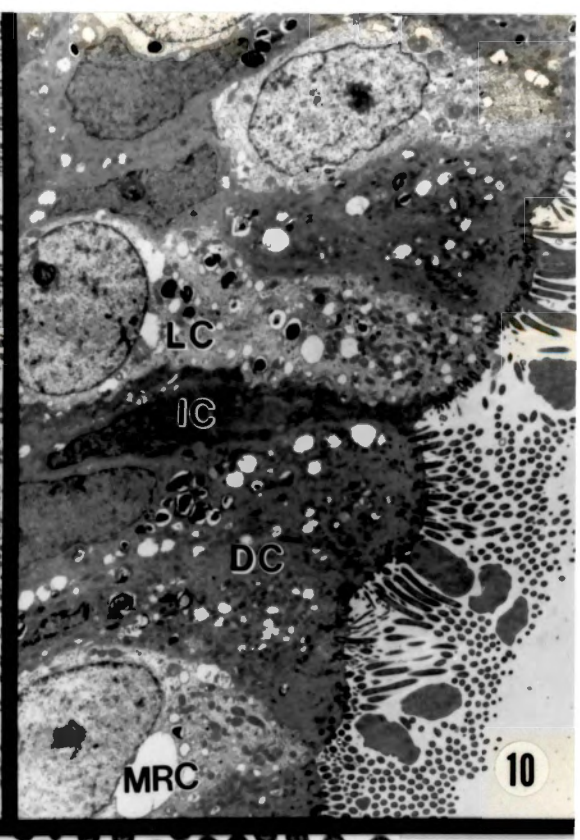
PLATE 3-3

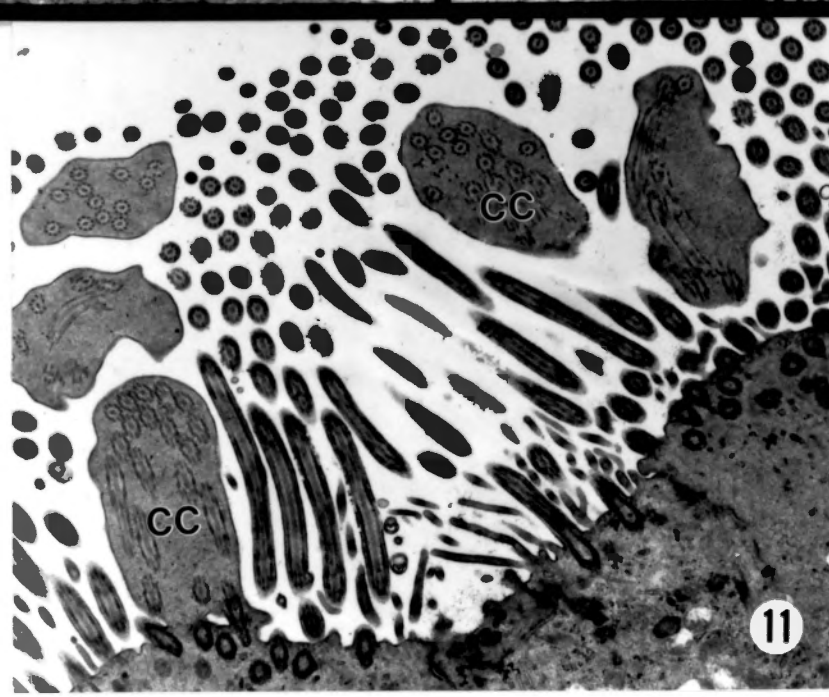
Fig. 3-9: Transmission electron micrograph of tracheal epithelium of 14-day-old kitten with a well-developed goblet cell (G) which contains abundant mucous granules and some granules contain electron-dense granules. X4,500.

Fig. 3-10: Transmission electron micrograph of tracheal epithelium of 21-day-old kitten. Light ciliated cells (LC) have a rounded nucleus, and the dark ciliated cells (DC) have compound cilia. Microvillus-rich cell (MRC), Intermediate cell (IC). X6,750

Fig. 3-11: Transmission electron micrograph of tracheal epithelium of 21-day-old kitten. The compound cilia (CC) contain between 10 to 20 cilia cut in longitudinal and cross sections. X15,000


G
9


LC
IC
DC
MRC
10


CC
CC
11

homogeneous material with electron-dense eccentric granules (Fig. 3-9).

In *21-day-old kittens*, the ciliated cells were well developed. The DC cells had compound cilia that contained 10 to 20 cilia (Figs. 3-10, 3-11). Large numbers of mitochondria and vacuoles were scattered throughout the cytoplasm. Dense-bodies were confined near the apical aspect of the nucleus. LC cells were increased in number and had lightly-stained, large, rounded, basally-located nuclei with a prominent nucleoli. The chromatin was evenly scattered throughout the nucleus. Abundant mitochondria were observed in the apical cytoplasm. A few dense-bodies, scattered throughout the cytoplasm, contained electron-dense material and electron-lucent vesicles. MRCs were also observed and had similar morphology to those of 7-day-old kittens, with the exception of their nucleus, which was rounded with evenly-distributed chromatin and a prominent nucleolus. Intermediate cells were similar to those in 7-day-old kittens (Fig. 3-10).

In the *adult*, only DC cells were present and contained oval and elongated mitochondria (Fig. 3-12). Immediately above the nucleus, large numbers of dense-bodies of differing electron density were present. Interdigitations between cells was not pronounced even in the basal area (Figs. 3-12, 3-14). Large irregular cytoplasmic extensions projected from the apical surface of some cells. These extensions had microvilli and blebs of different sizes and shape and contained a few basal bodies and ribosomes (Fig. 3-13).

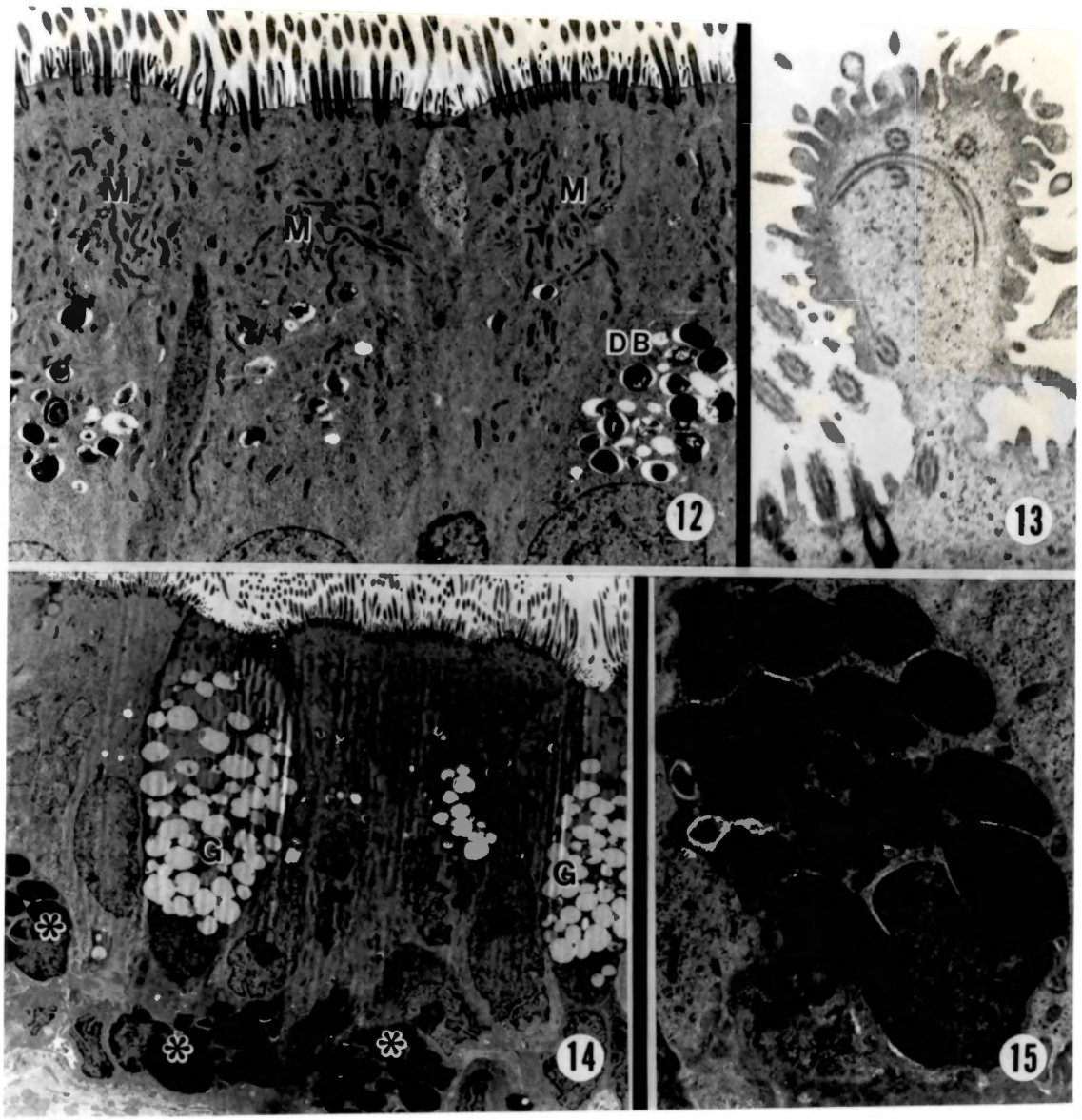
PLATE 3-4

Fig. 3-12: Transmission electron micrograph of tracheal epithelium of the adult cat. Note elongated mitochondria (M) and supranuclear dense-bodies (DB). X6,750.

Fig. 3-13: Transmission electron micrograph of ciliated cell of the adult tracheal epithelium with cytoplasmic extensions containing cilia and covered with microvilli and cytoplasmic blebs. X4,500

Fig. 3-14: Transmission electron micrograph of tracheal epithelium of the adult cat. Note well-developed goblet cells (G) packed with mucous granules of varying electron densities. Many serous cells (*) are basally located. X4,500

Fig. 3-15: Transmission electron micrograph of tracheal epithelium of the adult cat. Epithelial serous cell has large electron-dense granules, displacing the nucleus to one side of the cell. X10,500


M
M
M
DB
12
13
G
G
14
15

The goblet cells were well-developed and pear shaped. The apical surface was covered by microvilli. The cell had a small electron-dense nucleus which was pushed basally by the abundance of cytoplasmic mucous granules. The mucous granules were of different sizes and packed together so that the cytoplasmic matrix was hard to observe (Fig. 3-14). Granules were biphasic with darkly stained granules aggregated near the apical surface, whereas lightly stained granules were concentrated above the nucleus.

The epithelial serous cell (ESC) was observed only in the adult and was characterized by numerous electron-dense cytoplasmic granules of varying sizes which obscured the cytoplasmic matrix and left little space for other organelles. The mitochondria had an electron-dense matrix and abundant well-developed cristae (Figs. 3-14, 3-15). The often indented nucleus was small and eccentric, and had abundant heterochromatin attached to the inner nuclear membrane (Fig. 3-15).

DISCUSSION

The morphological features of postnatal development of tracheal epithelium was investigated by transmission electron microscopy (TEM) in prenatal, postnatal, and adult cats. Regardless of age, the lining epithelium was pseudostratified ciliated columnar with goblet cells. The degree of postnatal development was minimal; as the cats were born with nearly mature adult epithelium. It appears that most of the epithelial changes occur prior to 60 days postconception. Generally, the morphological appearance of the tracheal epithelium

was similar to that described for other species (Jeffery and Reid, 1975; Breeze and Wheeldon, 1977).

The ciliated cells were covered with microvilli prior to ciliogenesis. The early presence of microvilli leads to the presumption that the trachea commences its physiological function in utero. The microvilli might be involved in absorption of amniotic fluid and/or regulation of intracytoplasmic fluid.

Of the eight types of cells that have been identified in the epithelial lining of proximal conducting airways (Breeze and Wheeldon 1977; Jeffery, 1983), seven were identified in the trachea of the cat. These cells were ciliated and nonciliated cells. In the growing cats, the ciliated cells were further subdivided into two types according to the electron density of their cytoplasm: light and dark ciliated cells. These cells have not been described in other animals. The nonciliated cells include the following cells: microvillus-rich, goblet, intermediate, serous, and basal cells.

The ciliated cells of the growing cat were similar to those of other species including man (Rhodin, 1966; Frasca, 1968 a, b; Jeffery and Reid, 1975; Kawamata and Fujita, 1983). Ciliated cells form early in utero in mammals including man (Sorokin, 1961; Bucher and Reid, 1961). Ciliated cells of the cat apparently formed prior to the date of sample collection from prenatal kittens. Similar findings (Plopper et al., 1986) suggested that ciliated cells were already differentiated in 120 days gestational age in Rhesus monkeys. Although most of the ciliated cells mature before birth, ciliogenesis

was observed in some cells postnatally. Two forms of ciliogenic cells were observed prenatally. Those with basal bodies either lined up near the apical border with no cilia projecting from them or with basal bodies scattered throughout the cytoplasm, yet few cilia covered the apical cytoplasm. The second cell form had electron-lucent cytoplasm and lacked lateral interdigitations.

Lateral interdigitations of tracheal epithelium were well-developed except in those cells undergoing ciliogenesis. Similar interdigitations were observed by Inoue and Hogg (1974) and Breeze and Wheeldon (1977). Plopper and coworkers (1980a) observed lateral interdigitations in Clara cells of other animals. It has been speculated that the function of these lateral extensions in the trachea (Inoue and Hogg, 1974 and in bronchioles (Kilburn, 1974) is for fluid absorption.

The light ciliated cells were present only in growing cats. They have electron-lucent cytoplasm and are considered to be progenitor cells of the dark ciliated cells. Similar findings were observed by Frasca (1968) and Sorokin (1968) as cells became more electron-lucent with the onset of ciliogenesis. It is possible that these cells fall into an intermediate cell category classified by Jeffery (1983) and described earlier in the rat by Jeffery and Reid (1975).

Dark ciliated cells were the most frequent ciliated cell observed in pre- and post-natal kittens and were the only ciliated cell observed in the adult cat. These cells have an electron-dense

cytoplasm. The mitochondria of the adult Dc cells were elongated rods and aggregated in the apical cytoplasm. Occasional cytoplasmic vacuoles were observed in the ciliated cells of growing cats. No connections to the cell surface were observed; hence, these vacuoles do not appear to be intracytoplasmic lamina which occur in the early stages of ciliogenesis (Sorokin, 1968). Similar vacuoles have been described in human nasal epithelium (Boysen and Reith, 1980).

In the growing cat, dark cytoplasmic cells were ciliated and lacked granules. Therefore it is presumed that these cells were fully differentiated ciliated cells. Dark cytoplasmic cells have been described in the tracheobronchial tree of the ferret (Robinson et al., 1986). These cells were observed with or without granules and were proposed to constitute a single cell type. Previous reports had suggested that these cells could differentiate into goblet or ciliated cells (Rhodin, 1966). Dark cytoplasmic cells have been renamed as intermediate cells (Jeffery, 1983).

Occasional microvillus-rich cells were observed only in the growing cats. They were characterized by a few scattered mitochondria and numerous microvilli covering their luminal surface. Tandler and co-workers (1983 a, b) described these cells in adult cats. However, they were abundant in the membranous portion of the trachea.

Goblet cells were identified by the presence of mucous granules and the absence of cilia. They had dark cytoplasm, long profiles of rER, and a basally-located, irregular nucleus. Like ciliated cells,

goblet cells develop prior to birth. The exact time of their differentiation was prior to the ages used in this study. Goblet cells of prenatal kittens contained a few electron-dense granules, but granule numbers gradually increased until 21 days of age when the goblet cells became filled with mucous granules. Unlike the rat, many goblet cells of the cat contained a mixture of electron-lucent and electron-dense granules within the same cell. Jeffery (1978) had similar findings in the adult cat, and he suggested there was a mixture of distinct types of glycoproteins within the same cell.

The secretory granules of goblet cells show heterogenicity and often display an electron-dense core. These granules are similar to granules of the Paneth cells of the gut (Jeffery and Reid, 1975, 1977) and display an outer halo surrounding a dense core. The halo is composed of an acidic glycoprotein and the dense core of a neutral glycoprotein or noncarbohydrate osmiophilic material which may be lipid (Spicer et al., 1983, Jeffery, 1987).

Lamb (1975) observed that the goblet cells of the cat tracheal epithelium contained acidic glycosaminoglycans similar to those found in the dog (Wheeldon et al., 1976) and in the ferret (Robinson et al., 1986), in the bronchus of mouse (Pack et al., 1981) and in the upper trachea of the rat (Jeffery and Reid, 1975). However, their functional significance is not known.

In the cat, the most frequent epithelial secretory cell is the goblet cell, which comprises 7-15% of all epithelial cells in the larger airways (Jeffery, 1978). In the trachea of the mouse, the

abundant secretory cell is the nonciliated Clara cell, being virtually devoid of any mucous or serous cells (Breeze and Wheeldon, 1977). However, in the rat, the most frequent type of nonciliated secretory cell in the lower part of the trachea is the serous cell (Pack et al., 1981). In the ferret, the principle type of secretory cell in the upper trachea is the granule-containing dark cytoplasmic cells; whereas in lower trachea and bronchus, the goblet cell is the principle secretory cell.

The basal cell accounts for the pseudostratified appearance of the surface epithelium. Blenkinsopp (1967) has shown that basal cells act in a similar manner as the germinal layer of the epidermis. Division of this cell gives rise to more superficial layers (Inayama et al., 1988). Basal cells of the cat follow the developmental pattern of other cells and were observed in 60-day post conception kittens. Basal cells of the rat trachea appear at about 21 days gestation as a well-defined layer (Jeffery and Reid, 1977). In the rabbit, Leeson (1961) reported that basal cells were not present until term. In this study, the basal cells of the cat were attached by hemidesmosomes to the basal lamina and joined to superficial cells by desmosomes. In bronchi of both control and smoking dogs, Frasca et al. (1986 a, b) observed that basal cells were attached to the basal lamina via hemidesmosomes. Kawanami et al. (1979) observed hemidesmosomes in all of the structural components of canine respiratory epithelium.

In the rat, Lesson (1961) and Rhodin and Dalhamn (1956) proposed that basal cells were either lymphocytes or migrating macrophages. The presence of lymphocytes as a normal component of respiratory epithelium has been confirmed (Jeffery and Reid. 1975). It is now generally accepted that most of the basally situated cells are epithelial in origin and are not wandering cells from the mesenchyme (Rhodin, 1966; Baskerville, 1970, Jeffery and Reid, 1975). In the cat, it was morphologically confirmed that basal cells were part of the epithelial layer and not migratory lymphocytes.

Epithelial serous cells were observed only in the adult. They resemble serous cells of the submucosal gland, which are common in the rat (Jeffery and Reid, 1977) and in mouse (Pack et al., 1981). The serous cells of the adult cat, unlike those in the mouse which are near the lumen (Robinson et al, 1986), are in close apposition to the basal surface of the epithelium. They are characterized by the presence of large electron-dense granules filling the cytoplasm and pushing the nucleus toward the periphery of the cell. It has been shown that serous cell granules contain carbonic anhydrase enzyme which indicates that the serous cells function in the modulation of ions, pH and water. The serous secretion is normally of low viscosity and contribute to the sol phase (periciliary fluid layer) of airway secretions (Spicer et al., 1982).

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IV. POSTNATAL DEVELOPMENT OF FELINE BRONCHIAL EPITHELIUM: CORRELATIVE LIGHT, SCANNING AND TRANSMISSION ELECTRON MICROSCOPIC STUDY

ABSTRACT

The ciliated cells of the adult cat were covered with microvilli which often branched near or away from the cell surface. Although, the cats were born with epithelium which resembled that of the adult, ciliogensis was observed in both growing and adult cats. On SEM, the density and location of cilia and microvilli on the ciliogenic cell was variable. Goblet cells were covered with microvilli in the early stages of their secretory cycle but lost microvilli when their apical surface extended beyond adjacent cell surfaces. Serous cells were observed near the luminal surface of adult bronchi, and cytoplasmic islands were observed suggesting a group of granular secretion. Submucosal glands were present in all ages. However, they were best-developed in adult cats and extended distally into the medium-sized bronchioles. Bronchial-associated lymphoid tissue was not observed in growing cats. In the adult, it extended into the terminal bronchiole and into the adventitia of pulmonary vessels. It was of nodular and diffuse forms and was located below the muscular layer of the airways. The nodular form had distinct cortical and medullary zones. The diffuse form consisted of mononuclear cells scattered among the acini of submucosal glands of bronchi and bronchioles. Other cells were also observed including: nonciliated bronchiolar, microvillus-rich, and basal cells.

INTRODUCTION

The fetal respiratory epithelium begins as a single layer of columnar cells (Leeson, 1961) derived from an endothelial pouch. Subsequently, the epithelium becomes pseudostratified columnar consisting of double layer of nuclei (Rhodin and Dalhamn, 1956; Frasca et al., 1968a, b). The basal cells comprise the deepest layer; columnar cells (ciliated and microvilli covered cells) form the upper layer. All cells abut the basal lamina, but only the columnar cell reaches the airway.

Several reports on the fine structure of the bronchi of rats, man and dogs have been published (Rhodin and Dalhamn, 1956; Rhodin, 1959, 1966; Watson and Brinkman, 1964; Frasca et al., 1968a, b). Some differences are noted between respiratory epithelium of these species. The structure of bronchi has not been studied in cats, especially in the early postnatal periods. Consequently, this study, as part of a broad research project on pulmonary morphogenesis, was designed to describe the early postnatal growth of bronchial epithelium in cats and to provide a detailed baseline upon which to assess structural alternations in kittens during disease.

MATERIALS AND METHODS

Thirty-nine domestic cats (Felis catus) representing the six following age groups: prenatal (60 days post-conception), postnatal (1-, 7-, 14-, and 21-day-old), and adult were used in this study. The cats were anesthetized by the intramuscular injection of ketamine

(5 mg/kg) and xylazine (0.4 mg/kg). The trachea was cannulated and the lungs were fixed by infusion of 3% glutaraldehyde in phosphate buffer (pH 7.4, 0.1M) at a pressure of 20 cm H_2O. After fixation *in situ* for 30 minutes, a midline incision through the skin and subcutaneous tissue from the larynx to the xiphoid region was performed to expose the trachea and the sternum. The thorax was opened and the lungs with the trachea were carefully dissected free and removed. Tissue samples were taken from the principle bronchus and the caudal lobe of the lung, cut into small pieces, and immersed into the fixative for an additional four hours. The samples were then processed for light, scanning and transmission electron microscopy.

For light microscopy, glutaraldehyde fixed tissue was washed three times in phosphate buffer (pH 7.4, 0.1M). Subsequently, the samples were dehydrated in a graded series of ethanol (50%, 70%, 95%; two changes at 10 minutes each). Infiltration of the samples was done at room temperature using standard JB-4 methods (Polysciences Inc., Warrington, PA). Sections, 3μm thick, were taken at 25μm intervals from each block with glass knives using a Reichert Jung Autocut microtome, model 1140, and stained with hematoxylin and eosin and periodic acid-Schiff reagent.

Glutaraldehyde fixed samples for scanning electron microscopy were postfixed for 90 minutes in 1% osmium tetroxide in phosphate buffer (pH 7.4, 0.1M). They were washed three times in phosphate buffer for 10 minutes each, then dehydrated in a graded series of

acetone solutions (70%, 80%, 95%, with three changes in 100% acetone). The samples were dried in a critical point drying apparatus with liquid CO_2. Tissues were then mounted on aluminum stubs, coated with a 15nm gold-palladium layer, and examined with an ETEC Scanning Electron Microscope operating at 25 kV.

For transmission electron microscopy the samples were washed three times in phosphate buffer (pH 7.4, 0.1M), postfixed in 1% osmium tetroxide in phosphate buffer (pH 7.4, 0.1M) for 90 minutes, dehydrated in a graded series of ethanol and acetone, and embedded in an Epon-Araldite mixture. Thin sections were stained with 2% (w/v) aqueous uranyl acetate, contrasted with lead citrate, and viewed by Phillips electron microscope, model 20, working at 60 kV.

RESULTS

Light microscopic findings:

Bronchi and bronchioles were divided into large, medium, and small based on their diameter when cut in a transverse section (Table 4-1). In addition, Table 4-2 and Figure 4-1 present the morphological criteria by which each segment was identified.

In *prenatal kittens*, the lining epithelium of large bronchi was thrown into longitudinal folds of variable height. The epithelium was pseudostratified ciliated columnar with goblet cells which rested on a basement membrane and was surrounded by a lamina propria of

Table 4-1: Ranges of airway diameter of developing cat lung.

AGE	LARGE (mm)	MEDIUM (mm)	SMALL (mm)
		BRONCHI	
Prenatal	1.6-1.00	0.45-0.26	0.24-0.20
1-day-old	1.7-1.00	0.65-0.40	0.37-0.20
7-day-old	1.3-0.50	0.50-0.25	0.25-0.18
14-day-old	1.3-0.64	0.36-0.30	0.27-0.24
21-day-old	1.8-0.70	0.70-0.32	0.29-0.20
Adult	3.3-1.00	1.00-0.50	0.46-0.40
		BRONCHIOLES	
Prenatal	0.18-0.16	>0.15	>0.09
1-day-old	0.19-0.17	>0.16	>0.11
7-day-old	0.18-0.16	>0.16	>0.11
14-day-old	0.22-0.18	>0.18	>0.12
21-day-old	0.20-0.16	>0.16	>0.10
Adult	0.39-0.30	>0.30	>0.20

Table 4-2: Morphological features of feline air-conducting airways. Pseudostratified ciliated columnar (PSCC), Simple columnar (SCOL), simple cuboidal (SCUB). Parameters were graded using the following scale: 4+ = high, 1+ = low, - = not present.

	BRONCHI			BRONCHIOLES		
	LARGE	MEDIUM	SMALL	LARGE	MEDIUM	SMALL
EPITHELIUM	PSCC	PSCC	SCOL	SCOL	SCOL/SCUB	SCUB
CILIATED CELL	++++	+++	+++	+++	++	+
GOBLET CELL	++++	+++	++	++	+/-	-
FOLD	++++	+++	++	+++	++	+
SMOOTH MUSCLE	++++	+++	++	+++	++	+
GLAND	++++	+++	++	++	+	-
LYMPH NODE	+++	++	++	++	++	+
CARTILAGE	++++	+++	+	-	-	-

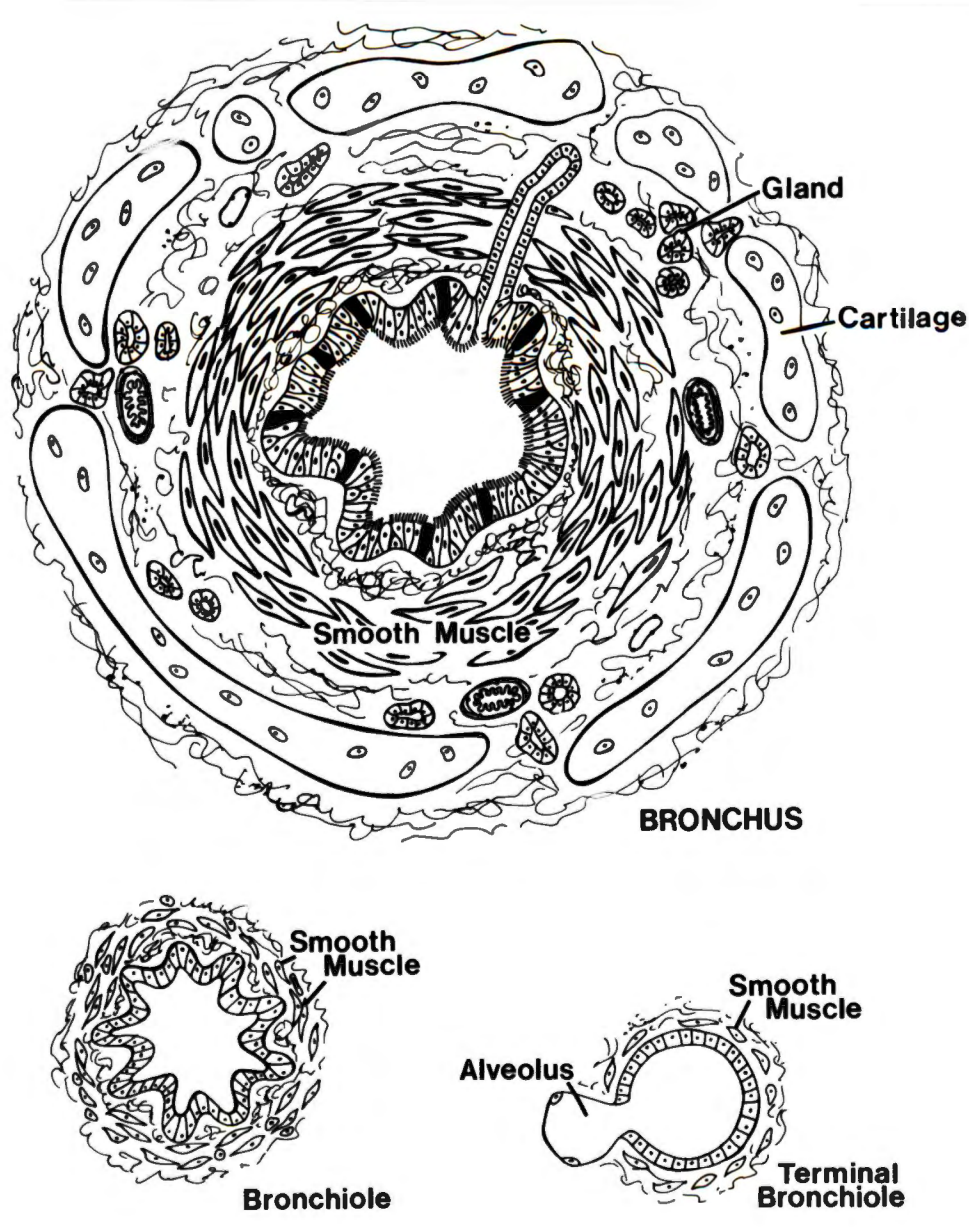


Fig. 4-1: Schematic drawing of bronchial generation, showing typical morphological features for their identification.

loose connective tissue. Subjacent to this layer was a layer of circularly oriented smooth muscle fibers (Fig. 4-3). Tubuloacinar submucosal glands were few and tended to be located below the smooth muscle layer and in the connective tissue peripheral to the cartilaginous plates. The epithelium gradually changed to columnar and simple cuboidal with a concomitant decrease in the number of goblet cells and folds, and in quantity of cartilage as a bronchus decreased in size (Table 4-2). Bronchioles were identified by their lack of cartilage and simple epithelial lining which varied in height. Goblet and ciliated cells were rare in medium and small size bronchioles (Fig. 4-2). The epithelium was surrounded by a layer of varying numbers of circularly oriented smooth muscle fibers. Terminal bronchioles were lined by simple cuboidal epithelium and characterized by the presence of alveolar outpockets in their wall.

Postnatally, most changes were observed in the submucosal glands. Acinus numbers increased and the glands extended into the submucosa especially between the cartilaginous plates (Figs. 4-4,4-6). Smooth muscle layers increased from two layers in the prenatal to four or five layers in *21-day-old kittens* (Fig. 4-6). In addition, lining epithelial cells of the bronchioles contained PAS-positive glycogen deposits (Fig. 4-5).

In the *adult*, the smooth muscle layer was thicker and in the small bronchi it tended to have a spiral course (Fig. 4-7). In small

PLATE 4-1

Fig. 4-2: Intrapulmonary bronchiole of a prenatal kitten lined by simple cuboidal cells with no cilia. Smooth muscle (SM). H&E Stain. X230

Fig. 4-3: Primary bronchus of prenatal kitten. Smooth muscle (SM), Cartilage (Ca). H&E Stain. X115

Fig. 4-4: Bronchus of a 1-day-old kitten showing submucosal glands (G) between the cartilaginous plates (Ca). H&E Stain. X230

Fig. 4-5: Bronchiole of a 1-day-old kitten, the lining epithelium is a combination of cuboidal and columnar cells that contain PAS-positive cytoplasmic inclusions. PAS Stain. X230

Fig. 4-6: Bronchus of a 21-day-old-kitten showing many submucosal glands (G). PAS Stain. X230

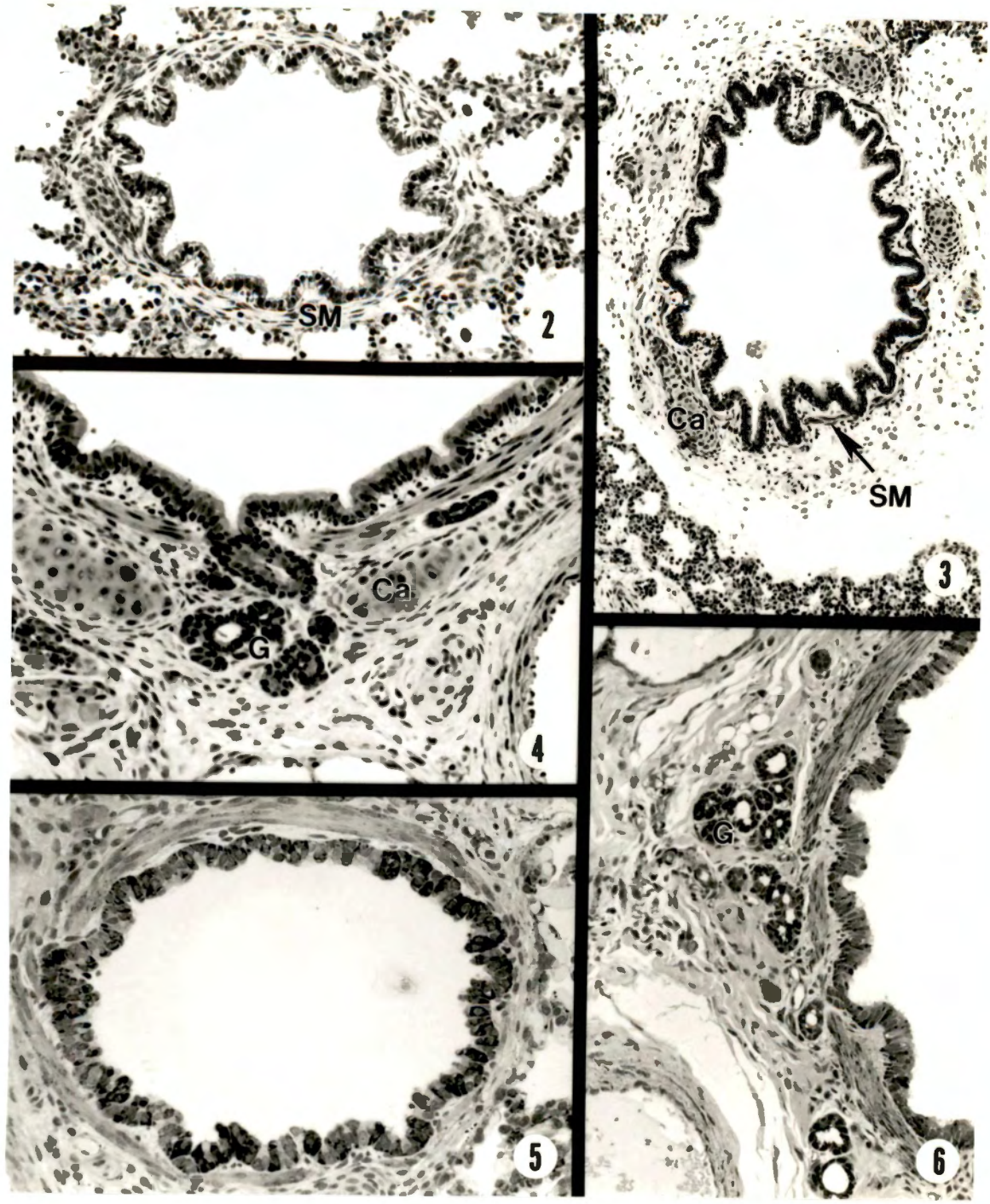

SM
2
Ca
SM
3
Ca
G
4
G
5
6

PLATE 4-2

Fig. 4-7: Small bronchus of an adult cat showing submucosal glands (G). H&E Stain. X115

Fig. 4-8: Medium bronchiole of an adult cat showing submucosa infiltrated by mononuclear cells among the submucosal gland acini. H&E Stain. X230

Fig. 4-9: Small bronchiole of an adult cat showing a acini of submucosal glands (G) below the smooth muscle layer (SM). H&E Stain. X115

Fig. 4-10: Large bronchus of an adult cat with submucosal infiltration by lymphoid tissue. H&E Stain. X115

Fig. 4-11: Large bronchus of an adult cat showing a nodule of lymphoid tissue in its adventitia. Submucosal gland (G). H&E Stain. X230

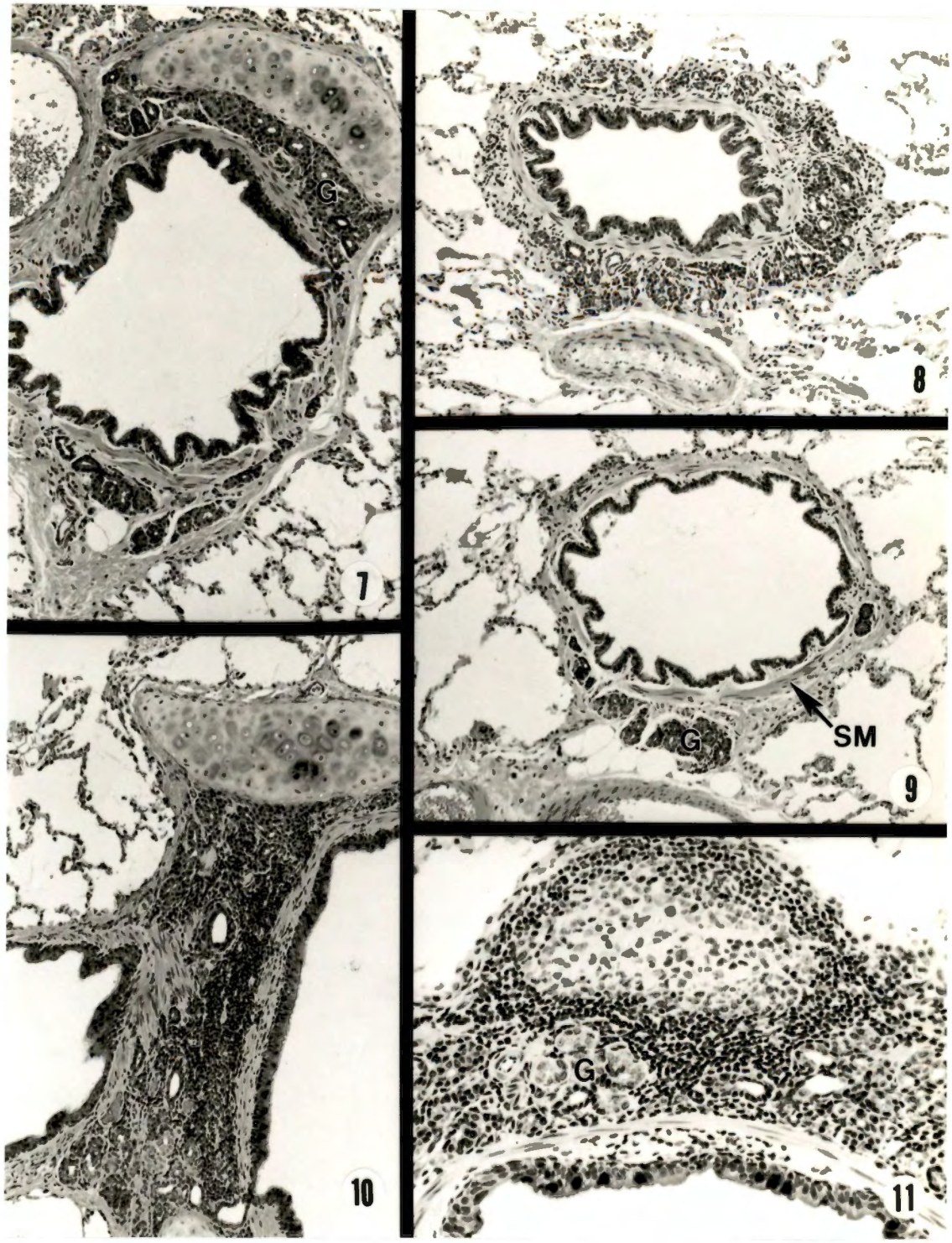

G
7
8
G
SM
9
10
G
11

PLATE 4-3

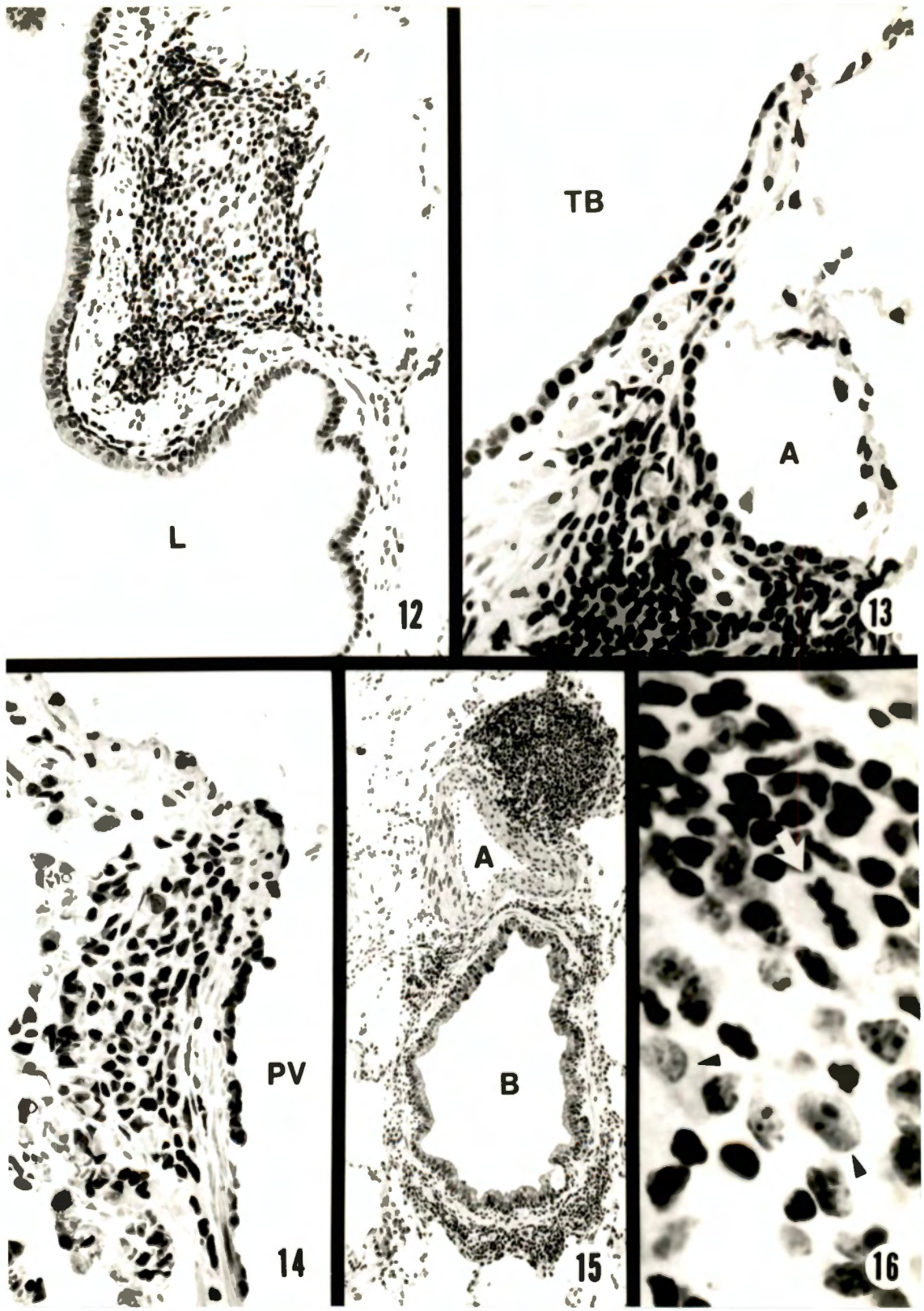
Fig. 4-12: Small bronchiole of an adult cat showing a lymphoid nodule covered by intact epithelium, which bulges into the lumen (L). H&E Stain. X230

Fig. 4-13: Lymphoid tissue of an adult cat in association with terminal bronchiole (TB) and alveolus (A). H&E Stain. X460

Fig. 4-14: Lymphoid tissue in the adventitia of a pulmonary vein (PV) of an adult cat. H&E Stain. X460

Fig. 4-15: Lymphoid tissue associated with a pulmonary artery (A) of an adult cat. Bronchiole (B). H&E Stain. X115

Fig. 4-16: High magnification of a lymph nodule of an adult cat showing a dividing cell (arrow) near the cortical area. Notice the medulla contains large mononuclear cells. (arrowheads). H&E Stain. X1,150


L
12
TB
A
13
PV
14
A
B
15
16

bronchioles, the smooth muscle layer was interrupted by glands (Fig. 4-9). The submucosal glands were well-developed, were located below the smooth muscle layer, were present in all divisions of bronchi, and extended into the medium-size bronchioles (Figs. 4-7 to 4-9).

Bronchus-associated lymphoid tissue (BALT) was observed only in the adult and was in two forms: diffuse and nodular (Figs. 4-8, 4-10). It lay outside the smooth muscle layer of the bronchi and bronchioles reaching to the terminal bronchiole (Fig. 4-13). The diffuse form was a collection of mononuclear cells which yielded an invasive appearance to the submucosa of bronchi and large bronchioles (Figs. 4-8, 4-10). The nodular form was usually associated with the bronchus, bronchiole and their divisions (Fig. 4-11). In bronchioles, the surface epithelium overlying the nodule was intact and bulged into the lumen (Fig. 4-12). However, the lymphoid tissue associated with the terminal bronchiole consisted of small aggregates of mononuclear cells that lacked distinct zones (Fig. 4-13). Nodular or diffuse lymphoid tissue was also observed outside the adventitia of the pulmonary vessels (Figs. 4-14, 4-15).

The lymphoid tissue consisted mainly of mononuclear cells and was scattered between the acini of submucosal gland (Figs. 4-10, 4-11). The nodules had a pronounced peripheral cortical zone surrounding the medulla (Figs. 4-11, 4-12). In the nodular form the majority of cells of the peripheral cortex appeared to be lymphocytes with darkly staining nuclei (Figs. 4-11, 4-12). Germinal centers were observed with large cells and lightly stained nuclei (Figs. 4-

11, 4-16). Occasionally, dividing cells were observed near the cortex (Fig. 4-16).

Scanning electron microscopic findings:

SEM shows only the surface topography of the epithelium; thus looking at the mucosal surface, it is not possible to classify the generation of each airway into large, medium, and small size. However, a gradual decrease in the density of ciliated cells versus nonciliated cells is noted as the bronchi decrease in size.

The surface epithelium of extrapulmonary bronchi had ciliated and nonciliated cells with interspersed goblet cells. The ciliated cells were predominant and cilia density increased with age but decreased in the distal airways (Figs. 4-17, 4-19 to 4-22). In *prenatal kittens* (Fig. 4-17), although the majority of epithelial cells had long cilia, short cilia were also observed. A large number of goblet cells and precipitated mucus were present. The goblet cells varied in size and had a smooth convex apical surface (Fig. 4-17). In addition, mucous droplets marked the opening of submucosal glands.

An equal ratio of ciliated and nonciliated cells was observed in the intrapulmonary bronchi of prenatal kittens (Fig. 4-18). Some cells had a microvilli covered surface with a few scattered cilia. The nonciliated bronchiolar cells (NBCs) were clearly delineated and of different sizes.

Postnatally, the surface epithelium of bronchi showed few changes. The surface was covered with ciliated cells whose cilia

PLATE 4-4

Fig. 4-17: Scanning electron micrograph of an extrapulmonary bronchus of prenatal kitten showing goblet cells (G) and precipitated mucus of submucosal glands on their openings (O). X2,100

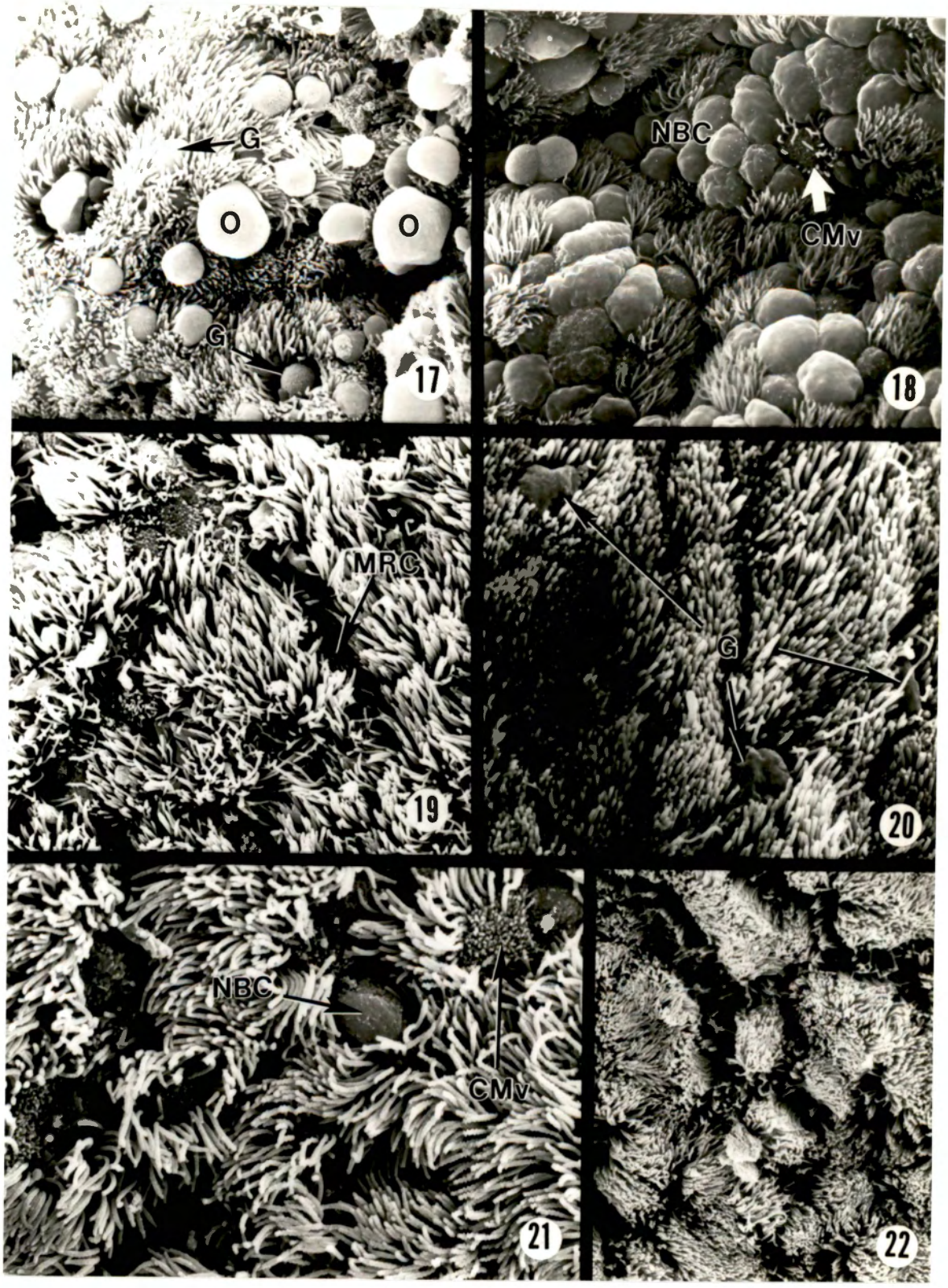
Fig. 4-18: Scanning electron micrograph of an extrapulmonary bronchus of prenatal kitten showing ciliated with microvilli (CMV) among ciliated cells and nonciliated bronchiolar cells (NBC). X2,040

Fig. 4-19: Scanning electron micrograph of a bronchus of a 7-day-old kitten showing a microvillus-rich cell (MRC). X2,700

Fig. 4-20: Scanning electron micrograph of a 14-day-old kitten bronchus showing well-developed goblet cells (G). X4,500

Fig. 4-21: Scanning electron micrograph of a 21-day-old kitten bronchiole showing nonciliated bronchiolar (NBC) and ciliated with microvilli (CMV) cells. X4,950

Fig. 4-22: Scanning electron micrograph of an adult bronchus. X2,250


G
O
O
G
17
NBC
CMv
18
MRC
19
G
20
NBC
CMv
21
22

were of a uniform length, distribution, and orientation. However, as the airways branched distally, the ratio of ciliated to nonciliated cells changed in favor of the nonciliated cells. Goblet cells of variable size and number, as well as mucous droplets, were observed on the epithelial surface. In *7-day-old kittens*, in addition to ciliated cells, a few cells covered with microvilli and cells with short and few cilia were observed (Fig. 4-19). In *14-day-old kittens*, some areas of extrapulmonary bronchi were covered with ciliated cells which lacked delineation. Their cilia were of a uniform length and orientation (Fig. 4-20). A few goblet cells, with smooth apical surfaces, were interspersed between ciliated cells. In *21-day-old kittens*, in addition to ciliated and nonciliated cells, a few ciliated with microvilli covered cells were observed in intrapulmonary bronchi. The NBCs were polygonal in shape and their apical surface bulged into the lumen and was covered with minute microvilli. The ciliated with microvilli cells were covered with abundant microvilli, relatively long cilia were aggregated either on one side of the cell or scattered near the cell perimeter, leaving the center covered with microvilli (Fig. 4-21). In the *adult cat*, the surface epithelium (Fig. 4-22) was similar to that observed in 21-day-old kittens.

Electron microscopic findings:

The ciliated cells of *prenatal kittens* were covered by well-developed cilia, and a few microvilli were scattered among the cilia (Fig. 4-23). In addition, cytoplasmic protrusions, with irregular

PLATE 4-5

Fig 4-23: Transmission electron micrograph of the prenatal bronchial ciliated cell with abundant elongated mitochondria (M). Dense-bodies (DB). X8,400

Fig. 4-24: Transmission electron micrograph of the prenatal bronchial ciliated cell with an apical protrusion (P). X18,000

Fig. 4-25: Transmission electron micrograph of a 1-day-old kitten bronchial epithelium with a cell undergoing ciliogenesis. Basal bodies (Arrowheads). X12,000

Fig. 4-26: Transmission electron micrograph of the prenatal bronchial goblet cell filled with electron-dense mucous granules. X8,400

Fig. 4-27: Transmission electron micrograph of a 7-day-old kitten goblet cell which contains large mucous granules of different electron density. X8,400

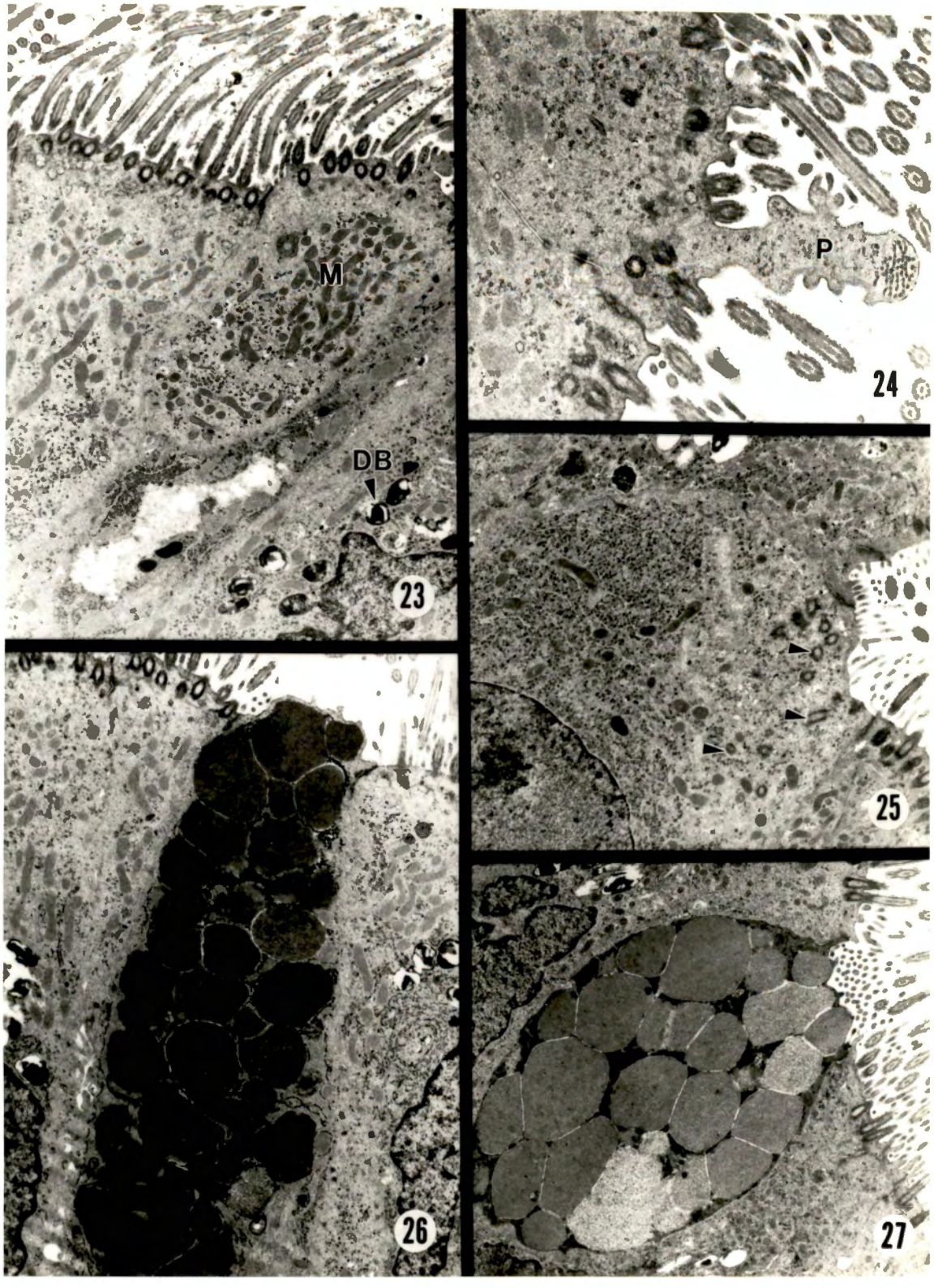

M
DB
23
P
24
25
26
27

PLATE 4-6

Fig. 4-28: Transmission electron micrograph of bronchial epithelium of 14-day-old kitten with a cell undergoing ciliogenesis. Basal bodies (arrowheads), Microvillus-rich cell (MRC). X12,000

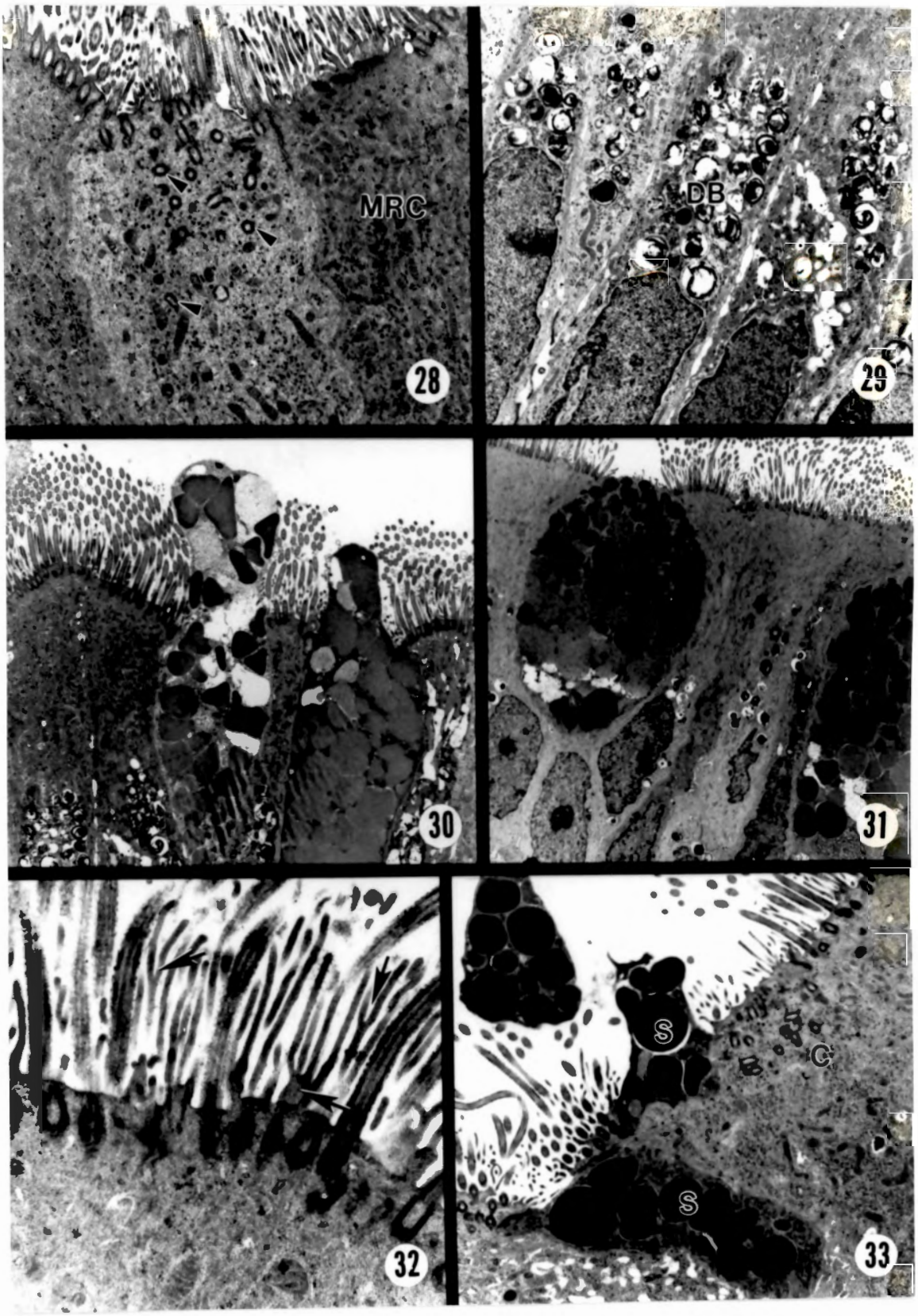
Fig. 4-29: Transmission electron micrograph of a 14-day-old kitten bronchial ciliated cells containing abundant dense-bodies (DB). X8,400

Fig. 4-30: Transmission electron micrograph of bronchial epithelium of 14-day-old kitten. Two goblet cells (G) have smooth apices and extend well beyond the adjacent cells. X5,400

Fig. 4-31: Transmission electron micrograph of bronchial epithelium of 21-day-old kitten. The goblet cell is well-developed and contains mucous granules of different electron-density. X36,000

Fig. 4-32: Transmission electron micrograph of a ciliated cell of an adult bronchus showing branched long slender microvilli (arrow). X24,000

Fig. 4-33: Transmission electron micrograph of adult bronchial epithelium. Serous cells (S) contain electron-dense granules. Notice the cytoplasmic island being shed into the bronchial lumen. Cell undergoing ciliogenesis (C). X8,400


MRC
28
DB
29
30
31
S
S
32
33

electron-dense cytoplasm. The ciliated cells had increased numbers of dense-bodies which were aggregated above the nucleus (Fig. 4-29). The goblet cells of 14-day-old kittens had a smooth apical surface which extended well-beyond adjacent cells (Fig. 4-30). The cytoplasm was filled with mucous granules of different shape, size, and electron density.

At *21-days-of-age*, the ciliated cells had morphology similar to that of younger kittens. Goblet cells were well-developed, ovoid in shape, and packed with mucous granules of different size and electron density (Fig. 4-31).

In the *adult cats*, the ciliated cells were covered with microvilli which often branched into two to four microvilli near or away from the luminal surface of the cell (Fig. 4-32). Cells undergoing ciliogenesis were observed similar to those in growing cats (Fig. 4-33). In addition, serous cells were located near the luminal surface and were characterized by large electron-dense granules of different size. Cytoplasmic islands, decapitated from serous cells, containing numerous secretory granules were observed in the lumen (Fig. 4-33).

DISCUSSION

The bronchi and bronchioles were divided into large, medium and small based on their cross-sectional size and their characteristics morphology of each segment. Previous studies by Engel (1953) divided the airways into primary, secondary and tertiary divisions.

Recently, each of these divisions has been subdivided into 2 to 16 generations (Nunn, 1987).

Besides cilia, adult ciliated cells were covered by microvilli which branched close to or away from the surface of the cell. This branching pattern has not been described in other species. Otherwise, the ciliated cells were similar to those of other species including man (Rhodin, 1966; Frasca 1968 a, b; Jeffery and Reid, 1975; Kawamata and Fujita, 1983). Each type of epithelial cell had developed by early prenatal life similar to other mammals including man (Sorokin, 1961; Bucher and Reid, 1961; Plopper et al., 1986).

Cells undergoing ciliogenesis were observed in some growing cats and in adult cats. They were considered an intermediate cell type with the potential of becoming ciliated cells. This suggests that cell differentiation continues postnatally and throughout life, but at a low level. Looking at the surface by scanning electron microscope, the cilia of these cells varied in density and distribution, reflecting a variable degree of differentiation. These cells have been described in both fetal (Leeson, 1961) and adult epithelium (Andrews, 1974, Jeffery and Reid, 1975).

Occasional microvillus-rich cells were observed in prenatal kittens. These cells were considered mature, since similar cells were described by Tandler and co-workers (1983 a, b) in the trachea of adult cats.

This investigation showed that fully packed goblet cell, with their apical surface protruding beyond adjacent cells, lacked

microvilli. This suggests that microvilli were present only in the earlier stages of the secretory cycle. However, when the cell surface was covered by a few microvilli, the cell was similar to those described in man (Rhodin, 1966) and in the rat (Rhodin and Dalhamn, 1956). Well-developed goblet cells contained large biphasic mucous granules that packed the cytoplasm. It has been suggested that such heterogeneity of the granules might be attributed to the presence of a mixture of distinct types of glycoproteins within the same cell (Jeffery, 1987). Goblet cells were numerous in bronchi and decreased in numbers in the bronchioles similar to the findings by Haller (1969) and Jeffery and Reid (1977a) in the cat and pig.

Submucosal glands were found in all ages. They were best-developed in adult cats and were observed distally to a medium-sized bronchiole, whereas, in growing cats, glands were only observed in bronchi. Contrasted to the earlier findings of Jeffery and Reid (1977a) observed glands extending into small intrapulmonary bronchi in the adult cat.

Epithelial serous cells were observed only in the adult extrapulmonary bronchi. Cytoplasmic islands, that contained a considerable number of secretory granules, were observed in the lumen suggesting a group secretion of this cell. It has been shown that serous cell granules contain carbonic anhydrase enzyme indicating that serous cells function in the modulation of ions, pH, and water. Serous secretion is normally of low viscosity and contributes to the sol phase (periciliary fluid layer) of airway secretions (Spicer et

al., 1982). The serous cells of the adult cat were similar to those observed by Robinson et al. (1986) in the mouse tracheobronchial tree in that they were located near the lumen. Ultrastructurally, they resemble serous cells of the submucosal gland, which are common in the rat (Jeffery and Reid, 1977b) and in mouse (Pack et al., 1981).

Bronchial-associated lymphoid tissue (BALT) was observed in adult cats only. The lymphoid tissue was of a nodular and diffuse form and was observed distally into the terminal bronchiole where it was also associated with alveoli. Anatomically, it has been described that lymph nodes are present along the primary bronchi of the cat (Schummer et al., 1981). In 1970, Jericho recognized lymphoid tissue in the following positions in the lungs of most species: subpleural, peribronchial, perivascular, and as diffuse pulmonary lymphoid tissue. He stated that lymphoid tissue was a consistent structure associated with pulmonary lymphatics. This investigation confirmed previous observations, except for the association of lymphoid tissue with pulmonary lymphatics. Scant literature exists on BALT and few functional studies have been reported (Bienenstock et al., 1973b; Kaltreider and Salmon, 1973). In 1875, Klein wrote:

> These lymphoid follicles in the bronchial walls are therefore in every respect analogous to the lymph follicles found in other mucous membranes, ex. tonsils and in the intestine....

Miller (1950) described this lymphoid tissue in several mammalian species including man as intrapulmonary lymphoid tissue in

association with the adventitia of pulmonary vessels (perivascular lymphoid tissue). Macklin (1955) referred to this type of lymphoid tissue as "sumps" or "pulmonary microtonsils" in which dust and organisms were trapped.

BALT is located far away from the bronchial epithelium, but reaches the epithelium through a narrow neck of lymphocytes. It has been described as consisting of lymphoid tissue closely associated with and infiltrating bronchial epithelium to form lymphoepithelium (Bienenstock et al., 1973a, 1976). Our findings show that the epithelium of terminal bronchioles over BALT was columnar, bulged into the lumen, and was not infiltrated with lymphocytes. Goblet cells were not correlated to BALT, but were correlated to the area of the bronchial tree. On the contrary, Bienenstock et al. (1973a) found that the epithelium overlying BALT and gut-associated lymphoid tissue was flattened, contained no goblet cells, and was heavily infiltrated with lymphocytes.

Lymphoid tissue was not present in growing cats. This indicates that most likely its appearance depends upon environmental antigenic stimulation. Previous reports (Bienenstock et al., 1973a) observed that 1-day-old rabbits lack BALT, although peripheral lymphoid structures such as spleen and lymph node were readily identified. On the contrary, Emery and Dinsdale (1973) reported that lymph nodes were present in lungs of children at birth, and increased in prominence, if not in numbers, throughout the first year of life.

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V. ULTRASTRUCTURE OF DEVELOPING FELINE NONCILIATED BRONCHIOLAR EPITHELIAL CELLS

ABSTRACT

The ultrastructure of the developing nonciliated bronchiolar cell was studied in six age groups: prenatal (60 days post-conception); postnatal (1-, 7-, 14-, and 21-day-old); and adult. Following intratracheal infusion of 3% glutaraldehyde in phosphate buffer at a standard pressure, lung tissue was processed for scanning and transmission electron microscopy. In the terminal bronchioles, the lining epithelium consists of numerous cuboidal to columnar nonciliated bronchiolar cells (NBCs) as well as exclusively ciliated and ciliated cells with microvilli. NBCs were characterized by a large, centrally located, indented nucleus. The apical surface of the NBCs extended beyond the surface of neighboring cells and was covered by minute microvilli, except in prenatal kittens. The lateral surface of NBCs interdigitated with neighboring cells. The NBC of the cat was characterized by abundant mitochondria and glycogen inclusions. In prenatal kittens, the cytoplasm was filled with patches of alpha and beta form of glycogen. Postnatally, glycogen was reduced in quantity, became scattered throughout the cytoplasm, and was predominantly of the beta form . The mitochondria varied in number, shape, and size in all ages. They contained a homogeneous electron-dense matrix with few to no cristae. They were scattered throughout the cytoplasm but were more abundant in the apical cytoplasm. Occasional dense-bodies of different size, shape,

and were electron density. Islands of cytoplasm, decapitated from the apical cytoplasm, were observed in the lumen of adult bronchioles. This suggests an apocrine mode of secretion. The NBCs attain maturity by three weeks of age.

INTRODUCTION

Three types of secretory cells are recognized by their ultrastructural features in airway epithelium: Clara, serous, and mucous cells. Most functional and structural studies of airway epithelium consider the mucous and serous cells to be the predominant nonciliated secretory cells of proximal conducting airways, whereas the Clara cell is the predominant secretory cell of the distal conducting airways (Smith et al., 1974; Breeze and Wheeldon, 1977; Reid and Jones, 1979).

Kolliker (1881) first noted these distal secretory cells; but Clara (1937), for whom the cells were named, gave a detailed description. Ultrastructure of the Clara cell was described in mice by Karrer (1956), in rabbits by Kisch (1958), and in man by Jarkovska (1970). Clara cells were characterized by light microscopy as having flame-shaped cytoplasmic processes (Azzopardi and Thurlbeck, 1969; Wang and Thurlbeck, 1970; Corrin et al., 1973), which has been interpreted as an indication of secretory function. Von Hayek (1960) thought the secretion to be mucus, but Niden (1967) suggested it to be pulmonary surfactant. Evans et al. (1978) described Clara cells as progenitor cells for the bronchiolar epithelium.

Two cellular characteristics typically distinguish this cell from other cell types of airway epithelium: the presence of apical membrane bound electron-dense ovoid granules, and abundant smooth endoplasmic reticulum (sER) in the apical cytoplasm (Breeze and Wheeldon, 1977). However, Plopper and co-workers reported considerable interspecies variation in the number, size, and morphology of the granules and in the extent and distribution of sER and cytoplasmic glycogen (Plopper et al., 1980a, b, c; 1983a, b). In rat lungs damaged by exposure to nitrogen dioxide, Evans et al. (1978) reported three morphologically distinct cells: one with granules and sER; one with granules and rough endoplasmic reticulum (rER); and one with variable amounts of ER and no granules. Young and co-workers reported similar findings in the rat (Young et al., 1986). Plopper et al. (1980a) reported considerable interspecies variations in the number, size, and morphology of the granules and in the extent and distribution of sER and cytoplasmic glycogen. Granules are scarce in steers and dogs, and absent in cats, while sER is scant and glycogen is the dominant cytoplasmic feature in all three (Plopper et al., 1980b).

Cytoplasmic glycogen is the primary cellular constituent of Clara cells prior to and for some weeks following parturition (Plopper et al., 1983c). Previous studies (Baskerville, 1970; Smith et al., 1974; Jeffery and Reid, 1977) have indicated that Clara cell differentiation was a postnatal phenomenon. However, granules have been described in lungs of neonatal rats (Smith et al., 1974) and

human fetuses (Rosan and Lauweryns, 1972). Despite numerous electron microscope studies of various species including the cat (Balasova, 1968; Wang et al., 1971; Cutz and Conen, 1971; Smith et al., 1974; Plopper et al., 1980b, c; 1983c; Lumsden et al., 1984; Young et al., 1986), results of studies are not available on the ultrastructure of developing nonciliated bronchiolar cells of the cat. The purpose of this investigation was to obtain more information on the ultrastructure of bronchiolar cells of the cat during development.

MATERIALS AND METHODS

Thirty-nine domestic cats (Felis catus) representing the six following age groups: prenatal (60 days post-conception); postnatal (1-, 7-, 14-, and 21-day-old); and adult.

The cats were anesthetized using intramuscular injections of ketamine (5 mg/kg) and xylazine (0.4 mg/kg). The trachea was cannulated, and the lungs were fixed by infusion of 3% glutaraldehyde in phosphate buffer (pH 7.4, 0.1M) at a pressure of 20 cm H_2O. After fixation for 30 minutes in situ, a midline incision through the skin and subcutaneous tissue from the larynx to the xiphoid region exposed the trachea and the sternum. The thorax was opened on the midline, and the lungs and the trachea were carefully dissected free and removed. Samples were taken from the caudal lobe of the lungs, cut into small pieces and immersed into the fixative for an additional four hours. The samples were then processed for both scanning and transmission electron microscopy.

Samples for scanning microscopy were postfixed for 90 minutes in 1% osmium tetroxide in phosphate buffer (pH 7.4, 0.2M). They were washed three times in phosphate buffer at 10 minutes each, then dehydrated in a graded series of acetone solutions (70%, 80%, 95%, with three changes in 100% acetone). Eventually, the samples were dried in a liquid CO_2 critical point drying apparatus. The samples were mounted on aluminum stubs and coated with a 15 nm gold-palladium layer and examined with an ETEC Scanning Electron Microscope operating at 25 kV.

For transmission electron microscopy the samples were washed three times in phosphate buffer (pH 7.4, 0.1M), postfixed in 1% osmium tetroxide in 0.1M phosphate buffer (pH 7.4) for 90 minutes, dehydrated in a graded series of ethanol and acetone, and embedded in an Epon-Araldite mixture. Thin sections were stained with 2% (w/v) aqueous uranyl acetate, contrasted with lead citrate, and viewed by a Phillips model 201 electron microscope working at 60 kV.

RESULTS

Scanning electron microscopic findings:

The nonciliated bronchiolar epithelial cells (NBCs) were the most common cells lining the bronchioli; other cells included ciliated cells with and without microvilli (Figs. 5-2 to 5-4). In *prenatal kittens*, the NBCs have clearly discernible boundaries and their surfaces reveal a characteristic smooth dome-shaped apical surface (Fig. 5-1).

PLATE 5-1

Fig. 5-1: Scanning micrograph of prenatal bronchiole. Nonciliated bronchiolar cells are delineated with a smooth irregular convex surface. 6,400X.

Fig. 5-2: Scanning micrograph of 7-day-old bronchiole. Nonciliated bronchiolar cells have a large apical protrusion (P). A few ciliated cells with microvilli (CMv) are present. 3,960X.

Fig. 5-3 Scanning micrograph of 21-day-old bronchiole. Many nonciliated bronchiolar cells have protrusions (P) from the center of their surfaces. Ciliated with microvilli (CMv). 3,190X.

Fig. 5-4 Scanning micrograph of adult bronchiole. Nonciliated bronchiolar cells (NBC) are large, delineated, vary in size, and their surfaces are covered with microvilli (Mv) which are concentrated near the cell perimeter. Ciliated with microvilli (CMv). 3,860X.

Postnatally, in *1-day-old kittens*, the surface epithelium had not changed remarkably. Later, the surface epithelium of the bronchioli looks like pebbles, rendering the surface rough and irregular (Figs. 5-2 to 5-4). However, by *7-days-old*, the NBC were covered by minute microvilli, and occasionally protrusions were observed coming from the center of their luminal surface (Fig. 5-2). By *21 days*, protrusions of different sizes were frequently observed (Fig. 5-3).

In the *adult* cat, the NBC varied in size and shape, but their boundaries were clearly discernible. The surface of the NBC was covered by minute microvilli, however there was a concentration of microvilli at the cell perimeter. A few NBC protruded into the lumen of bronchioli (Fig. 5-4).

Transmission electron microscopic findings:

The NBCs were cuboidal to columnar and lined the terminal bronchioles with the other epithelial cells (Figs. 5-5, 5-6, 5-8). The cells rested on a thin basement membrane. Their apical surface protruded beyond the neighboring cell surface, forming a rounded cap or a flame-like projection (Figs. 5-5, 5-7, 5-8). However, a few NBCs with a flat or concave apical surface were observed (Fig. 5-9). The NBC contained a large spherical centrally located nucleus. Occasional nuclear indentations were observed which often faced the basement membrane (Figs. 5-6, 5-8). The nucleus contained a well-developed nucleolus and patches of heterochromatin which were mainly attached to the nuclear membrane (Figs. 5-7, 5-8, 5-14).

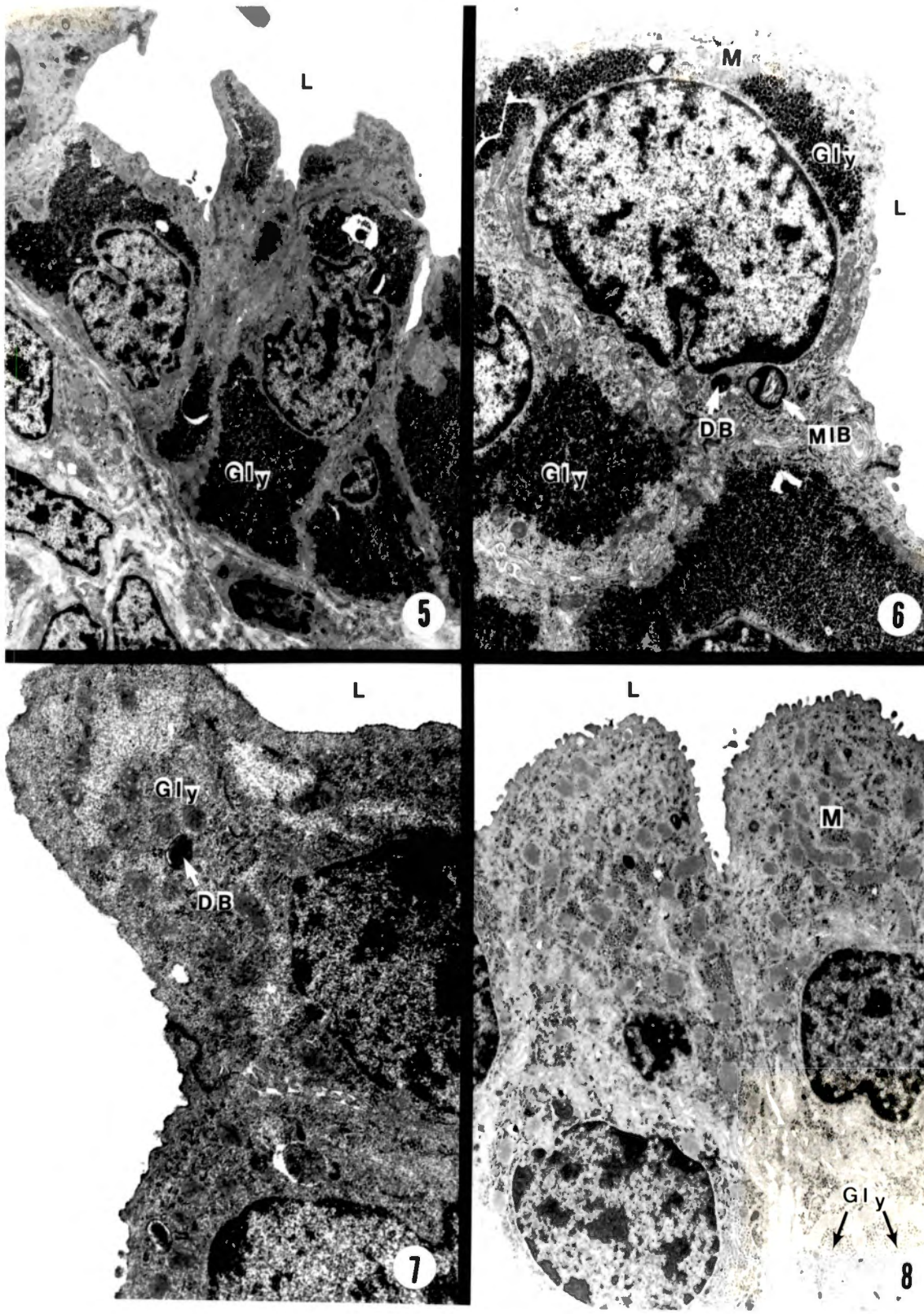
PLATE 5-2

Fig. 5-5: Electron micrograph of prenatal bronchiole. Nonciliated bronchiolar cells have apical projections into the lumen (L). Glycogen (Gly) dominates the cell cytoplasm. Uranyl acetate and lead citrate. 6,750X.

Fig. 5-6: Electron micrograph of prenatal bronchiole. Nonciliated bronchiolar cells have abundant glycogen (Gly), an occasional mitochondria (M), dense-body (DB) and osmiophilic multilamellated body (MlB). Uranyl acetate and lead citrate. 15,000X.

Fig. 5-7: Electron micrograph of 14-day-old bronchiole. Nonciliated bronchiolar cells have apical protrusions projecting into the lumen (L). Glycogen (Gly) surrounds electron dense-bodies (DB). Uranyl acetate and lead citrate. 22,500X.

Fig. 5-8: Electron micrograph of adult bronchiole. Nonciliated bronchiolar cells have a rounded apical cap projecting into the lumen (L). Abundant mitochondria (M) are in the apical cytoplasm, whereas the beta form of glycogen (Gly) is abundant in the basal cytoplasm. Uranyl acetate and lead citrate. 10,500X.


L
Gly
5
M
Gly
L
DB
MIB
Gly
6
L
Gly
DB
7
L
M
Gly
8

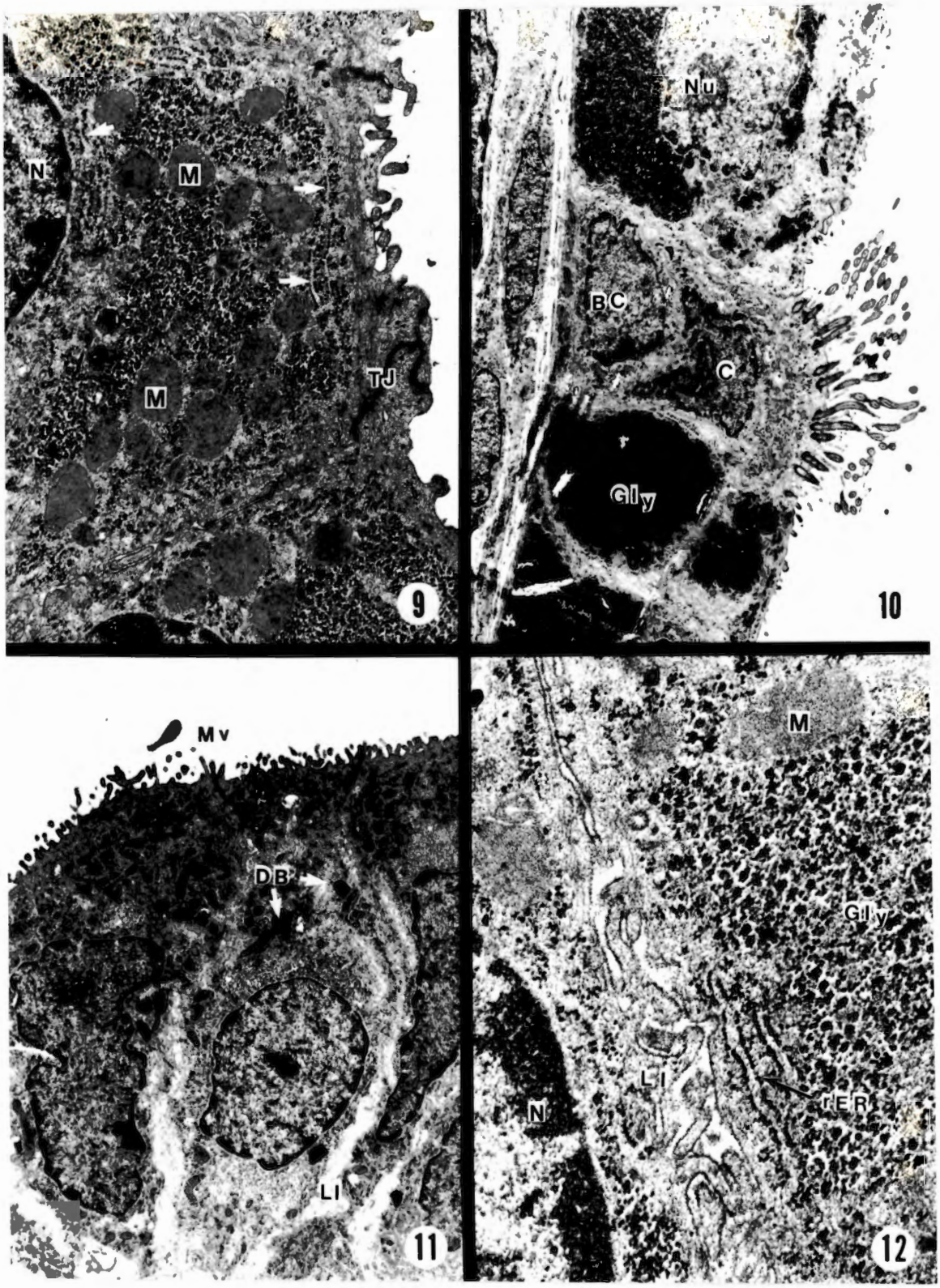

N
M
M
TJ
9
Nu
BC
C
Gly
10
Mv
DB
LI
11
M
Gly
LI
N
rER
12

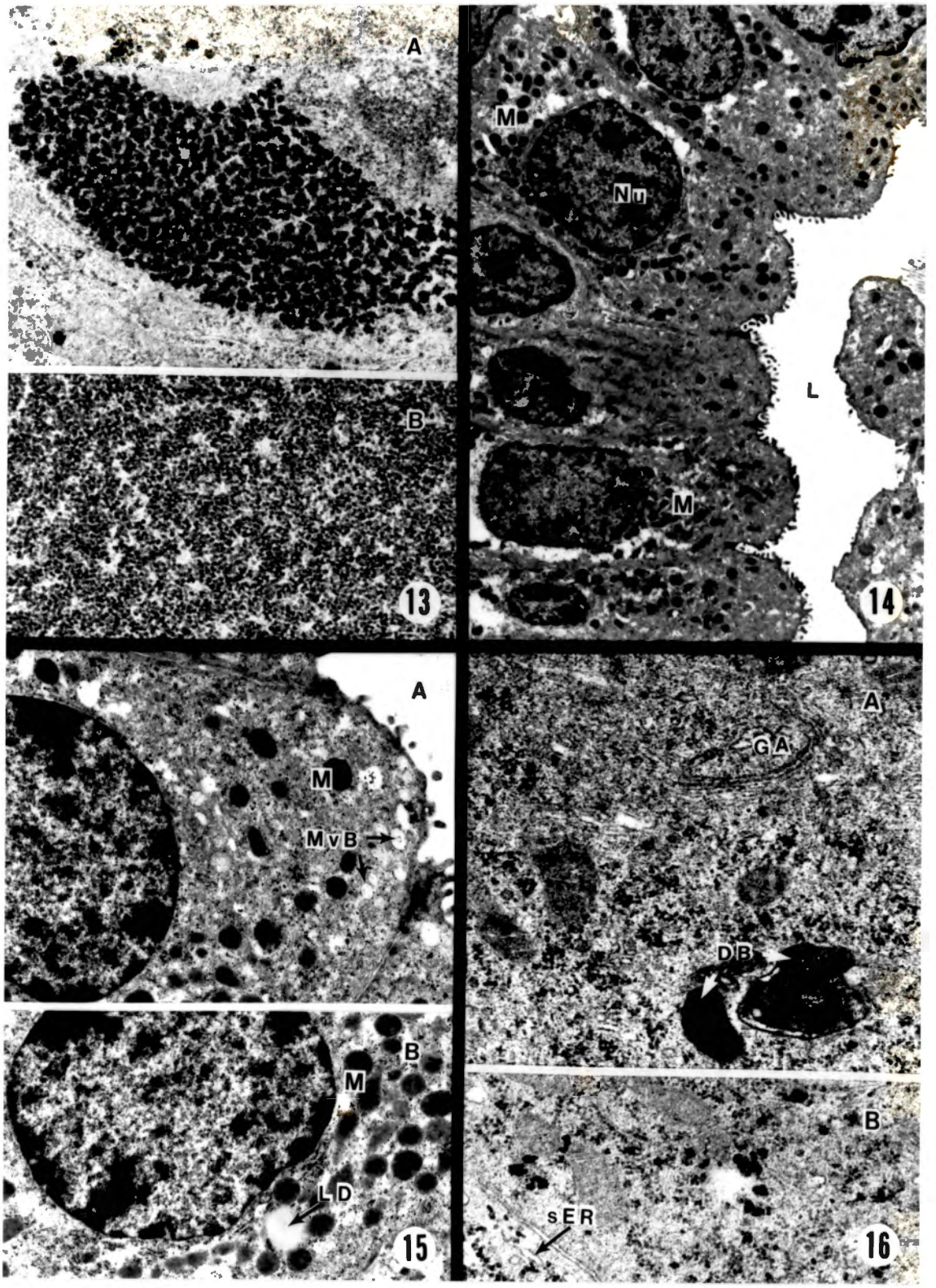
PLATE 5-4

Fig. 5-13: Electron micrograph of glycogen forms in 1-day-old kittens. A - alpha glycogen. B - beta glycogen. Uranyl acetate and lead citrate. 45,000X

Fig. 5-14: Electron micrograph of adult bronchiole. Nonciliated bronchiolar cells are columnar with a large centrally-located nucleus. Mitochondria (M) are abundant in the apical cytoplasm. Lumen (L), Nucleolus (Nu). Uranyl acetate and lead citrate. 45,000X.

Fig. 5-15: Electron micrograph of adult nonciliated bronchiolar cell. A - In the apical cytoplasm multivesicular-bodies (MvB) of different sizes are found. Mitochondria (M). B - Mitochondria (M), Lipid droplet (LD). Uranyl acetate and lead citrate. 10,500X.

Fig. 5-16: Electron micrograph of 1-day-old bronchiolar cell. A - Golgi apparatus (GA), Dense-bodies (DB). B - Smooth endoplasmic reticulum (sER). Uranyl acetate and lead citrate. 45,000X.


A
B
13
M
Nu
L
M
14
A
M
MvB
A
GA
DB
B
M
LD
15
B
sER
16

In *prenatal kittens*, the apical surface had irregular protrusions (Figs. 5-5, 5-6). *Postnatally*, the apical surface displayed minute microvilli of different sizes (Figs. 5-10, 5-11). The microvilli increased in number and size by adulthood, projecting in different directions and sometimes branched or joined at their bases (Fig. 5-11). There were no microvilli covering the apical surface of some NBC, yet the surface was irregular (Fig. 5-7).

The lateral surface of the NBC interdigitated (Figs. 5-9 to 5-12) with not only adjacent NBCs, but also with ciliated and basal cells (Fig. 5-10). The interdigitations were large and occupied the basal two-thirds of the lateral surface (Figs. 5-11, 5-12). The upper thirds of the cells were joined by tight junctions (Fig. 5-9).

The NBCs of cats were characterized by abundant glycogen inclusions and large mitochondria (Figs. 5-5 to 5-10). In *prenatal* and early *postnatal kittens*, more glycogen was present and there were fewer mitochondria. The glycogen was in large patches and located mainly above and below the nucleus (Figs. 5-5, 5-6, 5-10). The glycogen was observed either as electron-dense granules (beta particles or monoparticulate form) or as collections of such particles forming rosettes (alpha particles or rosette form) (Figs. 5-12, 5-13). The monoparticulate form was most common, being present in *all ages* and pronounced in the adult (Fig. 5-8). However, a few rosette forms were also present in the adult (Figs. 5-9, 5-12). Glycogen of the *adult* was present in small clusters and located

throughout the cytoplasm, but it was abundant in the basal cytoplasm (Fig. 5-8).

The mitochondria varied in size and shape (ovoid to elongate) (Figs. 5-8, 5-9, 5-19) and contained an electron-dense matrix with few cristae (Figs. 5-9, 5-19). They were scattered throughout the cytoplasm and were more abundant in the apical cytoplasm (Figs. 5-8, 5-11, 5-14). The mitochondria were few in number in prenatal kittens (Fig. 5-6), but gradually increased in number by adulthood (Figs. 5-8, 5-14).

Smooth (sER) and rough endoplasmic reticulum (rER) were in small clusters. The rER consisted of long cisternae located in the perinuclear region and in the apical cytoplasm (Fig. 5-9). Occasionally rER was also found in the cytoplasm lateral to the nucleus (Fig. 5-12). However, sER was scarce and consisted of a few small cisternae (Fig. 5-16B). The Golgi apparatus was not well developed and was located close to the luminal side of the nucleus (Fig. 5-17).

Multivesicular-bodies and dense-bodies of different electron densities were observed in *all ages* (Figs. 5-6, 5-7, 5-15A, 5-16A, 5-17, 5-20). However, in the *adult* they were abundant and concentrated in the apical cytoplasm, especially when the cap was separated from the apical cytoplasm of the cell during secretion (Fig. 5-20). A few multilamellated inclusions similar to type II surfactant were observed in the *prenatal kittens* (Fig. 5-6). In addition, a few small electron-dense and membrane-bound granules were observed in *21-*

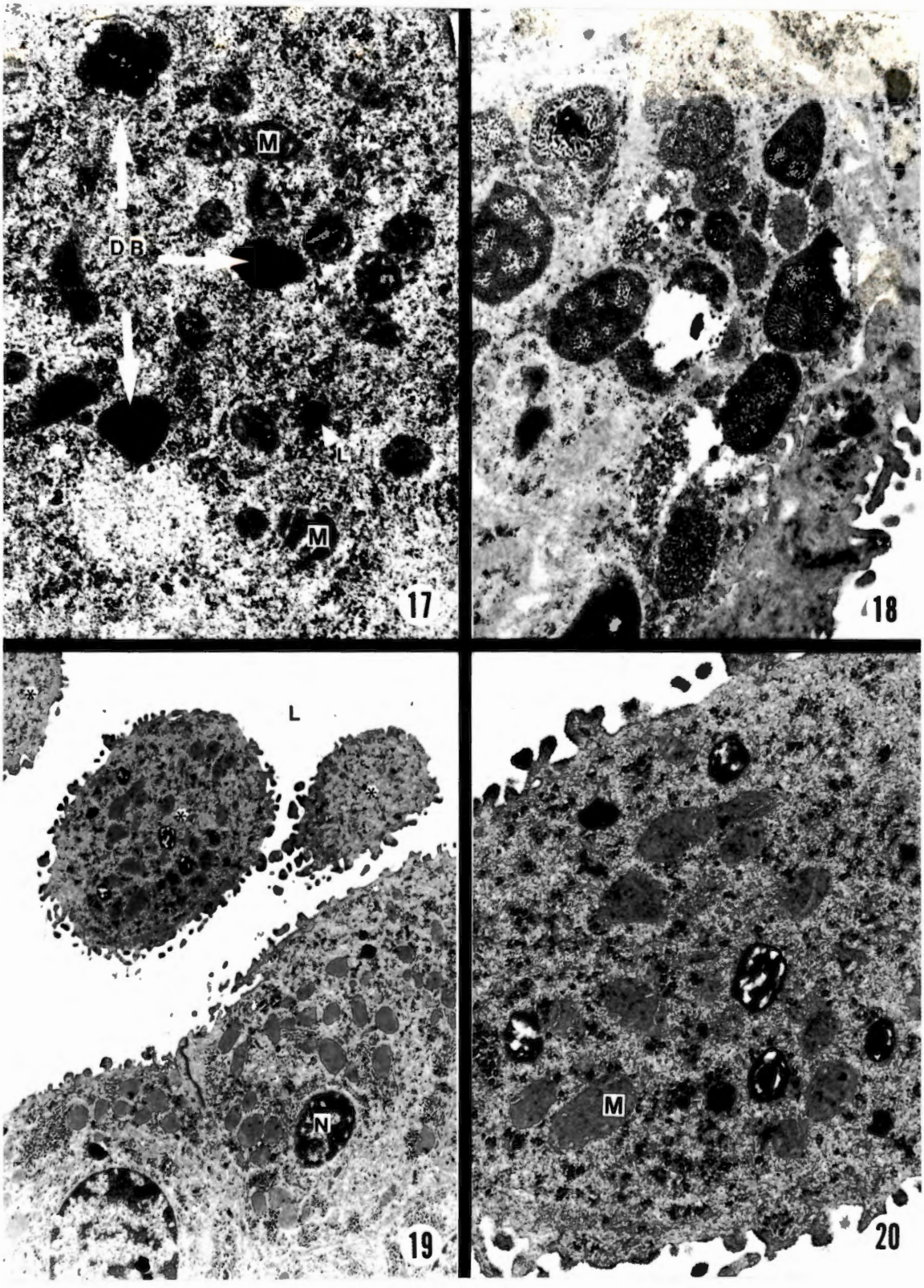
PLATE 5-5

Fig.5-17: Electron micrograph of 21-day-old bronchiolar cell. Dense-bodies (DB) vary in shape and electron density. Mitochondria (M), Lysosome (L). Uranyl acetate and lead citrate. 45,000X.

Fig. 5-18: Electron micrograph of adult bronchiolar cell. Abundant large lysosomes containing glycogen are observed. Uranyl acetate and lead citrate. 22,500X.

Fig. 5-19: Electron micrograph of adult bronchiole. The lumen (L) contains islands of cytoplasm (asterisks). Nucleus (N) of nonciliated bronchiolar cells. Uranyl acetate and lead citrate. 10,500X.

Fig. 5-20: A higher magnification of the island in figure 19 with many dense-bodies of different electron density. Mitochondria (M). Uranyl acetate and lead citrate 30,000X.


M
D B
L
M
17
18
L
N
19
M
20

day-old kittens (Fig. 5-17). In the *adult*, occasional large membrane-bound inclusions were observed which contained electron-dense materials. In addition, homogeneous granules of less electron density were seen in the adult (Fig. 5-15B).

DISCUSSION

The development of NBCs was studied, and several similarities and differences with other species were noted. The NBC is considered in many animals as the predominant epithelial cell of the distal conducting airways (bronchioles) (Plopper et al., 1980c). However, other cells (ciliated and ciliated with microvilli) are also encountered in the bronchioles of cats, except in prenatal kittens. Ciliated cells with microvilli have also been described in the rat (Andrews, 1974), in the dog (Frasca, 1968), and in the human (Greenwood and Holland, 1975).

Microvilli are abundant on the surface of ciliated cells, particularly on immature ciliated cells undergoing ciliogenesis. Only a few short cilia may project from their surface, which is covered by microvilli. In this study, the cilia of ciliated cells with microvilli were either at the cell periphery, leaving the center of the cell covered exclusively with microvilli, or the cilia were located near one side of the cell. Kilburn (1968) reported that a considerable volume of tracheobronchial secretion must be absorbed between the lung and the pharynx, and proposed that microvilli of ciliated cells might perform this function.

NBCs were identified using SEM by their irregular shape and blunt luminal projections distributed over their surface (Becci et al., 1978). These protrusions appear postnatally, and their number and size increase with age. Ultrastructurally these protrusions are accumulations of various organelles, mainly mitochondria, in the apical cytoplasm.

The NBCs of the cat share similar morphological characteristics, including microvilli on their luminal surface, with other animals (Plopper et al. 1980b, 1983a). The microvilli, which appeared postnatally and occasionally branched near their bases, presumably function to increase the surface area of the cell. They did not cover the protrusions; this might be explained by the stretching of the apical membrane because of the increase of the apical contents.

Lateral interdigitations of the NBCs of the cat were well developed. Interdigitations not only occurred with other NBCs but also with ciliated and basal cells. Similar interdigitations have been observed in other animals (Plopper et al., 1980a) and have been described also in the tracheal epithelium (Breeze and Wheeldon, 1977, Inoue and Hogg, 1974). It has been speculated that the function of these lateral extensions in the trachea (Inoue and Hogg 1974) and in bronchioles (Kilburn, 1974) are for fluid absorption.

Previous comparative studies (Plopper et al., 1980c) demonstrate that sER and electron-dense granules are not the major components of NBCs in adult animals. Our results confirmed these findings, with sER rarely found in the perinuclear cytoplasm and in small clusters.

In addition, the developing cytoplasm is free of electron-dense granules. Postnatally, rER is present as relatively long cisternae, but it is not abundant.

Glycogen inclusions consistently filled the cytoplasm of NBCs in the cat. In the prenatal, large patches of glycogen nearly fill the cytoplasm. Postnatally, glycogen content is reduced, and becomes finely granular resembling free ribosomes. Glycogen is also found in other laboratory animals and man (Plopper et al., 1980a, b). Two forms (alpha and beta) of glycogen were observed in all ages. Beta particles or monoparticulate form is more common and is encountered in most normal tissue (Ghadially, 1982). On the other hand alpha particles or the rosette form is the typical glycogen deposit in the liver, but occasionally is seen in other normal tissue (Ghadially, 1982) and in pathological conditions (Biava et al., 1966). Why large quantities of the rosette form glycogen are present in prenatal and early postnatal kittens is unclear.

Glycogen is a normal cytoplasmic constituent and has characteristic features for identification. However, it is occasionally found in other cell compartments such as the nucleus (Sobel et al., 1969), mitochondria (Kraus and Cain, 1980), rER (Ghadially et al., 1977), and lysosomes (Hers and Van Hoof, 1969). Glycogen was found in lysosomes of the adult cat, and its function is unknown. But lysosomes containing glycogen have been reported in CNS disease of the cat (Sandstrom et al., 1969) and in a hereditary disease (Pompe's disease) (Hers, 1963). The functional basis for the

accumulation of glycogen in lysosomes, especially in Pompe's disease, is due to a genetic defect leading to the absence of a single lysosomal enzyme (acid maltase), which is necessary for the breakdown of glycogen to glucose.

Glycogen is a metabolic fuel stored in cells; hence, its abundance may indicate a metabolically active cell. However, since accumulation of glycogen also occurs in disease, glycogen accumulation in the cell may indicate diminished usage rather than increased metabolic activity. Nevertheless, the abundant accumulation of glycogen and the scant amount of rER and mitochondria in the prenatal cells is normal and a sign of immaturity, similar to observations by Plopper (1983) and Plopper et al. (1983c).

Large mitochondria, a second characteristic of nonciliated bronchiolar cells of the cat, are present in small numbers in prenatal cells, but increase in number with age. The mitochondria are located mainly in the apical cytoplasm and have an electron-dense matrix with few cristae. These findings agree with those of Plopper et al. (1980b). Large mitochondria with similar morphological features were also observed in mice, rabbits, and guinea pigs (Plopper et al., 1980c).

Multivesicular bodies were found in the adult and have been considered to be stages in electron-dense granule genesis (Young et al., 1986). Lamellated osmiophilic inclusions were observed only in prenatal cells. They have similar morphology to those found in pulmonary type II pneumocytes (Kilburn, 1974). Lamellated

osmiophilic bodies have been observed in the apical cytoplasm of adult cats (Plopper et al., 1980b). It has been suggested that phospholipid was secreted by Clara cells of mice (Niden, 1967; Etherton et al., 1973; Petrik and Collet, 1974). Walker et al. (1986) recently confirmed that Clara cells of the rat lung produce the major surfactant element (apoprotein).

Several authors have concluded that nonciliated bronchiolar cells have a secretory function, although there is not substantial morphological evidence. Three roles have been linked to the function of this cell: (1) secretion of airway lining materials (Niden, 1967; Cutz and Conen, 1971; Etherton et al., 1973), (2) metabolism of xenobiotic compounds (Serabjit-Singh et al., 1980), and (3) being progenitor cells for bronchiolar epithelial regeneration (Evans et al., 1978). In this study, NBCs of the cat lacked substantial amounts of sER and secretory granules.

Conflicting reports exist on the mechanism of secretion of the NBC in various animals (Stinson and Loosli, 1978; Etherton et al., 1979; Al-Ugaily et al., 1980, Pack et al., 1980; 1981). Several studies suggest that Clara cells may undergo apocrine secretion due to the presence of apical cytoplasmic blebs (Niden, 1967, Etherton, 1979). The limited morphological evidence of these studies may substantiate only the merocrine mode of secretion. On the other hand, Etherton et al. (1973) observed decapitation of the apical cytoplasm of the cells and speculated that this represents the secretory mechanism of the Clara cell. The observed islands of

cytoplasm in the bronchiolar lumen support the observations of Etherton et al. (1973) and Smith et al. (1974).

The sER has been linked to synthesis of secretory granules and metabolism of xenobiotic compounds via the cytochrome p-450 monooxygenase system (Boyd, 1977). Therefore, we believe that this cell is not involved in metabolism of xenobiotic compounds. In addition, we found no evidence of cell multiplication which would indicate that these cells are progenitors.

Little information is available concerning the time and sequence of maturation of the NBCs in most mammalian species. Observations in laboratory animals suggest that NBCs do not achieve the adult state for a considerable period of time postnatally (Baskerville, 1970; Smith et al., 1979; Plopper et al., 1983c). In the rat (Smith et al., 1979) and rabbit (Plopper et al., 1983c), it took up to a month to attain the adult state, whereas it took three to six months in the pig (Baskerville, 1970). In the cat, NBC numbers were near those of the adult by the third week of postnatal life.

These observations emphasize the need for further studies of the NBCs of the cat, for better understanding of their function, and, since secretion and detoxification are not clearly understood, for investigations into their role in fluid balance.

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VI. MORPHOMETRIC EVALUATION OF POSTNATAL GROWTH OF FELINE LUNG

ABSTRACT

Lung growth was quantitatively investigated in prenatal (60 days post-conception); postnatal (1-, 7-, 14-, and 21-day-old); and adult cats by light and electron microscopic morphometry. Lung weight and lung volume increased proportionately with body weight and age. Lung volume also increased with lung weight. All tissue compartments showed pronounced decreases in mass. The volume fraction of alveolar space and capillaries and the surface area increased as the cats grew. The surface density increased from 648 cm^2/cm^3 in prenatal cats to 1218 cm^2/cm^3 in 21-day-old cats. Whereas, the harmonic mean thickness reduced significantly (from 0.54 μm to 0.31 μm) with age. The volume fraction of type I and type II cells decreased gradually with age. No proliferation of type II cells was observed. Variations between animals of the same age was significant and was most likely due to hereditary factors.

INTRODUCTION

Ethical and procedural considerations limit the study of pulmonary development and growth in man. Animal models have been used to study the mechanisms of normal and abnormal human lung development and growth; however, these models need to be closely allied to man. Additional studies will help identify similarities and differences of animal models and human lung.

Mammalian lungs have been extensively studied from both a morphologic (Alcorn et al., 1981; Thurlbeck, 1982; Lechner and Banchero, 1982; Fukuda et al., 1983) and a quantitative prospective (Burri, 1974; Thurlbeck, 1975; Maina and King, 1987; Timmwood et al., 1987). The growth of mammalian lungs has been divided into four phases: 1) lung expansion (where lung volume increases as a result of enlargement of existing air spaces), 2) tissue proliferation (where all tissue compartments show a mass increase), 3) equilibrated growth, and 4) simple expansion of the alveoli.

Morphometry is a sensitive quantitative tool which can be of great value in evaluating changes in lung tissue (Barry and Crapo, 1985). Systematic morphometric examination of pulmonary development has been reported in man (Thurlbeck, 1982; Zeltner et al., 1987), the rat (Vidic and Burri, 1983), the guinea pig (Lechner and Banchero, 1982), and the pig (Winkler and Cheville, 1985). Winkler and Cheville (1985) reported a remarkable immaturity of pig lungs at birth. The lungs of new born animals are quantitatively different from those of adults. Alveoli are either few or absent at birth in the human, rat, and opossum (Loosli and Potter, 1959; Lesson and Lesson, 1964; Krause and Lesson, 1973, 1975). Therefore, extensive differentiation occurs postnatally.

Many quantitative morphometric studies of lung growth have been reported for fetal life (Alcorn et al., 1981; Adamson and King, 1984; Bunton and Plopper, 1984; Langston et al., 1984; Collins et al., 1986; Moessinger et al., 1986). However, few quantitative

morphometric studies have been reported on the postnatal growth of mammalian lung (Burri et al., 1974; Hislop et al., 1984; Winkler and Cheville, 1985). Although cats have been used as a model (Tsunoda et al., 1974; Plopper et al., 1980, 1983a, b), little morphometric data is reported especially for the early postnatal period (Zeltner et al., 1987). For this reason, this study is done to characterize morphometrically the pulmonary parenchyma in the prenatal and early postnatal cat. Postnatal lung growth is recorded in the prenatal and up to 21-days-old. Variation between lung lobes is eliminated by preferably sampling from the large caudal lobe.

MATERIALS AND METHODS

Animals:

Thirty-nine domestic cats (Felis catus) representing the following six age groups: prenatal (60 days post-conception); postnatal (1-, 7-, 14-, and 21-day-old); and adult (Table 6-1).

The cats were weighed and anesthetized using intramuscular injection of ketamine (5 mg/kg) and xylazine (0.4 mg/kg). The trachea was cannulated, and the lungs were fixed by infusion of 3% glutaraldehyde in phosphate buffer (pH 7.4, 0.1M) at a pressure of 20 cm H_2O. After fixation *in situ* for 30 minutes, a midline incision through the skin and subcutaneous tissue from the larynx to the xiphoid region exposed the trachea and the sternum. The thorax was opened on the midline, and the lungs and the trachea were carefully dissected free, removed, weighed, and lung volume was measured by the

water displacement method and recorded (Table 1). Tissue samples from the caudal lobe of the lungs were cut into small pieces, immersed in the fixative for an additional four hours, and processed for light and electron microscopy.

Light Microscopic Procedure:

Glutaraldehyde fixed samples were washed three times in phosphate buffer (pH 7.4, 0.1M). Subsequently, the samples were dehydrated in a graded series of ethanol (50%, 70%, 95% two changes at 10 minutes each). Infiltration of the samples was done at room temperature using standard JB-4 methods (Polysciences Inc., Warrington, PA). The samples were embedded and precisely oriented in the mold to yield cross-sections through the lungs. A Reichert Jung autocut microtome model 1140 was used to cut 3 μm thick sections. Sections were taken at 25 μm intervals from each block with glass knives and stained with hematoxylin and eosin.

Electron Microscopic Procedure:

Fixed samples were washed three times in phosphate buffer (pH 7.4, 0.1M), postfixed in 1% osmium tetroxide in phosphate buffer (pH 7.4; 0.1M) for 90 minutes, dehydrated in a graded series of ethanol and acetone, and embedded in an Epon-Araldite mixture. Thin sections were stained with 2% (w/v) aqueous uranyl acetate, contrasted with lead citrate, and viewed by Phillips model 201 electron microscope working at 60 kV.

Table 6-1: Number and sex of the cats used in this study with their parameters (Mean ± SD).

Age	N	Sex	Body Weight (gm)	Lung Weight (gm)	Lung Volume (cm^3)
Prenatal	8	4F/4M	80.937 ±3.771	2.437 ±0.457	2.300 ±0.458
1-day-old	9	4F/5M	89.285 ±6.466	2.611 ±0.387	2.833 ±1.012
7-day-old	5	1F/4M	172.417 ±44.122	3.300 ±0.800	4.767 ±1.779
14-day-old	6	2F/4M	264.850 ±20.072	4.550 ±1.659	6.167 ±0.764
21-day-old	7	4F/3M	365.989 ±44.250	6.222 ±0.566	8.833 ±0.289
Adult 2-4 years	4	3F/1M	3317.333 ±792.314	31.867 ±5.954	50.333 ±0.577

Morphometry:

Three cats from each group were randomly selected for morphometric examination. Light microscope stereological data from the lung were derived by point counts, utilizing a clear plastic simple square lattice test system of 100 points via video overlay on the screen. Five fields were randomly selected and counted from each of six blocks per animal. A total of 600-2000 points were counted for each parameter examined.

Points on the lung sections were divided into those falling on alveolar wall, alveolar space, bronchial tree, and connective tissue. Volume densities were determined using the following formula:

$$VF = Pr/PT$$

Where VF is the volume fraction (density) of the area of interest. Pr is the number of test points falling over the reference area, and PT is the total number of test points falling over the tissue.

Electron microscope stereological data were collected from three randomly selected lung blocks from each animal. Ten electron micrographs were taken from each block at 2000X magnification. The negatives were printed on RC photographic paper to yield a total magnification of 5250X. A total of 30 micrographs per animal or 90 micrographs per age group were used to measure the surface density and the harmonic mean thickness of air-blood barrier. These parameters can be measured by using a 100 points simple square

lattice and counting the number and measuring the length of intersections crossing the entire barriers.

Volume densities were measured using a 100 points simple square lattice. Only points falling on connective tissue and on the endothelial cell nuclei were counted. In addition, volume fraction and number of type I and type II pneumocytes were counted by points falling on their nuclei, assuming that they were single cells having single nuclei. The following formulae were used in these measurements:

$$Th = 2/3\ Ih$$

$$Sv = 4 \times I/d \times PT$$

$$Nv = K/\beta \times \downarrow Na^3/Vv$$

Symbols:

Th = harmonic mean thickness

Ih = harmonic mean intercept length

Sv = surface density

I = number of intersections

d = unit length of a linear probe in a test system

PT = total number of points counted

Nv = number of cells per unit volume

Na = number of cells per unit area

Vv = volume fraction or density

K = size distribution coefficient = 1.07 (Weibel et al., 1966)

ß = shape distribution coefficient = 1.382 (Weibel et al., 1966)

Statistical Methods:

Body weight, lung weight, and lung volume were tabulated from thirty-nine cats of different age and sex in table 1. Numerical data were analyzed from three cats per group using General Linear Model (GLM) procedures contained in Statistics Analysis System (SAS) software package. The functional relationships among body weight and age were described by simple regression equations. Since these functional relationships change with age and adult cats were not precisely aged, data were regressed with and without the adult. Difference of less than 5% statistical probability of being due to chance ($P<0.05$) is considered as a level of significance. The coefficient of determination (r^2) is used to characterize the closeness of the fit. An $r^2 = 1$ indicates a perfect correlation whereas an $r^2 = 0$ indicates no correlation (Snedecor and Cochran 1980). In this study, r^2 greater than 0.500 was considered a significant correlation. In addition, the root mean squared error for each relationship was noted.

RESULTS

Lung growth:

Table 6-2 summarizes the regression equations of lung weight and lung volume as a function of body weight . Body weight is a highly significant predictor of both lung weight and lung volume, regardless of sample size. However, with the addition of *adult cats*, the slope of the relationship for both dependant variables is significantly

decreased. Similarly, body weight, lung weight and lung volume are each a linear function of age in the *growing cats* (Table 6-3). In addition, the lung volume with or without *adult cats* is strongly correlated to lung weight (Table 6-4).

Volume density of the lung:

The volume densities of alveolar wall, alveolar space, and bronchial tree and connective tissue were measured from light micrographs and are listed in Table 6-5. The greatest changes in the composition of the parenchyma were in the alveolar space and alveolar duct, which doubled with age. Volume density of alveolar wall, and bronchial tree and connective tissue decreased with age.

The volume densities of alveolar space, bronchial tree and connective tissue of *growing cats* were linearly correlated with body weight, but volume density of alveolar wall was statistically significant but not linear (Table 6-7). When all cats were included in the analyses (Table 6-6) no correlation was observed, but the volume densities of alveolar wall and space were statistically significant. Regression as a function of age indicated that the majority of volume densities show strong linear relationships with age (Table 6-8).

The volume densities of septal connective tissue, capillaries, type I cells, and type II cells were measured from electron micrographs and their means and standard deviations listed in Table 6-5. Composition of septal connective tissue did not change during the postnatal period, but its volume density decreased from 35% in

Table 6-2: Correlation of lung weight (LW) and lung volume (LV) as a function of body weight with (n = 39) and without (n = 35) adult cats.

Dependent Variables	n	Intercept	Slope	r^2	s	P<F
LW	39	1.748	0.009	0.945	2.324	0.0001
LV	39	2.846	0.009	0.916	2.913	0.0001
LW	35	1.071	0.014	0.854	0.676	0.0001
LV	35	0.618	0.022	0.880	0.978	0.0001

Table 6-3: Correlation of body weight (BW), lung weight (LW), and lung volume (LV) as a function of age of growing cats.

Dependent Variables	n	Intercept	Slope	r^2	s	P<F
BW	35	-637.505	11.525	0.921	33.507	0.0001
LW	35	-7.639	0.158	0.782	0.827	0.0001
LV	35	-13.869	0.261	0.840	1.127	0.0001

Table 6-4: Correlation of lung volume (LV) as a function of lung weight (LW) with (n = 39) and without (n = 35) adult cats.

Dependent Variables	n	Intercept	Slope	r^2	s	P<F
LV	39	1.060	1.005	0.981	1.393	0.0001
LV	35	-0.506	1.451	0.827	1.172	0.0001

Table 6-5: Mean and standard error of the mean of volume density of alveolar wall (AW), alveolar space (AS), bronchial tree and connective tissue (BCT), type I (TI) and type II (TII) cells, septal connective tissue (CT) and capillaries (CAP); harmonic thickness (HT); surface density (SD); and numerical densities of type I (NI) and type II (NII) cells. Postconception (PC).

VARIABLES	N	PREPARTUM	POSTPARTUM				ADULT
		60 DAYS PC	1 DAY	7 DAYS	14 DAYS	21 DAYS	2-4 YEARS
AW	18	0.414 ±0.015	0.250 ±0.012	0.244 ±0.005	0.244 ±0.001	0.212 ±0.010	0.186 ±0.027
AS	18	0.283 ±0.006	0.522 ±0.007	0.547 ±0.014	0.589 ±0.011	0.607 ±0.007	0.642 ±0.038
BCT	18	0.304 ±0.015	0.228 ±0.008	0.204 ±0.006	0.167 ±0.009	0.181 ±0.003	0.180 ±0.010
TI	18	0.028 ±0.004	0.012 ±0.002	0.012 ±0.001	0.007 ±0.001	0.006 ±0.001	0.004 ±0.001
TII	18	0.097 ±0.023	0.044 ±0.003	0.044 ±0.012	0.026 ±0.005	0.018 ±0.003	0.022 ±0.002
CT	18	0.347 ±0.032	0.290 ±0.014	0.262 ±0.025	0.232 ±0.005	0.201 ±0.016	0.142 ±0.031
CAP	18	0.120 ±0.008	0.134 ±0.024	0.106 ±0.022	0.064 ±0.013	0.117 ±0.016	0.219 ±0.014
HT (μm)	18	0.544 ±0.021	0.476 ±0.017	0.419 ±0.011	0.336 ±0.014	0.326 ±0.005	0.310 ±0.003
SD (cm^2/cm^3)	18	647.662 ±46.083	796.250 ±58.942	947.329 ±55.109	1041.250 ±74.187	1218.194 ±64.114	1463.194 ±59.004
NI/mm^3	18	317655 ±51028	213781 ±10319	139180 ±37923	1748471 ±18517	202799 ±30816	157013 ±12452
NII/mm^3	18	848644 ±135086	776117 ±9045	493911 ±83488	652544 ±62206	310085 ±62298	292471 ±25993

Table 6-6: Correlation of volume density of alveolar wall (AW), alveolar space (AS), bronchial tree and connective tissue (BCT), type I (TI) and type II (TII) cells, septal connective tissue (CT) and capillaries (CAP); and numerical densities of type I (NI) and type II (NII) cells as a function of body weight including the adult. Linear and/or significant relationship (*).

Dependent Variables	n	Intercept	Slope	r^2	s	P<F
AW	18	0.281	-0.000	0.252	0.069	0.0340*
AS	18	0.497	0.000	0.236	0.111	0.0412*
BCT	18	0.221	-0.000	0.125	0.047	0.1509
TI	18	0.014	-0.000	0.180	0.008	0.0794
TII	18	0.048	-0.000	0.112	0.031	0.1742
CT	18	0.276	-0.000	0.497	0.054	0.0011*
CAP	18	0.102	0.000	0.596*	0.035	0.0002*
HT (μm)	18	0.429	-0.000	0.244	0.085	0.0371*
SD (cm^2/cm^3)	18	908.613	0.161	0.379	261.049	0.0065*
NI/mm^3	18	213043.7	-17.009	0.079	73635.1	0.2578
NII/mm^3	18	638698.2	106.832	0.285	21484.9	0.0224*

prenatal kittens to 14% in *adults*. On the contrary, the volume density of capillaries increased from 12% in the *prenatal* to 22% in the *adult*. In addition, volume density of type I cells decreased from 3% in the *prenatal* to 0.4% in the *adult*. Similarly, volume density of type II cells decreased from 10% in *prenatal* to 2% in the *adult*.

Linear regression of volume densities of septal connective tissue, capillaries, type I cells, and type II cells are summarized in Table 6-6. The volume density of septal connective tissue was not correlated with body weight but was significant when all ages were included. However, it was significantly correlated in growing cats (Table 6-7). Furthermore, volume density of capillaries was neither significant nor correlated with body weight in growing cats, but it was significantly correlated when all ages were included (Table 6-6). Volume density of type I cells and type II cells were statistically significant as a function of body weight in growing cats(Table 6-7). Regression as a function of age indicated that only capillary volume fraction was not significantly correlated (Table 6-8).

Surface Density and Barrier Thickness:

The surface density of the lung increased significantly from *prenatal* 648 cm^2/cm^3 to *adulthood* 1463 cm^2/cm^3 (Table 6-5). The harmonic mean thickness of air-blood diffusion barrier reduced from 0.54 μm in the *prenatal* to 0.31 μm in the *adult*. Linear regression of surface density of the lung and harmonic mean thickness of air-

Table 6-7: Correlations of volume density of alveolar wall (AW), alveolar space (AS), bronchial tree and connective tissue (BCT), type I (TI) and type II (TII) cells, septal connective tissue (CT) and capillaries (CAP); and numerical densities of type I (NI) and type II (NII) cells as a function of body weight of growing cats. Linear and/or significant relationship (*).

Dependent Variables	n	Intercept	Slope	r^2	s	P<F
AW	15	0.354	-0.000	0.413	0.060	0.0098*
AS	15	0.363	0.001	0.508*	0.089	0.0029*
BCT	15	0.281	-0.000	0.546*	0.036	0.0017*
TI	15	0.023	-0.000	0.455	0.007	0.0058*
TII	15	0.084	-0.000	0.453	0.026	0.0060*
CT	15	0.349	-0.000	0.665*	0.036	0.0002*
CAP	15	0.128	-0.000	0.116	0.035	0.2153
HT (μm)	15	0.554	-0.000	0.706*	0.053	0.0001*
SD (cm^2/cm^3)	15	616.6	1.683	0.531*	183.828	0.0021*
NI/mm^3	15	261534.1	-226.4	0.152	75053.8	0.1507
NII/mm^3	15	900433.6	1459.3	0.514*	169382.2	0.0026*

Table 6-8: Correlation of volume density of alveolar wall (AW), alveolar space (AS), bronchial tree and connective tissue (BCT), type I (TI) and type II (TII) cells, septal connective tissue (CT) and capillaries (CAP); and numerical densities of type I (NI) and type II (NII) cells as a function of age of the growing cat. Most are linearly correlated (*).

Dependent Variables	n	Intercept	Slope	r^2	s	P<F
VW	15	0.695	-0.006	0.569*	0.051	0.0012*
AS	15	-0.242	0.010	0.680*	0.072	0.0002*
BCT	15	0.543	-0.005	0.716*	0.029	0.0001*
TI	15	0.065	-0.001	0.629*	0.006	0.0004*
TII	15	0.231	-0.003	0.556*	0.023	0.0014*
CT	15	0.643	0.005	0.714*	0.033	0.0001*
CAP	15	0.191	-0.001	0.098	0.035	0.2565
HT (μm)	15	1.049	-0.009	0.800*	0.044	0.0001*
SD (cm^2/cm^3)	15	-575.9	20.9	0.611*	67.4	0.0006*
NI/mm^3	15	485293.0	-3807.2	0.220	71979.9	0.0777
NII/mm^3	15	1931130.2	18161.2	0.564*	160398.6	0.0013*

blood diffusion barrier as a function of body weight was significant but not correlated (Table 6-6). Furthermore, a regression of harmonic mean thickness of the air-blood diffusion barrier indicated a strong correlation with body weight in *growing cats*. Also the surface density of the lung was significantly correlated (Table 6-7). Similarly, regression as a function of age improved the linearity of both the harmonic mean thickness of the air-blood diffusion barrier and the surface density of the lung (Table 6-8).

Numerical density:

The number of type I and type II cells per unit volume was calculated and listed in Table 6-5. The number of type I cells was reduced by a half from the *prenatal* to the *adult*, whereas type II cells decreased by more than one half. Linear regression, whether it includes the *adult* or not indicated no correlation with body weight for type I cells (Table 6-6, 6-7), but for type II cells, there was a significant correlation only in *growing cats*. In addition, regression as a function of age generated similar results (Table 6-8). Regressing the means of all parameters improved the linearity but not all were significant (Table 6-9).

Table 6-9: Correlation of the means of volume density of alveolar wall (AW), alveolar space (AS), bronchial tree and connective tissue (BCT), type I (TI) and type II (TII) cells, septal connective tissue (CT) and capillaries (CAP); and numerical densities of type I (NI) and type II (NII) cells as function of age of the growing cat. The linearity of most was strong but not necessarily significant(*).

Dependent Variables	n	Intercept	Slope	r^2	s	P<F
AW	5	0.701	-0.006	0.593*	0.059	0.1275
AS	5	-0.244	0.010	0.690*	0.084	0.0815
BCT	5	0.542	-0.004	0.767*	0.030	0.0515*
TI	5	0.063	-0.000	0.731*	0.005	0.0649
TII	5	0.233	-0.000	0.770*	0.017	0.0507*
CT	5	0.643	-0.005	0.955*	0.024	0.0042*
CAP	5	0.192	-0.001	0.208	0.027	0.4400
HT (μm)	5	1.047	-0.009	0.952*	0.023	0.0045*
SD (cm^2/cm^3)	5	-575.9	20.9	0.978*	38.0	0.0014*
NI/mm^3	5	485295.2	-3807.2	0.354	62099.8	0.2900
NII/mm^3	5	1931129.8	-18161.2	0.761*	122996.6	0.0538*

DISCUSSION

This study reports several features of normal growing lungs in the cat. This study also supports the hypothesis of Engel (1953) that mammalian lung morphology at birth reflects the level of general body development of the species. While numerous morphologic reports suggest this conclusion, few studies have provided morphometric data on functionally related parameters (Burri et al., 1974).

Lechner and Banchero (1982) reported that lung volume of guinea pigs increased exponentially with body weight from 56 days of gestation to 16 days postpartum. Similar relationships were reported in man (Langston et al., 1984). However, recent studies on rhesus monkeys (Bunton and Plopper, 1984; Hislop et al., 1984) and guinea pigs (Collins et al., 1986) suggest that the relationship of lung volume to body weight is linear. Our data shows a strong linear relationship between lung volume and body weight. In addition, increase in lung weight was also proportional to body weight.

Morphometric evaluation of growing cat lungs at both light and electron microscopic levels makes it possible to estimate relative volume, surface area, and the air blood barrier thickness. The volume density of alveolar wall in 21-day-old kittens is reduced to one-half of prenatal levels. It is possible that the gradual reduction of septal connective tissue may be attributed to the reduction of alveolar wall or to an increase of alveolar lumen space.

Winkler and Cheville (1985) observed significant increases in relative volume density of septal connective tissue in pig lungs.

The increase in connective tissue is reported to be from fibroblast proliferation and an increase in the amount of collagen (Brody and Vaccaro, 1979; Brody and Kaplan, 1983; Winkler and Cheville, 1984). The relatively large amount of connective tissue in cats may be from the presence of a double capillary bed in their walls. The connective tissue supports the capillaries by interlacing between them, giving a strong fibrous continuum to support their weight. The volume density of capillaries increases during postnatal life. Similar findings have been reported in pigs (Rendas et al., 1978; Haworth and Hislop, 1981).

Burri et al. (1974) reported the period of equilibrated growth commences between day 13 and 21 in the rat. During this phase, the newly formed respiratory units progressively enlarge due to stretching of the septal contours, as a result the volume density of the alveolar space is increased. In this report postnatal restructuring of the interstitium is presumed an important stage of growth, since the lung is probably in an equilibrated growth phase.

Tenney and Remmers (1963) have shown that the high metabolic rate and oxygen demand of small animals is accommodated by increased alveolar partitioning which results in decreased alveolar size. This conclusion was supported by Collins et al. (1986) showing that the increased surface area in guinea pigs is from increasing alveolar numbers. Our data indicates an increase in surface density of growing cat lungs. The increase in lung volume suggests alveolar proliferation resulted from both subdivision and enlargement of the

existing air spaces due to stretching of their walls. Gellhaar and Weibel (1971) suggest if this increase is due to an increase in the number of alveoli, which result from finer subdivision of the air spaces, then there will be no change in the lung volume.

There was no indication of Type II pneumocyte cell proliferation. This is consistent with earlier morphologic observations (Winkler and Cheville, 1984) and morphometric observations (Winkler and Cheville, 1985) in the pig. Regional differences of lung structure were described in dogs (Glazier et al., 1967) and were probably due to gravitation (Berendsen and DeFouw, 1982). Winkler and Cheville (1985) reported no significant variations between parameters on different lobes of porcine lungs. Although the cats were held in similar environments, variations among them were found. This was observed when the means of all parameters were regressed with age. These analyses show a strong linear relationship between age and most lung parameters but not all such relationships are significant. This is likely attributable to hereditary.

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VII. A CORRELATIVE LIGHT AND SCANNING ELECTRON MICROSCOPIC STUDY OF THE DEVELOPING FELINE PULMONARY ACINUS

ABSTRACT

To investigate the postnatal growth of feline lungs, lungs were fixed by intratracheal instillation of 3% glutaraldehyde in phosphate buffer at a pressure of 20cm H_2O. Samples, taken from the caudal lobe of prenatal, 1-, 7-, 14- and 21-day-old postnatal, and adults were processed for light and SEM. The cats were born with incomplete alveolar formation. The lungs have many saccules surrounded by thick primary septa. The primary septum was flanked by a double capillary network that remains throughout the postnatal period. Postnatally, the saccule expanded and was subdivided by secondary septa of variable height, which were similar in structure to primary septa. Alveolar formation by septation of the saccule, continued beyond 21 days of age. As development progressed, the secondary septum lengthened, became thin and yet contained a double capillary network, and its free end became blunt. The saccule was lined by cuboidal type II pneumocytes whose surfaces were covered with a few microvilli. The microvilli increased in number gradually with age thus covering the adult cell extensively. Type II pneumocytes were located at the base of secondary septa in growing cats, giving structural stability to the secondary septa. Transformation of type II pneumocytes into type I pneumocytes is suggested from the observation of cytoplasmic extensions and attenuations. Pores of Kohn were observed only in adult cats. It is concluded that the

basic pattern of alveolar formation, through septation of saccules, is similar to that of other mammalian species.

INTRODUCTION

There is controversy regarding alveolar development among investigators who study postnatal growth of the lungs of different species (Boyden and Tompsett, 1965; Burri, 1974; Amy et al., 1977; Antonia and Ten Have-Opbroek, 1981; Tyler et al., 1988). From these studies, one can categorize lung development into three modes: 1. Alveoli are well-developed in prenatal life as in the lungs of lambs (Engel, 1953). 2. Many of the alveoli form post partum as in man (Zeltner and Burri, 1987). 3. The alveoli develop totally in postnatal life, as in rats and mice (Burri et al., 1974; Thurlbeck, 1975). Lung growth has been extensively studied in rats and mice, as they remain a standard laboratory animal model (Ten Have-Opbroek, 1986; Scheuermann et al., 1988). In recent years, various animals including monkeys have been used and suggested to be the closest model to human lungs for study of diseases which affect the respiratory system (Winkler and Cheville, 1984; Maina, 1987; Tyler et al., 1988).

Cats were recently introduced as a laboratory animal. However, little data has been published concerning their lung development (Mercurio and Rhodin, 1976; 1984). Therefore, this study was undertaken to observe the method of alveolization in the domestic cat as seen with the aid of light and scanning electron microscopy. This

study will enable us to point-out similarities and differences with the other species whose lung development has been already documented.

MATERIALS AND METHODS

Thirty-nine domestic cats (Felis catus) representing the six following age groups: prenatal (60 days post-conception), postnatal (1-, 7-, 14-, and 21-day-old), and adult were used in this study. The cats were anesthetized by the intramuscular injection of ketamine (5 mg/kg) and xylazine (0.4 mg/kg). The trachea was cannulated and the lungs were fixed by infusion of 3% glutaraldehyde in phosphate buffer (pH 7.4, 0.1M) at a pressure of 20 cm H_2O. After fixation *in situ* for 30 minutes, a midline incision through the skin and subcutaneous tissue from the larynx to the xiphoid region was performed to expose the trachea and the sternum. The thorax was opened and the lungs with the trachea were carefully dissected free and removed. Tissue samples were taken from the caudal lobe of the lung and cut into small pieces and immersed into the fixative for an additional four hours. The samples were then processed for light and scanning electron microscopy.

For light microscopy, glutaraldehyde fixed tissue was washed three times in phosphate buffer (pH 7.4, 0.1M). Subsequently, the samples were dehydrated in a graded series of ethanol (50%, 70%, 95%; two changes at 10 minutes each). Infiltration of the samples was done at room temperature using standard JB-4 methods (Polysciences Inc., Warrington, PA). Sections, 3μm thick, were taken at 25μm

intervals from each block with glass knives using a Reichert Jung Autocut microtome, model 1140 and stained with hematoxylin and eosin.

Glutaraldehyde fixed samples for scanning electron microscopy were postfixed for 90 minutes in 1% osmium tetroxide in phosphate buffer (pH 7.4, 0.1M). They were washed three times in phosphate buffer for 10 minutes each, then dehydrated in a graded series of acetone solutions (70%, 80%, 95%, with three changes in 100% acetone). The samples were dried in a critical point drying apparatus with liquid CO_2. Tissues were then mounted on aluminum stubs, coated with a 15nm gold-palladium layer, and examined with an ETEC Scanning Electron Microscope operating at 25 kV.

RESULTS

Light Microscopic Findings:

The lungs of *prenatal kittens* had saccules of different sizes which were delineated by thick connective tissue septa. The wall of saccules, called primary septum, was irregular on the respiratory side and lined primarily by cuboidal type II pneumocytes, which bulged into the air spaces (Fig. 7-1). At higher magnification, these cells were recognized by their large centrally located nucleus with a prominent nucleolus. The cytoplasm was vesicular due to the presence of multilamellated inclusions. The cells were prominent where secondary septa were beginning to emerge from the primary septa (Fig. 7-2). All parenchymal septa were thick and cellular. The connective tissue of the primary septa was cellular and rich in blood

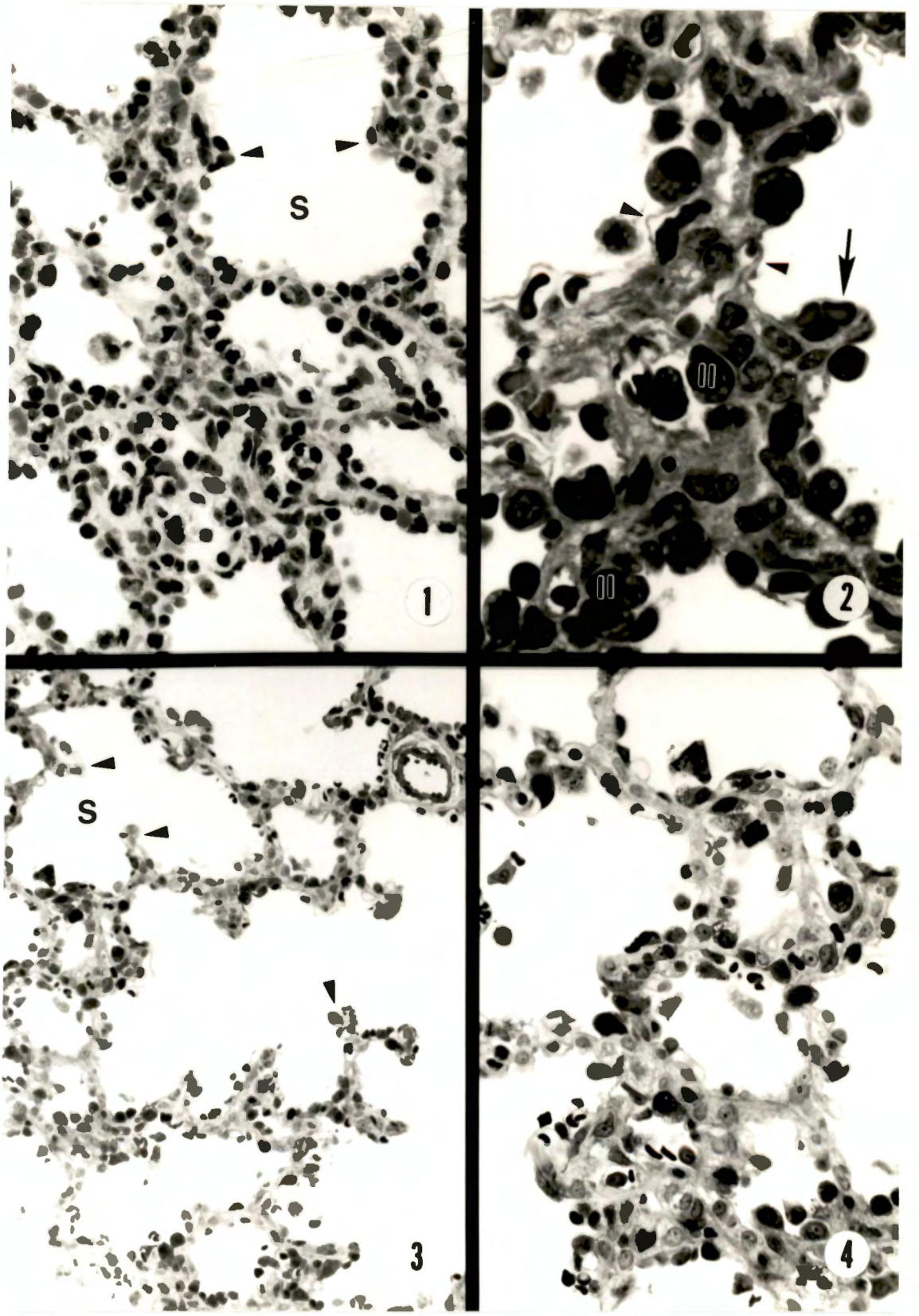
PLATE 7-1

Fig. 7-1: Lung section of prenatal kitten showing a saccule (S) surrounded by a thick wall. Growing secondary septa (arrowheads). X465

Fig. 7-2: High magnification of prenatal lung section showing cellular content of primary septa. Notice the double capillary network (arrowheads) and emergence of a secondary septum (arrow). Type II pneumocytes (II). X1,150

Fig. 7-3: Lung of 1-day-old kitten showing expanded saccules (S) and many secondary septa of variable heights (arrowheads). X115

Fig. 7-4: Light micrograph of the lung of 7-day-old kitten. Notice the thinning of the primary wall and the double capillary network. X465


S
1
2
S
3
4

capillaries. Numerous mesenchymal cells were scattered among the connective tissue fibers (Figs. 7-1, 7-2).

The primary septum was flanked by a double capillary network which bulged into the air spaces. Blunt ridges of variable heights protruded from the primary septa, marking the formation of secondary septa (Figs. 7-1, 7-2). The secondary septa were similar to the primary septa.

One day postnatally, the lungs were similar to the prenatal. The saccule was expanded and numerous secondary septa were beginning to divide them into multiple shallow alveoli (Fig. 7-3). *Seven days postnatally*, the stroma of the primary septum was still cellular and the wall contained a double capillary network (Fig. 7-4).

In *14-day-old kittens*, a marked decrease in the cellularity of the primary saccule, rendered the wall thinner than at younger ages. The saccule was divided into shallow alveoli by the growing secondary septa which were variable in height (Fig. 7-5). The respiratory surface of alveoli was smooth. Fibroblasts had increased in numbers at the junction of primary septa and a few mitotic cells were observed. The other features of the walls appeared similar to those of previous ages (Fig. 7-6).

Further maturation of the secondary septa was observed in *21-day-old kittens*. In some saccules, the septa had lengthened and expanded at the tip where it contained a capillary loop (Fig. 7-7). Septation was irregular due to the formation of new secondary septa. However, the wall of the saccule still had a double capillary

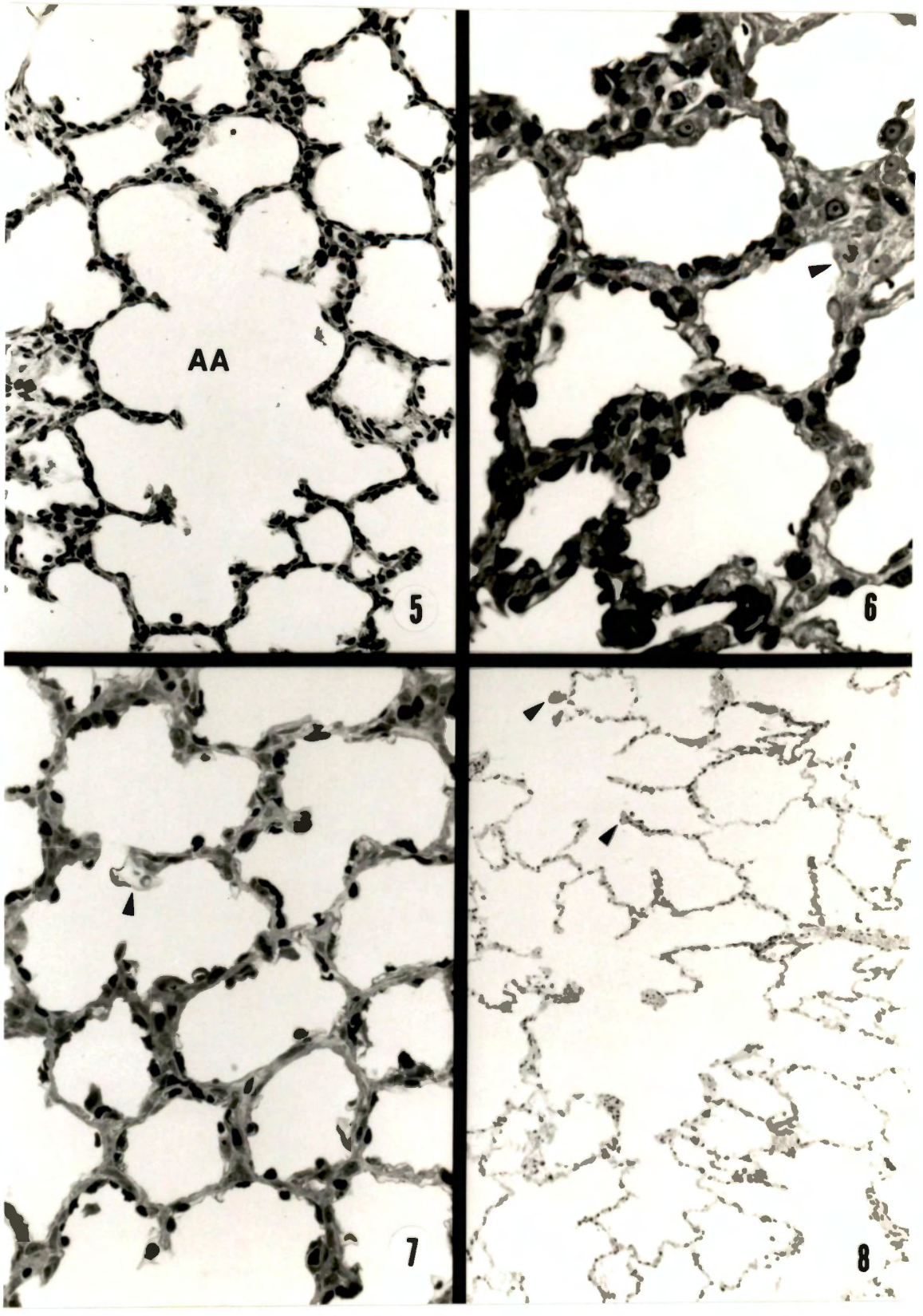
PLATE 7-2

Fig. 7-5: Light micrograph of a 14-day-old kitten showing a large alveolar antrum (AA) divided into many alveoli by secondary septa of variable heights. X230

Fig. 7-6: Light micrograph of a 14-day-old kitten. Note the thickened connective tissue present at the septal junction. Fibroblast in mitosis (arrowhead). X465

Fig. 7-7: Light micrograph of a 21-day-old kitten. Note the thin wall of the saccule and the expanded tip of the secondary septum (arrowheads) with blood capillaries. X465

Fig. 7-8: Adult lung showing thin alveolar walls and well-developed septa that end in a knob (arrowheads) with a single capillary network. X230


AA
5
6
7
8

network, but was reduced in relative thickness in comparison with younger ages. The *adult* lung had characteristically thin walls and a single capillary network which bulged alternatively into the alveolar space. The alveoli were deepened by the elongated septa which ended in an expanded blunt tip (Fig. 7-8).

Scanning Electron Microscopic Findings:

At low magnification, irregular expansions of different sizes were observed in *prenatal kittens* which represent the gas exchange unit or saccules. However, alveolization had begun in a few areas by saccules being divided into multicompartments by the growth of secondary septa (Fig. 7-9). At higher magnification, thick mesenchymal tissue with abundant connective tissue fibers surrounding the saccule was observed (Fig. 7-10). The saccule was lined by cuboidal type II pneumocytes identified by their characteristic shape and the presence of minute microvilli covering their surface (Fig. 7-10). In areas where alveoli were forming, low ridges divided the saccule into shallow alveoli (Fig. 7-11). These ridges, corresponding to the secondary septa, had a smooth surface.

By the *first day of postnatal life*, the saccule had expanded, and the wall appeared to have become thinner (Fig. 7-12). The secondary septum was a ridge running along the wall of the saccule dividing it into two or more air compartments. Cytoplasmic extensions of type I pneumocytes were observed. In other areas, the newly formed alveoli were well-developed and lined by type II pneumocytes which were covered with numerous microvilli. Occasional

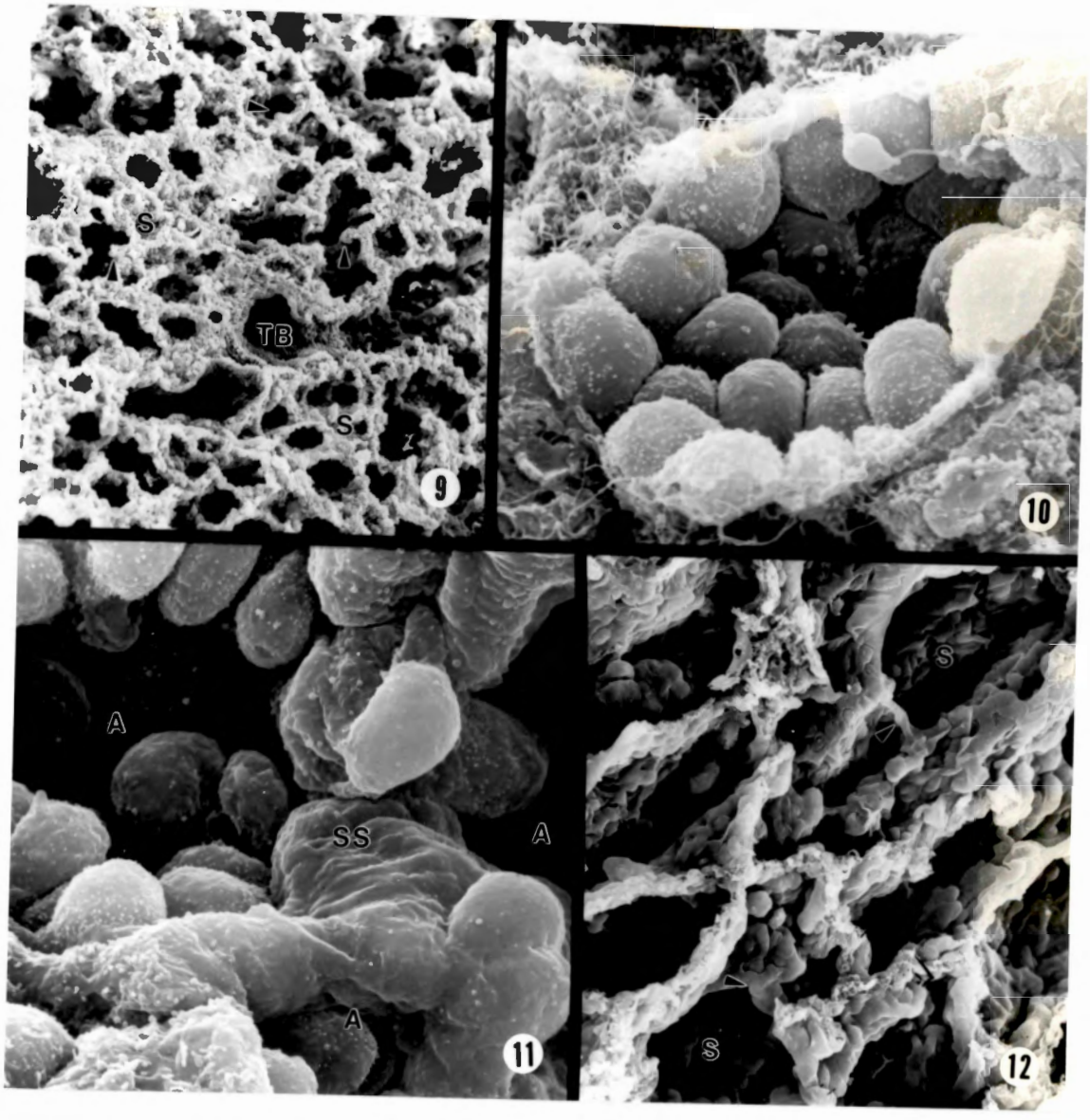
PLATE 7-3

Fig. 7-9: Lung of prenatal kitten with many saccules (S) surrounded by a thick wall. Note some saccules starting to develop secondary septa (arrowheads). Terminal bronchiole (TB). X216

Fig. 7-10: Lung of prenatal kittens with a saccule surrounded by a thick mesenchymal tissue which represents a primary septum. Cuboidal type II pneumocytes line the saccule. X4,800

Fig. 7-11: Lung of prenatal kitten with a portion of three shallow, primitive alveoli (A) separated by low secondary septa (SS). X4,800

Fig. 7-12: Lung of 1-day-old kitten with an expanded saccule (S) surrounded by thin walls and divided by low secondary septa (arrowhead). X540


S
TB
S
9
10
A
SS
A
A
11
S
S
12

macrophages, characterized by rough surfaces and elaborate pseudopodia, were observed on the surface of alveolar epithelium (Fig. 7-13).

In *7-day-old kittens*, the presence of many secondary septa emerging from the primary septum indicated maturation of the lung (Fig. 7-14). Although most of the parenchyma contained alveoli, in some areas an occasional saccule was observed. Primary septa, of thick connective tissue which surrounded the saccules, were thinner (Fig. 7-15). At higher magnification, three types of cells were identified: 1. Type I pneumocytes which have a smooth flat surface. 2. Type II pneumocytes which bulge into the lumen and were covered with numerous microvilli. 3. Macrophages similar to those of 1-day-old kittens (Fig. 7-16).

By *14 days*, the lung had changed markedly. The rapidly growing secondary septa had partitioned saccules into many alveoli of different sizes (Fig. 7-17). The secondary septa had increased in height thus deepening the previously shallow alveoli. The septa were thinner with a smooth surface (Fig. 7-18).

At *21 days of postnatal life*, a large number of thin-walled alveoli were observed and many alveoli opened into the alveolar antrum (Fig. 7-19). The lung was similar to adult lung, but it lacked pores of Kohn.

The *adult* lungs had large cup-shaped alveoli with interalveolar pores of Kohn (Fig. 7-20). The pores were ellipsoidal or rounded. The alveoli were separated by thin walls covered by simple squamous

PLATE 7-4

Fig. 7-13: High magnification of an alveoli of a 1-day-old kitten. Many cuboidal type II pneumocytes are covered by microvilli. Macrophage (M). X5,400

Fig. 7-14: Lung of a 7-day-old kitten with many large saccules divided by secondary septa (arrows) into small shallow alveoli. Alveolar duct (AD). X225

Fig. 7-15: Lung of a 7-day-old kitten with a few saccules (S) observed surrounding the primary wall among the alveoli (A). X1,800

Fig. 7-16: High magnification of an alveolus of a 7-day-old kitten. Three types of cells are observed: macrophage (M), type I pneumocyte (I), and type II (II) pneumocyte. X4,800

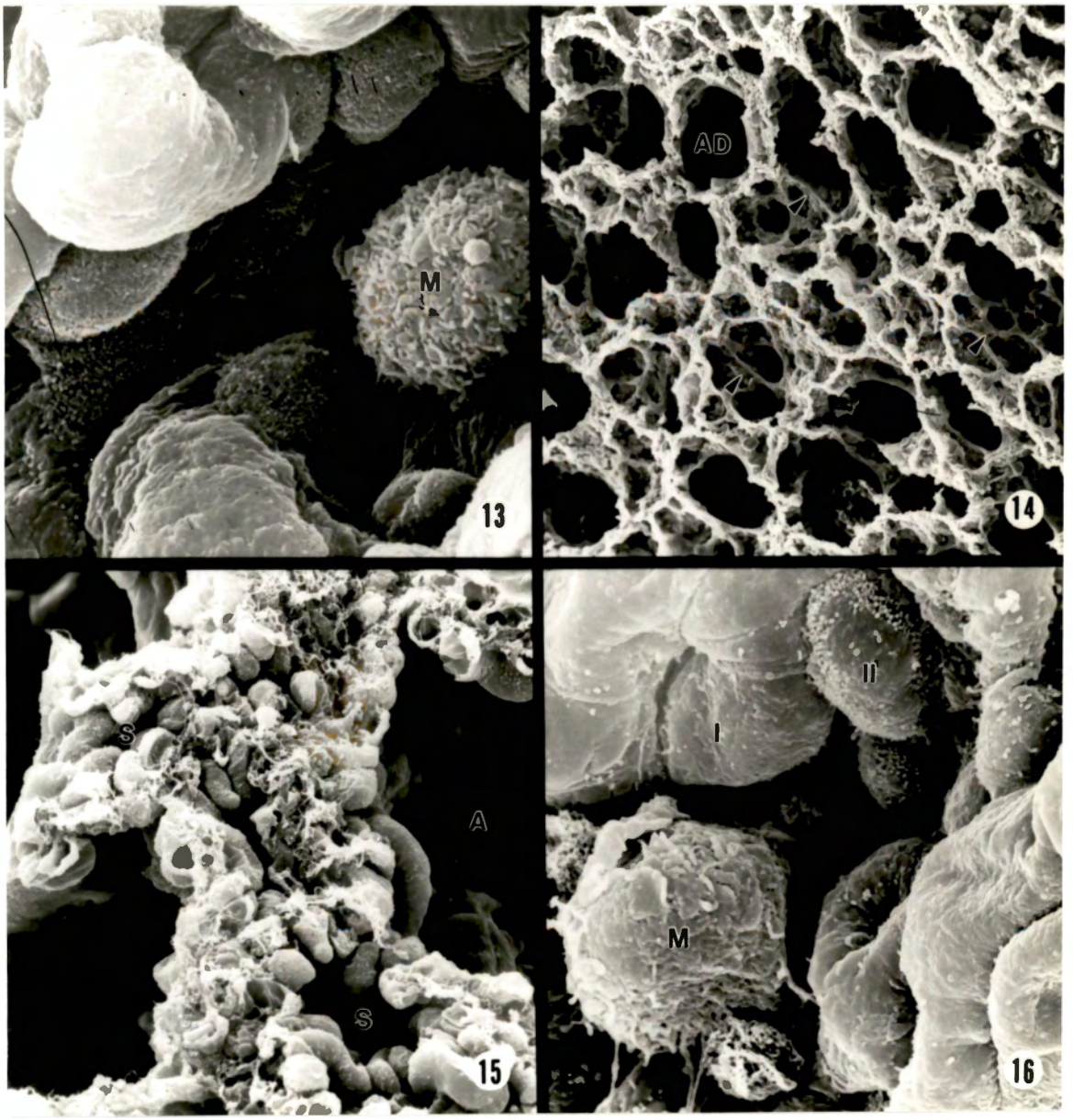

M
13
AD
14
S
A
S
15
II
I
M
16

PLATE 7-5

Fig. 7-17: Lung of a 14-day-old kitten, showing several alveoli (A). X203

Fig. 7-18: High magnification of a 14-day-old kitten with newly formed alveoli (A) which are deepened by the increasing height of the secondary septa (SS). X2,100

Fig. 7-19: Lung of a 21-day-old kitten with the alveolar antrum (AA) opening into many alveoli. Alveolar duct (AD). X390

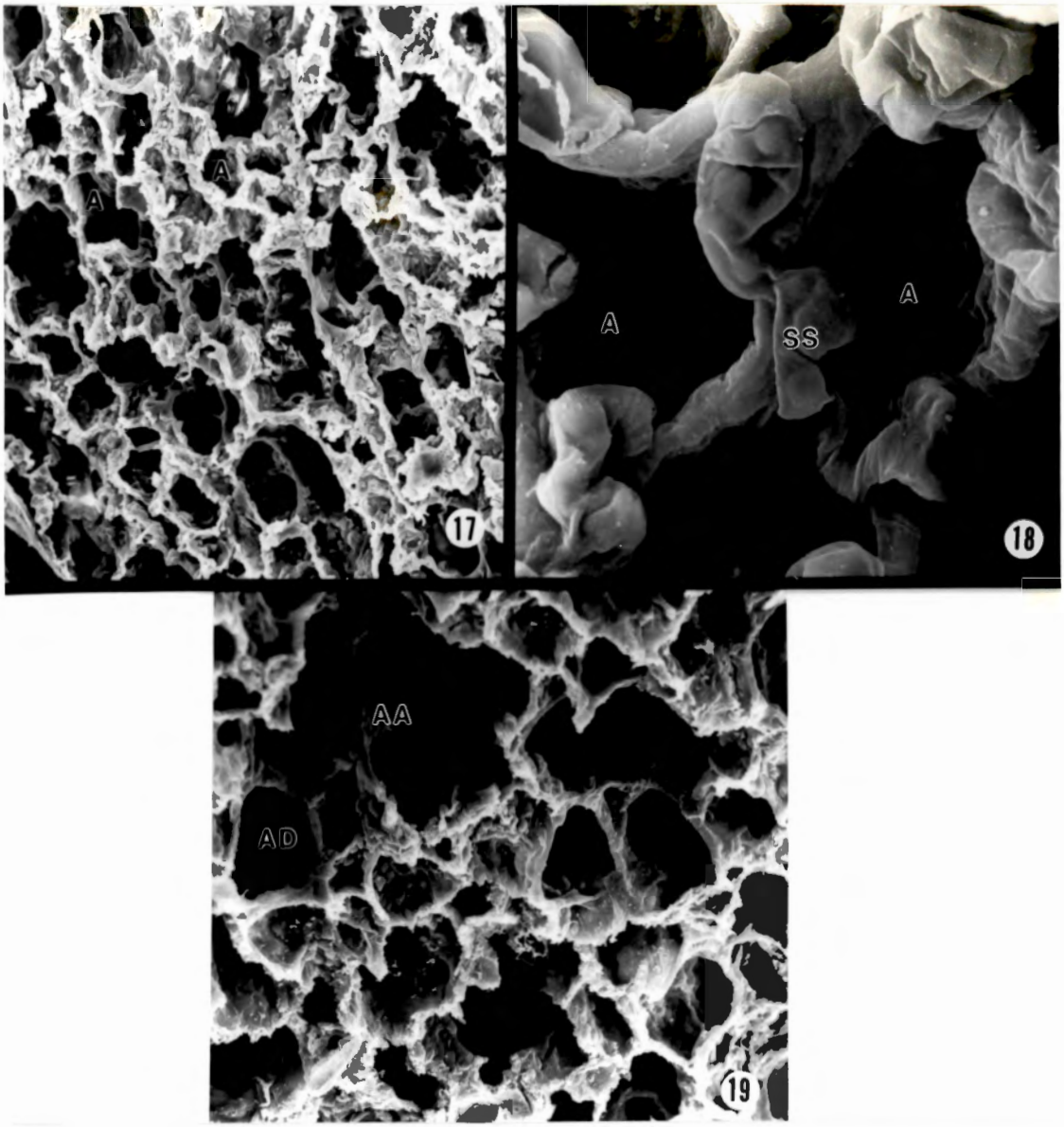
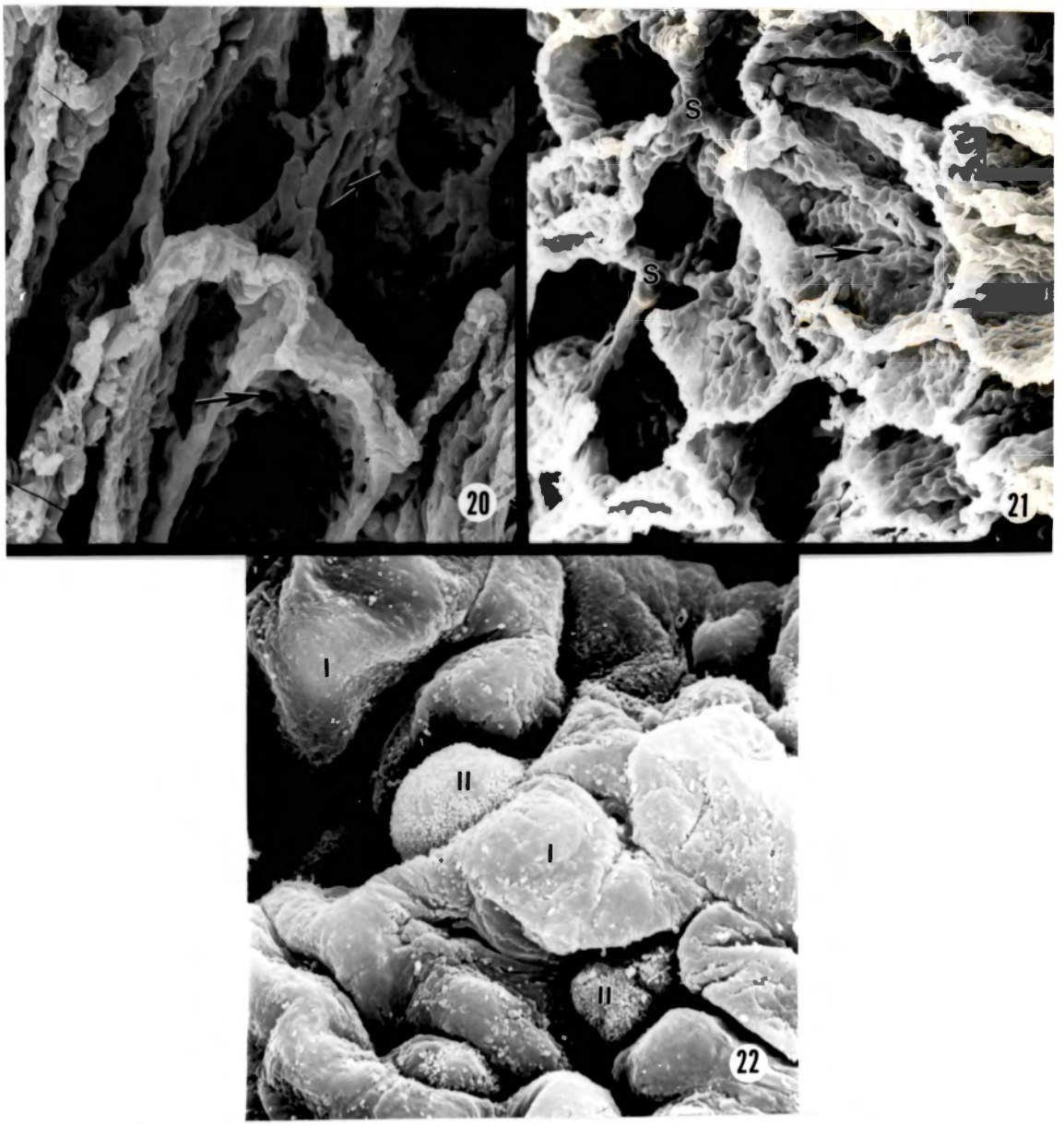

A
A
17
A
SS
A
18
AA
AD
19

PLATE 7-6

Fig. 7-20: Lung of an adult cat with its alveolar walls perforated by pores of Kohn (arrows). X660

Fig. 7-21: Lung of an adult cat with a well-developed septum (S) that has a smooth expanded surface. Pores of Kohn (arrow). X840

Fig. 7-22: High magnification of the alveolus of an adult cat showing well-developed type II pneumocytes (II) covered with numerous microvilli. Cytoplasmic extensions of type I pneumocyte (I). X4,800


20
S
S
21
I
II
I
II
22

type I pneumocytes. The septa were fully developed in length and terminated in a free, smooth, rounded knob (Fig. 7-21). At higher magnification, cuboidal type II pneumocytes were covered with numerous microvilli and were located at the entrance into the alveolus (Fig. 7-22).

DISCUSSION

Cats were born with incomplete alveolization of their lungs. The lung parenchyma of prenatal kittens had a large number of saccules. Although a few saccules had a few shallow alveoli, they were considered immature because of their thick wall and lining of cuboidal type II pneumocytes. The pattern of alveolization was similar to that described in the rat (Burri, 1974; Scheuermann, et al., 1988) and mouse (Amy et al., 1977). The process of alveolization started when numerous short blunt ridges, called secondary septa, commences to divide the saccule into many shallow alveoli. Alveolar variation is time dependant and due to irregular division by the septa. The formation of alveoli was not complete at 21 days of postnatal life which is consistent with observations of others who report variation in the time of alveolar formation in man (Dunnill, 1962; Emery and Wilcock, 1966; Thurlbeck, 1982; Zeltner and Burri, 1987).

Lung expansion was observed during the initial postnatal period. This is due to initial inspiration of air and not solely from the onset of alveolar formation. Burri (1974) related that alveolar

formation in humans is preceded by a phase of lung expansion similar to that found in rats, cats and rabbits.

As cats matured with age, Type II pneumocytes were observed at the base of the secondary septa. We postulate that this location would strengthen the septum and enable it to withstand the deformations that may occur during respiration. Adult type II pneumocytes were located at the entrance into the alveolar space. Type II pneumocytes, as seen by SEM, bulged into the lumen and were covered with microvilli. The numbers of microvilli gradually increased as development progressed until they extensively covered the cell surface. Often macrophages were present in the alveolar space of growing cats, as has been reported in the lungs of calves (Rybicka et al., 1974). Since these cats were not subjected to environmental stresses, low numbers of macrophages would be expected.

Type I pneumocytes had a smooth surface and most likely appeared by transformation from type II pneumocytes. This is in accordance with previous investigations (Evans et al., 1973; Bachofen and Weibel, 1974; Kauffman et al., 1974; Adamson and Bowden, 1975) who report on the ability of type II to proliferate and subsequently transform into type I pneumocytes. Bremer (1904) was the first to describe the transformation of the type I pneumocyte from its cuboidal form to a squamous form with attenuations which extended across the capillaries. More recently, Mercurio and Rhodin (1976) stated the differentiation of type I pneumocytes of the cat occurred

via the formation of one or more cytoplasmic attenuations of type II pneumocytes.

The interalveolar septum of an adult cat commonly had pores of Kohn communicating between adjacent alveoli. However, pores of Kohn were not present in growing cats. This is in agreement with other investigators (Macklin, 1936; Wang et al., 1973; Amy et al., 1977; Ranga and Kleinerman, 1980). This observation is also consistent with more recent findings (Kawakami, 1984; Henry and Ranga, 1985; Shimura et al., 1986) that pores are rare or absent before birth. They all agree that the pores develop postnatally. However, the time of their appearance is still a matter of controversy. Shimura and co-workers (1986), demonstrated that the number of alveolar pores increased significantly between 1 month and 10 years of age in monkeys ranging from 1 month to 31 years of age.

Previous reports (Loosli, 1937; Pump, 1976) stated that pores arise as a natural consequence of aging. Therefore, it would not be likely to observe pores at 21 days of age in cats. Recently, a positive correlation between age and occurrence of alveolar pores was observed in mice between 38 days - 28 months of age with major increases at 68 days (Kawakami et al., 1984). In addition, Mercurio and Rhodin (1984) reported the formation of alveolar pores after 90 days of age in cats. We speculate that the pores form later in life as part of an adaptive response to environmental stress. Thus, we agree with Henry and Ranga (1985) that one of the methods of alveolar pores is an adult complement.

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VIII. FINE STRUCTURAL ASPECTS OF POSTNATAL DEVELOPMENT OF FELINE LUNG

ABSTRACT

This study provides information on the postnatal development of the feline lung. Lungs were fixed by intratracheal instillation of 3% glutaraldehyde in phosphate buffer at a pressure of 20 cm H_2O. Samples were collected from the caudal lobe of prenatal, 1-, 7-, 14-, and 21-days-postnatal and adults and processed for TEM. The cats were born with a few alveoli, and the lungs appeared to have patches of primitive air spaces called saccules. The saccules of prenatal kittens were thick walled, very cellular, and lined by type II pneumocytes. Eosinophils were observed in the septum, intraepithelially, and in the alveolar space of growing cat. Secondary septa were flanked by a double capillary network and divided saccules into multiple shallow alveoli. The division was irregular and time dependent. Therefore, by day 21 of postnatal life septation was not completed. Elastic fibers accumulated at the tip of the septa, seemingly playing an important role in alveolar formation. Type II pneumocytes were located at the base of the secondary septa in growing cats, thus strengthening secondary septa to withstand the stresses of respiration. Pores of Kohn were not observed in growing cats. The basic patterns of lung growth in cats is similar to other mammalian species.

INTRODUCTION

Animals are either born with a full complement of adult alveoli as in lambs (Engel, 1953) or most of their alveoli are formed during early postnatal life as in man (Zeltner and Burri, 1987). In the rhesus monkey, alveolization commences at 95 days gestational age, with the formation of secondary septa in the terminal sacs (Tyler et al., 1988). This time sequence corresponds to formation of secondary septa in man (Langston and Thurlbeck, 1982). However, in rats and mice, the lungs have no alveoli at birth, but they develop entirely postnatally (Burri et al., 1974; Thurlbeck, 1975). This controversy encourages many investigators to study lung development and growth in the prenatal and postnatal periods. The morphological changes in the prenatal period have been studied successfully. These data have aided therapy and understanding of diseases of premature infants (Motoyama et al., 1988). Nevertheless, postnatal development has received little attention.

Recently, lung development has been described in the mouse (Ten Have-Opbroek, 1986), in the rat (Henry and Ranga, 1985; Scheuermann et al,. 1988), in the pig (Winkler and Cheville, 1984), in nonhuman primates (Hislop et al., 1984; Bunton and Plopper, 1984; Maina, 1987), in the primate (Tyler et al., 1988), and in man (Langston et al., 1984; Zeltner et al., 1987; Zeltner and Burri, 1987). However, little work has been done on postnatal development of the feline lung (Mercurio and Rhodin, 1976, 1984). The present study examines the postnatal development of feline lung to determine if differences

exist from other species. In addition, since cat lung is similar to human lung [in that alveolar multiplication is continued postnatally (Engel 1953)], the feline lung may constitute a model for the study of human lung development.

MATERIALS AND METHODS

Thirty-nine domestic cats (Felis catus) representing the six following age groups: prenatal (60 days post-conception), postnatal (1-, 7-, 14- and 21-day-old), and adult were used in this study. The cats were anesthetized by the intramuscular injection of ketamine (5 mg/kg) and xylazine (0.4 mg/kg). The trachea was cannulated and the lungs were fixed by infusion of 3% glutaraldehyde in phosphate buffer (pH 7.4, 0.1M) at a pressure of 20 cm H_2O. After fixation in situ for at least 30 minutes, a midline incision through the skin and subcutaneous tissue from the larynx to the xiphoid region was performed to expose the trachea and the sternum. The thorax was opened and the lungs with the trachea were carefully dissected free and removed. Tissue samples were taken from the caudal lobe of the lung and cut into small pieces and immersed in the fixative for an additional four hours. The samples were processed for transmission electron microscopy.

For transmission electron microscopy the samples were washed three times in phosphate buffer (pH 7.4, 0.1M), postfixed in 1% osmium tetroxide in 0.1M phosphate buffer (pH 7.4) for 90 minutes, dehydrated in a graded series of ethanol and acetone, and embedded in

an Epon-Araldite mixture. Thin sections were stained with 2% (w/v) aqueous uranyl acetate, contrasted with lead citrate, and viewed by Phillips electron microscope model 201 working at 60 kV.

RESULTS

Saccules of various sizes were observed in lungs of *prenatal kittens* and were separated by primary septa of variable height and thickness. They were lined by cuboidal type II pneumocytes which rested on a thin basement membrane. Numerous short microvilli covered the free surface of the pneumocytes. Often these cells were almost completely separated from each other by a wide intercellular space. Their cytoplasm contained a few darkly stained multilaminated inclusions located near the apical surface. Their nucleus was large, rounded, centrally-located, and slightly-indented and contained a prominent nucleolus (Fig. 8-1).

The primary septa were cellular and contained numerous rounded to oval mesenchymal cells which contained large rounded or elongated indented nuclei. Their cytoplasm contained a few mitochondria and rough endoplasmic reticulum. The mesenchymal cells were separated by a wide intercellular space except in an area where they were joined to each other by desmosomes. The wall of the saccules was flanked by a double capillary network which bulged into the saccular space. A few collagenous fibers were observed below the capillary network

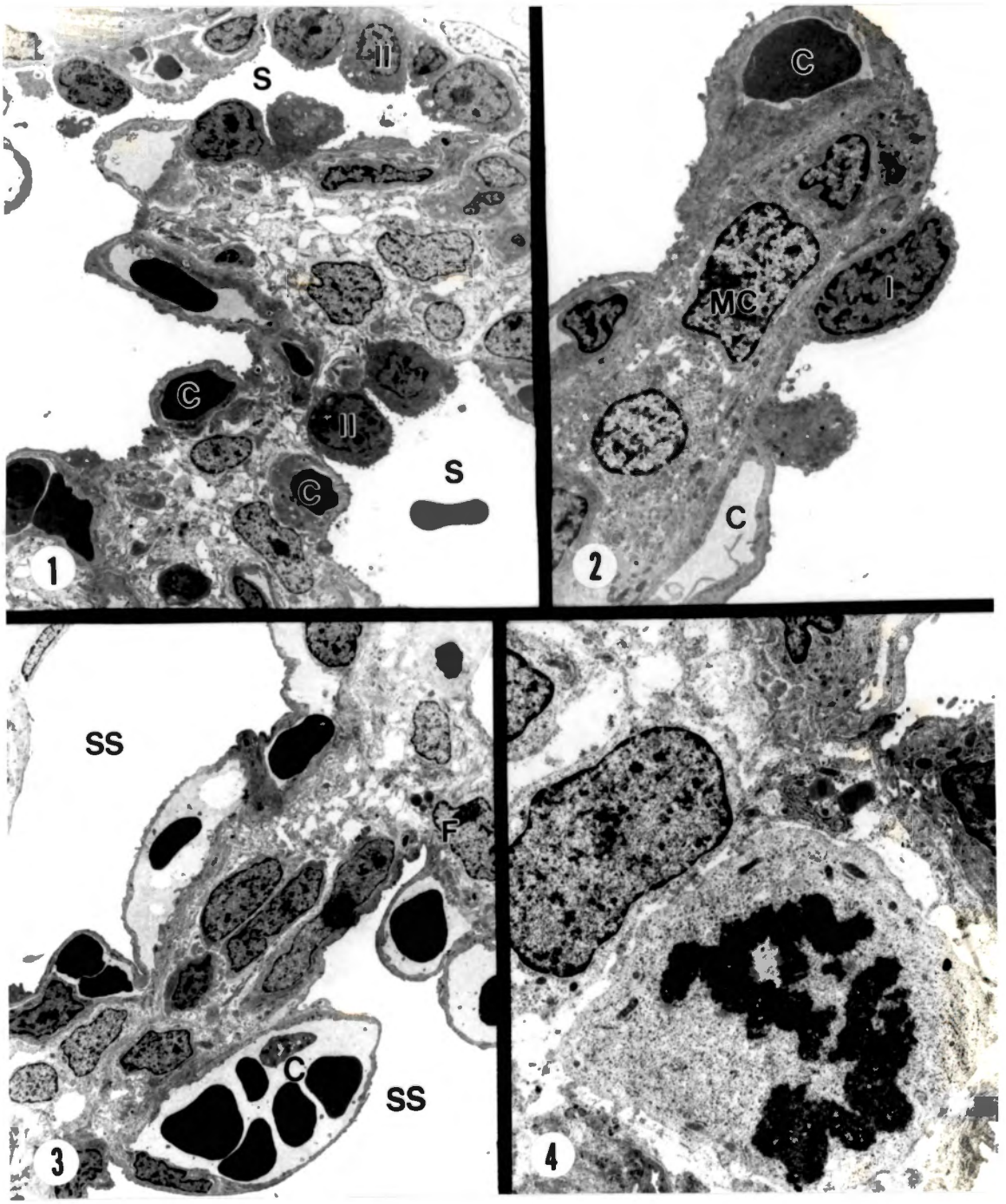
PLATE 8-1

Fig. 8-1: Transmission electron micrograph of the primary septum of a prenatal kitten with a thick wall and a double capillary network (C) separating saccules (S) lined by type II pneumocytes (II). X3,000.

Fig. 8-2: Transmission electron micrograph of the free end of the primary septum of a prenatal kitten which has a double capillary network (C) flanked by mesenchymal cells (MC) and is covered by type I pneumocyte (I). X6,750.

Fig. 8-3: Transmission electron micrograph of a 1-day-old kitten lung showing a large thin-walled capillary (C) bulging into the saccular space (SS). Fibroblast with lipid inclusions (F). X3,000

Fig. 8-4: Transmission electron micrograph of a 1-day-old kitten showing a fibroblast in mitosis. X10,500.


S
II
C
II
C
S
1
C
MC
I
C
2
SS
F
C
SS
3
4

(Fig. 8-1). The core of the primary septum contained large mesenchymal cells and a double capillary network. The tip of the septum was covered by squamous type I pneumocytes and contained a capillary loop that bulged into the saccular lumen. At the tip of the septa, the capillary wall was thick, whereas, near the base of the septa thickness varied (Fig. 8-2).

Postnatally, in *1-day-old kittens* the size of the saccule was increased. The wall had a double capillary network, whose thin wall bulged into the saccular lumen. Abundant fibroblasts were also observed (Fig. 8-3) and occasional dividing cells were observed in the intersaccular septa (Fig. 8-4). The free end of the secondary septa contained a capillary loop and fibroblasts with associated collagen and elastic fibers. Type II pneumocytes were located at the base of the secondary septa and contained numerous multilaminated inclusions and a few mitochondria in the apical cytoplasm (Fig. 8-5). Type I pneumocytes were similar to those of the prenatal.

In *7-day-old* kittens, progressive but irregular septation of saccules was observed. The septum was covered by type I and type II pneumocytes. In areas where secondary septa were developing from the primary septa, type II pneumocytes were located at their base. Type II pneumocytes were large, well-developed, and covered with numerous long microvilli. Numerous mitochondria were scattered throughout the cytoplasm among the multilaminated inclusions. These cells also exhibited lateral interdigitations with the neighboring cells (Fig. 8-6). The core of the secondary septum remained cellular with

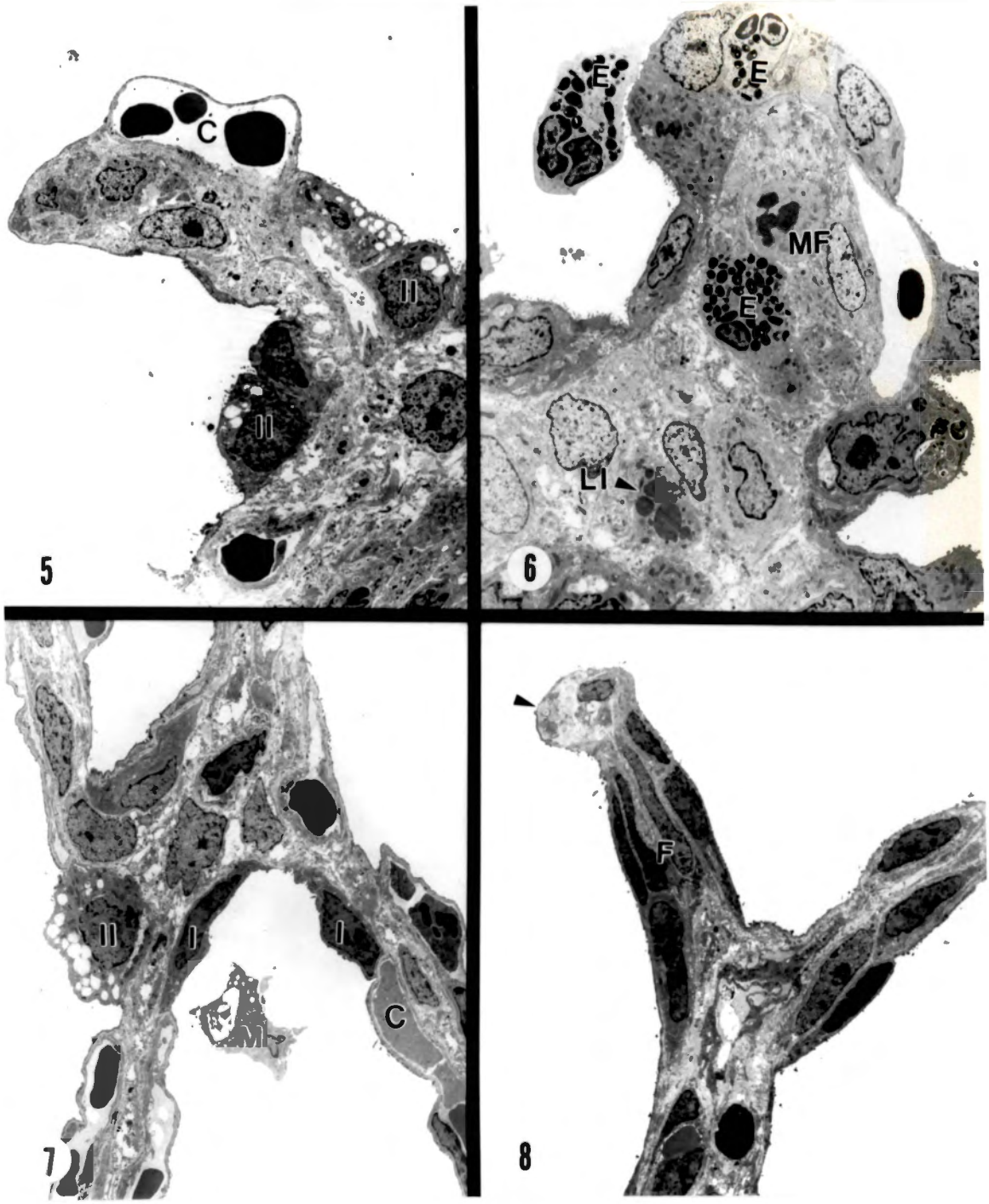
PLATE 8-2

Fig. 8-5: Transmission electron micrograph of the secondary septum of a 1-day-old kitten. Type II pneumocytes (II) are basally located. Capillary (C). X3,000.

Fig. 8-6: Transmission electron micrograph of the secondary septum of a 7-day-old kitten showing a mitotic figure (MF), a fibroblast with intracytoplasmic lipid inclusions (LI) and eosinophils (E) in its core. X3,000.

Fig. 8-7: Transmission electron micrograph of the septal junction of a 14-day-old kitten. The saccule is lined by type I pneumocytes (I) and contains macrophage (M). Capillary (C), Type II pneumocyte (II). X4,500.

Fig. 8-8: Transmission electron micrograph of the secondary septum of a 14-day-old kitten which is thin and ends in a blunt knob containing elastic fibers (arrowhead). Fibroblast (F). X2,400.


C
II
II
5
E
E
MF
E
LI
6
II
I
I
C
7
F
8

occasional mitotic cells observed. Occasional eosinophils were observed in the septal connective tissue, in the epithelium covering the secondary septa, and in the alveolar space. Fibroblasts occasionally contained lipid inclusions (Fig. 8-6).

By *day 14*, the lung had progressed in development. The saccules were larger, the primary septa were thinner, but a double capillary network was present. The saccules were lined primarily by type I pneumocytes which were characterized by their elongated or fusiform nucleus which bulged into the saccular lumen. When present, the organelles in the scant cytoplasm of type I pneumocytes were concentrated on either pole of the nucleus. Occasional macrophages were observed in the saccular space (Fig. 8-7).

Type II pneumocytes were large and contained numerous multilaminated inclusions in their apical cytoplasm. They were situated at the base of the septa. The wall of the primary septa was relatively thin and increased numbers of connective tissue cells were seen at the junction of the septa (Fig. 8-7). Secondary septa were thin, relatively long, ended in a blunt knob like structure, and contained a few large fibroblasts with large elongated nuclei. At the tip of the septa, elastic and collagenous fibers were observed (Fig. 8-8).

Development of *21-day-old* lung had progressed considerably, in some areas the septum contained a double capillary network. The capillary wall was thin, bulged into the saccular space, and covered with long cytoplasmic extensions of type I pneumocytes (Fig. 8-9).

In several areas of the lung, the primary septa were increased in length and become thinner than before (Figs. 8-9, 8-10). The wall of the primary septa was less cellular and contained abundant connective tissue fibers. However, a short blunt ridge indicated the onset of primitive secondary septa outgrowth. The newly formed secondary septa contained mostly elastin and a few collagenous fibers concentrated in the tip (Fig. 8-10). Each secondary septa had a single capillary loop which either was located near the base, abutted type II pneumocytes (Fig. 8-11), or capped the tip of the septum together with the type I pneumocytes (Fig. 8-12). Type II pneumocytes were well-developed and contained numerous mitochondria with electron-dense matrices and few cristae (Fig. 8-11). Multilamellated inclusions were observed in the apical cytoplasm.

The lungs of *adult cats* were characterized by alveoli lined by cytoplasmic extensions of type I pneumocytes. Their walls were thin and contained abundant collagen fibers and a few fibrocytes (Fig. 8-13). The walls of alveoli contained a single large capillary bed whose wall was thin and bulged into the alveolar space. Pores of Kohn interrupted the wall.

DISCUSSION

This investigation supports the general process of alveolization observed in rat (Burri, 1974, Scheuermann, et al. 1988) and in mice (Amy et al., 1977) through division of saccules by secondary septa. The lungs of growing cats contained both saccules

PLATE 8-3

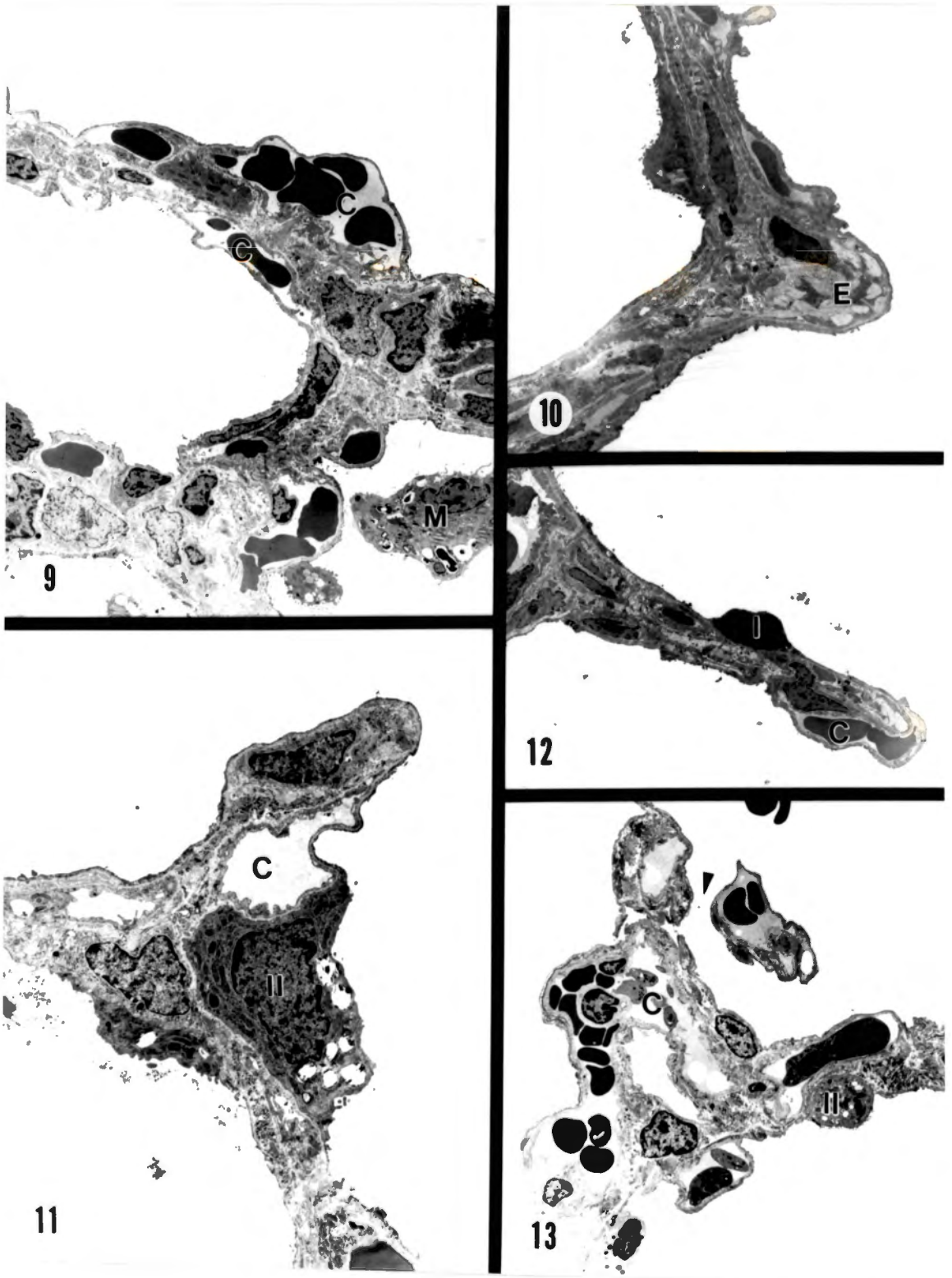
Fig. 8-9: Transmission electron micrograph of primary septa of a 21-day-old kitten which are thin and contain a double capillary network (C). Macrophage (M). X3,000.

Fig. 8-10: Transmission electron micrograph of a primitive secondary septum of a 21-day-old kitten with abundant elastic fibers (E). X3,000.

Fig. 8-11: Transmission electron micrograph of a secondary septum of a 21-day-old kitten with a type II pneumocyte (II) located at the base and abutting a capillary (C). X6,750.

Fig. 8-12: Transmission electron micrograph of a secondary septum of a 21-day-old kitten which is thin, lengthened, covered by type I pneumocytes (I), and a blood capillary (C) caps its tip. X3,000.

Fig. 8-13: Transmission electron micrograph of a septum of the adult lung showing a single capillary network (C), type II pneumocytes (II), and a pore of Kohn (arrowhead). X1,800.


C
C
M
9
E
10
I
C
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C
II
11
C
II
13

and alveoli. In areas where saccules were undergoing remodeling, secondary septa of variable heights subdivided the saccule into immature shallow alveoli. Primary saccules were characterized by a double capillary network similar to the findings of Short (1951) and Weibel (1967). Short (1951) pointed out that each developing airway in the fetus has a capillary network of its own. Our findings were similar in the growing cat. By 21 days of age, many alveoli were formed, yet the new growth of parenchymal septa is an indication that growth and remodeling of the lung is continued beyond this age.

Most investigators agree that alveolization in mammals, except in mice and rat, begins in the prenatal period and is completed postnatally. The time of complete alveolization in mammalian lungs in postnatal life is still a matter of controversy among many investigators. In humans, septa development continues up to 1¡ years of age (Zeltner et al., 1987; Zeltner and Burri, 1987). Thurlbeck (1982) concluded based on alveolar counting, that only limited or no alveolar formation occurred beyond 2 years of age. This is contrasted by Dunnill's (1962) earlier findings that alveolar formation could take place up to 8 years of age, while others up to 20 years (Emery and Wilcock, 1966).

Interseptal connective tissue gives the newly formed alveolar septum stability. Frequent mitotic connective tissue cells, fibroblasts, were observed. In agreement with Thurlbeck (1975), fibroblasts were characterized by the presence of intracytoplasmic lipid inclusions. He suggested that lipid inclusions were important

in the postnatal development of alveoli in rat and mice. In addition to fibroblasts, eosinophils were observed in the septa, intraepithelially, and in the alveolar space. The presence of eosinophils and their function in the lung remains unclear. Macrophages were present in the alveolar space of growing cats as similarly observed in lungs of the calf by Rybicka et al. (1974).

A substantial amount of connective tissue fibers, mainly elastin, were interwoven with the capillary network. This is consistent with earlier findings of Burri (1974) who stated that the development of secondary septa in rats seemed to be closely linked to the deposition of elastic fiber strands at the tips of the septa.

Type II pneumocyte were observed at the base of secondary septa in all ages. We speculate that their function is to stabilize the actively-growing delicate septa against the deformations that occur during respiration. No mitotic figures were observed in type II pneumocytes. This is in contrast to recent findings of numerous mitotic figures in type II pneumocytes by Scheuermann et al. (1988) during rapid proliferation of secondary alveolar septa of rats . Type II pneumocytes are known to be the stem cells of the alveolar epithelium having the capacity to proliferate and transform into type I pneumocytes (Evans et al., 1973; Bachofen and Weibel, 1974; Kauffman et al., 1974; Adamson and Bowden, 1975). Bremer (1904) was the first to describe the transformation of the type I pneumocyte from its cuboidal form to its squamous form with attenuations extending across the capillaries.

We speculate that transformation of type II pneumocytes into type I pneumocytes is the most likely method of formation of type I pneumocytes in the cat. This is consistent with the earlier findings in the cat by Mercurio and Rhodin (1976) who observed differentiation of type I pneumocytes via the formation of one or more cytoplasmic attenuations. Pores of Kohn were not observed in growing cat lung. Kawakami et al. (1984) showed a positive correlation between age and occurrence of alveolar pores in mice between 38 days and 28 months of life with a major increase at 68 days. Since pores arise as a natural consequence of aging (Loosli, 1937, Pump, 1976), it would be unlikely to observe them in growing cats in the early postnatal period. In addition, previous studies by Mercurio and Rhodin (1984) depicted the formation of alveolar pores in 90-day-old cats.

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APPENDIXES

APPENDIX A

TISSUE PROCESSING FOR PLASTIC SECTIONING

The specimens were cut into 3 to 5 mm pieces and fixed in 3% glutaraldehyde in phosphate buffer (pH 7.4, 0.01M for four hours. The specimens were then processed as follows:

A. Partial dehydration: The specimens were passed through a graded series of ethanol.

1. 50% ethanol: two changes, 10 minutes each.
2. 70% ethanol: two changes, 10 minutes each.
3. 95% ethanol: two changes, 10 minutes each.

B. Infiltration: The specimens were infiltrated with 100 ml JB-4 solution A and 0.9 gm catalyst. The infiltration of the catalyzed JB-4 solution was accomplished at room temperature with two to three changes ranging from 40 to 50 minutes each. The changes were continued until the samples became translucent and sank to the bottom of the container.

C. Embedding and Polymerization: The JB-4 mixture was polymerized at room temperature and prepared by the addition of one ml of JB-4 solution B to 40 ml of freshly prepared and catalyzed JB-4 solution A. All the mixtures were stirred thoroughly. The specimens were oriented and properly positioned in the plastic mold containing the JB-4 mixture. A stub was attached to each specimen and allowed to polymerize at room temperature.

APPENDIX B

TISSUE STAINING PROCEDURES

GILL'S HEMATOXYLIN AND EOSIN STAIN:

Reagents:

Gills' Hematoxylin #3

Eosin Y Alcohol 0.5 gram per ml in 70% aqueous ethanol

Scott's tap water substitute

Magnesium sulfate	4 gm
Sodium bicarbonate	0.8 gm
Distilled water	400 ml

Staining Procedure:

1. Dry slides in the oven at 60^{o} to 80^{o} for ten minutes, allow to cool to room temperature
2. Gill's Hematoxylin #3 for 45 minutes.
3. Water two changes, ten dips each.
4. Scott's tap water substitute for three minutes.
5. Water two changes, ten dips each.
6. 70% ethanol two changes, ten dips each.
7. Eosin Y Alcohol for 30 minutes.
8. Absolute ethanol two changes, ten dips each.
9. Clear in xylene.
10. Mount and cover slip

PERIODIC ACID-SCHIFF'S STAIN

Solution:

1% Aqueous periodic acid

Periodic acid	1 gm
Distilled water	100 ml

Schiff's reagent (Double strength)

Distilled water	200 ml
Basic fuchsin	2 gm
Sodium metabisulfite	4 gm
1N Hydrochloric acid*	40 ml

*1N Hydrochloric acid

Hydrochloric acid	83.5 ml
Distilled water	916.5 ml

Bring the water to boiling point and remove it from the flame. At once, dissolve basic fuchsin in the water by slowly adding the dye. Cool to 60°C and filter. Add 4 gm sodium metabisulfite and shake the solution well. Add 40 ml of 1N hydrochloric acid and shake the solution (the solution should be deep amber color) leave it in the dark, overnight (18 to 24 hours), at room temperature. In the morning, the solution should have a light amber color. Add 600 milligrams activated charcoal and shake for one minute. Filter through filter paper and store the solution in a dark bottle in the refrigerator. After filtering, the solution should be clear (water-white). Repeat filtration until clear, and allow reagent to reach room temperature before use. Discard the solution if precipitate forms or if pink color appears.

Staining Procedure:

1. Dry slides in hot oven at 60 to 80°C for ten minutes, allow to cool to room temperature.
2. Oxidize in 1% Periodic acid for ten minutes.
3. Rinse in distilled water.
4. Stain in Schiff's reagent for 30 minutes.
5. Wash well in running tap water and allow stain to develop dark pink color.
6. Counterstain with Gill's hematoxylin #3 for 40 minutes.
7. Water two changes, ten dips each
8. 70% ethanol two changes, ten dips each.
9. Absolute ethanol two changes, ten dips each.
10. Clear in xylene
11. Mount and cover slip.

Results:

Periodic acid-Schiff positive material will stain rose to purplish red.

WEIGERT'S METHOD FOR ELASTIC FIBERS

Fixation: Any well fixed tissue.

Technique: Cut paraffin sections at 6 microns.

Solutions:

WEIGERT'S IRON HEMATOXYLIN

Solution **A**

Hematoxylin crystals	1.0 gm
Alcohol, 95%	100.0 ml

Solution **B**

Ferric Chloride, 29% aqueous	4.0 ml
Distilled water	95.0 ml
Hydrochloric acid, concentrated	1.0 ml

Working Solution

Equal parts of Solution A and Solution B.

RESORCIN-FUCHSIN SOLUTION (stock)

Basic fuchsin	2.0 gm
Resorcinol	4.0 gm
Distilled water	200.0 ml

Mix in a porcelain dish, bring to a boil and let boil for 1 minute, then add 25 ml of 29% ferric chloride solution. Cool, filter, and leave precipitate on filter paper until dry. Return to porcelain dish, which should be dry but still contains whatever part of the precipitate remains adherent to it. Add 200 ml of 95% alcohol and heat very carefully dissolving all precipitate from filter paper before discarding it. Add 4 ml of hydrochloric acid. This solution keeps for several months.

RESORCIN-FUCHSIN SOLUTION (working)

Resorcin-fuchsin, stock	10.0 ml
Alcohol, 70%	100.0 ml
Hydrochloric acid, concentrated	2.0 ml

VAN GIESON'S SOLUTION

Acid fuchsin, 1% aqueous	2.5 ml
Picric acid, saturated aqueous	97.5 ml

Staining Procedure:

1. Deparaffinize and hydrate to distilled water.
2. Weigert's hematoxylin solution for 10 minutes.
3. Wash well in water. Intensifies stain.
4. Resorcin-fuchsin solution for 30 minutes or longer.
5. Rinse in 95% alcohol.
6. Wash in tap water.
7. Van Gieson's solution for 1 minute. Save solution. If overstained, water will remove excess van Gieson's solution.
8. Dehydrate in 95% alcohol, absolute alcohol, and clear in xylene, two changes each.
9. Mount with Permount or Histoclad.

Results:

Elastic fibers	blue-black to black
Nuclei	blue to black
Collagen	pink to red
Other tissue elements	yellow

APPENDIX C

PROCEDURE TO ESTIMATE SUFFICIENT SAMPLING

To ensure satisfactory sampling, the mean value of the count derived from a sample at any one site must have a standard error (RSE) that is acceptable statistically ($P<0.025$). For example, to ensure enough counting of the volume fraction of any reference area, the standard error should be less than 0.025. It was calculated as follows:

$$RSE = \sqrt{\frac{1 - V_v}{N}}$$

Where: N = the number of points that fell on the reference area.

A preliminary study was conducted. To ensure sufficient sampling from each cat, 2000 points were required.

APPENDIX D

MORPHOMETRIC PROCEDURES

Symbols:

P = number of points, regardless of their origin.

Pi = number of test points in reference area.

P_t = total number of points counted.

P_l = number of intersections per unit length of test time.

P_a = number of points per test area.

P_v = number of points per unit volume.

l = length of lineal element (L = El).

S_v = surface area per unit volume, surface fraction or density.

V_L = volume of lung before fixation.

V_v = volume fraction or density.

d = unit length of a linear probe in a test system.

L_t = total length of linear probe used.

T_h = harmonic mean thickness.

I = number of intersections.

T = arithmetic mean.

N = number of particles.

N_v = number of particles per unit volume.

N_a = number of profile per unit area.

ß = shape distribution coefficient.

K = size distribution coefficient.

l = test line lying between two surface intersections.

Average thickness:

The arithmetic mean thickness (T), which provides estimate for the tissue mass can be measured by short line lattice of length (d) on a random section of sheet (i) (Weibel and Knight, 1964). This can be done by recording the number of end points (Pi) lying on sheet tissue and number of surface intersections Ii, and by using this formula to estimate the thickness.

$$T = d * Pi/ 2 * Ii$$

For the transport across the membrane, the harmonic mean (T_h) is a more appropriate measure of effective average thickness. It is the sum of the reciprocals of the sample values divided by sample number. It is measured by placing a long line lattice on sections of sheet (tissue), record intercept length of l between entrance and exit points, and form the harmonic mean intercept length (l_h) then using the following formula to get the harmonic thickness.

$$T_h = 2/3\ l_h$$

According to Weibel (1979) there are two kinds of intercepts: 1. Those which enter the barrier at surface (a) (a = alveolar) and leave at surface (b) (b = blood capillary) respectively. In this case, these are "true intercepts" because they span the entire barrier. 2. The other intercepts which enter at the surface and leave again at the same surface are "false intercepts". These intercepts are due to the curvature of the barrier and will not be measured because they do not extend across the barrier.

Volume density (fraction):

The volume density is defined as the volume fraction of a given component within a tissue, and it is expressed as a percent. It is determined by point counting using simple square lattice test system A100. The following formula is used to find the value.

$$Vv = \frac{\text{test points over reference area}}{\text{total test point over the tissue}} \quad \text{or} \quad Pi/PT$$

Absolute volume (V_A) = V_V * V_L.

Where V is the volume of the tissue before embedding, measured by water displacement.

Surface density (fraction):

The surface density is defined as the surface area of the selected region per unit volume. It is expressed in area/volume. It is determined by using a simple square lattice test system A100 and the following formula to calculate the (S_V).

$$S_V = 4 \times I / d \times P_T$$

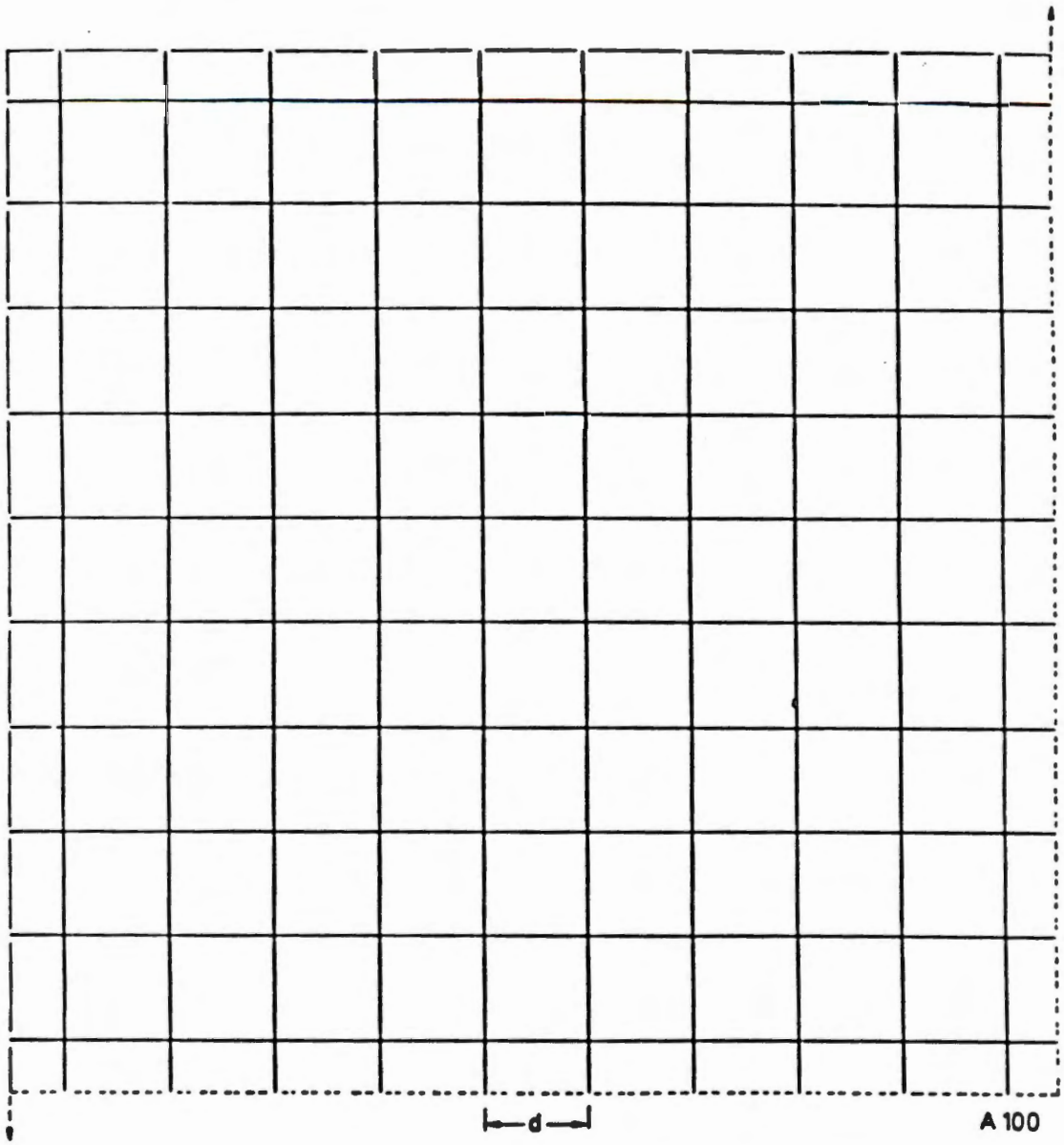
Total surface area (Stot) = $S_V \times V_L$.

Numerical density:

The alveoli of the lung can be regarded as randomly distributed small polyhedral bodies. This assumption allows the number of alveoli per unit volume to be counted, because it will be considered as a discrete structure and counted using this formula.

$$N_V = K/\beta \times \sqrt{NA^3/V_V}$$

Where K is the size distribution coefficient which is larger than one and falls between 1.01 to 1.1, for normal distribution with a standard deviation of ±25% of the mean, K = 1.07 (Weibel et al., 1966). β is the shape distribution coefficient with a value of (1.382) and is between sphere and cylinder.



Simple square lattice test system A100
Calibration d = distance of test lines
Frame width = 10d

Test point number $P_t = 100$
Test line length $L_t = P_t\ 2\ d = 200\ d$
Test area $A_t = P_t\ a^{12} = 100\ d$

APPENDIX E

Program used to print data

```
************************  ROW DATA PRINT OUT  **********;

DATA PRINT;

INFILE LM;

INPUT  AGE $  ANIMAL  BLOCK  FIELD
         ALVWALL   ALVSP   TRBTCT;

IF AGE='1' THEN AGE = 'PRENL';
IF AGE='2' THEN AGE = 'ONEDY';
IF AGE='3' THEN AGE = 'ONEWK';
IF AGE='4' THEN AGE = 'TWWKS';
IF AGE='5' THEN AGE = 'THWKS';
IF AGE='6' THEN AGE = 'ADULT';

PROC PRINT;

************************************************************;

************************  ROW DATA PRINT OUT  ********;

DATA ELECTRON;

INFILE EM;

INPUT  AGE $  ANIMAL  BLOCK  TYPEI  TYPEII
       NUMINT  LEINT   CONTIS   CAPEND ;

IF AGE='1' THEN AGE = 'PRENL';
IF AGE='2' THEN AGE = 'ONEDY';
IF AGE='3' THEN AGE = 'ONEWK';
IF AGE='4' THEN AGE = 'TWWKS';
IF AGE='5' THEN AGE = 'THWKS';
IF AGE='6' THEN AGE = 'ADULT';

PROC PRINT;
VAR AGE ANIMAL TYPEI TYPEII CONTIS;

PROC PRINT;
VAR AGE ANIMAL NUMINT LEINT CAPEND;

*************************************************************;
```

```
**********************   VOLUME FRACTIONS      ********;

DATA ELECTRON;

INFILE SUM;

INPUT  AGE $  ANIMAL  NUMTI   NUMTII   NPOTTI
           NPOTTII NPOTCON  NPOTCAP  NPOTINT;

IF AGE='1' THEN AGE = 'PRENL';
IF AGE='2' THEN AGE = 'ONEDY';
IF AGE='3' THEN AGE = 'ONEWK';
IF AGE='4' THEN AGE = 'TWWKS';
IF AGE='5' THEN AGE = 'THWKS';
IF AGE='6' THEN AGE = 'ADULT';

PROC PRINT;
VAR AGE ANIMAL NUMTI NUMTII NPOTTI NPOTTII;

PROC PRINT;
VAR AGE ANIMAL NPOTCON NPOTCAP NPOTINT;

*****************************************************;

************************  ROW DATA PRINT OUT  *******;

DATA PRINT;

INFILE LSUM;

INPUT  AGE $  ANIMAL  POTAVW   POTAWS  POTTRBC;

IF AGE='1' THEN AGE = 'PRENL';
IF AGE='2' THEN AGE = 'ONEDY';
IF AGE='3' THEN AGE = 'ONEWK';
IF AGE='4' THEN AGE = 'TWWKS';
IF AGE='5' THEN AGE = 'THWKS';
IF AGE='6' THEN AGE = 'ADULT';

PROC PRINT;
*****************************************************;
```

```
********************** DATA HARMONIC MEAN  ********;

DATA HARM;

INFILE HMEN;

INPUT AGE $ ANIMAL BODYWT LUNGWT LUNGVOL
          ARTHMEAN HMEAN HTHICK SURFDEN;

IF AGE='1' THEN AGE = 'PRENL';
IF AGE='2' THEN AGE = 'ONEDY';
IF AGE='3' THEN AGE = 'ONEWK';
IF AGE='4' THEN AGE = 'TWWKS';
IF AGE='5' THEN AGE = 'THWKS';
IF AGE='6' THEN AGE = 'ADULT';

PROC PRINT;
VAR AGE ANIMAL ARTHMEAN HMEAN HTHICK SURFDEN;

************************************************;

******************   DATA VOL. FRACTION   ********;

DATA VOL;

INFILE EVO;

INPUT AGE $ ANIMAL BODYWT LUNGWT LUNGVOL
      VFTI VFTII VFCON VFCAP NVTI NVTII;

IF AGE='1' THEN AGE = 'PRENL';
IF AGE='2' THEN AGE = 'ONEDY';
IF AGE='3' THEN AGE = 'ONEWK';
IF AGE='4' THEN AGE = 'TWWKS';
IF AGE='5' THEN AGE = 'THWKS';
IF AGE='6' THEN AGE = 'ADULT';

PROC PRINT;

VAR AGE ANIMAL VFTI VFTII VFCON;

PROC PRINT;
VAR AGE ANIMAL VFCAP NVTI NVTII;

************************************************;
```

```
**************************   DATA TRACHEA  ***************

DATA TRACH;

INPUT AGE $ ANIMAL EPITH  SUBMOC   SUBBC;

IF AGE ='1' THEN AGE = 'PRENL';
IF AGE ='2' THEN AGE = 'ONEDY';
IF AGE ='3' THEN AGE = 'ONEWK';
IF AGE ='4' THEN AGE = 'TWWKS';
IF AGE ='5' THEN AGE = 'THWKS';
IF AGE ='6' THEN AGE = 'ADULT';

CARDS;
1  1  21.166  36.118  116.267  4  1  29.578  49.543  108.040
1  1  21.819  39.895  141.948  4  1  27.136  52.744  109.636
1  1  26.235  34.515  132.711  4  1  27.703  49.829   81.972
1  2  26.691  66.575  119.695  4  2  27.884  48.278  150.264
1  2  19.216  75.161  141.516  4  2  27.054  53.852  188.618
1  2  23.139  71.533  144.936  4  2  28.261  49.600  156.604
1  3  24.353  31.131  111.128  4  3  29.242  34.085   98.468
1  3  26.114  24.954   81.755  4  3  29.362  61.384   92.105
1  3  24.471  31.534   69.344  4  3  28.581  65.252   97.812
2  1  32.672  34.570  266.384  5  1  29.262  54.100   56.079
2  1  28.843  30.504  238.638  5  1  28.999  60.689  204.022
2  1  23.800  36.272  255.225  5  1  34.839  73.954  225.664
2  2  32.636  40.690  282.897  5  2  37.068  71.785  163.132
2  2  30.359  46.987  232.892  5  2  26.954  46.641  146.635
2  2  33.625  42.844  223.624  5  2  28.660  60.142  144.200
2  3  32.462  26.879  224.280  5  3  36.454  69.974  145.178
2  3  29.222  25.838  220.054  5  3  34.080  61.343  126.756
2  3  30.742  28.826  223.570  5  3  34.839  73.709  139.003
3  1  26.533  89.336  262.926  6  1  39.016  78.918  301.507
3  1  20.454  83.830  222.583  6  1  44.095  67.884  282.315
3  1  22.693  30.381  248.382  6  1  41.787  72.607  187.937
3  2  33.540  33.222  201.313  6  2  35.880  82.391  302.171
3  2  41.211  53.358  139.304  6  2  39.002  53.677  307.203
3  2  38.235  33.483  186.396  6  2  41.468  73.724  247.052
3  3  33.202  63.335  132.152  6  3  42.683  42.370  276.544
3  3  26.195  51.683  130.636  6  3  36.965  65.358  338.651
3  3  32.349  33.203  164.124  6  3  37.838  59.211  378.286
;
PROC PRINT;

*********************************
```

```
DATA CILIA;

INPUT AGE $ ANIMAL CILIA BODYWT LUNGWT LUNGVOL EPITHICK  SUBOC  SUBBC;

IF AGE ='1' THEN AGE = 'PRENL';
IF AGE ='2' THEN AGE = 'ONEDY';
IF AGE ='3' THEN AGE = 'ONEWK';
IF AGE ='4' THEN AGE = 'TWWKS';
IF AGE ='5' THEN AGE = 'THWKS';
IF AGE ='6' THEN AGE = 'ADULT';

CARDS;

1  1  5.673    82.333    2.667   2.8  23.17  33.749  55.255
1  2  5.268    76.667    1.911   1.9  22.98  43.507  151.322
1  3  4.343    83.811    2.733   2.2  24.98  27.282  136.979
2  1  5.533    95.000    2.967   4.0  28.40  36.816  126.642
2  2  4.938    90.587    2.200   2.3  27.73  71.156  135.149
2  3  5.133    82.267    2.667   2.2  29.06  28.839  84.176
3  1  6.028    223.350   4.100   6.8  23.24  50.706  99.883
3  2  4.699    145.900   2.500   4.0  33.66  50.577  165.162
3  3  4.945    148.000   3.300   3.5  30.58  53.574  96.128
4  1  5.270    280.500   6.300   6.0  28.22  62.914  244.630
4  2  5.666    271.830   3.000   5.5  32.22  59.523  189.140
4  3  5.044    242.220   4.350   7.0  30.44  68.344  168.931
5  1  6.006    372.307   6.067   8.5  31.03  82.636  253.379
5  2  5.943    318.920   5.750   9.0  30.89  68.154  246.238
5  3  4.945    406.740   6.850   9.0  34.82  50.835  229.302
6  1  5.876    4082.00   35.60   50.0 41.63  73.136  257.253
6  2  6.274    2500.00   25.00   50.0 39.90  69.931  285.475
6  3  5.321    3370.00   35.00   51.0 38.78  65.646  331.160
;

PROC PRINT;
VAR AGE ANIMAL CILIA EPITHICK SUBOC SUBBC;

**********************************************************************;
```

```
********************  LIGHT VOL FRATION  *****;

DATA LIGHT;

INFILE LVO;

INPUT AGE $ ANIMAL BODYWT LUNGWT LUNGVOL
                    VFAVW VFAVS VFTRBC;

IF AGE='1' THEN AGE = 'PRENL';
IF AGE='2' THEN AGE = 'ONEDY';
IF AGE='3' THEN AGE = 'ONEWK';
IF AGE='4' THEN AGE = 'TWWKS';
IF AGE='5' THEN AGE = 'THWKS';
IF AGE='6' THEN AGE = 'ADULT';

PROC PRINT;
VAR AGE ANIMAL BODYWT LUNGWT LUNGVOL;

PROC PRINT;
VAR AGE ANIMAL VFAVW VFAVS VFTRBC;
*************************************************************************;
```

APPENDIX F

Data collected on thickness of epithelium (EPITH), submucosa of the tracheal epithelium over cartilage (SUBOC) and submucosa of the tracheal epithelium between cartilage (SUBBC).

OBS	AGE	ANIMAL	EPITH	SUBMOC	SUBBC
1	PRENL	1	21.166	36.118	116.267
2	PRENL	1	21.819	39.895	141.948
3	PRENL	1	26.235	34.515	132.711
4	PRENL	2	26.691	66.575	119.695
5	PRENL	2	19.216	75.161	141.516
6	PRENL	2	23.139	71.533	144.936
7	PRENL	3	24.353	31.131	111.128
8	PRENL	3	26.114	24.954	81.755
9	PRENL	3	24.471	31.534	69.344
10	ONEDY	1	32.672	34.570	266.384
11	ONEDY	1	28.843	30.504	238.638
12	ONEDY	1	23.800	36.272	255.225
13	ONEDY	2	32.636	40.690	282.897
14	ONEDY	2	30.359	46.987	232.892
15	ONEDY	2	33.625	42.844	223.624
16	ONEDY	3	32.462	26.879	224.280
17	ONEDY	3	29.222	25.838	220.054
18	ONEDY	3	30.742	28.826	223.570
19	ONEWK	1	26.533	89.336	262.926
20	ONEWK	1	20.454	83.830	222.583
21	ONEWK	1	22.693	30.381	248.382
22	ONEWK	2	33.540	33.222	201.313
23	ONEWK	2	41.211	53.358	139.304
24	ONEWK	2	38.235	33.483	186.398
25	ONEWK	3	33.202	63.335	132.152
26	ONEWK	3	26.195	51.683	130.636
27	ONEWK	3	32.349	33.203	164.124
28	TWWKS	1	29.578	49.543	108.040
29	TWWKS	1	27.136	52.744	109.636
30	TWWKS	1	27.703	49.829	81.972
31	TWWKS	2	27.884	48.278	150.264
32	TWWKS	2	27.054	53.852	188.618
33	TWWKS	2	28.261	49.600	156.604
34	TWWKS	3	29.242	34.085	98.468
35	TWWKS	3	29.362	61.384	92.105
36	TWWKS	3	28.581	65.252	97.812
37	THWKS	1	29.262	54.100	56.079
38	THWKS	1	28.999	60.689	204.022
39	THWKS	1	34.839	73.954	225.664
40	THWKS	2	37.068	71.785	163.132
41	THWKS	2	26.954	46.641	146.635
42	THWKS	2	28.660	60.142	144.200

OBS	AGE	ANIMAL	EPITH	SUBMOC	SUBBC
43	THWKS	3	36.454	69.974	145.178
44	THWKS	3	34.080	61.343	126.756
45	THWKS	3	34.839	73.709	139.003
46	ADULT	1	39.016	78.918	301.507
47	ADULT	1	44.095	67.884	282.315
48	ADULT	1	41.787	72.607	187.937
49	ADULT	2	35.880	82.391	302.171
50	ADULT	2	39.002	53.677	307.203
51	ADULT	2	41.468	73.724	247.052
52	ADULT	3	42.683	42.370	276.544
53	ADULT	3	36.965	65.358	338.651
54	ADULT	3	37.838	59.211	378.286

APPENDIX G

Data collected on number of points falling on alveolar wall (ALVWALL), alveolar space (ALVSP), and Tracheobronchial tree and associated connective tissue (TRVTCT) at light microscopic level.

OBS	AGE	ANIMAL	BLOCK	FIELD	ALVWALL	ALVSP	TRBTCT
1	PRENL	1	1	1	31	39	30
2	PRENL	1	1	2	56	31	14
3	PRENL	1	1	3	37	43	21
4	PRENL	1	1	4	58	30	10
5	PRENL	1	1	5	40	32	28
6	PRENL	1	2	1	20	13	60
7	PRENL	1	2	2	46	15	39
8	PRENL	1	2	3	44	50	5
9	PRENL	1	2	4	57	31	13
10	PRENL	1	2	5	40	37	19
11	PRENL	1	3	1	33	26	39
12	PRENL	1	3	2	36	37	26
13	PRENL	1	3	3	45	28	28
14	PRENL	1	3	4	45	41	13
15	PRENL	1	3	5	15	9	74
16	PRENL	1	4	1	36	37	27
17	PRENL	1	4	2	39	26	34
18	PRENL	1	4	3	34	19	48
19	PRENL	1	4	4	16	15	68
20	PRENL	1	4	5	26	17	58
21	PRENL	1	5	1	34	35	31
22	PRENL	1	5	2	31	7	63
23	PRENL	1	5	3	41	26	33
24	PRENL	1	5	4	48	17	36
25	PRENL	1	5	5	28	26	45
26	PRENL	1	6	1	32	16	52
27	PRENL	1	6	2	56	37	8
28	PRENL	1	6	3	44	30	26
29	PRENL	1	6	4	35	40	27
30	PRENL	1	6	5	55	23	22
31	PRENL	2	1	1	53	25	25
32	PRENL	2	1	2	65	22	14
33	PRENL	2	1	3	50	34	17
34	PRENL	2	1	4	43	27	32
35	PRENL	2	1	5	39	14	49
36	PRENL	2	2	1	48	33	19
37	PRENL	2	2	2	66	24	10
38	PRENL	2	2	3	45	26	29
39	PRENL	2	2	4	35	23	42
40	PRENL	2	2	5	35	32	27
41	PRENL	2	3	1	42	20	39
42	PRENL	2	3	2	40	29	30
43	PRENL	2	3	3	50	19	31
44	PRENL	2	3	4	62	16	20

OBS	AGE	ANIMAL	BLOCK	FIELD	ALVWALL	ALVSP	TRBTOT
45	PRENL	2	3	5	47	22	30
46	PRENL	2	4	1	52	21	28
47	PRENL	2	4	2	43	21	36
48	PRENL	2	4	3	41	20	40
49	PRENL	2	4	4	47	28	26
50	PRENL	2	4	5	43	20	37
51	PRENL	2	5	1	47	40	6
52	PRENL	2	5	2	20	19	64
53	PRENL	2	5	3	31	35	34
54	PRENL	2	5	4	36	46	15
55	PRENL	2	5	5	37	42	20
56	PRENL	2	6	1	41	47	12
57	PRENL	2	6	2	42	29	28
58	PRENL	2	6	3	38	33	28
59	PRENL	2	6	4	29	35	37
60	PRENL	2	6	5	46	27	27
61	PRENL	3	1	1	41	25	34
62	PRENL	3	1	2	38	11	51
63	PRENL	3	1	3	56	29	15
64	PRENL	3	1	4	46	24	30
65	PRENL	3	1	5	38	31	48
66	PRENL	3	2	1	35	28	37
67	PRENL	3	2	2	47	33	22
68	PRENL	3	2	3	50	31	20
69	PRENL	3	2	4	42	36	23
70	PRENL	3	2	5	47	35	20
71	PRENL	3	3	1	55	22	21
72	PRENL	3	3	2	52	25	24
73	PRENL	3	3	3	35	14	53
74	PRENL	3	3	4	48	34	18
75	PRENL	3	3	5	52	17	32
76	PRENL	3	4	1	59	30	11
77	PRENL	3	4	2	49	31	20
78	PRENL	3	4	3	35	25	41
79	PRENL	3	4	4	51	23	26
80	PRENL	3	4	5	38	39	22
81	PRENL	3	5	1	27	18	55
82	PRENL	3	5	2	39	19	42
83	PRENL	3	5	3	47	43	10
84	PRENL	3	5	4	42	33	25
85	PRENL	3	5	5	41	20	40
86	PRENL	3	6	1	19	48	34
87	PRENL	3	6	2	29	43	28
88	PRENL	3	6	3	32	44	24
89	PRENL	3	6	4	32	41	27
90	PRENL	3	6	5	31	37	32
91	ONEDY	1	1	1	29	57	14
92	ONEDY	1	1	2	35	57	8
93	ONEDY	1	1	3	26	52	22
94	ONEDY	1	1	4	34	61	5
95	ONEDY	1	1	5	22	45	33

OBS	AGE	ANIMAL	BLOCK	FIELD	ALVWALL	ALVSP	TRBTCT
96	ONEDY	1	2	1	25	40	35
97	ONEDY	1	2	2	30	53	17
98	ONEDY	1	2	3	14	42	45
99	ONEDY	1	2	4	20	41	39
100	ONEDY	1	2	5	28	37	35
101	ONEDY	1	3	1	24	53	23
102	ONEDY	1	3	2	22	58	20
103	ONEDY	1	3	3	35	38	27
104	ONEDY	1	3	4	27	61	12
105	ONEDY	1	3	5	21	49	30
106	ONEDY	1	4	1	28	42	30
107	ONEDY	1	4	2	20	60	20
108	ONEDY	1	4	3	21	50	29
109	ONEDY	1	4	4	31	42	27
110	ONEDY	1	4	5	19	42	34
111	ONEDY	1	5	1	20	63	17
112	ONEDY	1	5	2	17	65	18
113	ONEDY	1	5	3	22	63	15
114	ONEDY	1	5	4	14	67	19
115	ONEDY	1	5	5	21	59	20
116	ONEDY	1	6	1	33	54	13
117	ONEDY	1	6	2	13	52	35
118	ONEDY	1	6	3	16	65	19
119	ONEDY	1	6	4	13	59	29
120	ONEDY	1	6	5	16	48	38
121	ONEDY	2	1	1	24	35	42
122	ONEDY	2	1	2	21	53	26
123	ONEDY	2	1	3	32	64	4
124	ONEDY	2	1	4	20	71	9
125	ONEDY	2	1	5	18	53	29
126	ONEDY	2	2	1	32	53	15
127	ONEDY	2	2	2	18	62	20
128	ONEDY	2	2	3	24	46	30
129	ONEDY	2	2	4	12	58	30
130	ONEDY	2	2	5	24	66	10
131	ONEDY	2	3	1	35	49	16
132	ONEDY	2	3	2	23	51	26
133	ONEDY	2	3	3	16	68	16
134	ONEDY	2	3	4	20	43	37
135	ONEDY	2	3	5	19	59	22
136	ONEDY	2	4	1	35	32	33
137	ONEDY	2	4	2	40	60	0
138	ONEDY	2	4	3	25	28	47
139	ONEDY	2	4	4	26	38	36
140	ONEDY	2	4	5	34	45	21
141	ONEDY	2	5	1	54	46	0
142	ONEDY	2	5	2	34	35	31
143	ONEDY	2	5	3	24	44	32
144	ONEDY	2	5	4	37	37	26
145	ONEDY	2	5	5	41	30	29
146	ONEDY	2	6	1	29	59	12
147	ONEDY	2	6	2	25	70	5

OBS	AGE	ANIMAL	BLOCK	FIELD	ALVWALL	ALVSP	TRBICT
148	ONEDY	2	6	3	26	65	9
149	ONEDY	2	6	4	21	63	16
150	ONEDY	2	6	5	28	45	27
151	ONEDY	3	1	1	35	48	17
152	ONEDY	3	1	2	25	37	38
153	ONEDY	3	1	3	33	46	21
154	ONEDY	3	1	4	24	46	30
155	ONEDY	3	1	5	31	41	28
156	ONEDY	3	2	1	29	61	10
157	ONEDY	3	2	2	16	67	17
158	ONEDY	3	2	3	27	61	12
159	ONEDY	3	2	4	21	50	29
160	ONEDY	3	2	5	23	63	14
161	ONEDY	3	3	1	12	59	29
162	ONEDY	3	3	2	15	49	36
163	ONEDY	3	3	3	19	56	25
164	ONEDY	3	3	4	12	48	40
165	ONEDY	3	3	5	20	57	23
166	ONEDY	3	4	1	19	68	13
167	ONEDY	3	4	2	22	53	25
168	ONEDY	3	4	3	15	49	36
169	ONEDY	3	4	4	15	57	28
170	ONEDY	3	4	5	29	61	10
171	ONEDY	3	5	1	15	64	21
172	ONEDY	3	5	2	19	65	16
173	ONEDY	3	5	3	17	77	6
174	ONEDY	3	5	4	21	64	15
175	ONEDY	3	5	5	20	53	27
176	ONEDY	3	6	1	42	56	2
177	ONEDY	3	6	2	32	35	33
178	ONEDY	3	6	3	37	34	29
179	ONEDY	3	6	4	41	48	11
180	ONEDY	3	6	5	52	25	23
181	ONEWK	1	1	1	31	45	24
182	ONEWK	1	1	2	25	55	20
183	ONEWK	1	1	3	33	64	3
184	ONEWK	1	1	4	20	34	41
185	ONEWK	1	1	5	23	64	13
186	ONEWK	1	2	1	30	65	5
187	ONEWK	1	2	2	26	43	31
188	ONEWK	1	2	3	14	75	11
189	ONEWK	1	2	4	28	57	15
190	ONEWK	1	2	5	28	56	16
191	ONEWK	1	3	1	21	67	12
192	ONEWK	1	3	2	35	55	10
193	ONEWK	1	3	3	24	63	13
194	ONEWK	1	3	4	13	79	8
195	ONEWK	1	3	5	14	58	28
196	ONEWK	1	4	1	18	51	31
197	ONEWK	1	4	2	21	44	35

OBS	AGE	ANIMAL	BLOCK	FIELD	ALVWALL	ALVSP	TRBTCT
198	ONEWK	1	4	3	21	68	11
199	ONEWK	1	4	4	23	47	30
200	ONEWK	1	4	5	38	51	11
201	ONEWK	1	5	1	22	70	8
202	ONEWK	1	5	2	25	54	21
203	ONEWK	1	5	3	25	50	25
204	ONEWK	1	5	4	17	24	59
205	ONEWK	1	5	5	14	67	19
206	ONEWK	1	6	1	22	71	7
207	ONEWK	1	6	2	19	60	21
208	ONEWK	1	6	3	23	64	13
209	ONEWK	1	6	4	21	59	20
210	ONEWK	1	6	5	30	54	16
211	ONEWK	2	1	1	25	64	11
212	ONEWK	2	1	2	31	52	17
213	ONEWK	2	1	3	23	61	18
214	ONEWK	2	1	4	25	57	16
215	ONEWK	2	1	5	19	59	22
216	ONEWK	2	2	1	26	27	47
217	ONEWK	2	2	2	32	57	11
218	ONEWK	2	2	3	30	48	22
219	ONEWK	2	2	4	31	55	14
220	ONEWK	2	2	5	29	57	14
221	ONEWK	2	3	1	21	46	33
222	ONEWK	2	3	2	23	63	14
223	ONEWK	2	3	3	20	27	53
224	ONEWK	2	3	4	19	58	23
225	ONEWK	2	3	5	19	49	32
226	ONEWK	2	4	1	11	47	42
227	ONEWK	2	4	2	27	54	19
228	ONEWK	2	4	3	31	55	14
229	ONEWK	2	4	4	24	52	24
230	ONEWK	2	4	5	29	50	21
231	ONEWK	2	5	1	18	58	24
232	ONEWK	2	5	2	24	68	8
233	ONEWK	2	5	3	34	41	25
234	ONEWK	2	5	4	24	62	14
235	ONEWK	2	5	5	29	52	19
236	ONEWK	2	6	1	25	35	7
237	ONEWK	2	6	2	35	48	17
238	ONEWK	2	6	3	30	54	16
239	ONEWK	2	6	4	24	73	3
240	ONEWK	2	6	5	23	38	39
241	ONEWK	3	1	1	38	62	0
242	ONEWK	3	1	2	40	46	14
243	ONEWK	3	1	3	38	35	27
244	ONEWK	3	1	4	26	13	61
245	ONEWK	3	1	5	41	55	4
246	ONEWK	3	2	1	29	59	12
247	ONEWK	3	2	2	18	33	49
248	ONEWK	3	2	3	29	63	8
249	ONEWK	3	2	4	30	69	1

OBS	AGE	ANIMAL	BLOCK	FIELD	ALVWALL	ALVSP	TRBTCT
250	ONEWK	3	2	5	26	60	14
251	ONEWK	3	3	1	24	55	21
252	ONEWK	3	3	2	20	60	20
253	ONEWK	3	3	3	30	36	34
254	ONEWK	3	3	4	11	41	42
255	ONEWK	3	3	5	20	50	30
256	ONEWK	3	4	1	26	62	12
257	ONEWK	3	4	2	22	66	12
258	ONEWK	3	4	3	19	61	20
259	ONEWK	3	4	4	19	47	34
260	ONEWK	3	4	5	19	60	21
261	ONEWK	3	5	1	24	70	6
262	ONEWK	3	5	2	20	58	22
263	ONEWK	3	5	3	14	54	32
264	ONEWK	3	5	4	23	70	7
265	ONEWK	3	5	5	19	69	12
266	ONEWK	3	6	1	24	71	5
267	ONEWK	3	6	2	15	47	38
268	ONEWK	3	6	3	20	62	18
269	ONEWK	3	6	4	30	50	20
270	ONEWK	3	6	5	19	54	27
271	TWWKS	1	1	1	17	81	2
272	TWWKS	1	1	2	15	75	10
273	TWWKS	1	1	3	23	59	18
274	TWWKS	1	1	4	15	64	21
275	TWWKS	1	1	5	11	73	16
276	TWWKS	1	2	1	45	48	7
277	TWWKS	1	2	2	25	44	31
278	TWWKS	1	2	3	26	50	24
279	TWWKS	1	2	4	25	53	22
280	TWWKS	1	2	5	30	54	16
281	TWWKS	1	3	1	22	61	17
282	TWWKS	1	3	2	27	59	14
283	TWWKS	1	3	3	20	55	25
284	TWWKS	1	3	4	22	60	18
285	TWWKS	1	3	5	21	57	22
286	TWWKS	1	4	1	21	50	29
287	TWWKS	1	4	2	24	53	23
288	TWWKS	1	4	3	23	47	30
289	TWWKS	1	4	4	25	62	13
290	TWWKS	1	4	5	32	49	19
291	TWWKS	1	5	1	30	64	6
292	TWWKS	1	5	2	22	59	19
293	TWWKS	1	5	3	30	49	21
294	TWWKS	1	5	4	27	43	30
295	TWWKS	1	5	5	25	58	11
296	TWWKS	1	6	1	24	59	17
297	TWWKS	1	6	2	15	71	14
298	TWWKS	1	6	3	33	48	19
299	TWWKS	1	6	4	36	52	12
300	TWWKS	1	6	5	21	53	26

OBS	AGE	ANIMAL	BLOCK	FIELD	ALVWALL	ALVSP	TRBTCT
301	TWWKS	2	1	1	25	70	5
302	TWWKS	2	1	2	23	62	15
303	TWWKS	2	1	3	20	61	19
304	TWWKS	2	1	4	23	59	18
305	TWWKS	2	1	5	24	68	8
306	TWWKS	2	2	1	18	76	6
307	TWWKS	2	2	2	22	67	11
308	TWWKS	2	2	3	23	70	7
309	TWWKS	2	2	4	27	62	11
310	TWWKS	2	2	5	22	50	28
311	TWWKS	2	3	1	32	53	15
312	TWWKS	2	3	2	26	45	29
313	TWWKS	2	3	3	32	52	16
314	TWWKS	2	3	4	22	62	16
315	TWWKS	2	3	5	31	58	11
316	TWWKS	2	4	1	40	58	2
317	TWWKS	2	4	2	35	35	30
318	TWWKS	2	4	3	32	36	32
319	TWWKS	2	4	4	27	26	47
320	TWWKS	2	4	5	31	62	7
321	TWWKS	2	5	1	18	56	26
322	TWWKS	2	5	2	7	70	23
323	TWWKS	2	5	3	30	61	9
324	TWWKS	2	5	4	19	66	15
325	TWWKS	2	5	5	22	72	6
326	TWWKS	2	6	1	25	69	6
327	TWWKS	2	6	2	19	72	9
328	TWWKS	2	6	3	19	63	18
329	TWWKS	2	6	4	15	81	4
330	TWWKS	2	6	5	29	64	7
331	TWWKS	3	1	1	25	67	8
332	TWWKS	3	1	2	21	67	12
333	TWWKS	3	1	3	27	57	16
334	TWWKS	3	1	4	22	55	23
335	TWWKS	3	1	5	18	56	26
336	TWWKS	3	2	1	38	56	6
337	TWWKS	3	2	2	27	58	15
338	TWWKS	3	2	3	32	51	17
339	TWWKS	3	2	4	29	54	17
340	TWWKS	3	2	5	30	67	3
341	TWWKS	3	3	1	17	69	14
342	TWWKS	3	3	2	33	54	15
343	TWWKS	3	3	3	21	67	12
344	TWWKS	3	3	4	15	52	33
345	TWWKS	3	3	5	24	71	5
346	TWWKS	3	4	1	22	64	14
347	TWWKS	3	4	2	19	51	30
348	TWWKS	3	4	3	22	65	13
349	TWWKS	3	4	4	17	54	29
350	TWWKS	3	4	5	23	64	13
351	TWWKS	3	5	1	17	71	12
352	TWWKS	3	5	2	15	71	14

OBS	AGE	ANIMAL	BLOCK	FIELD	ALVWALL	ALVSP	TRBTCT
353	TWWKS	3	5	3	21	67	12
354	TWWKS	3	5	4	15	64	21
355	TWWKS	3	5	5	20	69	11
356	TWWKS	3	6	1	25	55	20
357	TWWKS	3	6	2	21	34	45
358	TWWKS	3	6	3	46	48	6
359	TWWKS	3	6	4	34	64	2
360	TWWKS	3	6	5	30	39	31
361	THWKS	1	1	1	34	47	19
362	THWKS	1	1	2	30	47	23
363	THWKS	1	1	3	24	62	14
364	THWKS	1	1	4	21	59	20
365	THWKS	1	1	5	22	53	25
366	THWKS	1	2	1	18	62	20
367	THWKS	1	2	2	18	63	19
368	THWKS	1	2	3	21	63	16
369	THWKS	1	2	4	23	74	3
370	THWKS	1	2	5	26	65	9
371	THWKS	1	3	1	24	56	20
372	THWKS	1	3	2	18	63	19
373	THWKS	1	3	3	14	59	27
374	THWKS	1	3	4	26	67	7
375	THWKS	1	3	5	22	75	3
376	THWKS	1	4	1	21	59	20
377	THWKS	1	4	2	25	56	19
378	THWKS	1	4	3	24	64	12
379	THWKS	1	4	4	19	63	18
380	THWKS	1	4	5	19	55	26
381	THWKS	1	5	1	25	55	20
382	THWKS	1	5	2	30	56	14
383	THWKS	1	5	3	17	53	30
384	THWKS	1	5	4	18	37	45
385	THWKS	1	5	5	20	66	14
386	THWKS	1	6	1	8	60	32
387	THWKS	1	6	2	18	76	6
388	THWKS	1	6	3	21	68	11
389	THWKS	1	6	4	22	65	13
390	THWKS	1	6	5	32	63	5
391	THWKS	2	1	1	22	38	40
392	THWKS	2	1	2	29	62	9
393	THWKS	2	1	3	26	58	16
394	THWKS	2	1	4	31	33	36
395	THWKS	2	1	5	18	42	40
396	THWKS	2	2	1	27	62	11
397	THWKS	2	2	2	23	43	34
398	THWKS	2	2	3	29	53	18
399	THWKS	2	2	4	22	63	15
400	THWKS	2	2	5	32	60	8
401	THWKS	2	3	1	32	59	9
402	THWKS	2	3	2	28	56	16
403	THWKS	2	3	3	25	60	15
404	THWKS	2	3	4	29	63	8
405	THWKS	2	3	5	33	61	6

OBS	AGE	ANIMAL	BLOCK	FIELD	ALVWALL	ALVSP	TRBTCT
406	THWKS	2	4	1	23	64	13
407	THWKS	2	4	2	30	44	26
408	THWKS	2	4	3	25	44	31
409	THWKS	2	4	4	23	64	13
410	THWKS	2	4	5	13	67	20
411	THWKS	2	5	1	20	62	18
412	THWKS	2	5	2	21	66	13
413	THWKS	2	5	3	14	73	13
414	THWKS	2	5	4	14	64	22
415	THWKS	2	5	5	10	65	25
416	THWKS	2	6	1	15	81	4
417	THWKS	2	6	2	12	84	4
418	THWKS	2	6	3	11	54	35
419	THWKS	2	6	4	23	69	8
420	THWKS	2	6	5	13	78	9
421	THWKS	3	1	1	17	73	10
422	THWKS	3	1	2	10	78	12
423	THWKS	3	1	3	14	68	18
424	THWKS	3	1	4	28	51	21
425	THWKS	3	1	5	18	71	11
426	THWKS	3	2	1	24	59	17
427	THWKS	3	2	2	10	46	44
428	THWKS	3	2	3	19	46	35
429	THWKS	3	2	4	14	56	30
430	THWKS	3	2	5	26	59	15
431	THWKS	3	3	1	16	72	12
432	THWKS	3	3	2	18	69	13
433	THWKS	3	3	3	16	68	16
434	THWKS	3	3	4	20	50	30
435	THWKS	3	3	5	24	67	9
436	THWKS	3	4	1	24	72	4
437	THWKS	3	4	2	27	66	7
438	THWKS	3	4	3	20	65	15
439	THWKS	3	4	4	23	64	13
440	THWKS	3	4	5	22	46	32
441	THWKS	3	5	1	18	57	25
442	THWKS	3	5	2	24	65	11
443	THWKS	3	5	3	18	69	12
444	THWKS	3	5	4	18	66	16
445	THWKS	3	5	5	23	63	14
446	THWKS	3	6	1	12	51	37
447	THWKS	3	6	2	18	55	27
448	THWKS	3	6	3	12	70	18
449	THWKS	3	6	4	23	48	29
450	THWKS	3	6	5	23	68	9
451	ADULT	1	1	1	10	87	3
452	ADULT	1	1	2	17	75	8
453	ADULT	1	1	3	10	85	6
454	ADULT	1	1	4	14	82	26
455	ADULT	1	1	5	11	63	26
456	ADULT	1	2	1	13	65	22

OBS	AGE	ANIMAL	BLOCK	FIELD	ALVWALL	ALVSP	TRBTCT
457	ADULT	1	2	2	15	78	7
458	ADULT	1	2	3	11	77	12
459	ADULT	1	2	4	16	70	14
460	ADULT	1	2	5	11	72	17
461	ADULT	1	3	1	16	70	14
462	ADULT	1	3	2	14	70	16
463	ADULT	1	3	3	25	77	0
464	ADULT	1	3	4	14	48	38
465	ADULT	1	3	5	8	73	19
466	ADULT	1	4	1	8	82	10
467	ADULT	1	4	2	18	82	0
468	ADULT	1	4	3	9	49	42
469	ADULT	1	4	4	18	47	35
470	ADULT	1	4	5	11	59	30
471	ADULT	1	5	1	13	84	3
472	ADULT	1	5	2	9	77	14
473	ADULT	1	5	3	14	75	11
474	ADULT	1	5	4	9	54	37
475	ADULT	1	5	5	15	71	16
476	ADULT	1	6	1	13	72	15
477	ADULT	1	6	2	16	74	11
478	ADULT	1	6	3	15	83	3
479	ADULT	1	6	4	11	64	25
480	ADULT	1	6	5	14	86	1
481	ADULT	2	1	1	7	68	26
482	ADULT	2	1	2	19	72	9
483	ADULT	2	1	3	14	80	6
484	ADULT	2	1	4	9	69	23
485	ADULT	2	1	5	11	88	1
486	ADULT	2	2	1	18	72	10
487	ADULT	2	2	2	17	72	13
488	ADULT	2	2	3	8	76	18
489	ADULT	2	2	4	9	74	18
490	ADULT	2	2	5	12	67	22
491	ADULT	2	3	1	26	61	15
492	ADULT	2	3	2	20	53	29
493	ADULT	2	3	3	35	62	3
494	ADULT	2	3	4	34	49	18
495	ADULT	2	3	5	20	43	37
496	ADULT	2	4	1	35	54	11
497	ADULT	2	4	2	26	61	13
498	ADULT	2	4	3	24	52	24
499	ADULT	2	4	4	26	72	1
500	ADULT	2	4	5	15	48	37
501	ADULT	2	5	1	34	55	11
502	ADULT	2	5	2	19	35	45
503	ADULT	2	5	3	20	56	24
504	ADULT	2	5	4	33	52	15
505	ADULT	2	5	5	22	46	32
506	ADULT	2	6	1	27	53	20
507	ADULT	2	6	2	21	64	15

OBS	AGE	ANIMAL	BLOCK	FIELD	ALVWALL	ALVSP	TRBTCT
508	ADULT	2	6	3	26	65	9
509	ADULT	2	6	4	23	56	21
510	ADULT	2	6	5	25	43	32
511	ADULT	3	1	1	41	36	23
512	ADULT	3	1	2	29	49	22
513	ADULT	3	1	3	40	47	13
514	ADULT	3	1	4	30	65	4
515	ADULT	3	1	5	33	58	9
516	ADULT	3	2	1	19	81	0
517	ADULT	3	2	2	14	70	16
518	ADULT	3	2	3	14	56	30
519	ADULT	3	2	4	21	71	8
520	ADULT	3	2	5	13	73	14
521	ADULT	3	3	1	18	78	4
522	ADULT	3	3	2	14	80	6
523	ADULT	3	3	3	16	66	18
524	ADULT	3	3	4	17	56	30
525	ADULT	3	3	5	14	56	27
526	ADULT	3	4	1	12	82	6
527	ADULT	3	4	2	15	77	8
528	ADULT	3	4	3	18	61	21
529	ADULT	3	4	4	7	50	43
530	ADULT	3	4	5	24	71	5
531	ADULT	3	5	1	29	50	21
532	ADULT	3	5	2	22	38	40
533	ADULT	3	5	3	17	58	45
534	ADULT	3	5	4	31	47	11
535	ADULT	3	5	5	20	38	33
536	ADULT	3	6	1	16	74	10
537	ADULT	3	6	2	20	43	37
538	ADULT	3	6	3	24	60	16
539	ADULT	3	6	4	27	58	15
540	ADULT	3	6	5	27	60	13

APPENDIX H

Data collected on number of points falling on type I and type II pneumocytes and connective tissue (CONTIS) at electron microscopic level.

OBS	AGE	ANIMAL	TYPEI	TYPEII	CONTIS
1	PRENL	1	1	12	19
2	PRENL	1	2	0	34
3	PRENL	1	1	9	31
4	PRENL	1	1	6	29
5	PRENL	1	5	10	39
6	PRENL	1	2	16	37
7	PRENL	1	0	6	25
8	PRENL	1	2	7	15
9	PRENL	1	4	8	31
10	PRENL	1	5	5	31
11	PRENL	1	0	7	26
12	PRENL	1	2	0	25
13	PRENL	1	0	0	19
14	PRENL	1	1	5	36
15	PRENL	1	2	11	20
16	PRENL	1	1	5	27
17	PRENL	1	1	8	57
18	PRENL	1	9	11	29
19	PRENL	1	1	7	30
20	PRENL	1	0	6	28
21	PRENL	1	1	2	20
22	PRENL	1	3	3	35
23	PRENL	1	0	0	16
24	PRENL	1	2	6	31
25	PRENL	1	5	10	16
26	PRENL	1	2	1	20
27	PRENL	1	4	7	34
28	PRENL	1	3	9	30
29	PRENL	1	0	2	20
30	PRENL	1	1	8	39
31	PRENL	2	1	12	53
32	PRENL	2	4	12	7
33	PRENL	2	4	25	27
34	PRENL	2	5	10	24
35	PRENL	2	0	8	30
36	PRENL	2	2	15	37
37	PRENL	2	2	9	37
38	PRENL	2	3	11	41
39	PRENL	2	5	10	36
40	PRENL	2	0	8	27
41	PRENL	2	2	24	26
42	PRENL	2	3	13	57
43	PRENL	2	7	13	28
44	PRENL	2	1	24	50

OBS	AGE	ANIMAL	TYPEI	TYPEII	CONTIS
45	PRENL	2	4	17	24
46	PRENL	2	3	14	52
47	PRENL	2	1	15	49
48	PRENL	2	3	12	40
49	PRENL	2	5	21	42
50	PRENL	2	7	13	23
51	PRENL	2	4	16	31
52	PRENL	2	2	7	49
53	PRENL	2	3	12	44
54	PRENL	2	2	2	43
55	PRENL	2	1	18	47
56	PRENL	2	5	17	45
57	PRENL	2	0	19	32
58	PRENL	2	3	8	49
59	PRENL	2	0	16	20
60	PRENL	2	1	18	37
61	PRENL	3	2	9	24
62	PRENL	3	3	10	43
63	PRENL	3	2	8	38
64	PRENL	3	4	21	37
65	PRENL	3	5	16	40
66	PRENL	3	5	11	19
67	PRENL	3	7	6	26
68	PRENL	3	4	2	22
69	PRENL	3	0	3	40
70	PRENL	3	2	12	28
71	PRENL	3	7	10	38
72	PRENL	3	6	15	35
73	PRENL	3	4	15	63
74	PRENL	3	4	6	39
75	PRENL	3	4	5	56
76	PRENL	3	3	2	44
77	PRENL	3	4	13	44
78	PRENL	3	7	14	58
79	PRENL	3	2	9	54
80	PRENL	3	2	10	55
81	PRENL	3	8	6	39
82	PRENL	3	2	6	60
83	PRENL	3	3	15	42
84	PRENL	3	3	7	47
85	PRENL	3	1	2	31
86	PRENL	3	3	9	37
87	PRENL	3	2	8	23
88	PRENL	3	5	9	38
89	PRENL	3	0	3	33
90	PRENL	3	2	2	10
91	ONEDY	1	1	0	20
92	ONEDY	1	1	3	25
93	ONEDY	1	1	6	37
94	ONEDY	1	3	1	23
95	ONEDY	1	4	0	21

OBS	AGE	ANIMAL	TYPEI	TYPEII	CONTIS
96	ONEDY	1	0	11	30
97	ONEDY	1	0	7	16
98	ONEDY	1	0	9	25
99	ONEDY	1	0	0	50
100	ONEDY	1	0	0	50
101	ONEDY	1	2	2	31
102	ONEDY	1	1	5	17
103	ONEDY	1	1	3	47
104	ONEDY	1	1	4	24
105	ONEDY	1	1	2	26
106	ONEDY	1	1	5	25
107	ONEDY	1	0	4	40
108	ONEDY	1	3	1	25
109	ONEDY	1	0	1	32
110	ONEDY	1	1	7	22
111	ONEDY	1	5	7	34
112	ONEDY	1	1	3	30
113	ONEDY	1	1	6	66
114	ONEDY	1	10	6	45
115	ONEDY	1	0	4	19
116	ONEDY	1	3	10	25
117	ONEDY	1	0	5	25
118	ONEDY	1	1	8	20
119	ONEDY	1	4	6	33
120	ONEDY	1	4	9	25
121	ONEDY	2	2	0	45
122	ONEDY	2	0	0	26
123	ONEDY	2	0	6	44
124	ONEDY	2	3	4	23
125	ONEDY	2	0	0	36
126	ONEDY	2	0	12	60
127	ONEDY	2	0	13	29
128	ONEDY	2	2	7	39
129	ONEDY	2	0	7	28
130	ONEDY	2	3	3	20
131	ONEDY	2	0	10	28
132	ONEDY	2	0	1	31
133	ONEDY	2	2	3	27
134	ONEDY	2	1	0	23
135	ONEDY	2	2	1	20
136	ONEDY	2	1	0	18
137	ONEDY	2	0	4	33
138	ONEDY	2	0	0	13
139	ONEDY	2	1	2	19
140	ONEDY	2	1	0	16
141	ONEDY	2	1	1	42
142	ONEDY	2	0	2	37
143	ONEDY	2	2	2	44
144	ONEDY	2	0	8	45
145	ONEDY	2	2	2	45
146	ONEDY	2	0	3	42

OBS	AGE	ANIMAL	TYPEI	TYPEII	CONTIS
147	ONEDY	2	1	5	22
148	ONEDY	2	1	6	16
149	ONEDY	2	0	12	24
150	ONEDY	2	2	2	21
151	ONEDY	3	1	5	16
152	ONEDY	3	0	13	12
153	ONEDY	3	1	11	11
154	ONEDY	3	0	0	25
155	ONEDY	3	1	9	28
156	ONEDY	3	0	2	22
157	ONEDY	3	2	0	21
158	ONEDY	3	0	0	17
159	ONEDY	3	3	9	34
160	ONEDY	3	0	0	44
161	ONEDY	3	1	5	28
162	ONEDY	3	2	3	28
163	ONEDY	3	5	1	54
164	ONEDY	3	2	0	39
165	ONEDY	3	2	6	30
166	ONEDY	3	1	10	29
167	ONEDY	3	0	14	20
168	ONEDY	3	1	2	28
169	ONEDY	3	1	1	20
170	ONEDY	3	1	4	23
171	ONEDY	3	5	4	20
172	ONEDY	3	1	10	33
173	ONEDY	3	0	10	26
174	ONEDY	3	0	0	21
175	ONEDY	3	2	5	26
176	ONEDY	3	0	2	32
177	ONEDY	3	0	3	39
178	ONEDY	3	0	7	28
179	ONEDY	3	1	2	17
180	ONEDY	3	0	3	19
181	ONEWK	1	0	8	25
182	ONEWK	1	1	6	28
183	ONEWK	1	0	0	34
184	ONEWK	1	1	3	42
185	ONEWK	1	0	1	15
186	ONEWK	1	1	2	30
187	ONEWK	1	2	7	16
188	ONEWK	1	1	2	18
189	ONEWK	1	2	4	20
190	ONEWK	1	2	6	30
191	ONEWK	1	0	3	16
192	ONEWK	1	1	2	16
193	ONEWK	1	1	1	10
194	ONEWK	1	0	0	20
195	ONEWK	1	0	3	12
196	ONEWK	1	2	4	11
197	ONEWK	1	2	0	10

OBS	AGE	ANIMAL	TYPEI	TYPEII	CONTIS
198	ONEWK	1	0	7	11
199	ONEWK	1	2	0	5
200	ONEWK	1	1	3	21
201	ONEWK	1	3	2	31
202	ONEWK	1	2	1	20
203	ONEWK	1	2	0	31
204	ONEWK	1	1	7	25
205	ONEWK	1	1	0	61
206	ONEWK	1	2	1	18
207	ONEWK	1	1	6	47
208	ONEWK	1	4	5	23
209	ONEWK	1	0	2	39
210	ONEWK	1	1	2	19
211	ONEWK	2	1	0	19
212	ONEWK	2	1	0	28
213	ONEWK	2	0	1	50
214	ONEWK	2	1	0	59
215	ONEWK	2	0	0	36
216	ONEWK	2	3	4	24
217	ONEWK	2	1	1	39
218	ONEWK	2	3	8	27
219	ONEWK	2	0	4	32
220	ONEWK	2	2	0	30
221	ONEWK	2	1	8	25
222	ONEWK	2	2	6	21
223	ONEWK	2	0	0	41
224	ONEWK	2	0	8	26
225	ONEWK	2	2	4	18
226	ONEWK	2	6	0	39
227	ONEWK	2	0	6	34
228	ONEWK	2	1	1	18
229	ONEWK	2	0	3	33
230	ONEWK	2	1	2	45
231	ONEWK	2	3	2	62
232	ONEWK	2	1	11	30
233	ONEWK	2	2	10	30
234	ONEWK	2	1	5	27
235	ONEWK	2	2	0	18
236	ONEWK	2	0	0	33
237	ONEWK	2	0	0	28
238	ONEWK	2	3	11	32
239	ONEWK	2	0	0	12
240	ONEWK	2	0	6	16
241	ONEWK	3	1	2	23
242	ONEWK	3	1	0	50
243	ONEWK	3	2	0	17
244	ONEWK	3	1	15	29
245	ONEWK	3	0	0	15
246	ONEWK	3	2	5	42
247	ONEWK	3	2	4	50

OBS	AGE	ANIMAL	TYPEI	TYPEII	CONTIS
248	ONEWK	3	1	5	25
249	ONEWK	3	3	1	15
250	ONEWK	3	1	4	14
251	ONEWK	3	2	15	14
252	ONEWK	3	2	16	23
253	ONEWK	3	2	11	13
254	ONEWK	3	4	13	34
255	ONEWK	3	2	9	8
256	ONEWK	3	1	2	6
257	ONEWK	3	3	7	34
258	ONEWK	3	0	19	27
259	ONEWK	3	0	13	24
260	ONEWK	3	2	5	26
261	ONEWK	3	0	13	34
262	ONEWK	3	0	3	36
263	ONEWK	3	0	0	18
264	ONEWK	3	1	2	26
265	ONEWK	3	0	9	27
266	ONEWK	3	1	0	13
267	ONEWK	3	0	13	16
268	ONEWK	3	4	12	19
269	ONEWK	3	1	3	23
270	ONEWK	3	0	5	17
271	TWWKS	1	1	3	27
272	TWWKS	1	0	1	16
273	TWWKS	1	0	1	20
274	TWWKS	1	3	7	26
275	TWWKS	1	0	2	17
276	TWWKS	1	0	1	15
277	TWWKS	1	0	3	23
278	TWWKS	1	1	3	23
279	TWWKS	1	0	1	43
280	TWWKS	1	0	0	25
281	TWWKS	1	0	6	22
282	TWWKS	1	1	9	9
283	TWWKS	1	2	3	19
284	TWWKS	1	0	8	6
285	TWWKS	1	1	1	16
286	TWWKS	1	3	7	19
287	TWWKS	1	0	7	24
288	TWWKS	1	0	3	20
289	TWWKS	1	0	2	21
290	TWWKS	1	3	4	17
291	TWWKS	1	2	4	46
292	TWWKS	1	2	4	20
293	TWWKS	1	0	3	29
294	TWWKS	1	2	6	26
295	TWWKS	1	0	3	25
296	TWWKS	1	0	5	24
297	TWWKS	1	0	0	24
298	TWWKS	1	1	3	16
299	TWWKS	1	2	5	19

OBS	AGE	ANIMAL	TYPEI	TYPEII	CONTIS
300	TWWKS	1	0	0	32
301	TWWKS	2	0	5	20
302	TWWKS	2	0	2	20
303	TWWKS	2	0	5	19
304	TWWKS	2	1	0	24
305	TWWKS	2	0	5	27
306	TWWKS	2	0	0	34
307	TWWKS	2	0	2	23
308	TWWKS	2	0	6	48
309	TWWKS	2	0	2	18
310	TWWKS	2	0	0	15
311	TWWKS	2	0	5	20
312	TWWKS	2	2	1	15
313	TWWKS	2	1	0	6
314	TWWKS	2	0	0	14
315	TWWKS	2	1	3	29
316	TWWKS	2	1	4	24
317	TWWKS	2	0	3	23
318	TWWKS	2	2	2	37
319	TWWKS	2	1	1	29
320	TWWKS	2	0	3	36
321	TWWKS	2	1	1	36
322	TWWKS	2	0	2	10
323	TWWKS	2	1	2	11
324	TWWKS	2	3	4	23
325	TWWKS	2	0	0	17
326	TWWKS	2	1	0	24
327	TWWKS	2	1	1	10
328	TWWKS	2	1	2	13
329	TWWKS	2	0	1	38
330	TWWKS	2	0	0	39
331	TWWKS	3	1	6	22
332	TWWKS	3	3	0	32
333	TWWKS	3	0	1	32
334	TWWKS	3	2	4	28
335	TWWKS	3	0	0	25
336	TWWKS	3	2	0	21
337	TWWKS	3	1	3	14
338	TWWKS	3	0	3	15
339	TWWKS	3	1	1	25
340	TWWKS	3	3	0	15
341	TWWKS	3	0	6	36
342	TWWKS	3	1	6	23
343	TWWKS	3	1	0	20
344	TWWKS	3	0	0	35
345	TWWKS	3	0	7	36
346	TWWKS	3	0	3	16
347	TWWKS	3	0	2	16
348	TWWKS	3	0	3	20
349	TWWKS	3	1	2	26
350	TWWKS	3	0	0	16

OBS	AGE	ANIMAL	TYPEI	TYPEII	CONTIS
351	TWWKS	3	0	7	23
352	TWWKS	3	1	0	21
353	TWWKS	3	0	1	24
354	TWWKS	3	1	2	20
355	TWWKS	3	0	0	18
356	TWWKS	3	0	3	16
357	TWWKS	3	0	2	18
358	TWWKS	3	2	0	43
359	TWWKS	3	0	5	37
360	TWWKS	3	0	0	26
361	THWKS	1	0	0	10
362	THWKS	1	0	1	15
363	THWKS	1	0	6	11
364	THWKS	1	0	5	17
365	THWKS	1	0	0	13
366	THWKS	1	0	0	21
367	THWKS	1	1	0	9
368	THWKS	1	2	3	22
369	THWKS	1	1	0	14
370	THWKS	1	0	0	14
371	THWKS	1	0	1	14
372	THWKS	1	2	1	8
373	THWKS	1	0	1	12
374	THWKS	1	0	4	16
375	THWKS	1	0	0	10
376	THWKS	1	0	4	34
377	THWKS	1	0	0	20
378	THWKS	1	2	0	14
379	THWKS	1	0	1	15
380	THWKS	1	1	2	27
381	THWKS	1	3	0	23
382	THWKS	1	1	0	16
383	THWKS	1	1	0	20
384	THWKS	1	1	3	12
385	THWKS	1	2	0	24
386	THWKS	1	0	0	19
387	THWKS	1	0	0	20
388	THWKS	1	0	0	19
389	THWKS	1	0	1	19
390	THWKS	1	1	3	26
391	THWKS	2	0	10	19
392	THWKS	2	0	2	31
393	THWKS	2	0	5	36
394	THWKS	2	1	2	12
395	THWKS	2	2	0	21
396	THWKS	2	1	7	24
397	THWKS	2	0	2	26
398	THWKS	2	0	4	29
399	THWKS	2	1	1	28
400	THWKS	2	1	2	20
401	THWKS	2	1	0	26
402	THWKS	2	1	6	15

OBS	AGE	ANIMAL	TYPEI	TYPEII	CONTIS
403	THWKS	2	0	2	18
404	THWKS	2	0	1	10
405	THWKS	2	0	1	17
406	THWKS	2	0	3	30
407	THWKS	2	1	4	36
408	THWKS	2	0	0	17
409	THWKS	2	2	0	25
410	THWKS	2	1	3	20
411	THWKS	2	0	2	36
412	THWKS	2	0	0	25
413	THWKS	2	0	0	19
414	THWKS	2	1	2	13
415	THWKS	2	0	0	18
416	THWKS	2	0	0	17
417	THWKS	2	0	0	19
418	THWKS	2	0	1	11
419	THWKS	2	0	0	48
420	THWKS	2	1	1	9
421	THWKS	3	2	1	25
422	THWKS	3	1	0	33
423	THWKS	3	2	8	35
424	THWKS	3	1	0	35
425	THWKS	3	2	8	35
426	THWKS	3	1	6	32
427	THWKS	3	0	6	9
428	THWKS	3	0	3	37
429	THWKS	3	0	0	12
430	THWKS	3	3	0	18
431	THWKS	3	0	0	11
432	THWKS	3	0	0	30
433	THWKS	3	0	8	29
434	THWKS	3	2	0	5
435	THWKS	3	1	1	12
436	THWKS	3	0	0	9
437	THWKS	3	1	1	8
438	THWKS	3	0	3	24
439	THWKS	3	0	0	24
440	THWKS	3	0	2	14
441	THWKS	3	0	3	14
442	THWKS	3	3	1	36
443	THWKS	3	1	6	13
444	THWKS	3	0	1	14
445	THWKS	3	0	2	19
446	THWKS	3	1	2	16
447	THWKS	3	1	2	26
448	THWKS	3	0	1	20
449	THWKS	3	0	0	12
450	THWKS	3	0	0	20
451	ADULT	1	1	0	4
452	ADULT	1	0	4	6
453	ADULT	1	0	3	7

OBS	AGE	ANIMAL	TYPEI	TYPEII	CONTIS
454	ADULT	1	0	4	3
455	ADULT	1	0	1	5
456	ADULT	1	0	0	1
457	ADULT	1	0	4	0
458	ADULT	1	0	2	2
459	ADULT	1	0	4	3
460	ADULT	1	0	3	11
461	ADULT	1	0	4	9
462	ADULT	1	0	0	4
463	ADULT	1	0	4	0
464	ADULT	1	2	0	0
465	ADULT	1	0	0	1
466	ADULT	1	1	0	13
467	ADULT	1	0	0	3
468	ADULT	1	0	4	5
469	ADULT	1	1	0	30
470	ADULT	1	4	5	16
471	ADULT	1	0	2	15
472	ADULT	1	2	1	26
473	ADULT	1	2	0	17
474	ADULT	1	0	1	14
475	ADULT	1	0	0	9
476	ADULT	1	0	0	13
477	ADULT	1	0	3	6
478	ADULT	1	0	0	4
479	ADULT	1	2	1	23
480	ADULT	1	0	6	3
481	ADULT	2	0	3	7
482	ADULT	2	0	1	22
483	ADULT	2	0	0	13
484	ADULT	2	0	2	10
485	ADULT	2	0	0	6
486	ADULT	2	0	1	34
487	ADULT	2	1	0	15
488	ADULT	2	0	4	27
489	ADULT	2	0	3	22
490	ADULT	2	1	2	21
491	ADULT	2	0	3	16
492	ADULT	2	0	2	7
493	ADULT	2	1	1	14
494	ADULT	2	1	3	11
495	ADULT	2	0	1	19
496	ADULT	2	0	3	12
497	ADULT	2	1	3	12
498	ADULT	2	1	9	16
499	ADULT	2	0	6	32
500	ADULT	2	0	4	16
501	ADULT	2	0	0	10
502	ADULT	2	2	1	16
503	ADULT	2	2	1	1
504	ADULT	2	2	0	16
505	ADULT	2	1	0	25

OBS	AGE	ANIMAL	TYPEI	TYPEII	CONTIS
506	ADULT	2	1	2	10
507	ADULT	2	0	2	9
508	ADULT	2	0	1	8
509	ADULT	2	0	2	21
510	ADULT	2	0	2	3
511	ADULT	3	1	0	9
512	ADULT	3	0	7	44
513	ADULT	3	1	0	12
514	ADULT	3	0	2	19
515	ADULT	3	0	0	16
516	ADULT	3	0	1	12
517	ADULT	3	0	2	14
518	ADULT	3	1	0	20
519	ADULT	3	0	5	14
520	ADULT	3	0	1	21
521	ADULT	3	0	2	13
522	ADULT	3	0	3	19
523	ADULT	3	1	3	32
524	ADULT	3	1	1	27
525	ADULT	3	0	4	16
526	ADULT	3	1	0	8
527	ADULT	3	0	4	12
528	ADULT	3	0	3	17
529	ADULT	3	0	1	24
530	ADULT	3	0	0	11
531	ADULT	3	0	12	17
532	ADULT	3	1	3	20
533	ADULT	3	0	5	16
534	ADULT	3	2	2	25
535	ADULT	3	1	1	33
536	ADULT	3	0	0	25
537	ADULT	3	0	6	18
538	ADULT	3	1	3	19
539	ADULT	3	0	7	24
540	ADULT	3	0	2	15

APPENDIX I

Data collected on number of intercepts (NUMINT), Length of intercept (LEINT) and number of points falling on capillary endothelium (CAPEND).

OBS	AGE	ANIMAL	NUMINT	LEINT	CAPEND
1	PRENL	1	0	0.00	4
2	PRENL	1	8	4.82	14
3	PRENL	1	4	4.19	9
4	PRENL	1	1	4.16	13
5	PRENL	1	5	4.97	12
6	PRENL	1	1	3.77	14
7	PRENL	1	7	3.97	6
8	PRENL	1	5	3.15	8
9	PRENL	1	2	4.50	7
10	PRENL	1	2	4.92	11
11	PRENL	1	2	4.09	9
12	PRENL	1	8	3.67	5
13	PRENL	1	6	4.60	6
14	PRENL	1	6	3.35	19
15	PRENL	1	5	3.86	5
16	PRENL	1	11	4.17	11
17	PRENL	1	8	4.51	11
18	PRENL	1	6	3.64	7
19	PRENL	1	8	4.18	11
20	PRENL	1	4	3.47	7
21	PRENL	1	4	3.77	12
22	PRENL	1	12	4.08	17
23	PRENL	1	6	3.52	11
24	PRENL	1	15	3.14	12
25	PRENL	1	12	3.56	16
26	PRENL	1	12	3.20	14
27	PRENL	1	12	3.93	17
28	PRENL	1	8	4.55	19
29	PRENL	1	10	4.53	13
30	PRENL	1	5	3.73	17
31	PRENL	2	8	3.95	14
32	PRENL	2	3	4.55	15
33	PRENL	2	8	5.07	15
34	PRENL	2	14	6.02	9
35	PRENL	2	14	5.46	18
36	PRENL	2	8	5.49	18
37	PRENL	2	10	4.27	3
38	PRENL	2	5	5.44	11
39	PRENL	2	4	5.30	6
40	PRENL	2	10	6.30	7
41	PRENL	2	14	6.10	1
42	PRENL	2	10	4.50	8
43	PRENL	2	15	4.15	7
44	PRENL	2	0	0.00	13
45	PRENL	2	11	4.58	20
46	PRENL	2	3	3.75	4
47	PRENL	2	15	4.46	13

OBS	AGE	ANIMAL	NUMINT	LEINT	CAPEND
48	PRENL	2	9	4.58	7
49	PRENL	2	10	4.56	6
50	PRENL	2	2	4.37	10
51	PRENL	2	7	4.67	12
52	PRENL	2	6	4.06	11
53	PRENL	2	9	4.67	19
54	PRENL	2	12	5.38	12
55	PRENL	2	3	5.40	13
56	PRENL	2	4	5.12	7
57	PRENL	2	5	5.14	17
58	PRENL	2	9	5.78	21
59	PRENL	2	8	4.57	3
60	PRENL	2	8	5.15	11
61	PRENL	3	17	4.94	19
62	PRENL	3	3	5.54	13
63	PRENL	3	0	0.00	13
64	PRENL	3	2	5.03	10
65	PRENL	3	0	0.00	10
66	PRENL	3	4	4.27	19
67	PRENL	3	5	5.89	40
68	PRENL	3	3	5.83	8
69	PRENL	3	8	4.47	12
70	PRENL	3	3	5.25	18
71	PRENL	3	6	2.91	10
72	PRENL	3	0	0.00	14
73	PRENL	3	8	5.25	5
74	PRENL	3	5	4.59	20
75	PRENL	3	4	4.38	18
76	PRENL	3	0	0.00	9
77	PRENL	3	1	5.50	12
78	PRENL	3	0	0.00	12
79	PRENL	3	2	5.25	12
80	PRENL	3	3	5.00	23
81	PRENL	3	9	5.70	15
82	PRENL	3	2	6.00	11
83	PRENL	3	0	0.00	6
84	PRENL	3	13	6.21	13
85	PRENL	3	8	6.00	12
86	PRENL	3	10	5.97	10
87	PRENL	3	8	5.75	9
88	PRENL	3	0	5.59	15
89	PRENL	3	4	6.66	4
90	PRENL	3	4	4.94	16
91	ONEDY	1	5	6.40	24
92	ONEDY	1	21	5.74	15
93	ONEDY	1	4	5.71	17
94	ONEDY	1	9	4.31	16
95	ONEDY	1	5	4.69	19
96	ONEDY	1	6	6.84	12
97	ONEDY	1	14	4.51	17
98	ONEDY	1	6	4.47	5
99	ONEDY	1	2	3.66	16

OBS	AGE	ANIMAL	NUMINT	LEINT	CAPEND
100	ONEDY	1	7	3.40	44
101	ONEDY	1	0	0.00	0
102	ONEDY	1	7	2.94	4
103	ONEDY	1	8	3.00	5
104	ONEDY	1	0	0.00	0
105	ONEDY	1	8	2.95	1
106	ONEDY	1	8	2.54	4
107	ONEDY	1	3	3.26	12
108	ONEDY	1	0	0.00	6
109	ONEDY	1	5	3.44	3
110	ONEDY	1	5	4.03	3
111	ONEDY	1	2	4.15	2
112	ONEDY	1	2	4.43	3
113	ONEDY	1	0	0.00	0
114	ONEDY	1	2	3.74	5
115	ONEDY	1	1	4.20	4
116	ONEDY	1	7	3.60	7
117	ONEDY	1	4	2.98	5
118	ONEDY	1	1	3.20	0
119	ONEDY	1	1	3.55	4
120	ONEDY	1	3	3.12	8
121	ONEDY	2	4	5.15	8
122	ONEDY	2	12	4.47	13
123	ONEDY	2	21	3.75	23
124	ONEDY	2	16	4.38	8
125	ONEDY	2	0	0.00	36
126	ONEDY	2	18	3.85	11
127	ONEDY	2	8	4.56	10
128	ONEDY	2	15	3.75	9
129	ONEDY	2	23	3.41	15
130	ONEDY	2	8	4.26	11
131	ONEDY	2	8	4.59	13
132	ONEDY	2	13	3.54	11
133	ONEDY	2	14	2.88	19
134	ONEDY	2	16	3.52	25
135	ONEDY	2	14	3.65	25
136	ONEDY	2	10	2.87	30
137	ONEDY	2	5	4.78	22
138	ONEDY	2	7	4.83	26
139	ONEDY	2	7	2.98	10
140	ONEDY	2	7	4.84	28
141	ONEDY	2	12	4.48	10
142	ONEDY	2	4	4.26	19
143	ONEDY	2	13	4.50	12
144	ONEDY	2	11	4.68	9
145	ONEDY	2	4	5.63	6
146	ONEDY	2	5	4.24	26
147	ONEDY	2	3	3.90	11
148	ONEDY	2	2	3.45	16
149	ONEDY	2	7	4.85	20
150	ONEDY	2	5	5.85	22

OBS	AGE	ANIMAL	NUMINT	LEINT	CAPEND
151	ONEDY	3	4	3.20	12
152	ONEDY	3	12	6.30	27
153	ONEDY	3	10	4.83	25
154	ONEDY	3	14	3.75	25
155	ONEDY	3	8	5.04	8
156	ONEDY	3	9	4.00	15
157	ONEDY	3	2	3.89	11
158	ONEDY	3	5	4.93	20
159	ONEDY	3	2	4.48	18
160	ONEDY	3	3	4.04	25
161	ONEDY	3	7	3.13	18
162	ONEDY	3	5	3.22	23
163	ONEDY	3	18	3.33	9
164	ONEDY	3	7	4.33	12
165	ONEDY	3	2	3.10	17
166	ONEDY	3	10	3.24	5
167	ONEDY	3	1	3.63	14
168	ONEDY	3	6	3.27	5
169	ONEDY	3	19	2.83	5
170	ONEDY	3	7	2.79	4
171	ONEDY	3	6	3.08	9
172	ONEDY	3	9	3.48	8
173	ONEDY	3	7	3.54	7
174	ONEDY	3	12	3.70	19
175	ONEDY	3	12	3.67	24
176	ONEDY	3	12	3.17	24
177	ONEDY	3	18	3.10	16
178	ONEDY	3	17	3.41	9
179	ONEDY	3	9	3.25	17
180	ONEDY	3	11	3.17	6
181	ONEWK	1	6	2.30	9
182	ONEWK	1	19	4.08	8
183	ONEWK	1	7	2.91	15
184	ONEWK	1	1	2.90	10
185	ONEWK	1	12	3.33	4
186	ONEWK	1	14	3.46	6
187	ONEWK	1	7	3.18	5
188	ONEWK	1	6	3.75	3
189	ONEWK	1	3	2.93	4
190	ONEWK	1	8	4.15	9
191	ONEWK	1	3	3.20	2
192	ONEWK	1	11	3.15	4
193	ONEWK	1	7	3.31	3
194	ONEWK	1	11	2.79	9
195	ONEWK	1	9	2.70	4
196	ONEWK	1	10	2.62	5
197	ONEWK	1	10	2.85	19
198	ONEWK	1	11	2.95	5
199	ONEWK	1	0	0.00	22

OBS	AGE	ANIMAL	NUMINT	LEINT	CAPEND
200	ONEWK	1	18	3.82	10
201	ONEWK	1	22	4.30	2
202	ONEWK	1	9	3.10	1
203	ONEWK	1	0	0.00	2
204	ONEWK	1	12	1.80	3
205	ONEWK	1	11	2.84	5
206	ONEWK	1	12	2.90	5
207	ONEWK	1	8	2.30	3
208	ONEWK	1	5	2.10	9
209	ONEWK	1	13	3.63	2
210	ONEWK	1	13	3.45	3
211	ONEWK	2	20	2.62	16
212	ONEWK	2	8	2.45	18
213	ONEWK	2	4	2.78	21
214	ONEWK	2	10	2.63	11
215	ONEWK	2	14	2.79	23
216	ONEWK	2	8	3.35	8
217	ONEWK	2	15	2.53	24
218	ONEWK	2	13	2.90	22
219	ONEWK	2	8	2.76	10
220	ONEWK	2	4	2.80	13
221	ONEWK	2	7	2.97	6
222	ONEWK	2	7	2.91	7
223	ONEWK	2	13	3.39	15
224	ONEWK	2	7	4.31	7
225	ONEWK	2	15	3.38	13
226	ONEWK	2	10	2.87	15
227	ONEWK	2	14	3.36	13
228	ONEWK	2	16	3.41	14
229	ONEWK	2	4	2.93	19
230	ONEWK	2	13	2.70	11
231	ONEWK	2	11	4.31	12
232	ONEWK	2	15	4.00	5
233	ONEWK	2	4	4.20	12
234	ONEWK	2	7	3.80	8
235	ONEWK	2	3	3.73	4
236	ONEWK	2	22	4.70	28
237	ONEWK	2	5	3.50	8
238	ONEWK	2	4	5.12	7
239	ONEWK	2	12	4.11	19
240	ONEWK	2	10	5.00	18
241	ONEWK	3	8	3.28	4
242	ONEWK	3	14	2.86	9
243	ONEWK	3	9	2.32	5
244	ONEWK	3	2	2.75	2
245	ONEWK	3	5	2.46	3
246	ONEWK	3	7	2.95	5
247	ONEWK	3	3	3.97	5
248	ONEWK	3	2	3.80	3
249	ONEWK	3	5	3.72	5

OBS	AGE	ANIMAL	NUMINT	LEINT	CAPEND
250	ONEWK	3	3	2.73	3
251	ONEWK	3	18	4.49	32
252	ONEWK	3	7	3.13	8
253	ONEWK	3	10	4.50	10
254	ONEWK	3	1	3.20	40
255	ONEWK	3	6	4.05	10
256	ONEWK	3	19	4.24	22
257	ONEWK	3	7	3.89	6
258	ONEWK	3	9	4.42	15
259	ONEWK	3	6	4.08	10
260	ONEWK	3	7	5.17	17
261	ONEWK	3	12	3.93	7
262	ONEWK	3	17	3.82	19
263	ONEWK	3	17	3.58	14
264	ONEWK	3	12	3.74	12
265	ONEWK	3	12	3.46	10
266	ONEWK	3	18	3.63	19
267	ONEWK	3	16	3.57	21
268	ONEWK	3	6	3.63	13
269	ONEWK	3	11	3.55	13
270	ONEWK	3	9	3.08	12
271	TWWKS	1	2	3.50	7
272	TWWKS	1	0	0.00	3
273	TWWKS	1	5	3.44	0
274	TWWKS	1	3	2.50	2
275	TWWKS	1	1	2.71	3
276	TWWKS	1	5	2.56	1
277	TWWKS	1	4	3.50	1
278	TWWKS	1	1	2.40	5
279	TWWKS	1	4	2.25	5
280	TWWKS	1	9	3.10	5
281	TWWKS	1	2	2.92	8
282	TWWKS	1	14	3.70	5
283	TWWKS	1	19	3.50	11
284	TWWKS	1	0	0.00	12
285	TWWKS	1	8	3.71	13
286	TWWKS	1	6	3.50	8
287	TWWKS	1	8	4.38	11
288	TWWKS	1	8	2.87	6
289	TWWKS	1	4	4.43	5
290	TWWKS	1	20	3.17	10
291	TWWKS	1	8	2.70	15
292	TWWKS	1	9	3.00	19
293	TWWKS	1	13	2.79	15
294	TWWKS	1	16	3.11	12
295	TWWKS	1	12	3.24	12
296	TWWKS	1	17	5.49	12
297	TWWKS	1	23	2.94	10
298	TWWKS	1	10	3.05	11
299	TWWKS	1	15	2.80	2
300	TWWKS	1	5	3.10	9

OBS	AGE	ANIMAL	NUMINT	LEINT	CAPEND
301	TWWKS	2	20	2.40	3
302	TWWKS	2	12	2.55	4
303	TWWKS	2	5	2.64	5
304	TWWKS	2	3	2.49	6
305	TWWKS	2	9	2.48	5
306	TWWKS	2	4	2.77	13
307	TWWKS	2	5	2.58	4
308	TWWKS	2	13	3.02	7
309	TWWKS	2	2	2.48	4
310	TWWKS	2	16	2.51	7
311	TWWKS	2	9	2.45	6
312	TWWKS	2	9	2.57	6
313	TWWKS	2	6	2.56	16
314	TWWKS	2	11	2.65	3
315	TWWKS	2	3	2.07	4
316	TWWKS	2	8	1.80	13
317	TWWKS	2	3	2.86	5
318	TWWKS	2	10	2.68	7
319	TWWKS	2	11	2.60	6
320	TWWKS	2	11	2.24	4
321	TWWKS	2	12	3.13	10
322	TWWKS	2	10	3.44	11
323	TWWKS	2	16	3.93	9
324	TWWKS	2	7	3.83	13
325	TWWKS	2	18	2.68	5
326	TWWKS	2	22	3.62	2
327	TWWKS	2	10	2.94	7
328	TWWKS	2	16	2.53	7
329	TWWKS	2	13	2.43	10
330	TWWKS	2	18	2.84	21
331	TWWKS	3	0	0.00	1
332	TWWKS	3	12	2.93	6
333	TWWKS	3	0	0.00	5
334	TWWKS	3	17	2.15	3
335	TWWKS	3	0	0.00	3
336	TWWKS	3	28	2.85	2
337	TWWKS	3	18	2.74	2
338	TWWKS	3	24	3.80	4
339	TWWKS	3	17	3.68	8
340	TWWKS	3	19	3.10	2
341	TWWKS	3	13	2.73	3
342	TWWKS	3	15	2.88	8
343	TWWKS	3	13	3.93	1
344	TWWKS	3	9	2.00	1
345	TWWKS	3	13	3.25	2
346	TWWKS	3	0	0.00	0
347	TWWKS	3	11	2.00	1
348	TWWKS	3	0	0.00	0
349	TWWKS	3	17	3.55	6

OBS	AGE	ANIMAL	NUMINT	LEINT	CAPEND
350	TWWKS	3	14	3.73	4
351	TWWKS	3	7	2.99	5
352	TWWKS	3	5	2.10	4
353	TWWKS	3	0	0.00	0
354	TWWKS	3	19	2.87	12
355	TWWKS	3	6	2.60	6
356	TWWKS	3	14	2.49	6
357	TWWKS	3	19	2.64	7
358	TWWKS	3	19	2.25	4
359	TWWKS	3	0	0.00	6
360	TWWKS	3	26	2.50	3
361	THWKS	1	12	2.78	11
362	THWKS	1	21	2.34	11
363	THWKS	1	15	2.20	3
364	THWKS	1	10	2.07	12
365	THWKS	1	8	3.01	5
366	THWKS	1	18	2.56	11
367	THWKS	1	10	2.48	9
368	THWKS	1	7	2.41	8
369	THWKS	1	11	2.74	12
370	THWKS	1	11	2.28	7
371	THWKS	1	16	2.94	7
372	THWKS	1	8	2.48	7
373	THWKS	1	8	2.39	3
374	THWKS	1	11	2.15	6
375	THWKS	1	12	2.60	10
376	THWKS	1	3	2.43	5
377	THWKS	1	3	3.77	4
378	THWKS	1	8	3.11	7
379	THWKS	1	11	2.45	3
380	THWKS	1	6	2.10	7
381	THWKS	1	17	2.45	17
382	THWKS	1	6	2.50	5
383	THWKS	1	8	2.60	10
384	THWKS	1	9	2.66	11
385	THWKS	1	11	2.74	5
386	THWKS	1	4	1.83	4
387	THWKS	1	19	2.37	16
388	THWKS	1	13	2.22	5
389	THWKS	1	5	2.14	19
390	THWKS	1	6	2.80	12
391	THWKS	2	11	2.70	9
392	THWKS	2	3	2.46	18
393	THWKS	2	33	2.33	21
394	THWKS	2	5	2.86	12
395	THWKS	2	18	2.49	15
396	THWKS	2	13	2.35	23
397	THWKS	2	16	2.56	17
398	THWKS	2	16	2.36	25
399	THWKS	2	8	3.16	10

OBS	AGE	ANIMAL	NUMINT	LEINT	CAPEND
400	THWKS	2	3	2.27	8
401	THWKS	2	5	1.74	11
402	THWKS	2	14	2.15	12
403	THWKS	2	7	2.27	4
404	THWKS	2	5	2.50	6
405	THWKS	2	8	2.33	9
406	THWKS	2	4	2.18	9
407	THWKS	2	3	2.20	16
408	THWKS	2	12	3.05	10
409	THWKS	2	8	1.85	15
410	THWKS	2	20	2.51	15
411	THWKS	2	12	3.50	11
412	THWKS	2	4	2.75	5
413	THWKS	2	16	2.23	11
414	THWKS	2	18	2.62	13
415	THWKS	2	22	2.56	21
416	THWKS	2	20	2.38	24
417	THWKS	2	10	1.78	6
418	THWKS	2	9	2.46	17
419	THWKS	2	17	2.38	17
420	THWKS	2	10	2.39	7
421	THWKS	3	6	3.67	7
422	THWKS	3	12	3.53	8
423	THWKS	3	9	3.25	13
424	THWKS	3	5	3.10	9
425	THWKS	3	8	2.36	5
426	THWKS	3	6	3.42	7
427	THWKS	3	11	3.30	15
428	THWKS	3	12	2.78	16
429	THWKS	3	12	2.67	14
430	THWKS	3	12	2.89	14
431	THWKS	3	21	2.33	17
432	THWKS	3	13	2.96	12
433	THWKS	3	16	2.42	22
434	THWKS	3	17	2.07	10
435	THWKS	3	10	2.47	6
436	THWKS	3	22	2.33	16
437	THWKS	3	18	2.47	19
438	THWKS	3	16	2.84	15
439	THWKS	3	13	2.56	7
440	THWKS	3	7	2.40	11
441	THWKS	3	24	3.37	21
442	THWKS	3	17	2.74	18
443	THWKS	3	6	2.40	12
444	THWKS	3	13	2.47	5
445	THWKS	3	13	2.22	11
446	THWKS	3	19	2.58	13
447	THWKS	3	16	2.89	16
448	THWKS	3	23	2.60	20
449	THWKS	3	24	2.26	17

OBS	AGE	ANIMAL	NUMINT	LEINT	CAPEND
450	THWKS	3	16	2.56	25
451	ADULT	1	17	2.22	18
452	ADULT	1	20	2.82	25
453	ADULT	1	7	2.27	12
454	ADULT	1	14	2.57	28
455	ADULT	1	15	2.25	20
456	ADULT	1	12	2.13	11
457	ADULT	1	21	2.33	18
458	ADULT	1	10	2.22	12
459	ADULT	1	18	2.11	13
460	ADULT	1	10	2.19	16
461	ADULT	1	13	2.22	13
462	ADULT	1	14	2.11	34
463	ADULT	1	19	2.11	12
464	ADULT	1	20	2.02	19
465	ADULT	1	21	2.42	21
466	ADULT	1	17	2.78	24
467	ADULT	1	21	2.96	28
468	ADULT	1	14	2.81	20
469	ADULT	1	9	3.18	27
470	ADULT	1	5	3.00	23
471	ADULT	1	15	2.41	28
472	ADULT	1	13	2.63	26
473	ADULT	1	13	2.58	26
474	ADULT	1	9	2.52	33
475	ADULT	1	13	2.73	33
476	ADULT	1	10	2.87	20
477	ADULT	1	10	2.83	26
478	ADULT	1	9	2.54	44
479	ADULT	1	15	2.49	30
480	ADULT	1	10	2.40	15
481	ADULT	2	23	2.21	22
482	ADULT	2	15	2.43	29
483	ADULT	2	32	2.25	28
484	ADULT	2	15	2.45	16
485	ADULT	2	16	2.01	7
486	ADULT	2	29	2.52	32
487	ADULT	2	29	2.07	31
488	ADULT	2	20	2.07	33
489	ADULT	2	20	2.30	23
490	ADULT	2	10	2.25	19
491	ADULT	2	14	2.38	19
492	ADULT	2	27	2.23	20
493	ADULT	2	16	2.28	21
494	ADULT	2	18	2.66	22
495	ADULT	2	13	2.52	27
496	ADULT	2	18	2.57	20
497	ADULT	2	12	2.80	18
498	ADULT	2	12	2.26	14
499	ADULT	2	14	2.48	17

OBS	AGE	ANIMAL	NUMINT	LEINT	CAPEND
500	ADULT	2	24	2.55	18
501	ADULT	2	14	2.64	19
502	ADULT	2	7	2.27	5
503	ADULT	2	12	2.94	19
504	ADULT	2	13	3.26	15
505	ADULT	2	9	2.53	11
506	ADULT	2	21	2.53	14
507	ADULT	2	11	2.37	13
508	ADULT	2	19	2.44	14
509	ADULT	2	9	2.47	7
510	ADULT	2	18	2.51	24
511	ADULT	3	5	2.32	12
512	ADULT	3	8	3.01	15
513	ADULT	3	15	2.44	20
514	ADULT	3	13	2.43	24
515	ADULT	3	11	2.43	24
516	ADULT	3	10	2.33	22
517	ADULT	3	10	2.28	29
518	ADULT	3	13	2.21	22
519	ADULT	3	8	2.20	19
520	ADULT	3	20	2.45	35
521	ADULT	3	25	2.32	33
522	ADULT	3	10	2.30	33
523	ADULT	3	4	2.38	17
524	ADULT	3	14	2.38	20
525	ADULT	3	10	2.53	21
526	ADULT	3	15	2.65	29
527	ADULT	3	10	2.32	19
528	ADULT	3	13	2.40	19
529	ADULT	3	14	2.32	30
530	ADULT	3	6	2.47	24
531	ADULT	3	11	2.20	25
532	ADULT	3	17	2.24	29
533	ADULT	3	10	2.39	23
534	ADULT	3	8	2.35	17
535	ADULT	3	15	2.49	19
536	ADULT	3	16	2.01	21
537	ADULT	3	13	2.19	21
538	ADULT	3	13	2.45	30
539	ADULT	3	15	2.72	34
540	ADULT	3	14	2.24	32

APPENDIX J

Data collected on body weight (BODYWT), lung weight (LUNGWT), and lung volume (LUNGVOL).

OBS	AGE	ANIMAL	BODYWT	LUNGWT	LUNGVOL
1	PRENL	1	82.83	2.667	2.8
2	PRENL	2	76.67	1.911	1.9
3	PRENL	3	83.81	2.733	2.2
4	ONEDY	1	95.00	2.967	4.0
5	ONEDY	2	90.59	2.200	2.3
6	ONEDY	3	82.27	2.667	2.2
7	ONEWK	1	223.35	4.100	6.8
8	ONEWK	2	145.90	2.500	4.0
9	ONEWK	3	148.00	3.300	3.5
10	TWWKS	1	280.50	6.300	6.0
11	TWWKS	2	271.83	3.000	5.5
12	TWWKS	3	242.22	4.350	7.0
13	THWKS	1	372.31	6.067	8.5
14	THWKS	2	318.92	5.750	9.0
15	THWKS	3	406.74	6.850	9.0
16	ADULT	1	4082.00	35.600	50.0
17	ADULT	2	2500.00	25.000	50.0
18	ADULT	3	3370.00	35.000	51.0

APPENDIX K

Program used to obtain means, standard deviation and sum of cilia and thickness of epithelium and submucosa over and between cartilages.

```
********** MEANS OF THE TRACHEA **********;

DATA TRACHEA;

INPUT AGE ANIMAL CILIA BODYWT LUNGWT LUNGVOL EPITHICK SUBOC SUBBC;

CARDS;

1 1 5.673 82.333 2.667 2.8 23.17 33.749 55.255
1 2 5.268 76.667 1.911 1.9 22.98 43.507 151.322
1 3 4.343 83.811 2.733 2.2 24.98 27.282 136.979
2 1 5.533 95.000 2.967 4.0 28.40 36.816 126.642
2 2 4.938 90.587 2.200 2.3 27.73 71.156 135.149
2 3 5.133 82.267 2.667 2.2 29.06 28.839 84.176
3 1 6.028 223.350 4.100 6.8 23.24 50.706 99.883
3 2 4.699 145.900 2.500 4.0 33.66 50.577 165.162
3 3 4.945 148.000 3.300 3.5 30.58 53.574 96.128
4 1 5.270 280.500 6.300 6.0 28.22 62.914 244.630
4 2 5.666 271.830 3.000 5.5 32.22 59.523 189.140
4 3 5.044 242.220 4.350 7.0 30.44 68.344 168.931
5 1 6.006 372.307 6.067 8.5 31.03 82.636 253.379
5 2 5.943 318.920 5.750 9.0 30.89 68.154 246.238
5 3 4.945 406.740 6.850 9.0 34.82 50.835 229.302
6 1 5.876 4082.00 35.60 50.0 41.63 73.136 257.253
6 2 6.274 2500.00 25.00 50.0 39.90 69.931 285.475
6 3 5.321 3370.00 35.00 51.0 38.78 65.646 331.160
;

PROC PRINT;

PROC MEANS MEAN STD SUM;
BY AGE;
**********;
```

APPENDIX L

Data collected on length of cilia (CILIA), thickness of epithelium (EPITHICK), submucosa over cartilage (SUBOC) and submucosa between cartilage (SUBBC) at light microscopic level.

OBS	AGE	ANIMAL	CILIA	EPITHICK	SUBOC	SUBBC
1	PRENL	1	5.673	23.17	33.749	55.255
2	PRENL	2	5.268	22.98	43.507	151.322
3	PRENL	3	4.343	24.98	27.282	136.979
4	ONEDY	1	5.533	28.40	36.816	126.642
5	ONEDY	2	4.938	27.73	71.156	135.149
6	ONEDY	3	5.133	29.06	28.839	84.176
7	ONEWK	1	6.028	23.24	50.706	99.883
8	ONEWK	2	4.699	33.66	50.577	165.162
9	ONEWK	3	4.945	30.58	53.574	96.128
10	TWWKS	1	5.270	28.22	62.914	244.630
11	TWWKS	2	5.666	32.22	59.523	189.140
12	TWWKS	3	5.044	30.44	68.344	168.931
13	THWKS	1	6.006	31.03	82.636	253.379
14	THWKS	2	5.943	30.89	68.154	246.238
15	THWKS	3	4.945	34.82	50.835	229.302
16	ADULT	1	5.876	41.63	73.136	257.253
17	ADULT	2	6.274	39.90	69.931	285.475
18	ADULT	3	5.321	38.78	65.646	331.160

```
     4 ***************************  MEANS   ********************;
     5 DATA MEAN;
     6
     7 INFILE NEW;
     8
     9 INPUT AGE ANIMAL NPNTI NPCI NUMTI
    10                   NPNTII NUMTII NPCII TOT;
    11
NOTE: INFILE NEW IS FILE SYS$USER2:[HENRY]NEW.DAT.
NOTE: 180 LINES WERE READ FROM INFILE NEW.
      THE MINIMUM LINE LENGTH IS 18.
      THE MAXIMUM LINE LENGTH IS 18.
NOTE: THE DATA SET WORK.MEAN HAS 180 OBSERVATIONS AND 9 VARIABLES.
NOTE: THE DATA STEP USED 00:00:00.61 CPU SECONDS, 291 PAGEFAULTS.
    12 PROC PRINT;
    13 VAR AGE ANIMAL NPNTI NPCI NUMTI;
    14
NOTE: THE PROCEDURE PRINT USED 00:00:00.44 CPU SECONDS, 108 PAGEFAULTS.
NOTE: THE PROCEDURE PRINTED PAGES 1 THROUGH 4.
    15 PROC PRINT;
    16 VAR AGE ANIMAL NPNTII NUMTII NPCII TOT;
    17
NOTE: THE PROCEDURE PRINT USED 00:00:00.43 CPU SECONDS, 3 PAGEFAULTS.
NOTE: THE PROCEDURE PRINTED PAGES 5 THROUGH 8.
    18 PROC SORT;
    19 BY  AGE ANIMAL;
    20
NOTE: THE DATA SET WORK.MEAN HAS 180 OBSERVATIONS AND 9 VARIABLES.
NOTE: THE PROCEDURE SORT USED 00:00:00.45 CPU SECONDS, 89 PAGEFAULTS.
    21 PROC MEANS SUM;
    22 BY AGE ANIMAL;
NOTE: THE PROCEDURE MEANS USED 00:00:00.36 CPU SECONDS, 18 PAGEFAULTS.
NOTE: THE PROCEDURE PRINTED PAGES 9 THROUGH 12.
NOTE: SAS INSTITUTE INC., SAS CIRCLE, BOX 8000, CARY, N. C., 27512-8000.
```

VARIABLE	SUM
-- AGE=1	ANIMAL=1 ---
NPNTI	13.00000000
NPCI	24.00000000
NUMTI	15.00000000
NPNTII	23.00000000
NUMTII	36.00000000
NPCII	45.00000000
TOT	495.00000000
-- AGE=1	ANIMAL=2 ---
NPNTI	9.00000000
NPCI	16.00000000
NUMTI	11.00000000
NPNTII	22.00000000
NUMTII	28.00000000
NPCII	49.00000000
TOT	491.00000000
-- AGE=1	ANIMAL=3 ---
NPNTI	9.00000000
NPCI	36.00000000
NUMTI	16.00000000
NPNTII	40.00000000
NUMTII	50.00000000
NPCII	75.00000000
TOT	639.00000000
-- AGE=2	ANIMAL=1 ---
NPNTI	4.00000000
NPCI	40.00000000
NUMTI	8.00000000
NPNTII	21.00000000
NUMTII	32.00000000
NPCII	53.00000000
TOT	505.00000000
-- AGE=2	ANIMAL=2 ---
NPNTI	7.00000000
NPCI	40.00000000
NUMTI	10.00000000
NPNTII	13.00000000
NUMTII	28.00000000
NPCII	58.00000000
TOT	399.00000000

VARIABLE	SUM
-- AGE=2	ANIMAL=3 ---
NPNTI	5.00000000
NPCI	45.00000000
NUMTI	8.00000000
NPNTII	26.00000000
NUMTII	35.00000000
NPCII	59.00000000
TOT	477.00000000
-- AGE=3	ANIMAL=1 ---
NPNTI	7.00000000
NPCI	41.00000000
NUMTI	6.00000000
NPNTII	21.00000000
NUMTII	25.00000000
NPCII	53.00000000
TOT	495.00000000
-- AGE=3	ANIMAL=2 ---
NPNTI	5.00000000
NPCI	34.00000000
NUMTI	5.00000000
NPNTII	21.00000000
NUMTII	28.00000000
NPCII	69.00000000
TOT	545.00000000
-- AGE=3	ANIMAL=3 ---
NPNTI	8.00000000
NPCI	31.00000000
NUMTI	10.00000000
NPNTII	16.00000000
NUMTII	17.00000000
NPCII	23.00000000
TOT	481.00000000
-- AGE=4	ANIMAL=1 ---
NPNTI	1.00000000
NPCI	41.00000000
NUMTI	4.00000000
NPNTII	18.00000000
NUMTII	27.00000000
NPCII	39.00000000
TOT	327.00000000

VARIABLE	SUM
-- AGE=4	ANIMAL=2 ---
NPNTI	6.00000000
NPCI	33.00000000
NUMTI	9.00000000
NPNTII	7.00000000
NUMTII	18.00000000
NPCII	33.00000000
TOT	316.00000000
-- AGE=4	ANIMAL=3 ---
NPNTI	5.00000000
NPCI	31.00000000
NUMTI	7.00000000
NPNTII	11.00000000
NUMTII	26.00000000
NPCII	23.00000000
TOT	351.00000000
-- AGE=5	ANIMAL=1 ---
NPNTI	9.00000000
NPCI	44.00000000
NUMTI	11.00000000
NPNTII	8.00000000
NUMTII	16.00000000
NPCII	20.00000000
TOT	336.00000000
-- AGE=5	ANIMAL=2 ---
NPNTI	9.00000000
NPCI	51.00000000
NUMTI	11.00000000
NPNTII	8.00000000
NUMTII	11.00000000
NPCII	11.00000000
TOT	377.00000000
-- AGE=5	ANIMAL=3 ---
NPNTI	4.00000000
NPCI	54.00000000
NUMTI	6.00000000
NPNTII	8.00000000
NUMTII	11.00000000
NPCII	18.00000000
TOT	372.00000000

VARIABLE	SUM
-- AGE=6	ANIMAL=1 ---
NPNTI	5.00000000
NPCI	54.00000000
NUMTI	7.00000000
NPNTII	7.00000000
NUMTII	13.00000000
NPCII	26.00000000
TOT	482.00000000
-- AGE=6	ANIMAL=2 ---
NPNTI	7.00000000
NPCI	54.00000000
NUMTI	7.00000000
NPNTII	6.00000000
NUMTII	11.00000000
NPCII	15.00000000
TOT	324.00000000
-- AGE=6	ANIMAL=3 ---
NPNTI	6.00000000
NPCI	76.00000000
NUMTI	8.00000000
NPNTII	3.00000000
NUMTII	8.00000000
NPCII	16.00000000
TOT	346.00000000

```
*************************************************************************;
***********************  ROW DATA PRINT OUT  ***********************;

DATA PRINT;

INFILE LSUM;

INPUT  AGE  ANIMAL  POTAVW   POTAVS  POTTRBC;


VFAVW = POTAVW/3000;
VFAVS = POTAVS/3000;
VFTRBC = POTTRBC/3000;


PROC PRINT;
VAR AGE ANIMAL VFAVW VFAVS VFTRBC;

PROC SORT;
BY AGE;

PROC MEANS N MEAN STD STDERR;
VAR VFAVW VFAVS VFTRBC;
BY AGE;
```

OBS	AGE	ANIMAL	VFAVW	VFAVS	VFTRBC
1	1	1	0.386000	0.277667	0.332333
2	1	2	0.437667	0.276333	0.284000
3	1	3	0.417667	0.296333	0.295000
4	2	1	0.232000	0.525000	0.242667
5	2	2	0.272333	0.509333	0.218667
6	2	3	0.246000	0.532667	0.221333
7	3	1	0.234667	0.571333	0.192333
8	3	2	0.253667	0.522333	0.213000
9	3	3	0.244333	0.546000	0.207667
10	4	1	0.244000	0.570000	0.184000
11	4	2	0.246000	0.602000	0.152000
12	4	3	0.242000	0.593667	0.165000
13	5	1	0.220000	0.603667	0.176333
14	5	2	0.224333	0.597333	0.178333
15	5	3	0.193000	0.619333	0.187333
16	6	1	0.132667	0.717000	0.160333
17	6	2	0.211667	0.606000	0.186000
18	6	3	0.214000	0.603000	0.194667

APPENDIX M

Program used to obtain arithmetic mean, harmonic mean, harmonic mean thickness, and surface density.

```
*************************** SURFACE AREA ***********************************;

DATA SURFACE;

INFILE EM;

INPUT AGE ANIMAL BLOCK TYPEI TYPEII NUMINT LEINT CONTIS CAPEND;

TOTAL = TYPEI+TYPEII+CONTIS+CAPEND;

D = 18*1000/5250;

ARTHMEAN = LEINT/2*1000/5250;
SURFDEN = 4*NUMINT/D*TOTAL;
HMEAN = LEINT*1000/5250;
HTHICK = 2/3*HMEAN;

*************************************************************************;
```

APPENDIX N

Data collected on arithmetic mean (ARTHMEAN), harmonic mean (HMEAN), harmonic mean thickness (HTHICK), and surface density (SURFDEN).

OBS	AGE	ANIMAL	ARTHMEAN	HMEAN	HTHICK	SURFDEN
1	PRENL	1	0.36825	0.73651	0.49101	353.617
2	PRENL	2	0.45346	0.90692	0.60461	405.572
3	PRENL	3	0.40292	0.80584	0.53723	421.944
4	ONEDY	1	0.33289	0.66578	0.44385	365.050
5	ONEDY	2	0.38698	0.77397	0.51598	600.522
6	ONEDY	3	0.35206	0.70413	0.46942	315.856
7	ONEWK	1	0.27556	0.55111	0.36741	255.500
8	ONEWK	2	0.32479	0.64959	0.43306	603.283
9	ONEWK	3	0.34286	0.68571	0.45714	480.472
10	TWWKS	1	0.28686	0.57371	0.38248	368.511
11	TWWKS	2	0.25959	0.51917	0.34612	589.750
12	TWWKS	3	0.20876	0.41752	0.27835	473.161
13	THWKS	1	0.24000	0.48000	0.32000	324.256
14	THWKS	2	0.23292	0.46584	0.31056	556.500
15	THWKS	3	0.26003	0.52006	0.34671	592.161
16	ADULT	1	0.23721	0.47441	0.31628	513.994
17	ADULT	2	0.23254	0.46508	0.31005	757.867
18	ADULT	3	0.22683	0.45365	0.30243	669.278

APPENDIX O

Program used to obtain volume fraction and number of type I and type II pneumocytes per unit volume.

```
**************   VOLUME FRACTIONS     **************************;

DATA VOLUME;

INFILE NEW;

INPUT  AGE NPNTI NPCI NUMTI NPNTII NUMTII NPCTII TOT;

VFNI = NPNTI/100;
VFCI = NPCI/100;
VFNII = NPNTII/100;
VFCII = NPCTII/100;

D = 18**2/5250**2;
AREA = 100*D;

NATI = NUMTI/AREA;
NATII = NUMTII/AREA;

FRATI = NATI**3/VFNI;
FRATII = NATII**3/VFNII;

TYI  = SQRT(FRATI);
TYII = SQRT(FRATII);

NVTI = 1.07/1.382*TYI;
NVTII = 1.07/1.382*TYII;

PROC SORT;
BY AGE;

PROC PRINT;
 VAR AGE NPNTI NPCI NUMTI NPNTII NUMTII NPCTII TOT;

PROC PRINT;
VAR AGE VFNI VFCI VFNII VFCII;

PROC MEANS  MEAN STD SUM N MAXDEC=5;
VAR VFNI VFCI VFNII VFCII;
BY AGE;

PROC PRINT;
VAR AGE D AREA NATI NATII FRATI FRATII;

PROC MEANS MEAN STD  N MAXDEC=5;
VAR D AREA NATI NATII FRATI FRATII;
BY AGE;

PROC MEANS N MEAN STD SUM MAXDEC=5;
VAR VFNI VFCI VFNII VFCII NVTI NVTII;
BY AGE;
```

OBS	AGE	ANIMAL	NPNTI	NPCI	NUMTI	NPNTII	NUMTII	NPCTII	TOT
1	1	1	13	24	15	23	36	45	495
2	1	2	9	16	11	22	28	49	491
3	1	3	9	36	16	40	50	75	639
4	2	1	4	40	8	21	32	53	505
5	2	2	7	40	10	13	28	58	399
6	2	3	5	45	8	26	35	59	477
7	3	1	7	41	6	21	25	53	495
8	3	2	5	34	5	21	28	69	545
9	3	3	8	31	10	16	17	23	481
10	4	1	1	41	4	18	27	39	327
11	4	2	6	33	9	7	18	33	316
12	4	3	5	31	7	11	26	23	351
13	5	1	9	44	11	8	16	20	336
14	5	2	9	51	11	8	11	11	377
15	5	3	4	54	6	8	11	18	372
16	6	1	5	54	7	7	13	26	482
17	6	2	7	54	7	6	11	15	324
18	6	3	6	76	8	3	8	16	346

OBS	AGE	ANIMAL	D	AREA	NATI	NATII	FRATI	FRATII
1	1	1	0.0000117551	0.0117551	1276.04	3062.50	159827392665	1.24882E+12
2	1	2	0.0000117551	0.0117551	935.76	2381.94	91045049392	614288242548
3	1	3	0.0000117551	0.0117551	1361.11	4253.47	280180707971	1.92385E+12
4	2	1	0.0000117551	0.0117551	680.56	2722.22	78800824117	960619570187
5	2	2	0.0000117551	0.0117551	850.69	2381.94	87947348345	1.03956E+12
6	2	3	0.0000117551	0.0117551	680.56	2977.43	63040659294	1.01520E+12
7	3	1	0.0000117551	0.0117551	510.42	2126.74	18996627242	458059105963
8	3	2	0.0000117551	0.0117551	425.35	2381.94	15390785960	643540063622
9	3	3	0.0000117551	0.0117551	850.69	1446.18	76953929802	189037328558
10	4	1	0.0000117551	0.0117551	340.28	2296.88	39400412058	673192977905
11	4	2	0.0000117551	0.0117551	765.63	1531.25	74799219767	512908935547
12	4	3	0.0000117551	0.0117551	595.49	2211.81	42232316675	983667105596
13	5	1	0.0000117551	0.0117551	935.76	1361.11	91045049392	315203296468
14	5	2	0.0000117551	0.0117551	935.76	935.76	91045049392	102425680566
15	5	3	0.0000117551	0.0117551	510.42	935.76	33244097674	102425680566
16	6	1	0.0000117551	0.0117551	595.49	1105.90	42232316675	193220324314
17	6	2	0.0000117551	0.0117551	595.49	935.76	30165940482	136567574088
18	6	3	0.0000117551	0.0117551	680.56	680.56	52533882745	105067765489

```
**************   VOLUME FRACTIONS      **************************;

DATA VOLUME;

INFILE AVG;

INPUT  AGE ANIMAL NPNTI NPCI NUMTI NPNTII NUMTII NPCTII TOT;

VFNI = NPNTI/1000;
VFCI = NPCI/1000;
VFNII = NPNTII/1000;
VFCII = NPCTII/1000;

D = 18**2/5250**2;
AREA = 1000*D;

NATI = NUMTI/AREA;
NATII = NUMTII/AREA;

FRATI = NATI**3/VFNI;
FRATII = NATII**3/VFNII;

TYI  = SQRT(FRATI);
TYII = SQRT(FRATII);

NVTI = 1.07/1.382*TYI;
NVTII = 1.07/1.382*TYII;

PROC SORT;
BY AGE;

PROC PRINT;
 VAR AGE ANIMAL NPNTI NPCI NUMTI NPNTII NUMTII NPCTII TOT;

PROC PRINT;
VAR AGE ANIMAL VFNI VFCI VFNII VFCII;

PROC MEANS  MEAN STD STDERR SUM N MAXDEC=5;
VAR VFNI VFCI VFNII VFCII;
BY AGE;

PROC PRINT;
VAR AGE ANIMAL D AREA NATI NATII FRATI FRATII;

PROC MEANS MEAN STD  N MAXDEC=5;
VAR D AREA NATI NATII FRATI FRATII;
BY AGE;

PROC MEANS N MEAN STD STDERR SUM MAXDEC=5;
VAR VFNI VFCI VFNII VFCII NVTI NVTII;
BY AGE;

PROC SORT;
BY AGE ANIMAL;

PROC MEANS N MEAN MAXDEC=5;
VAR VFNI VFCI VFNII VFCII NVTI NVTII;
BY AGE ANIMAL;
```

OBS	AGE	ANIMAL	NPNTI	NPCI	NUMTI
1	1	1	2	5	2
2	1	1	0	7	0
3	1	1	1	0	1
4	1	1	2	1	2
5	1	1	1	2	1
6	1	1	0	0	1
7	1	1	0	0	0
8	1	1	1	2	1
9	1	1	2	1	2
10	1	1	4	6	5
11	1	2	0	2	0
12	1	2	1	2	1
13	1	2	1	1	2
14	1	2	2	0	2
15	1	2	1	2	1
16	1	2	1	1	1
17	1	2	1	3	1
18	1	2	1	1	1
19	1	2	0	2	0
20	1	2	1	2	2
21	1	3	2	5	2
22	1	3	0	4	1
23	1	3	1	2	1
24	1	3	0	2	2
25	1	3	2	5	2
26	1	3	0	5	1
27	1	3	1	1	1
28	1	3	0	5	2
29	1	3	1	2	1
30	1	3	2	5	3
31	2	1	0	3	1
32	2	1	0	4	0
33	2	1	0	4	0
34	2	1	1	3	2
35	2	1	1	4	1
36	2	1	0	5	1
37	2	1	0	4	1
38	2	1	1	4	1
39	2	1	1	3	1
40	2	1	0	4	0
41	2	1	0	2	0
42	2	2	0	4	0
43	2	2	1	6	1
44	2	2	2	3	3
45	2	2	1	5	3
46	2	2	0	4	0
47	2	2	0	5	0
48	2	2	0	2	0
49	2	2	0	4	1
50	2	2	0	2	1
51	2	2	3	5	1
52	2	3	2	5	3
53	2	3	0	4	1
54	2	3	0	5	0
55	2	3	0	9	0

OBS	AGE	ANIMAL	NPNTI	NPCI	NUMTI
56	2	3	0	7	1
57	2	3	0	7	0
58	2	3	3	2	2
59	2	3	0	4	0
60	2	3	0	2	1
61	3	1	0	2	1
62	3	1	0	9	0
63	3	1	0	3	0
64	3	1	1	6	0
65	3	1	1	1	1
66	3	1	0	4	0
67	3	1	2	4	1
68	3	1	0	3	0
69	3	1	1	6	1
70	3	1	2	3	2
71	3	2	0	7	0
72	3	2	1	2	1
73	3	2	0	5	1
74	3	2	2	4	1
75	3	2	0	2	0
76	3	2	0	3	0
77	3	2	0	2	0
78	3	2	1	3	1
79	3	2	0	0	0
80	3	2	1	6	1
81	3	3	1	2	1
82	3	3	0	3	0
83	3	3	3	2	2
84	3	3	0	1	0
85	3	3	0	5	0
86	3	3	0	6	0
87	3	3	1	5	2
88	3	3	1	3	2
89	3	3	2	3	3
90	3	3	0	1	0
91	4	1	0	4	0
92	4	1	0	6	1
93	4	1	0	4	0
94	4	1	0	3	1
95	4	1	1	2	1
96	4	1	0	4	0
97	4	1	0	6	1
98	4	1	0	5	0
99	4	1	0	4	0
100	4	1	0	3	0
101	4	2	0	2	0
102	4	2	0	3	0
103	4	2	2	4	2
104	4	2	1	3	1
105	4	2	0	4	0
106	4	2	0	2	1
107	4	2	1	4	2
108	4	2	0	2	0
109	4	2	1	4	2
110	4	2	1	5	1

OBS	AGE	ANIMAL	NPNTI	NPCI	NUMTI
111	4	3	0	5	0
112	4	3	0	1	0
113	4	3	0	5	1
114	4	3	0	3	0
115	4	3	1	2	1
116	4	3	0	4	0
117	4	3	1	2	1
118	4	3	1	4	2
119	4	3	0	3	0
120	4	3	2	2	2
121	5	1	0	6	0
122	5	1	1	0	2
123	5	1	0	3	0
124	5	1	3	6	2
125	5	1	1	4	1
126	5	1	2	4	3
127	5	1	0	5	1
128	5	1	0	5	0
129	5	1	1	5	1
130	5	1	1	6	1
131	5	2	0	4	0
132	5	2	1	5	1
133	5	2	2	5	2
134	5	2	0	2	1
135	5	2	1	5	1
136	5	2	2	6	2
137	5	2	0	5	1
138	5	2	1	5	1
139	5	2	2	5	2
140	5	2	0	9	0
141	5	3	0	6	1
142	5	3	0	5	0
143	5	3	1	5	1
144	5	3	0	7	0
145	5	3	0	6	1
146	5	3	1	3	1
147	5	3	0	6	0
148	5	3	0	7	1
149	5	3	2	5	1
150	5	3	0	4	0
151	6	1	1	3	1
152	6	1	0	8	0
153	6	1	0	4	0
154	6	1	0	5	1
155	6	1	1	3	1
156	6	1	1	4	1
157	6	1	0	5	1
158	6	1	1	8	1
159	6	1	0	9	0
160	6	1	1	5	1
161	6	2	1	8	1
162	6	2	0	4	1
163	6	2	0	3	0
164	6	2	0	6	0
165	6	2	1	2	1

OBS	AGE	ANIMAL	NPNTI	NPCI	NUMTI
166	6	2	1	5	1
167	6	2	2	7	1
168	6	2	2	7	2
169	6	2	0	4	0
170	6	2	0	8	0
171	6	3	1	6	1
172	6	3	1	9	1
173	6	3	0	8	1
174	6	3	1	6	1
175	6	3	1	8	1
176	6	3	1	9	1
177	6	3	1	5	2
178	6	3	0	8	0
179	6	3	0	9	0
180	6	3	0	8	0

OBS	AGE	ANIMAL	VFNI	VFCI	VFNII	VFCII
1	1	1	0.013	0.024	0.023	0.045
2	1	2	0.009	0.016	0.022	0.049
3	1	3	0.009	0.036	0.040	0.075
4	2	1	0.004	0.040	0.021	0.053
5	2	2	0.007	0.040	0.013	0.058
6	2	3	0.005	0.045	0.026	0.059
7	3	1	0.007	0.041	0.021	0.053
8	3	2	0.005	0.034	0.021	0.069
9	3	3	0.008	0.031	0.016	0.023
10	4	1	0.001	0.041	0.018	0.039
11	4	2	0.006	0.033	0.007	0.033
12	4	3	0.005	0.031	0.011	0.023
13	5	1	0.009	0.044	0.008	0.020
14	5	2	0.009	0.051	0.008	0.011
15	5	3	0.004	0.054	0.008	0.018
16	6	1	0.005	0.054	0.007	0.026
17	6	2	0.007	0.054	0.006	0.015
18	6	3	0.006	0.076	0.003	0.016

OBS	AGE	ANIMAL	VFCAP	NVTI	NVTII
1	1	1	0.11233	309528	865218
2	1	2	0.11000	233616	606822
3	1	3	0.13600	409821	1073893
4	2	1	0.08700	217340	758842
5	2	2	0.16800	229608	789407
6	2	3	0.14567	194395	780102
7	3	1	0.06367	106712	524006
8	3	2	0.13567	96051	621102
9	3	3	0.11800	214778	336627
10	4	1	0.07933	153683	635251
11	4	2	0.07433	211750	554492
12	4	3	0.03833	159110	767891
13	5	1	0.08400	233616	434681
14	5	2	0.13233	233616	247787
15	5	3	0.13367	141166	247787
16	6	1	0.22500	159110	340331
17	6	2	0.19233	134472	286120
18	6	3	0.23933	177457	250963

OBS	AGE	ANIMAL	VFTI	VFTI	VFCON
1	1	1	0.02033	0.02033	0.28300
2	1	2	0.02767	0.02767	0.36900
3	1	3	0.03533	0.03533	0.38767
4	2	1	0.01667	0.01667	0.30267
5	2	2	0.00900	0.00900	0.30533
6	2	3	0.01100	0.01100	0.26333
7	3	1	0.01200	0.01200	0.23467
8	3	2	0.01233	0.01233	0.31067
9	3	3	0.01300	0.01300	0.23933
10	4	1	0.00800	0.00800	0.22300
11	4	2	0.00667	0.00667	0.23400
12	4	3	0.00567	0.00567	0.23967
13	5	1	0.00600	0.00600	0.17133
14	5	2	0.00467	0.00467	0.22500
15	5	3	0.00733	0.00733	0.20900
16	6	1	0.00500	0.00500	0.08433
17	6	2	0.00467	0.00467	0.15033
18	6	3	0.00367	0.00367	0.19067

OBS	AGE	ANIMAL	NPNTII	NUMTII	NPCII	TOT
1	1	1	3	4	4	58
2	1	1	4	5	7	37
3	1	1	1	0	0	51
4	1	1	5	4	5	51
5	1	1	0	4	9	68
6	1	1	5	9	6	51
7	1	1	3	3	2	37
8	1	1	1	1	0	44
9	1	1	1	4	8	51
10	1	1	0	2	4	47
11	1	2	4	3	0	43
12	1	2	3	3	7	52
13	1	2	4	6	5	50
14	1	2	1	1	5	70
15	1	2	2	2	2	32
16	1	2	2	4	7	64
17	1	2	2	2	5	49
18	1	2	0	0	0	30
19	1	2	3	4	9	48
20	1	2	1	3	9	53
21	1	3	5	3	4	75
22	1	3	2	4	7	56
23	1	3	5	8	9	75
24	1	3	3	6	9	71
25	1	3	3	4	5	58
26	1	3	5	6	9	84
27	1	3	4	3	9	55
28	1	3	4	5	7	50
29	1	3	4	7	9	55
30	1	3	5	4	7	60
31	2	1	3	5	8	40
32	2	1	2	4	5	39
33	2	1	2	2	3	40
34	2	1	2	2	3	42
35	2	1	0	0	2	62
36	2	1	1	1	2	26
37	2	1	3	4	9	72
38	2	1	3	6	9	68
39	2	1	1	2	5	60
40	2	1	1	2	3	30
41	2	1	3	4	4	26
42	2	2	2	2	2	62
43	2	2	0	1	4	53
44	2	2	1	1	2	28
45	2	2	3	3	7	41
46	2	2	4	4	9	42
47	2	2	1	4	8	29
48	2	2	2	6	5	30
49	2	2	0	2	7	26
50	2	2	0	1	5	12
51	2	2	0	4	9	76
52	2	3	2	1	1	31
53	2	3	3	3	9	35
54	2	3	5	6	9	60
55	2	3	4	5	9	61

OBS	AGE	ANIMAL	NPNTII	NUMTII	NPCII	TOT
56	2	3	1	5	8	55
57	2	3	4	4	7	50
58	2	3	1	1	3	54
59	2	3	2	5	7	77
60	2	3	4	5	6	54
61	3	1	2	2	3	37
62	3	1	0	0	0	40
63	3	1	1	2	5	62
64	3	1	0	1	1	46
65	3	1	3	7	8	30
66	3	1	5	4	9	64
67	3	1	4	3	9	69
68	3	1	3	3	9	34
69	3	1	1	1	2	47
70	3	1	2	2	7	66
71	3	2	1	3	9	67
72	3	2	1	2	5	62
73	3	2	1	1	6	52
74	3	2	2	2	9	52
75	3	2	5	3	9	72
76	3	2	3	5	8	69
77	3	2	3	5	8	48
78	3	2	1	1	5	46
79	3	2	2	3	5	40
80	3	2	2	3	5	37
81	3	3	0	1	1	41
82	3	3	1	2	4	60
83	3	3	0	0	0	45
84	3	3	4	3	5	45
85	3	3	3	2	2	41
86	3	3	2	1	1	55
87	3	3	3	5	4	61
88	3	3	0	0	0	49
89	3	3	0	0	0	49
90	3	3	3	3	6	35
91	4	1	1	2	5	29
92	4	1	1	1	0	23
93	4	1	1	3	5	31
94	4	1	2	5	7	36
95	4	1	1	1	1	28
96	4	1	2	2	3	27
97	4	1	1	1	1	39
98	4	1	4	5	4	31
99	4	1	1	3	5	36
100	4	1	4	4	8	47
101	4	2	0	3	6	21
102	4	2	0	1	4	22
103	4	2	0	0	0	26
104	4	2	0	2	3	28
105	4	2	2	3	6	33
106	4	2	2	2	2	34
107	4	2	2	2	2	37
108	4	2	0	3	7	34
109	4	2	0	1	2	51
110	4	2	1	1	1	30

OBS	AGE	ANIMAL	NPNTII	NUMTII	NPCII	TOT
111	4	3	0	2	4	21
112	4	3	0	3	2	43
113	4	3	0	1	1	26
114	4	3	2	2	1	35
115	4	3	0	1	1	42
116	4	3	1	5	3	27
117	4	3	0	2	2	38
118	4	3	5	4	3	40
119	4	3	3	4	3	43
120	4	3	0	2	3	36
121	5	1	0	1	2	25
122	5	1	0	0	0	21
123	5	1	0	3	3	22
124	5	1	0	0	0	35
125	5	1	0	0	0	23
126	5	1	2	2	3	42
127	5	1	2	2	2	54
128	5	1	2	4	4	34
129	5	1	0	1	3	42
130	5	1	2	3	3	38
131	5	2	3	4	5	40
132	5	2	0	1	1	28
133	5	2	0	0	0	36
134	5	2	2	1	0	43
135	5	2	0	0	0	44
136	5	2	1	1	0	18
137	5	2	0	1	2	39
138	5	2	1	1	1	39
139	5	2	1	1	1	46
140	5	2	0	1	1	44
141	5	3	0	1	1	39
142	5	3	0	0	0	30
143	5	3	0	0	2	24
144	5	3	2	2	4	58
145	5	3	0	0	0	16
146	5	3	1	1	0	21
147	5	3	1	1	2	49
148	5	3	0	0	0	42
149	5	3	3	3	3	50
150	5	3	1	3	6	43
151	6	1	2	4	7	51
152	6	1	1	1	3	55
153	6	1	1	2	4	46
154	6	1	0	1	2	46
155	6	1	0	0	0	44
156	6	1	1	1	2	44
157	6	1	1	1	2	58
158	6	1	1	2	3	57
159	6	1	0	0	0	37
160	6	1	0	1	3	44
161	6	2	0	0	0	32
162	6	2	0	1	1	40
163	6	2	1	1	3	24
164	6	2	0	1	1	25
165	6	2	0	0	0	27

OBS	AGE	ANIMAL	NPNTII	NUMTII	NPCII	TOT
166	6	2	0	0	0	24
167	6	2	1	1	0	31
168	6	2	2	3	4	36
169	6	2	1	3	3	46
170	6	2	1	1	3	39
171	6	3	0	1	2	44
172	6	3	0	0	0	42
173	6	3	1	1	3	26
174	6	3	0	0	0	47
175	6	3	0	0	0	62
176	6	3	0	0	0	43
177	6	3	0	0	0	21
178	6	3	0	3	5	18
179	6	3	1	2	4	22
180	6	3	1	1	2	21

```
OPTIONS LS=80 PS=40;
*************************************************************************;
*************************************************************************;

********************   VOLUME FRACTIONS     ***************************;

DATA VOLUME;

INFILE SUM;

INPUT  AGE  ANIMAL  NUMTI   NUMTII   NPOTTI   NPOTTII
       NPOTCON  NPOTCAP  NPOTINT;


VFTI = NPOTTI/3000;
VFTII = NPOTTII/3000;
VFCON = NPOTCON/3000;
VFCAP = NPOTCAP/3000;


PROC PRINT;
VAR AGE ANIMAL NUMTI NUMTII NPOTTI NPOTTII NPOTCON NPOTCAP NPOTINT;

PROC PRINT ;
VAR AGE ANIMAL VFTI VFTII VFCON VFCAP;

PROC SORT;
BY AGE;

PROC MEANS N MEAN STD STDERR;
VAR VFTI VFTII VFCON VFCAP ;
BY AGE;
```

OBS	AGE	VFNI	VFCI	VFNII	VFCII
1	1	0.02	0.05	0.03	0.04
2	1	0.00	0.07	0.04	0.07
3	1	0.01	0.00	0.01	0.00
4	1	0.02	0.01	0.05	0.05
5	1	0.01	0.02	0.00	0.09
6	1	0.00	0.00	0.05	0.06
7	1	0.00	0.00	0.03	0.02
8	1	0.01	0.02	0.01	0.00
9	1	0.02	0.01	0.01	0.08
10	1	0.04	0.06	0.00	0.04
11	1	0.00	0.02	0.04	0.00
12	1	0.01	0.02	0.03	0.07
13	1	0.01	0.01	0.04	0.05
14	1	0.02	0.00	0.01	0.05
15	1	0.01	0.02	0.02	0.02
16	1	0.01	0.01	0.02	0.07
17	1	0.01	0.03	0.02	0.05
18	1	0.01	0.01	0.00	0.00
19	1	0.00	0.02	0.03	0.09
20	1	0.01	0.02	0.01	0.09
21	1	0.02	0.05	0.05	0.04
22	1	0.00	0.04	0.02	0.07
23	1	0.01	0.02	0.05	0.09
24	1	0.00	0.02	0.03	0.09
25	1	0.02	0.05	0.03	0.05
26	1	0.00	0.05	0.05	0.09
27	1	0.01	0.01	0.04	0.09
28	1	0.00	0.05	0.04	0.07
29	1	0.01	0.02	0.04	0.09
30	1	0.02	0.05	0.05	0.07
31	2	0.00	0.03	0.03	0.08
32	2	0.00	0.04	0.02	0.05
33	2	0.00	0.04	0.02	0.03
34	2	0.01	0.03	0.02	0.03
35	2	0.01	0.04	0.00	0.02
36	2	0.00	0.05	0.01	0.02
37	2	0.00	0.04	0.03	0.09
38	2	0.01	0.04	0.03	0.09
39	2	0.01	0.03	0.01	0.05
40	2	0.00	0.04	0.01	0.03
41	2	0.00	0.02	0.03	0.04
42	2	0.00	0.04	0.02	0.02
43	2	0.01	0.06	0.00	0.04
44	2	0.02	0.03	0.01	0.02
45	2	0.01	0.05	0.03	0.07
46	2	0.00	0.04	0.04	0.09
47	2	0.00	0.05	0.01	0.08
48	2	0.00	0.02	0.02	0.05
49	2	0.00	0.04	0.00	0.07
50	2	0.00	0.02	0.00	0.05
51	2	0.03	0.05	0.00	0.09
52	2	0.02	0.05	0.02	0.01
53	2	0.00	0.04	0.03	0.09
54	2	0.00	0.05	0.05	0.09
55	2	0.00	0.09	0.04	0.09

OBS	AGE	VFNI	VFCI	VFNII	VFCII
56	2	0.00	0.07	0.01	0.08
57	2	0.00	0.07	0.04	0.07
58	2	0.03	0.02	0.01	0.03
59	2	0.00	0.04	0.02	0.07
60	2	0.00	0.02	0.04	0.06
61	3	0.00	0.02	0.02	0.03
62	3	0.00	0.09	0.00	0.00
63	3	0.00	0.03	0.01	0.05
64	3	0.01	0.06	0.00	0.01
65	3	0.01	0.01	0.03	0.08
66	3	0.00	0.04	0.05	0.09
67	3	0.02	0.04	0.04	0.09
68	3	0.00	0.03	0.03	0.09
69	3	0.01	0.06	0.01	0.02
70	3	0.02	0.03	0.02	0.07
71	3	0.00	0.07	0.01	0.09
72	3	0.01	0.02	0.01	0.05
73	3	0.00	0.05	0.01	0.06
74	3	0.02	0.04	0.02	0.09
75	3	0.00	0.02	0.05	0.09
76	3	0.00	0.03	0.03	0.08
77	3	0.00	0.02	0.03	0.08
78	3	0.01	0.03	0.01	0.05
79	3	0.00	0.00	0.02	0.05
80	3	0.01	0.06	0.02	0.05
81	3	0.01	0.02	0.00	0.01
82	3	0.00	0.03	0.01	0.04
83	3	0.03	0.02	0.00	0.00
84	3	0.00	0.01	0.04	0.05
85	3	0.00	0.05	0.03	0.02
86	3	0.00	0.06	0.02	0.01
87	3	0.01	0.05	0.03	0.04
88	3	0.01	0.03	0.00	0.00
89	3	0.02	0.03	0.00	0.00
90	3	0.00	0.01	0.03	0.06
91	4	0.00	0.04	0.01	0.05
92	4	0.00	0.06	0.01	0.00
93	4	0.00	0.04	0.01	0.05
94	4	0.00	0.03	0.02	0.07
95	4	0.01	0.02	0.01	0.01
96	4	0.00	0.04	0.02	0.03
97	4	0.00	0.06	0.01	0.01
98	4	0.00	0.05	0.04	0.04
99	4	0.00	0.04	0.01	0.05
100	4	0.00	0.03	0.04	0.08
101	4	0.00	0.02	0.00	0.06
102	4	0.00	0.03	0.00	0.04
103	4	0.02	0.04	0.00	0.00
104	4	0.01	0.03	0.00	0.03
105	4	0.00	0.04	0.02	0.06
106	4	0.00	0.02	0.02	0.02
107	4	0.01	0.04	0.02	0.02
108	4	0.00	0.02	0.00	0.07
109	4	0.01	0.04	0.00	0.02
110	4	0.01	0.05	0.01	0.01

OBS	AGE	VFNI	VFCI	VFNII	VFCII
111	4	0.00	0.05	0.00	0.04
112	4	0.00	0.01	0.00	0.02
113	4	0.00	0.05	0.00	0.01
114	4	0.00	0.03	0.02	0.01
115	4	0.01	0.02	0.00	0.01
116	4	0.00	0.04	0.01	0.03
117	4	0.01	0.02	0.00	0.02
118	4	0.01	0.04	0.05	0.03
119	4	0.00	0.03	0.03	0.03
120	4	0.02	0.02	0.00	0.03
121	5	0.00	0.06	0.00	0.02
122	5	0.01	0.00	0.00	0.00
123	5	0.00	0.03	0.00	0.03
124	5	0.03	0.06	0.00	0.00
125	5	0.01	0.04	0.00	0.00
126	5	0.02	0.04	0.02	0.03
127	5	0.00	0.05	0.02	0.02
128	5	0.00	0.05	0.02	0.04
129	5	0.01	0.05	0.00	0.03
130	5	0.01	0.06	0.02	0.03
131	5	0.00	0.04	0.03	0.05
132	5	0.01	0.05	0.00	0.01
133	5	0.02	0.05	0.00	0.00
134	5	0.00	0.02	0.02	0.00
135	5	0.01	0.05	0.00	0.00
136	5	0.02	0.06	0.01	0.00
137	5	0.00	0.05	0.00	0.02
138	5	0.01	0.05	0.01	0.01
139	5	0.02	0.05	0.01	0.01
140	5	0.00	0.09	0.00	0.01
141	5	0.00	0.06	0.00	0.01
142	5	0.00	0.05	0.00	0.00
143	5	0.01	0.05	0.00	0.02
144	5	0.00	0.07	0.02	0.04
145	5	0.00	0.06	0.00	0.00
146	5	0.01	0.03	0.01	0.00
147	5	0.00	0.06	0.01	0.02
148	5	0.00	0.07	0.00	0.00
149	5	0.02	0.05	0.03	0.03
150	5	0.00	0.04	0.01	0.06
151	6	0.01	0.03	0.02	0.07
152	6	0.00	0.08	0.01	0.03
153	6	0.00	0.04	0.01	0.04
154	6	0.00	0.05	0.00	0.02
155	6	0.01	0.03	0.00	0.00
156	6	0.01	0.04	0.01	0.02
157	6	0.00	0.05	0.01	0.02
158	6	0.01	0.08	0.01	0.03
159	6	0.00	0.09	0.00	0.00
160	6	0.01	0.05	0.00	0.03
161	6	0.01	0.08	0.00	0.00
162	6	0.00	0.04	0.00	0.01
163	6	0.00	0.03	0.01	0.03
164	6	0.00	0.06	0.00	0.01
165	6	0.01	0.02	0.00	0.00

OBS	AGE	VFNI	VFCI	VFNII	VFCII
166	6	0.01	0.05	0.00	0.00
167	6	0.02	0.07	0.01	0.00
168	6	0.02	0.07	0.02	0.04
169	6	0.00	0.04	0.01	0.03
170	6	0.00	0.08	0.01	0.03
171	6	0.01	0.06	0.00	0.02
172	6	0.01	0.09	0.00	0.00
173	6	0.00	0.08	0.01	0.03
174	6	0.01	0.06	0.00	0.00
175	6	0.01	0.08	0.00	0.00
176	6	0.01	0.09	0.00	0.00
177	6	0.01	0.05	0.00	0.00
178	6	0.00	0.08	0.00	0.05
179	6	0.00	0.09	0.01	0.04
180	6	0.00	0.08	0.01	0.02

APPENDIX P

Data collected on total number of that points fell on alveolar wall (POTAVN), alveolar space (POTAWS), and tracheobronchial tree and associated connective tissue (POTTRBC) of each animal at light microscopic level.

OBS	AGE	ANIMAL	POTAVW	POTAWS	POTTRBC
1	PRENL	1	1158	833	997
2	PRENL	2	1313	829	852
3	PRENL	3	1253	889	885
4	ONEDY	1	696	1575	728
5	ONEDY	2	817	1528	656
6	ONEDY	3	738	1598	664
7	ONEWK	1	704	1714	577
8	ONEWK	2	761	1567	639
9	ONEWK	3	733	1638	623
10	TWWKS	1	732	1710	552
11	TWWKS	2	738	1806	456
12	TWWKS	3	726	1781	495
13	THWKS	1	660	1811	529
14	THWKS	2	673	1792	535
15	THWKS	3	579	1858	562
16	ADULT	1	398	2151	481
17	ADULT	2	635	1818	558
18	ADULT	3	642	1809	584

APPENDIX Q

Data collected on total number of points that fell on connective tissue (NPOTCON), capillaries (NPOTCAP), and intercepts (NPOTINT) of each animal at electron microscopic level.

OBS	AGE	ANIMAL	NPOTCON	NPOTCAP	NPOTINT
1	PRENL	1	849	337	195
2	PRENL	2	1107	331	244
3	PRENL	3	1163	408	132
4	ONEDY	1	908	261	146
5	ONEDY	2	916	504	292
6	ONEDY	3	790	437	264
7	ONEWK	1	704	191	278
8	ONEWK	2	932	407	303
9	ONEWK	3	718	354	278
10	TWWKS	1	669	238	251
11	TWWKS	2	702	223	312
12	TWWKS	3	719	115	355
13	THWKS	1	514	252	307
14	THWKS	2	675	397	350
15	THWKS	3	627	401	417
16	ADULT	1	253	675	414
17	ADULT	2	451	577	510
18	ADULT	3	572	718	366

APPENDIX R

Data collected on total number of type I (NUMTI) and type II (NUMTII) pneumocytes and number of points falling on type I (NPOTTI) and type II (NPOTTII) pneumocytes of each animal at electron microscopic level.

OBS	AGE	ANIMAL	NUMTI	NUMTII	NPOTTI	NPOTTII
1	PRENL	1	33	164	61	187
2	PRENL	2	23	146	83	419
3	PRENL	3	31	135	106	264
4	ONEDY	1	21	45	50	135
5	ONEDY	2	36	52	27	116
6	ONEDY	3	43	65	33	141
7	ONEWK	1	36	15	36	88
8	ONEWK	2	27	52	37	101
9	ONEWK	3	25	40	39	206
10	TWWKS	1	33	46	24	105
11	TWWKS	2	30	38	20	67
12	TWWKS	3	27	43	17	62
13	THWKS	1	16	23	18	36
14	THWKS	2	22	29	14	61
15	THWKS	3	15	26	22	65
16	ADULT	1	14	22	15	56
17	ADULT	2	17	28	14	62
18	ADULT	3	10	28	11	80

APPENDIX S

Data collected on volume fraction (VF) of alveolar wall (AVW), alveolar space (AVS), and tracheobronchial tree (TRBC) at light microscopic level.

OBS	AGE	ANIMAL	VFAVW	VFAVS	VFTRBC
1	PRENL	1	0.38696	0.27858	0.33445
2	PRENL	2	0.43875	0.27783	0.28342
3	PRENL	3	0.41452	0.29391	0.29156
4	ONEDY	1	0.23214	0.52509	0.24277
5	ONEDY	2	0.27225	0.50922	0.21853
6	ONEDY	3	0.24600	0.53267	0.22133
7	ONEWK	1	0.23502	0.57193	0.19305
8	ONEWK	2	0.25779	0.52807	0.21414
9	ONEWK	3	0.24457	0.54687	0.20856
10	TWWKS	1	0.24453	0.57123	0.18423
11	TWWKS	2	0.24600	0.60200	0.15200
12	TWWKS	3	0.24178	0.59331	0.16490
13	THWKS	1	0.22000	0.60367	0.17633
14	THWKS	2	0.22433	0.59733	0.17833
15	THWKS	3	0.19306	0.61957	0.18737
16	ADULT	1	0.13138	0.71002	0.15860
17	ADULT	2	0.21112	0.60357	0.18531
18	ADULT	3	0.21507	0.60322	0.18171

APPENDIX T

Data collected on volume fraction (VF) of type I (TI) and type II (TII) pneumocytes and connective tissue (CON) at electron microscopic level.

OBS	AGE	ANIMAL	VFTI	VFTII	VFCON
1	PRENL	1	0.04254	0.13040	0.59205
2	PRENL	2	0.04278	0.21598	0.57062
3	PRENL	3	0.05461	0.13601	0.59918
4	ONEDY	1	0.03693	0.09971	0.67061
5	ONEDY	2	0.01727	0.07422	0.58605
6	ONEDY	3	0.02355	0.10064	0.56388
7	ONEWK	1	0.03533	0.08636	0.69087
8	ONEWK	2	0.02505	0.06838	0.63101
9	ONEWK	3	0.02961	0.15642	0.54518
10	TWWKS	1	0.02317	0.10135	0.64575
11	TWWKS	2	0.01977	0.06621	0.69368
12	TWWKS	3	0.01862	0.06791	0.78751
13	THWKS	1	0.02195	0.04390	0.62683
14	THWKS	2	0.01221	0.05318	0.58849
15	THWKS	3	0.01974	0.05830	0.56233
16	ADULT	1	0.01502	0.05606	0.25325
17	ADULT	2	0.01268	0.05616	0.40851
18	ADULT	3	0.00797	0.05793	0.41419

APPENDIX U

Data collected on volume fraction of capillary (VFCAP), number of type I (NVTI) and type II (NVTII) pneumocytes of each animals at electron microscopic level.

OBS	AGE	ANIMAL	VFCAP	NVTI	NVTII
1	PRENL	1	0.23501	0.85478	5.40870
2	PRENL	2	0.17062	0.31517	2.24346
3	PRENL	3	0.21020	0.43618	2.51171
4	ONEDY	1	0.19276	0.50760	0.96901
5	ONEDY	2	0.32246	1.34311	1.12490
6	ONEDY	3	0.31192	1.76931	1.59081
7	ONEWK	1	0.18744	1.78413	0.30692
8	ONEWK	2	0.27556	0.78861	1.27574
9	ONEWK	3	0.26879	0.76752	0.67588
10	TWWKS	1	0.22973	1.88627	1.48416
11	TWWKS	2	0.22036	1.83351	1.42809
12	TWWKS	3	0.12596	1.88212	1.98077
13	THWKS	1	0.30732	0.92902	1.13220
14	THWKS	2	0.34612	1.21424	0.88037
15	THWKS	3	0.35964	0.56098	0.74478
16	ADULT	1	0.67568	0.68372	0.69706
17	ADULT	2	0.52265	0.85691	0.86073
18	ADULT	3	0.51991	0.34866	0.60575

APPENDIX V

Programs used to obtain regression analysis.

```
************************  MEAN REGRESSION  ****************

DATA COMP;

INPUT  AGE BODYWT  NUMC  NUMG ;

CARDS;
60  81.103  20.11  14.33
65  89.287  22.00  13.00
72  172.42  18.67  16.11
79  264.85  21.67  13.00
86  365.99  21.67  11.00
;
PROC PRINT;

PROC REG DATA=COMP;
MODEL NUMC NUMG = BODYWT;

PROC REG DATA=COMP;
MODEL NUMC NUMG=AGE;

*********************************************************************
***************************   NUMBER OF CELLS ***************

DATA SURF;

INPUT  AGE BODYWT  NUMC  NUMG ;

CARDS;
60  81.103  20.11  14.33
65  89.287  22.00  13.00
72  172.42  18.67  16.11
79  264.85  21.67  13.00
86  365.99  21.67  11.00
730 3317.33 20.78  12.11
;
PROC PRINT;

PROC REG DATA=SURF;
MODEL NUMC NUMG = BODYWT;

*************************************************************;
```

```
DATA CC;

INPUT AGE ANIMAL BODYWT NUMC NUMG;

CARDS;

60  1   82.333    21   15.667
60  2   76.667    17   14
60  3   83.811    22.3 13.667
65  1   95.000    22   13
65  2   90.587    23   16
65  3   82.267    23.3 13.7
72  1   223.350   16   17.7
72  2   145.900   20.7 18
72  3   148.000   19.3 12.7
79  1   280.500   21.7 13
79  2   271.830   23.3 12.7
79  3   242.220   19   21.3
86  1   372.307   22.7 10
86  2   318.920   19.7 13.3
86  3   406.740   22.7 9.7
;

PROC PRINT;

PROC REG DATA=CC;
MODEL NUMC NUMG  = BODYWT;

PROC REG;
MODEL NUMC NUMG = AGE;
*******************************************
```

```
*****************  REGRESSION-----LIGHT  ****************;

DATA REGRESS;
INPUT AGE ANIMAL BODYWT LUNGWT LUNGVOL;

CARDS;

60    1     82.830     2.667      2.80
60    2     76.670     1.911      1.90
60    3     83.810     2.733      2.20
60    4     81.250     2.100      2.00
60    5     81.250     1.600      2.00
60    6     67.500     2.000      1.70
60    7     84.500     2.800      2.10
60    8     86.000     3.000      2.20
65    1     100.00     2.500      4.00
65    2     89.500     1.900      4.00
65    3     103.59     1.600      3.00
65    4     111.60     1.900      3.00
65    5     56.570     1.100      1.00
65    6     70.500     1.500      1.00
65    7     95.000     2.967      4.00
65    8     90.590     2.200      2.30
65    9     82.270     2.667      2.20
72    1     223.35     4.100      6.80
72    2     145.90     2.500      4.00
72    3     148.00     3.300      3.50
72    4     221.00     4.000      7.00
72    5     150.80     4.100      3.00
79    1     280.50     6.300      6.00
79    2     271.83     3.000      5.50
79    3     242.22     4.350      7.00
79    4     293.00     6.800      9.00
79    5     267.26     4.500      5.00
79    6     255.11     4.400      7.00
86    1     397.31     6.067      8.50
86    2     357.47     5.900      8.50
86    3     329.04     5.900      8.00
86    4     430.41     6.400      9.00
86    5     323.52     5.600      8.00
86    6     314.32     5.900      10.00
86    7     416.48     6.500      9.00
;

PROC PRINT;

PROC REG;
MODEL BODYWT LUNGWT LUNGVOL=AGE;

PROC REG;
MODEL LUNGWT LUNGVOL=BODYWT;

PROC REG;
MODEL LUNGVOL=LUNGWT;
```

```
************  WITH ADULT  ******************************;

DATA ADULT;

INPUT AGE ANIMAL BODYWT LUNGWT LUNGVOL;

CARDS;

60   1    82.830    2.667     2.80
60   2    76.670    1.911     1.90
60   3    83.810    2.733     2.20
60   4    81.250    2.100     2.00
60   5    81.250    1.600     2.00
60   6    67.500    2.000     1.70
60   7    84.500    2.800     2.10
60   8    86.000    3.000     2.20
65   1    100.00    2.500     4.00
65   2    89.500    1.900     4.00
65   3    103.59    1.600     3.00
65   4    111.60    1.900     3.00
65   5    56.570    1.100     1.00
65   6    70.500    1.500     1.00
65   7    95.000    2.967     4.00
65   8    90.590    2.200     2.30
65   9    82.270    2.667     2.20
72   1    223.35    4.100     6.80
72   2    145.90    2.500     4.00
72   3    148.00    3.300     3.50
72   4    221.00    4.000     7.00
72   5    150.80    4.100     3.00
79   1    280.50    6.300     6.00
79   2    271.83    3.000     5.50
79   3    242.22    4.350     7.00
79   4    293.00    6.800     9.00
79   5    267.26    4.500     5.00
79   6    255.11    4.400     7.00
86   1    397.31    6.067     8.50
86   2    357.47    5.900     8.50
86   3    329.04    5.900     8.00
86   4    430.41    6.400     9.00
86   5    323.52    5.600     8.00
86   6    314.32    5.900     10.00
86   7    416.48    6.500     9.00
730  1    4082.00   50.20     52.00
730  2    2948.33   23.20     25.00
730  3    2500.00   20.50     21.20
730  4    3950.00   35.40     34.40
;

PROC PRINT;

PROC REG;
MODEL LUNGWT LUNGVOL=BODYWT;

PROC REG;
MODEL LUNGVOL=LUNGWT;
```

Program used to obtain general linear model (GLM).

```
****************** REGRESSION TRACHEA ****************;
DATA CILIA;

INPUT AGE ANIMAL CILIA BODYWT LUNGWT LUNGVOL
                  EPITHICK  SUBOC  SUBBC;

CARDS;

60  1  5.673    82.333    2.667   2.8  23.17  33.749  55.255
60  2  5.268    76.667    1.911   1.9  22.98  43.507  151.322
60  3  4.343    83.811    2.733   2.2  24.98  27.282  136.979
65  1  5.533    95.000    2.967   4.0  28.40  36.816  126.642
65  2  4.938    90.587    2.200   2.3  27.73  71.156  135.149
65  3  5.133    82.267    2.667   2.2  29.06  28.839  84.176
72  1  6.028    223.350   4.100   6.8  23.24  50.706  99.883
72  2  4.699    145.900   2.500   4.0  33.66  50.577  165.162
72  3  4.945    148.000   3.300   3.5  30.58  53.574  96.128
79  1  5.270    280.500   6.300   6.0  28.22  62.914  244.630
79  2  5.666    271.830   3.000   5.5  32.22  59.523  189.140
79  3  5.044    242.220   4.350   7.0  30.44  68.344  168.931
86  1  6.006    372.307   6.067   8.5  31.03  82.636  253.379
86  2  5.943    318.920   5.750   9.0  30.89  68.154  246.238
86  3  4.945    406.740   6.850   9.0  34.82  50.835  229.302
;

PROC PRINT;

PROC GLM DATA=CILIA;
MODEL CILIA EPITHICK SUBOC SUBBC=AGE/SOLUTION;

*************************************************************;
```

```
****************  REGRESSION-----LIGHT  ****************;

DATA REGRESS;

INPUT AGE ANIMAL BODYWT LUNGWT LUNGVOL VFAVW VFAVS VFTRBC;

CARDS;
60   1    82.830    2.667    2.80    0.38696    0.27858    0.33445
60   2    76.670    1.911    1.90    0.43875    0.27783    0.28342
60   3    83.810    2.733    2.20    0.41452    0.29391    0.29156
65   1    95.000    2.967    4.00    0.23214    0.52509    0.24277
65   2    90.590    2.200    2.30    0.27225    0.50922    0.21853
65   3    82.270    2.667    2.20    0.24600    0.53267    0.22133
72   1    223.35    4.100    6.80    0.23502    0.57193    0.19305
72   2    145.90    2.500    4.00    0.25779    0.52807    0.21414
72   3    148.00    3.300    3.50    0.24457    0.54687    0.20856
79   1    280.50    6.300    6.00    0.24453    0.57123    0.18423
79   2    271.83    3.000    5.50    0.24600    0.60200    0.15200
79   3    242.22    4.350    7.00    0.24178    0.59331    0.16490
86   1    372.31    6.067    8.50    0.22000    0.60367    0.17633
86   2    318.92    5.750    9.00    0.22433    0.59733    0.17833
86   3    406.74    6.850    9.00    0.19306    0.61957    0.18737
;
PROC PRINT;

PROC GLM DATA=REGRESS;
MODEL VFAVW VFAVS VFTRBC=AGE/SOLUTION;
*************************************************************;
```

```
***************   REGRESSION------ELECTRON MICRO  ***************;

DATA MICRO;

INPUT AGE ANIMAL BODYWT LUNGWT LUNGVOL
      VFTI VFTII VFCON VFCAP NVTI NVTII;

CARDS;

60 1 82.83    2.667 2.80  0.04254  0.13040  0.59205  0.23501  0.85478  5.40870
60 2 76.67    1.911 1.90  0.04278  0.21598  0.57062  0.17062  0.31517  2.24346
60 3 83.81    2.733 2.20  0.05461  0.13601  0.59918  0.21020  0.43618  2.51171
65 1 95.00    2.967 4.00  0.03693  0.09971  0.67061  0.19276  0.50760  0.96901
65 2 90.59    2.200 2.30  0.01727  0.07422  0.58605  0.32246  1.34311  1.12490
65 3 82.27    2.667 2.20  0.02355  0.10064  0.56388  0.31192  1.76931  1.59081
72 1 223.35   4.100 6.80  0.03533  0.08636  0.69087  0.18744  1.78413  0.30692
72 2 145.90   3.300 4.00  0.02505  0.06838  0.63101  0.27556  0.78861  1.27574
72 3 148.00   2.500 3.50  0.02961  0.15642  0.54518  0.26879  0.76752  0.67588
79 1 280.50   6.300 6.00  0.02317  0.10135  0.64575  0.22973  1.88627  1.48416
79 2 271.83   3.000 5.50  0.01977  0.06621  0.69368  0.22036  1.83351  1.42809
79 3 242.22   4.350 7.00  0.01862  0.06791  0.78751  0.12596  1.88212  1.98077
86 1 372.31   6.067 8.50  0.02195  0.04390  0.62683  0.30732  0.92902  1.13220
86 2 318.92   5.750 9.00  0.01221  0.05318  0.58849  0.34612  1.21424  0.88037
86 3 406.74   6.850 9.00  0.01974  0.05830  0.56233  0.35964  0.56098  0.74478
;
PROC PRINT;

PROC GLM DATA=MICRO;
MODEL VFTI VFTII VFCON VFCAP NVTI NVTII=AGE/SOLUTION;

*************************************************************************;
```

```
******************    REGRESSION---SURFACE DENSITY  ********************;

DATA SURF;

INPUT AGE ANIMAL BODYWT LUNGWT LUNGVOL
        ARTHMEAN HMEAN HTHICK SURFDEN;

CARDS;

60 1  82.83     2.667  2.80   0.36825    0.73651   0.49101   353.6167
60 2  76.67     1.911  1.90   0.45346    0.90692   0.60461   405.5722
60 3  83.81     2.733  2.20   0.40292    0.80584   0.53723   421.9444
65 1  95.00     2.967  4.00   0.33289    0.66578   0.44385   365.0500
65 2  90.59     2.967  2.30   0.38698    0.77397   0.51598   600.5222
65 3  82.27     2.667  2.20   0.35206    0.70413   0.46942   315.8556
72 1  223.35    4.100  6.80   0.27556    0.55111   0.36741   255.5000
72 2  145.90    2.500  4.00   0.32479    0.64959   0.43306   603.2833
72 3  148.00    3.300  3.50   0.34286    0.68571   0.45714   480.4722
79 1  280.50    6.300  6.00   0.28686    0.57371   0.38248   368.5111
79 2  271.83    3.000  5.50   0.25959    0.51917   0.34612   589.7500
79 3  242.22    4.350  7.00   0.20876    0.41752   0.27835   473.1611
86 1  372.31    6.067  8.50   0.24000    0.48000   0.32000   324.2555
86 2  318.92    5.750  9.00   0.23292    0.46584   0.31056   556.5000
86 3  406.74    6.850  9.00   0.26003    0.52006   0.34671   592.1611
;
PROC PRINT;

PROC GLM DATA=SURF;
MODEL HTHICK SURFDEN=AGE/SOLUTION;

**********************************************************;
```

APPENDIX X

Program used to obtain general linear model (GLM) of the mean.

```
********************  NO ADULT ******************************;
DATA COMP;

INPUT AGE BODYWT LUNGWT LUNGVOL VFAVW VFAVS VFTRBC;

CARDS;

60  81.103  2.437    2.300  0.413  0.283  0.303
65  89.287  2.611    2.833  0.250  0.522  0.228
72  172.42  3.300    4.767  0.246  0.549  0.205
79  264.85  4.550    6.167  0.244  0.589  0.167
86  365.99  6.222    8.833  0.212  0.607  0.181
;
PROC PRINT;

PROC GLM DATA=COMP;
MODEL VFAVW VFAVS VFTRBC = AGE/SOLUTION;

*****************************************************************
```

```
****************** REG. MEAN TRACHEA ****************************;

DATA MEAN;

INPUT AGE CILIA BODYWT LUNGWT LUNGVOL EPITHICK  SUBOC SUBBC;

CARDS;

60  5.094  80.937  2.437  2.3  23.71  34.846  114.519
65  5.201  89.285  2.611  2.8  28.40  45.607  115.322
72  5.224  172.417 3.300  4.8  29.16  51.619  120.391
79  5.327  264.850 4.550  6.2  30.29  63.594  200.900
86  5.631  365.989 6.222  8.8  32.25  67.208  242.973
;

PROC PRINT;

PROC GLM DATA-MEAN;
MODEL CILIA EPITHICK SUBOC SUBBC-AGE/SOLUTION;

************************************************************************;
```

```
*****************  MEAN.REGRESSION  *******************;
DATA MEN;

INPUT  AGE BODYWT  LUNGWT  LUNGVOL  VFTI
       VFTII VFCON VFCAP NVTI NVTII;

CARDS;
60  81.103  2.437    2.300   0.047  0.161  0.587  0.205  0.535  3.388
65  89.287  2.611    2.833   0.026  0.092  0.607  0.276  1.207  1.228
72  172.42  3.300    4.767   0.030  0.104  0.622  0.244  1.113  0.753
79  264.85  4.550    6.167   0.021  0.078  0.709  0.192  1.867  1.631
86  365.99  6.222    8.833   0.018  0.052  0.593  0.338  0.901  0.919
;
PROC PRINT;

PROC GLM DATA=MEN;
MODEL VFTI VFTII VFCON VFCAP NVTI NVTII=AGE/SOLUTION;


*************************************************************************;
```

```
******************   REGRESSION---SURFACE DENSITY  *******

DATA SURF;

INPUT AGE BODYWT LUNGWT LUNGVOL ARTHMEAN HMEAN HTHICK SURFDEN;

CARDS;
60   81.103  2.437    2.300    0.408  0.816  0.544   393.711
65   89.287  2.611    2.833    0.357  0.715  0.476   427.143
72   172.42  3.300    4.767    0.314  0.629  0.419   446.419
79   264.85  4.550    6.167    0.252  0.503  0.336   477.141
86   365.99  6.222    8.833    0.244  0.489  0.326   490.972
;
PROC PRINT;

PROC GLM DATA=SURF;
MODEL HTHICK SURFDEN=AGE/SOLUTION;

*********************************************************;
```

```
OPTION LS=80;
*********************************************************;
*********************************************************;

********    REGRESSION------ELECTRON MICRO  ***************;

DATA MICRO;

INFILE EVO;

INPUT AGE ANIMAL BODYWT LUNGWT LUNGVOL
            VFTI VFTII VFCON VFCAP NVTI NVTII;

PROC SORT;
BY AGE;

PROC PRINT;

PROC MEANS N MEAN STD STDERR;
 VAR NVTI NVTII;
 BY AGE;

PROC REG DATA=MICRO;
MODEL NVTI NVTII=BODYWT ;

*********************************************************;
```

```
*****************  REGRESSION-----LIGHT  ****************;

DATA REGRESS;

INFILE NAD;

INPUT AGE ANIMAL BODYWT LUNGWT LUNGVOL VFAVW VFAVS VFTRBC;


PROC REG DATA=REGRESS;
MODEL VFAVW VFAVS VFTRBC = AGE;

PROC REG DATA=REGRESS;
MODEL VFAVW VFAVS VFTRBC=BODYWT;

*****************************************************************;
*****************************************************************;

***************   REGRESSION------ELECTRON MICRO  ***************;

DATA MICRO;

INFILE CAT;

INPUT AGE ANIMAL BODYWT LUNGWT LUNGVOL
             VFTI VFTII VFCON VFCAP NVTI NVTII;


PROC REG DATA=MICRO;
MODEL VFTI VFTII VFCON VFCAP=AGE;

PROC REG DATA=MICRO;
MODEL VFTI VFTII VFCON VFCAP=BODYWT;
```

```
**********    NO ADULT  ****************************;

DATA CAT;

INPUT AGE ANIMAL BODYWT LUNGWT LUNGVOL
            VFTI VFTII VFCON VFCAP NVTI NVTII;

CARDS;
60 1 82.83    2.667 2.80  0.02033  0.06233  0.28300  0.11233  309528   865218
60 2 76.67    1.911 1.90  0.02767  0.13967  0.36900  0.11000  233616   606822
60 3 83.81    2.733 2.20  0.03533  0.08800  0.38767  0.13600  409821   1073893
65 1 95.00    2.967 4.00  0.01667  0.04500  0.30267  0.08700  217340   758842
65 2 90.59    2.200 2.30  0.00900  0.03867  0.30533  0.16800  229608   789407
65 3 82.27    2.667 2.20  0.01100  0.04700  0.26333  0.14567  194395   780102
72 1 223.35   4.100 6.80  0.01200  0.02933  0.23467  0.06367  106712   524006
72 2 145.90   3.300 4.00  0.01233  0.03367  0.31067  0.13567  96051    621102
72 3 148.00   2.500 3.50  0.01300  0.06867  0.23933  0.11800  214778   336627
79 1 280.50   6.300 6.00  0.00800  0.03500  0.22300  0.07933  153683   635251
79 2 271.83   3.000 5.50  0.00667  0.02233  0.23400  0.07433  211750   554492
79 3 242.22   4.350 7.00  0.00567  0.02067  0.23967  0.03833  159110   767891
86 1 372.31   6.067 8.50  0.00600  0.01200  0.17133  0.08400  233616   434681
86 2 318.92   5.750 9.00  0.00467  0.02033  0.22500  0.13233  233616   247787
86 3 406.74   6.850 9.00  0.00733  0.02167  0.20900  0.13367  141166   247787
;

PROC PRINT;

PROC REG DATA=CAT;
MODEL NVTI NVTII=BODYWT ;

PROC REG DATA=CAT;
MODEL NVTI NVTII=AGE;
```

APPENDIX Y

PHOTOGRAPHIC PROCEDURES

ILFORD PAN F 35mm Film:

Purpose: for black and white negatives of light microscopic slides.

Solution:

Rodinal's developing solution

Rodinal ---------- 12 ml
Water ---------- 300 ml

Develop film:

1. Rodinal solution ---------- 5 min
2. Wash ---------- 30 sec
3. Fixative ---------- 3 min
4. Wash ---------- 30 sec
5. Hypoclear ---------- 1 min
6. Wash ---------- 1 min
7. Photoflo 1:200 ml
8. Hang and dry

LPD-4 35mm Film:

Purpose: for black and white positive slides of black and white drawings, line graphics generated on plotters, and tables.

Solution:

D 19 developing solution
D 19 developer ---------- 1 part
Water ---------- 1 part

Develop film:

1. Developing solution --------- 2.5 min
2. Stop solution ---------- 30 sec
3. Fixative ---------- 3 min
4. Wash ---------- 1 min
5. Hypoclear ---------- 1 min
6. Wash ---------- 15 sec
7. Final was add 1 cap photo flo 200 in the water

KODAK RAPID PROCESS COPY FILM 2064:

Purpose: direct-reversal film used for production of black and white slides of black and white transparencies, black and white reflection copy, and radiographs.

Develop film:

1. may be used in the radiographic processor with 90 sec longer cycles.
2. Conventional black and white chemicals.

VITA

Mohammed Shaker Al-Tikriti was born January 13, 1951, in Baghdad, Iraq, where he received his elementary and preparatory education. He graduated from Al-Adhamia High School in 1968. Following high school graduation, he enrolled in the University of Baghdad at Baghdad where he received a Bachelor of Veterinary Medicine and Surgery on June 30, 1973. Following graduation from Baghdad University, he joined the military service as an equine clinician for one year. In 1974 he was appointed as a demonstrator in the College of Veterinary Medicine, Department of Anatomy, Baghdad University, for four years. He was selected by Ministry of Higher Education and Scientific Research to pursue a graduate program in the United States of American in 1979. He graduated from Iowa State University at Ames, Iowa where he received Master of Science in Veterinary Anatomy in May, 1982.

The author was admitted to Louisiana State University in June of 1982 where he received Master of Science in Veterinary Anatomy and Fine Structure.

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