

PRENATAL DEVELOPMENT OF BOVINE LUNG

by

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## INTRODUCTION

Most studies on pulmonary development have been done on rat or human fetal lungs. Other species studied included the dog, mouse, guinea pig, rabbit, pig, and sheep. Few studies have been reported on the large domestic animals, which have a preponderancy of respiratory disease problems. Thus, additional information about respiratory system development may be important to our understanding of respiratory disease processes in the bovine species. In addition, a more complete knowledge of histological changes relative to age may aid in histopathological interpretation between normal and disease states. A better understanding of fetal histology may yield valuable information concerning behavior of neonatal and adult lungs.

Most texts and articles use the human lung as a development model and assume that other mammals follow the same or similar patterns. In these descriptions a system of nomenclature has evolved and has been formalized by the International Congress of Anatomists meeting in Leningrad in 1970 for inclusion in *Nomina Embriologica*.

Because the human fetal lung development has been used as a model for all mammals, there is no text available to veterinary students which emphasizes the developmental anatomy of domestic animals (with the possible exception of Patten's *Embryology of the Pig*).

With current progress in teratologic, viral, bacterial, and environmental diseases of the bovine and other domesticated species, it is important that efforts be directed towards documenting inter-species differences and stages of occurrence.

Constant change throughout fetal development and differences between species add importance to the knowledge of the normal fetal histology in order to evaluate fetal tissue. Because of these differences and lack of relative information, fetal histology is often difficult to interpret.

## LITERATURE REVIEW

Malpighi first examined lungs using light microscopy in the 17th century (1628-1694). He and Lewenhoeck (1632-1723) described capillaries whose presence was previously assumed by men such as Harvey (1578-1657). During the last quarter of a century much has been learned of prenatal and postnatal lung development in the rat, human, rabbit, sheep, and guinea pig.

The possibility of a continuous epithelial layer lining the alveolus was once the subject of much controversy (8,9,10). Many authors assumed that capillaries were devoid of any epithelial covering and thus came into direct contact with the alveolar air (11). Some early authors, using mainly pathologic specimens, described a continuous alveolar lining (12). However, these observations were challenged on the basis that epithelium develops quickly in diseased tissues. Colberg (1967), Kuttner (1876), Flint (1906), Ozawa (1920) and Bender (1925) reported that a continuous layer of cuboidal epithelium lined the fetal lung alveolus throughout fetal development and became squamous before or immediately after birth. Rose (1928), studying human fetal lungs, questioned if alveoli develop from endodermal buds, and suggested that they originated as spaces in a capillary network.

Clements (1938) reported that epithelium was discontinuous in the 200 millimeter fetal pig, that breaks occurred in the tubes leading to the end-buds, and that alveolar epithelium was largely absent before

term. Bensley and Groff (1935) and Cooper (1938) gave support to an earlier view that alveolar epithelium exists as a continuous layer throughout fetal life. These controversies were resolved by the additional resolution of the electron microscope which showed the presence of a continuous alveolar epithelial lining (13) and that the alveolar lining in man and many other species is composed of two different cell types (14).

Many names have been applied to the two cell types since their discovery by Policard and associates (1954). The terms type I and type II cells were suggested (15) and the term pneumocyte was added to avoid confusion with cells of other organs (16). Considerable interest has been shown in ultrastructural development of the lung in recent years (17) in several species of animals including the lamb (18), mouse (19), and rabbit (20).

It is now recognized that knowledge of the microscopic structure of the fetal lung adds valuable information about the behavior of the neonatal and adult lung in relation to disease and physiological processes. For example, the production of pulmonary surfactant by type II pneumocytes which is required for survival at birth.

Three stages of fetal lung development have been recognized (21): the glandular (22) or pseudoglandular (23), the canalicular, and the alveolar or terminal sac period. Occurrence times for these phases in the human, rabbit, sheep, and rat have been established. Many authors have discussed the terms applied and the extent of these three lung development stages in man, and the suggestions of Loosli and Potter (1959) have been frequently followed. However, since the alveoli appear

primitive and simple in outline in the alveolar phase, it was felt by other authors that the term terminal sac period best described the third stage, and the term alveolar period should be applied to structures seen in the perinatal and postnatal animal.

The irregularity of lung maturation has been discussed and differences in maturity between the upper and lower lung lobes of fetal rabbits have been reported (20). Lung development has been reported to occur in a centrifugal manner from hilum to periphery (24) followed by centripetal lung differentiation in the later stages of development (23). Experimental in vitro studies showed that budding and branching in a lung explant occurred only in the presence of mesoderm and suggested the existence of a growth stimulant which originated from the mesoderm (21,25,26).

Lung organogenesis in chickens was also promoted from mesenchyme that had not originated from the lung (27) but from various organs such as the digestive tract, dermis, and mesonephron. Two levels of mesodermal control in lung development have been determined (28). At the first level, the formation of lung buds results from interactions between gut endoderm and non-specific mesoderm. At the second level, the branching morphogenesis is produced by an interaction between specific bronchial mesoderm with the lung bud endoderm.

#### Embryology

The human lung begins as an epithelial bud, arising from the caudal end of the laryngo-tracheal groove. The lung bud appears on day 26 after ovulation (29). It divides, then elongates into two lung buds.

These grow dorsally on either side of the esophagus (30). Meanwhile, upper, middle, and lower lobar buds have developed which give rise to primary buds of the ten bronchopulmonary segments and become the future airways (31).

The International Congress of Anatomists meeting in Leningrad in 1970 (Nomina Embryological, 1970) and the Commission on Embryological Terminology (Arey and Mossman, 1970) named the stages of lung development: embryonic, in the human during the first 5 weeks after ovulation; pseudoglandular; canalicular; and terminal sac or alveolar stage.

The pseudoglandular stage was named for the resemblance of lung to an exocrine gland. In this stage the lung appears as buds of endodermal cells surrounded by mesenchymal tissue and establishes the future air-conducting system. Vascularization of the mesenchymal tissue is not apparent. Some elastic fibers are found associated with the main bronchi, arteries, and pleura (22).

The canalicular stage is characterized by enlargement of bronchi and bronchioles, and the lung tissue becomes highly vascularized. The arteries migrate toward the distal endoderm buds and capillaries push into the epithelium, causing it to become thin. The terminal buds branch and grow to form future terminal air sacs, while the distal airway transforms into respiratory bronchioles.

The terminal sac period is characterized by the formation of thin-walled terminal air sacs from alveolar ducts. Capillary proliferation and concurrent active development of lymphatic capillaries occurs. By 26 to 28 weeks, sufficient terminal air sacs and surfactant substance are present to permit survival of a prematurely born infant. Before

this time, the fetal lungs are incapable of providing adequate gas-exchange, partly due to underdeveloped vascularity, a lack of surfactant substance, and insufficient saccular surface area.

The alveolar stage is characterized by formation of alveoli within saccules. Approximately  $1/8$  to  $1/6$  of the future alveolar population is present at birth and increases until about the eighth year. Before birth, the primitive alveoli are seen as indentations in the saccule walls and growth of secondary septa into the saccular lumen. During the first few months after birth, the indentations (secondary septa) seen in the saccule wall deepen to form alveolar sacs. A number of alveolar sacs are formed by a process of budding of terminal saccules, which gives rise to additional alveoli. Alveoli also form along the alveolar duct and the terminal saccule takes on the appearance of the adult atrium (32). Few elastic fibers are found in the walls of the terminal sacs but are seen, at birth, around the alveolar openings. Elastic tissue increases as alveoli grow in size. The number of alveoli increases logarithmically from about 24 million at birth to the adult number of about 280 million at 8 years of age in man (33).

The alveolar surface area in rats increases much faster during the first 90 days of life than can be accounted for by the increase in lung volume during this period and an explosive proliferation of alveoli occurs between the fifth and tenth days of life (34). After birth the lung undergoes three distinct phases of growth: (1) a phase of lung expansion where the lung volume increases due to a gain in the volume of the air spaces without an increase in tissue (31,35,36); (2) a phase of tissue proliferation with an increase of the alveolar surface area



per unit volume of lung tissue; (3) a phase of proportionate growth which involves enlargement of existing structures.

### Anatomy

The lungs are the paired organs, with each invaginated into an ipsilateral pleural sac, and occupy much of the thoracic cavity. In bovine the right lung is almost twice as large as the left lung, and is subdivided into lobes of interlobar fissures, namely: the apical (cranial), the middle (cardiac), the diaphragmatic (caudal), and the accessory (intermediate). The left lung is divided into two lobes: an apical (cranial) lobe and a diaphragmatic (caudal) lobe.

The cow, sheep, and pig have been designated as subgross lung type I. The monkey, dog, and cat lungs have been designated as type II, and the horse lung as type III (37). The pleura and interlobular septa are thick in types I and III, extremely thin in type II and septa are absent. Lobularity is well developed in type I, absent in type II, and imperfectly developed in type III. True terminal bronchioles comprise the most frequent form taken by the distal airways in types I and III, although small numbers of poorly developed respiratory bronchioles are present. Well developed respiratory bronchioles appear to be the only form present in type II. The type III lungs of these animals most closely compare with the human being. A need exists to understand these differences and provides a reason for further study in the fields of comparative mammalian physiology, pathology, and histology.

The pulmonary passageways consist of an air-conduction portion, the bronchi and bronchioles, and a respiratory portion (gas exchange), the respiratory bronchioles, alveolar ducts, alveolar sacs, and alveoli (38). The portion of lung supplied by a terminal bronchiolus, respiratory bronchioli, alveolar ducts, and alveoli, is defined as an acinus. A lobule is a part of the lung supplied by three to five terminal bronchioli. The air-conducting system of the lung is lined by ciliated epithelium and the respiratory regions is lined by non-ciliated squamous or type I pneumocytes alveolar type epithelium. A mature lung is characterized by alveolar ducts (a conducting airspace with alveoli opening into it), alveolar sacs (final alveolar duct from which only alveoli arise), and alveolus (cup-shaped final respiratory airspace). An immature lung is characterized by the presence of saccules which are thin-walled structures, simple in outline, and containing some rudimentary alveoli. These comprise the respiratory region during fetal life and for some time after birth. Saccules are transformed into the alveolar ducts, alveolar sacs, and alveoli.

#### Electron Microscopic Appearance

Lung development stages have been described based on studies with the transmission electron microscope (17,39,40,41).

#### Pseudoglandular Stage

The glandular buds are composed of columnar cells that rest on a continuous basement membrane. The cells contain glycogen and most of the organelles are found in the supranuclear region. Collagen fibers

have been identified but not elastic fibers. Immature muscle cells and cells that resemble fibroblasts are present beneath the basement membrane.

#### Canalicular Stage

The canalicular stage epithelium is flatter, the cells still contain large amounts of glycogen and organelles are now located at the cell border. The stromal walls are thinner, contain capillaries, fibroblasts, and abundant collagen fibers. By 26 days of gestation, type I and type II pneumocytes can be distinguished by their respective shapes in rabbits (41). Further differentiation of the epithelial cells in two morphologically different types, as observed in adult lung, takes place during the sixth month of gestation in man. Cytologic immaturity exists until lamb fetuses reach the 135th day of gestation and is thought to be the basis for increased susceptibility of immature lambs to respiratory distress (18). The columnar epithelial cells of human fetuses contain large deposits of glycogen which become less abundant between the fourth and the sixth months (17).

#### Terminal Sac Period

All cells in the terminal sac period are fully differentiated, glycogen has disappeared, the blood-gas barrier is present, and tight junctions are present between epithelial cells (41). Histologically, the fetal pig lung shows a sequence of developmental stages similar to that which occurs in other mammals. Maturation is not uniform

throughout the lung at any one time and areas showing different degrees of development can be found (42).

A gradual transition between the columnar or cuboidal epithelium and the attenuated cytoplasmic lining of fully developed alveoli starts during the fifth month in man, when the first contacts between capillaries and epithelium are established (17).

An additional cell type, termed Kultschitsky or neurosecretory cell, is found in the human fetus at about 15 weeks of gestation within the endodermal bud (43). These cells are superficial to the basement membrane, but do not reach the lumen. Two functions suggested for the Kultschitsky cell include serotonin secretion or regulation of lobular growth. They have not been identified in rat or rabbit fetal lung tissue.

Most authors agree that the type I and type II pneumocytes are derived from the epithelial cells of the endodermal bud and fibroblasts, muscle cells, and capillary endothelium from the mesoderm. The presence of muscle cells surrounding glandular buds indicates that this region matures into a conducting airway.

The specific function of the glycogen in fetal lungs is, as yet, unknown. Some theories suggest that in the type II pneumocyte, glycogen may be associated with glycogenesis for the CDP-choline pathway of surfactant production (44). It might also represent an energy source for cell proliferation and mitotic activity (24). Another theory places lung glycogen into a regulatory role for blood glucose levels and carbohydrate metabolism in other organs (45). A type III pneumocyte has been described in the rat (46), more recently in the dog (47), but

not yet in man. This cell contributes little to the alveolar lining as most of its surface is covered by cytoplasmic extensions from a type I pneumocyte. The type III pneumocyte is characterized by microvilli at the free alveolar edge and glycogen is abundant. The function of the cell is unknown but the similarity between its brush border and that of the brush border of the small intestine suggests an absorptive function. Similar cells have been described in the trachea (Rhodin and Dalhamn, 1956), in the gallbladder (Riches, 1969), and in the submaxillary gland (Shackleford and Schneyer, 1971).

The alveolar lining in man and many other species (excluding rat and dog) is composed of two cell types: Type I pneumocyte and type II pneumocyte.

#### Type I Pneumocyte

The type I pneumocyte covers the major part of the alveolus, is characterized by its long cytoplasmic extensions, central nucleus, organelles such as ribosomes, RER, Golgi apparatus and occasionally, small mitochondria. The diameter of the type I pneumocyte is approximately 50 micrometers. Its alveolar surface is smooth and the cytoplasmic extensions include numerous pinocytotic vesicles that are seen to bud from the lumen and basal plasma membrane. The large size of the epithelial pinocytotic vesicles distinguishes bovine lung from most other mammalian and human lung tissue in which the endothelial and epithelial vesicles are similar (48). The epithelial cells are joined by tight junctions. Type I pneumocytes are part of the blood-gas exchange barrier.

### Type II Pneumocyte

The type II pneumocyte is cuboidal in shape, measuring approximately 9 micrometers in diameter, has cytoplasmic projections along its free alveolar edge, contains more organelles than the type I and contains osmiophilic, lamellated bodies thought to be associated with the production of pulmonary surfactant.

Osmiophilic inclusion bodies first appear at about 121 days of gestation in lambs. A dense osmiophilic alveolar lining layer has been described in mammals which may consist of surface active substance (18). The type II pneumocyte is generally considered to be the site of pulmonary surfactant production and secretion.

Type I and II pneumocytes are present in the alveoli in the proportion of 40%-60%, respectively. However, approximately 95% of the alveolus is covered by type I pneumocyte and only 5% by the type II (Weibel, 1963). The blood-gas barrier is comprised mainly of type I pneumocyte.

### Interalveolar Wall

The alveolar wall is composed of connective tissue, bounded by the basement membranes of the epithelium and the capillary endothelium. Within the connective tissue there are bundles of collagen, elastic, microfibrils (49), fibroblasts, muscle cells, adventitial cells and macrophages, as well as occasional mast cells, monocytes, neutrophils and lymphocytes. In adult mammals only one system of capillaries is present in the alveolar wall, but two sets have been seen in septa

during the saccular period. The fibroblasts' function is maintenance and production of the connective tissue fibers.

Alveolar macrophages are located within the alveolar space and are characterized by an eccentrically located nucleus with a prominent nucleolus plus a discrete Golgi apparatus. The most obvious feature of the macrophage is the presence of osmiophilic cytoplasmic phagocytized foreign debris. Alveolar macrophages were first thought to be desquamated type II pneumocytes (Bertalanffy, 1964; Spencer, 1968). However, electron microscopic studies have established that the type II pneumocyte is endodermal in origin and the macrophage is mesenchymal. Recent experimental studies have shown that pulmonary macrophages are derived from hemopoietic tissue (Pintrett et al., 1966; van Furth, 1970; Godleski and Brain, 1972).

#### Adventitial Cell

The adventitial cell or pericyte closely resembles the fibroblast. It is located around capillaries by the presence of a basement membrane around the cell and tubules within the cytoplasm (50). Processes from the adventitial cell are adjacent to the endothelium. The function of this cell is obscure. Since adventitial cells are found around non-muscular vessels only, it has been suggested that these cells are immature muscle cells (Rhodin, 1968) and may play a part in capillary constriction. Muscle cells, identical to smooth muscle cells in other regions, are sometimes found in the alveolar wall and around the larger blood vessels.

Mast cells, monocytes, neutrophils, and lymphocytes are wandering cells which are sometimes encountered in the alveolar walls and are morphologically similar to those found at other sites.

### Capillaries

Lung capillaries arise from small arteries and drain into small veins. They are lined by endothelial cells that rest on a continuous basement membrane. The endothelial cells are similar in shape to type I pneumocyte, have thin cytoplasmic extensions, organelles in the vicinity of the nucleus and pinocytotic vesicles are seen along the inner and outer surfaces of the membranes. The vesicles seem to act as a carrier for macromolecules, the so-called pinocytotic activity (Schneeberger and Karnovsky, 1968, 1971; Clements, 1970). Fenestrated endothelium is not seen in normal pulmonary vessels. However, fenestrae have been reported in several pathologic conditions (Suzuki, 1969; Mayrick et al., 1974).

Bovine lung capillaries have the same morphologic characteristics as described for other species, but without adventitial cells. Capillary numbers per unit area of section appear to be less in the bovine than in other species (51). Large macrophage-like cells in the vascular lumen, termed intracapillary macrophages, and unique to the calf lung have been described (48).

### Lymphatics

Lymphatics have not been identified in the alveolar walls of mammalian lung either with light or electron microscope. Lymphatics



are present in the connective tissue septa, around arteries, airways, veins, and under the pleura.

The pulmonary lymph vessels have a larger lumen than blood capillaries. The endothelium is thin and shows discontinuities, and the basement membrane is discontinuous or absent (52).

#### Surfactant Substance

Surfactant is a surface tension-reducing substance which forms an acellular layer that lines the alveolus. It reduces surface tension, thus aiding the stability of the alveoli. Surfactant substance has been demonstrated at 92 days of gestation in pig (42), at 160 days of gestation in man, 148 days in sheep, 31 days in rabbit, 22 days in rat, and 20 days in mouse. Three variations in the appearance of lamellated inclusion bodies have been described (53). In the fetal rabbit, sheep, rat, dog, and mouse, the inclusion bodies contained heavily osmiophilic coarsely lamellar material; in the hamster and guinea pig, lightly osmiophilic material was found; and vacuolated inclusions in the postnatal rabbit and postnatal sheep. Recently, Creasey and associates (1973) reported in primates (human, rhesus and squirrel monkey) that lamellae were spirally arranged. Lamellar and lattice structures identified in the lumens of normal bovine pulmonary alveoli might be a result of death and degeneration of alveolar epithelial cells or be related to the production of the pulmonary surfactant (51).

In addition to the presently recognized type II pneumocyte (50), the non-ciliated Clara cell (secretory granules are not recognized

during fetal life) and the alveolar macrophage have been suggested as sources of pulmonary surfactant.

Surfactant has been shown to be a mixture of phospholipids bound to both proteins and carbohydrate, which forms a barrier layer between the alveolar epithelial cells and alveolar gas. Surfactant is a smooth, osmiophilic layer which has been identified with the electron microscope (Groniowski and Bczyskova, 1969; Kalitaf et al., 1968) and the fluorescent microscope using an antibody technique (Bolande and Klaus, 1964). A dense osmiophilic alveolar lining layer, described in lambs, has been suggested to be a layer of surfact active substance (18).

#### Scanning Microscopic Appearance of the Developing Lung

Recently several reports have been published on the appearance of the developing fetal rabbit lung revealed by scanning electron microscope (41,54). Before 26 days the surface of the epithelium has a rather lumpy appearance. Later an attenuation of some cells is seen and type I and II pneumocytes are recognized near to term (28th day of gestation, 2 or 3 days prior to term), when the alveoli are covered by the smooth, thin covering of type I pneumocytes. Structural alteration takes place without any evidence of cell lysis or desquamation into the alveolar lumen (54).

A characteristic concentration of microvilli along cellular borders on the luminal surface of the 20 to 22-day fetal rabbit lung has been described; the function of these microvilli is unknown (41). The transition from the canalicular into the alveolar stage is gradual and follows a centrifugal pattern (41). Inter-alveolar pores of Kohn are not present in the lung of newborn rabbit (41).

## MATERIALS AND METHODS

Bovine lung tissue from sixty fetuses was collected from an abattoir (Jack Polen Beef Co., Kansas City, Kansas). Fetal ages were based on breeding dates or estimated, using published criteria (1). All lungs were removed immediately after death. Strips approximately one centimeter wide were cut from the right diaphragmatic lobe and extending from the basal border towards the hilus. Tissue strips from some fetal lungs were then divided into one centimeter blocks. These blocks were sectioned parallel to the long axis of the strip, so that the entire length could be studied microscopically. Fetal lungs with strips less than five centimeters in length were sectioned in one piece.

### Light Microscope

Fetal lungs were fixed either in 10% buffered neutral formalin (BNF) or in 2.5% gluteraldehyde in 0.05 M sodium cacodylate, pH 7.4, 350 m Osm and dehydrated in graded concentrations of ethanols. Tissue samples were embedded in paraffin, sectioned at 6 micrometers, and stained with one of four different staining techniques: Harrys' (2) hematoxylin and eosin, McManus' (3) method for basal membrane, Weigert's (4) and Verhoeff's (5) methods for elastic fibers. Photomicrographs were taken with an Olympus Photomicrographic system camera model PM-10-M, using color Kodak film number 2483.

### Scanning Electron Microscopy

Tissue samples were fixed in 10% buffered neutral formalin, dehydrated in graded ethanol concentrations for about 30 minutes, critical point dried with DCP-1 Critical Point Dryer<sup>a</sup>, and gold paladium coated with KSE-2A-M Vacuum Evaporator<sup>b</sup>. Some samples were embedded in paraffin, trimmed, and the blocks then exposed to one of the three different methods: (1) four toluene rinses at room temperature, (2) three toluene rinses at room temperature and one in hot toluene (40°C), or (3) three toluene rinses and three with thrichloroethylene-ethanol (50-50%). Other samples were washed first in Ringer's solution (90 ml) and heparin (1000  $\mu$ /ml, 10 ml), then in pure Ringer's solution and fixed in 10% buffered neutral formalin. Samples were dried and coated as previously described. Photomicrographs were taken with ETEC-Auto-Scan<sup>c</sup> at accelerating voltage of 10 KV, using Polaroid type 55 Kodak film.

### Transmission Electron Microscopy

Fetal lung samples of 1 mm<sup>3</sup> were fixed in 5% gluteraldehyde with 0.05 M sodium cacodylate buffer, pH 7.4, 690 m OsM. Tissues were transferred after 10 minutes to 2.5% gluteraldehyde in 0.05 M sodium cacodylate, pH 7.4, 350 m OsM, rinsed three times in 0.1 M sodium cacodylate buffer solution, postfixed in 1% cacodylate buffered

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<sup>a</sup>Denton Vacuum, Inc., Cherry Hill, New Jersey.

<sup>b</sup>Kinney Vacuum Company, Boston, Mass.

<sup>c</sup>ETEC Corporation, Hayward, California 94545.

osmium tetroxide for 2 hours at room temperature, briefly rinsed in distilled water, dehydrated through a graded series of ethanol and acetone, and embedded in Epon/Araldite (6).

Other samples were fixed in cool 2.5% gluteraldehyde in 0.05 M sodium cacodylate, pH 7.3, and 5 mM KCl and 2.5 mM MgCl<sub>2</sub> (7), rinsed three times in cool 0.1 M sodium cacodylate buffer, pH 7.3, postfixed in 1% cacodylate buffered osmium tetroxide for 2 hours, rinsed in distilled water, and stained in block with 1% aqueous uranyl acetate for 2 hours before dehydration through a graded series of ethanol and acetone. Fixation and prestain procedures were done in ice buckets. Tissues were embedded in Epon/Araldite (6) as before.

The blocks were trimmed and sectioned with glass knives in a Reichert Om-U2 ultramicrotome. The thin sections (60-150 nanometers) were stained for 10 minutes with 5% uranyl acetate-50% ethanol and with Reynolds lead citrate for 5 minutes.

All sections were examined in a Philips 201 transmission electron microscope at 60 KV. Pictures were taken by using Cronar Ortho S Litho film. Thin sections (1.0 micrometers) were cut and stained with toluidine blue; photomicrographs were taken with an Olympus Photomicrographic system camera model PM-10-M, using color Kodak film number 2483.

## RESULTS

### Light Microscope

#### Embryonic Period (30th to 50th day of gestation)

The primitive respiratory system by the 30th day of gestation was seen as an elongated outgrowth of the foregut (figures 1 and 2). A stratified epithelium covered both the digestive and respiratory tract. Cilia were not present in the future respiratory epithelium. The respiratory bud was surrounded by mesenchymal cells, some of which had differentiated into pre-cartilage tissue (figure 1). Early blood vessels were present, containing nucleated red blood cells (figure 1). The respiratory primordium, future trachea and extrapulmonary bronchi had a tubular origin and were expanded distal extremity (figure 2) (region of the future lung parenchyma). Glycogen content was prominent in the epithelial cells.

#### Pseudoglandular Period (50th to 120th day of gestation)

By the 50th day of gestation the lung parenchyma appeared similar to a developing gland with the presence of branching endodermal buds (figure 3). A small lumen was present in some of the earlier formed portions but had not formed in the advancing portions. Most of the endodermal buds were surrounded by a thin layer of smooth muscle (figure 4). Mesenchymal cells in different degrees of development

surrounded the endodermal buds, separating them from neighboring buds. Thick pseudostratified columnar or simple columnar epithelium covered the luminal surfaces of the first and second order endodermal buds (figure 4). The epithelial cell nuclei mainly occupied a luminal position and displayed a clear cytoplasm with Harrys' hematoxylin and eosin stain. With McManus' stain, the epithelial cell cytoplasm stained an intense red, indicating a probable glycogen presence, and a primitive basement membrane appeared at their base.

Intrapulmonary bronchi were lined with pseudostratified columnar non-ciliated epithelium and were surrounded by smooth muscle fibers plus plaques of hyaline cartilage. Sections of arteries, veins (with unnucleated red cells) and nerves were present (figure 5). No elastic fibers were seen with Verhoeff's staining method (figure 6). The pleural mesothelium was very thin (approximately 2 micrometers) with a scant amount of connective tissue separating it from lung parenchyma. Vascularization was not prominent. Around the endodermal buds, lymph channels were seen in a subpleural position (figure 3), and no lobulation was present.

Primitive or early separation of the lung parenchyma into lobules was apparent at the 70th gestational day (figure 7). The lumens of endodermal buds were enlarged and branching was progressing. An increase in vascularization appeared around the endodermal buds, epithelial cell glycogen was still present in large amounts and an imprecise basement membrane was present (figure 9). Lymph channels were found both in a subpleural position and between lobules.

Mesenchymal connective tissue was still abundant between endodermal buds, but no elastic fibers were seen at this age.

Different degrees of endodermal bud development were present by the 90th gestational day (figure 8). Vascularization increased, glycogen content slightly decreased, and the pleura composed of mesothelium and collagen fibers was thicker. No elastic fibers were present at this age, but pleural indentations indicated the future division into lobules (figure 8).

There was a marked increase of pulmonary parenchyma by the 120th gestational day and an architectural change representing an advance into the Pseudoglandular Period (figure 10). The lumens were more enlarged and vascularization increased. The epithelial cells of the endodermal buds were lower and showed less glycogen (figure 12). No elastic fibers were present at this age (figure 11), but lymph channels were better organized in the mesenchymal tissue.

#### Canalicular Period (120th to 180th day of gestation)

By the 150th day of gestation the lung parenchyma changed from a glandular appearance into the canalicular architecture (figure 13). The lumens were enlarged; the epithelial cells were reduced in height and organized into pseudostratified columnar, simple columnar and simple cuboidal arrangements. The larger conducting tubes (future bronchi and bronchioles) were established. The epithelium in the future terminal air sac was lost or degenerating. In some places the epithelium became flatter (figure 14).



Vascularization was increased by a process in which capillaries surrounded the air-ways and pushed into the epithelium, making it thinner. Mesenchymal tissue was abundant between air-ways (figure 14). The epithelial cells were non-ciliated, their glycogen content decreased and the basement membrane was more precise. Very thin elastic fibers were seen in the artery walls as a component of the internal elastic lamina. The pleura was thicker due to the presence of more connective tissue fibers (mainly collagen and some elastic fibers).

Advanced development of the Canalicular Period was apparent by the 165th gestational day (figure 15). The lung parenchyma showed characteristics of the previously described stages and in the older development sites there was enlargement of the future air-way lumens, reduced mesenchymal tissue, increased vascularization, and decreased epithelial cell glycogen content.

#### Terminal Sac Period (180th to 220th day of gestation)

Between the 180th to 220th day of gestation a change in architecture occurred, typical of the Terminal Sac Period (figures 16 and 17). Primitive sacculles formed, by the protrusion of thick septa (approximately 9-14 micrometers) from the walls, dividing the future air-space into sacculles with irregular outlines (figure 18). The intersaccular septa were characterized by the presence of capillaries, connective tissue cells, and the possible presence of type I and type II pneumocytes (figure 18).

The connective tissue between future air-ways decreased (figure 17). Cilia were evident in the main bronchi. Lymph channels were present in

a subpleural position and between lobules. The pleural mesothelium was associated with additional connective tissue fibers making the pleura thicker. Respiratory and terminal bronchioles and saccules were evident at this stage (figure 21).

Branching air-passages developed into alveolar ducts and saccules later in this period. Air-spaces were separated by thick walls of abundant mesenchyme.

Blood vessels began to appear in closer relationship to air-passages (figure 20). Glycogen content decreased in the epithelial cells and a precise basement membrane was seen at this stage (figure 19).

#### Alveolar Period (220th to 260th day of gestation)

By the 240th day of gestation a change in architecture appeared which was characterized by the presence of alveolar ducts and early alveoli (figure 22). The lung parenchyma acquired a definitive shape, and primitive alveoli formed by the presence of thin septa (approximately 4-6 micrometers) derived from the intersaccular septa and the alveolar duct walls (figure 23). Mesenchymal stroma around the alveoli was gradually replaced by a thin network of fibers, fibroblasts, macrophages, type I and type II pneumocytes, and capillaries (figure 24). Vascularization was very prominent at this age. The pleura had an adult histologic appearance. Elastic fibers were present in the walls of blood vessels and in the tips of interalveolar septa (figure 23). The intrapulmonary bronchi and orders of bronchioles presented the characteristic epithelium of adult lungs.

By the 260th day of gestation a more advanced stage in the Alveolar Period appeared, characterized by increased alveolarization of the lung parenchyma (figures 25 and 27). Thinner interalveolar septa (approximately 2-4 micrometers) (figure 26) containing elastic fibers in their tips were seen at this age (figure 28).

### Transmission Electron Microscope

#### Canalicular Period (150th day of gestation)

The epithelial lining of the endodermal bud was composed of columnar cells with irregular shaped nuclei located adjacent to luminal surfaces. Nucleoli and dispersed chromatin were present in the nuclei. Junctional complexes between epithelial cells were located close to their luminal surface (figure 29). Epithelial cell cytoplasm had few or no organelles characteristic of adult cells. No cilia were present at this age. Interdigitations occurred between the basal part of the epithelial cells and a primitive basement membrane, which contained collagen fibers (figure 30). Fibroblasts, muscle cells, capillaries and endothelial cells were present in the pulmonary interstium. Stromal cells showed a well structured cytoplasm containing mitochondria and other organelles (figure 31).

#### Terminal Sac Period (210th day of gestation)

Type I and type II pneumocytes lined the saccular wall (figure 32). Type I pneumocytes were characterized by large nuclei with small indentations surrounded by a thin cytoplasm containing mainly mitochondria

and rough endoplasmic reticulum plus thin, long cytoplasmic extensions which covered most of the saccular wall. Vesicles were prominent in the type I pneumocyte cytoplasm.

Type II pneumocytes protruded into the lumen of a sacculle, had regular nuclei, each with a nucleolus, and no cytoplasmic extensions. Their cytoplasm contained rounded structures similar to lamellar bodies but not differentiated into the adult form. These cells had few organelles and an appearance of cytoplasmic degeneration. Junctional complexes were present between type I and type II pneumocytes (figure 32). The thick intersaccular septa contained capillaries with red blood cells, agranular and granular leutocytes (figure 33). The endothelial cells contained cytoplasmic vesicles. Cytoplasmic extensions of type I pneumocytes covered the intersaccular septa and were joined by junctional complexes to other type I pneumocytes. A basement membrane separated the capillary walls from type I cytoplasmic extensions. Collagen fibers were seen in the intersaccular septa tip (figure 33).

#### Alveolar Period (240th day of gestation)

Thin irregular interalveolar septa appeared by the 240th day of gestation, characterized by the presence of irregular contours made by small evaginations and invaginations of the type I pneumocyte. Cytoplasmic extensions contained different sized vesicles (figure 34). Junctional complexes between epithelial cells were seen and a basement membrane separated capillaries and other cells from the type I pneumocyte. Capillaries present in the septa showed vesicles in

the endothelial cell cytoplasm. Fibroblasts contained elongated nuclei and a cytoplasmic fibrillar component. Collagen and elastic fibers occurred close to the septal tip and deeper in the septa (figure 35). A type II pneumocyte in an interalveolar septum had characteristic lamellar bodies in its cytoplasm (figure 35). Junctional complexes were seen between the basal part of the type II pneumocyte and the cytoplasmic extension of type I pneumocyte. Developing alveolar buds arose from the saccular septa (figure 35).

(260th day of gestation)

Thin interalveolar septa were seen at the 260th day of gestation protruding into formed alveolar lumens (figure 36). These septa were characterized by the presence of connective fibers (collagens and elastics) in their tips and capillaries (with blood cells) composed of the endothelial cells with cytoplasmic vesicles. Cytoplasmic extensions of type I pneumocytes covered most surfaces of the interalveolar septa. Junctional complexes were present. Type I pneumocytes located in the septa were characterized by irregular nuclei surrounded by a small amount of cytoplasm, containing vesicles and very long cytoplasmic extensions (figure 37). A type II pneumocyte in the interalveolar septum contained a round nucleus which had a nucleolus and compacted chromatin located close to the nuclear envelope. The cytoplasm had distinct organelles, including many lamellar bodies. The type II pneumocyte was covered by type I pneumocyte cytoplasmic extensions which presented evaginations (figure 38). Tight junctions were present between these two epithelial cells.

## Scanning Electron Microscope

### Pseudoglandular Period

By the 70th day of gestation the lung tissue had a rough appearance caused by the loose nature of the mesenchymal connective tissue (figure 39). A few lumens were seen and epithelium was very difficult to distinguish.

By the 90th day of gestation the cut fetal lung surfaces were rough with scattered holes and narrow crypts. Cross sections of future air-way tubules showed the presence of epithelial cells surrounded by mesenchymal connective tissue (figure 40). Cilia were not apparent.

### Canalicular Period

After 150 days of gestation the cut fetal lung surfaces presented different sized holes and narrow crypts, characteristic of structures during the Canalicular Period (figures 41 and 42). A change in architecture was seen at this period of lung development. Increased amounts of mesenchymal connective tissue had developed between holes and crypts and presented a rough appearance to the cut surface. Branching tubes were seen.

By 180 days of gestation the number of future air-way tubules had increased greatly (figure 43). Although substantial interstitial tissue was present, the lumina of some air-way tubules had enlarged (figure 44).

### Terminal Sac Period

By 220 days of gestation another morphologic change was seen in the lung parenchyma (figure 45). Primitive saccules could be identified but air-way tubules similar to those of earlier gestation periods persisted. The saccular walls were thick, some cells (possibly macrophages) protruded into the saccular lumen, and a ridged surface was seen due to the presence of underlying capillaries (figure 46). Interstitial tissue was considerably reduced.

### Alveolar Period

Different degrees of lung parenchyma maturation were revealed at 250 days of gestation. Alveolar-like structures could be identified which had thin interalveolar septa walls and interstitial stroma (figure 47). Cut surfaces were very irregular because of the presence of connective fibers. In some places, especially close to the pleura, thick walls and irregular shaped saccules were present (figure 48).

The presence of large irregular shaped cells (probably amniotic desquamated cells) without cytoplasmic extension were seen in the primitive alveolar walls. The epithelial cellular margins were slightly raised above the surface in the alveoli (figures 49 and 50).

At 270 days of gestation thicker interalveolar walls showed, indicating that development is a gradual process and different degrees of maturity can be seen (figures 51 and 52).

## DISCUSSION

The approximate times and morphologic appearances of the different bovine fetal lung developmental stages were defined for the first time using light, transmission, and scanning electron microscope. Little differences occurred between times of onset and range for the human and bovine lung development stages, except in the Terminal Sac Period, where marked differences appeared between the two species.

Bovine stages can be compared with results previously established for other domestic animals and man (38), which are shown in table 1.

In man, the Embryonic Period appears during the 4th week of gestation and, at birth, only 8% of the total number of adult alveoli are present. By the 30th day of gestation in the bovine, the Embryonic Period of lung development was seen as an elongated endodermal growth from the primitive foregut, surrounded by mesenchymal tissue with early differentiation of precartilage and blood vessels.

Morphologic changes from embryonic structures to those characteristic of the Pseudoglandular Period were seen by approximately the 50th day of gestation. Endodermal buds had the appearance of a gland with rounded, branching structures covered by pseudostratified columnar or simple cuboidal epithelium without cilia. Some of the endodermal buds were surrounded by muscle cells, which probably represents the development of these structures into future air-conducting portions of the



TABLE 1

Onset and Range of Times for Fetal Lung Development Stages

Species	Pseudoglandular	Canalicular	Terminal Sac
-----Days of Gestation-----			
Human	112	112-168	168
Rabbit	124	24-24	27
Sheep	95	95-120	120
Rat	18	19-20	21
Bovine	50-120	120-180	180-220

respiratory tract. Intrapulmonary bronchi surrounded by hyaline cartilage plaques, blood vessels, lymphatic channels in a subpleural position, nervous components, and a large amount of glycogen were present at this stage. The glycogen content may be needed as an energy source for the rapid mitotic process going on during the first stages of fetal lung development.

The characteristic morphology of the Pseudoglandular Period showed progressive developmental advancement until the 120th day of gestation when the lumens enlarged and the pulmonary parenchyma increased.

A completely different architecture was seen during the Canalicular Period, which started approximately at the 120th day of gestation and continued until the 180th day of gestation. The pulmonary parenchyma was more vascular and the enlarged lumens were characterized by epithelium which had nuclei in an apical position, absence of cilia, and degeneration of superficial cells. Intracellular glycogen concentration decreased and may be due to the decreased mitotic rate. No elastic fibers were present during the developmental stages previously cited. The connective tissue component was mainly restricted to the presence of collagen fibers.

After the 180th day of gestation the Terminal Sac Period became apparent by another change in architecture. The appearance was completely changed by the formation of the saccules separated by thick septa. The septa were composed mainly of connective tissue cells, collagen fibers, capillaries, elastic fibers which appeared for the first time during this stage, and type I and type II pneumocytic epithelial cells. The type II pneumocytes of the intersaccular septa

had cytoplasmic lamellar bodies undifferentiated and could be distinguished from the macrophage by the presence of epithelial junctional complexes. Transport vesicles were present in the cytoplasm of endothelial cells and type I pneumocytes.

The Alveolar Period started during fetal life at approximately the 220th day of gestation and continued, for an as yet undetermined time, into the bovine postnatal period. Differences between the Terminal Sac Period and the Alveolar Period could be identified based on the different histologic appearances of these stages. In the Alveolar Period, the interalveolar septa were thinner and were characterized by an increase of connective tissue fibers close to the septal tips, where they play a supporting role of the septa; and type I and type II pneumocytic cells appeared in the septa and primitive alveolar walls. Lamellar bodies showed characteristics typical of those seen in the type II pneumocytes of mature lungs.

Through all lung developmental stages, several progressive development phases could be seen. When subsequent periods of lung development appear in one area of the lung, the earlier stages of development continue in other areas.

Scanning electron microscopy studies supported this concept and showed that by the 270th day of gestation, not only were alveoli present in the lung parenchyma but also saccule formation was seen (figures 51 and 52). Different stages of development in the same fetal lung support the idea that the lung differentiation process occurs in a centrifugal manner (from the hilus to the periphery) especially in the first stages of lung development, and a centripetal manner during the alveolarization

process. Thus, lung maturation is a progressive process in which all stages may appear in the Alveolar Period, with the later stages seen throughout the central portions and the earlier stages at the periphery.

Problems with sectioning and fixation were confronted. The fixation in 2.5% gluteraldehyde in 0.05 M sodium cacodylate, pH 7.3, and 5 mM KCl and 2.5 mM MgCl<sub>2</sub> (7) was the best fixation method for bovine fetal lung tissues. Temperature of the fixative was critical in the fixation procedure. Cool temperatures (approximately 4°C) gave the best cellular preservation. Problems in fixation were more accentuated in earlier stages of gestation than the later ones, possibly because the cytoplasmic organelles were not mature enough or were more susceptible to environmental changes. In all stages of development, good preservation of nuclei (nuclear membranes) and plasma membranes was evident. During the Canalicular Period, fixation problems were seen in the epithelial cells which covered the future air-ways tubes, but interstitial cells were well preserved showing good fixation of mitochondria and other cytoplasmic organelles (figure 31). Fixative agents first act on the surfaces of an organ or in this case on the cells which are in contact with lumen. Problems in fixation presented difficulties because of the natural epithelial degeneration which seems to be the mechanism by which lumens are enlarged. This mechanism is similar to the process by which exocrine gland ducts are formed.

Weigert's method for elastic fibers was not useful in our laboratory as a specific stain of this kind of connective tissue fibers, but Verhoeff's method showed elastic fibers in known histological structures and in some stages of lung development.

Light microscope fixation using 2.5 gluteraldehyde in 0.05 M sodium cacodylate, pH 7.4, accentuated the staining capacity of tissues; therefore, a blue filter was needed for taking photomicrographs in order to attenuate the reddish stained tissue. It was not feasible to put fixative into fetal lungs via the trachea because the pressure of the fixative could cause distortion of normal morphology.

Problems also occurred in preparation of tissues for the scanning electron microscope in which a cut, smooth surface was required. The extreme fragility of fetal lung tissue made it difficult to obtain a smooth, cut surface. The best results were obtained by washing the fetal lungs with heparin and Ringer's solution and then fixing the tissue. This permitted a better view of the tissue.

These studies increase the understanding of the developmental stages of the bovine lung and permit a better comprehension of the fetal physiology, pathologic and teratologic problems associated with the developing bovine lung.

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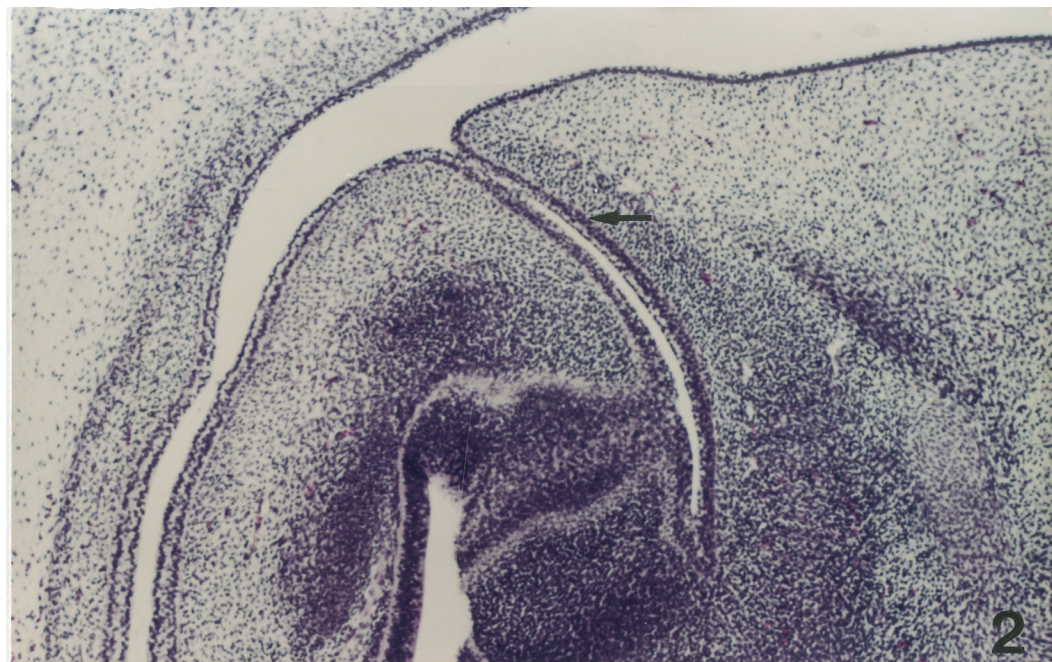
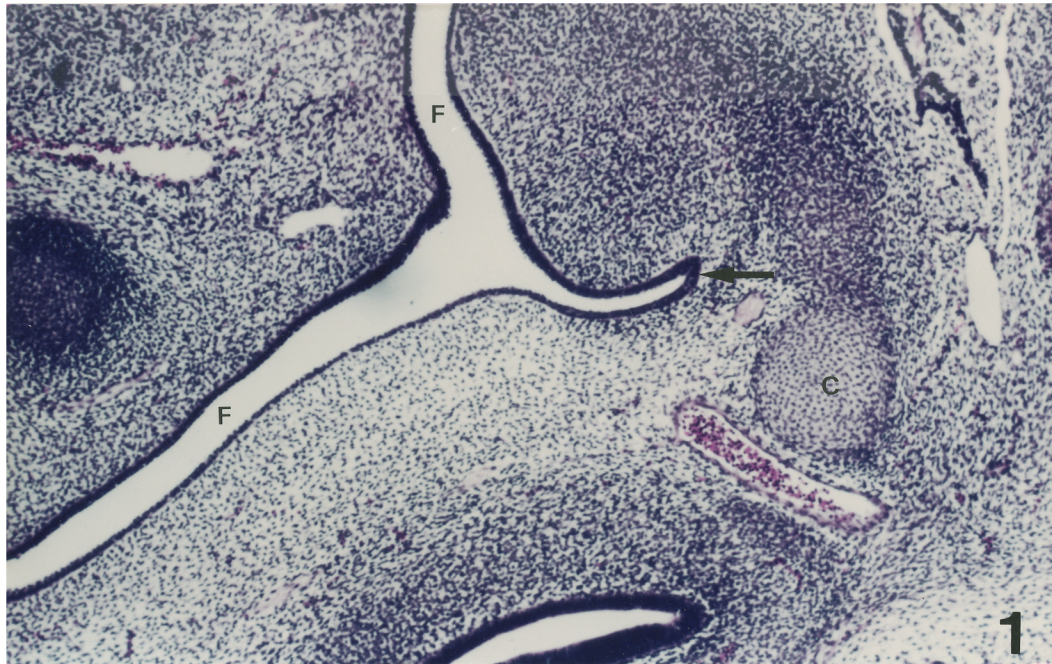
## APPENDIX

### FIGURES WITH EXPLANATIONS

## Explanation of Figures

Fig. 1. Light photomicrograph of approximately 30 day old bovine embryonic lung, showing the primitive foregut (F), respiratory diverticulum (arrow), and embryonic cartilage (C). (Harrys' hematoxylin and eosin, X40)

Fig. 2. Light photomicrograph of approximately 30 day old bovine embryonic lung, a more advanced development of the future respiratory system (arrow). (Harrys' hematoxylin and eosin, X40)

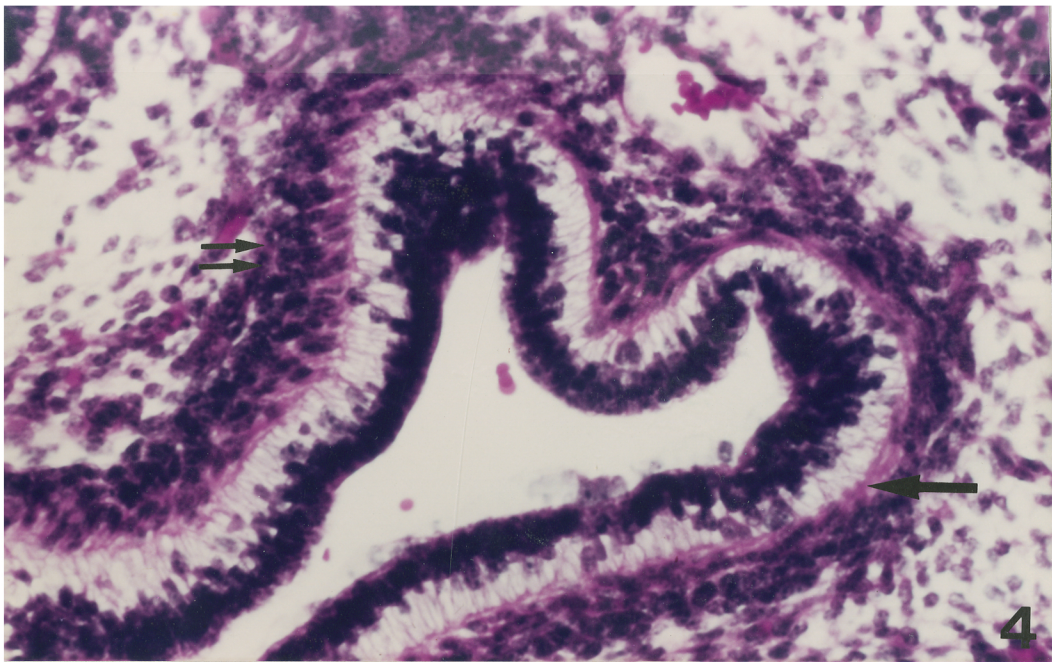
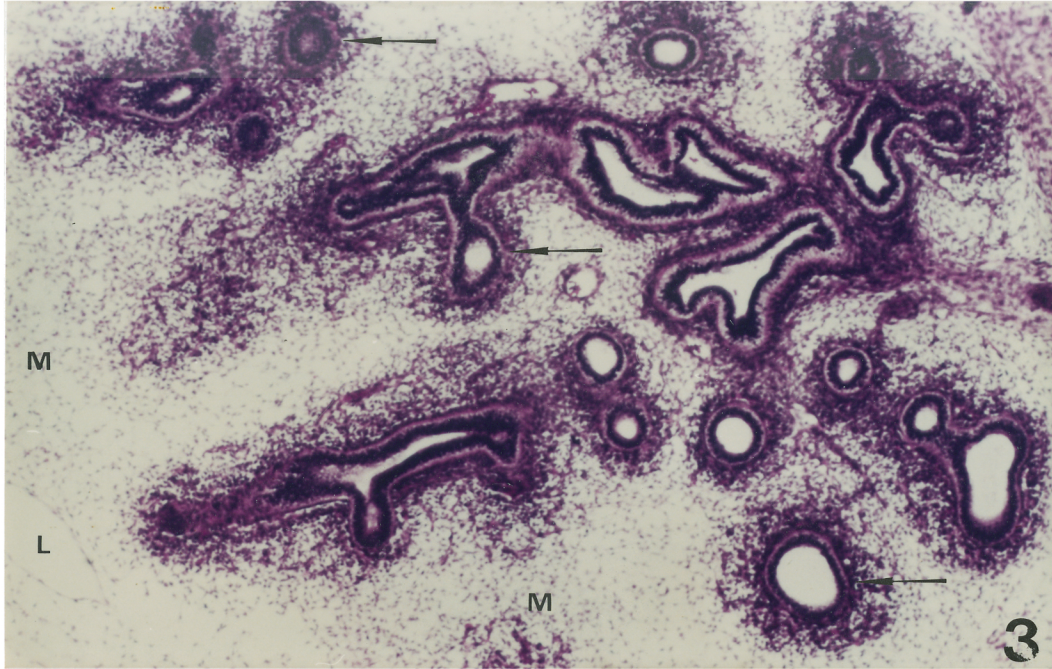


### Explanation of Figures

Fig. 3. Light photomicrograph of 50 day old bovine fetal lung, showing the characteristic architecture of Pseudoglandular Stage. Endodermal buds (arrows) surrounded by mesenchymal tissue (M), and a lymph channel (L). (Harrys' hematoxylin and eosin, X40)

Fig. 4. Light photomicrograph of 50 day old bovine fetal lung (Pseudoglandular Stage), showing a future air-conducting tube surrounded by a muscular layer (arrow) and packed mesenchymal cells (double arrow). (Harrys' hematoxylin and eosin, X200)



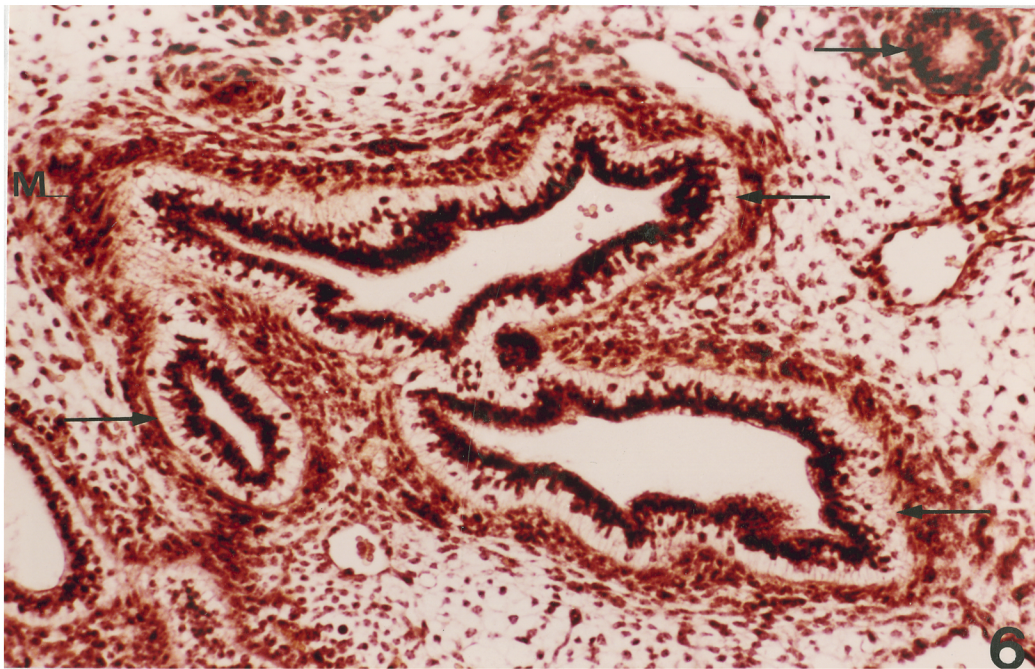
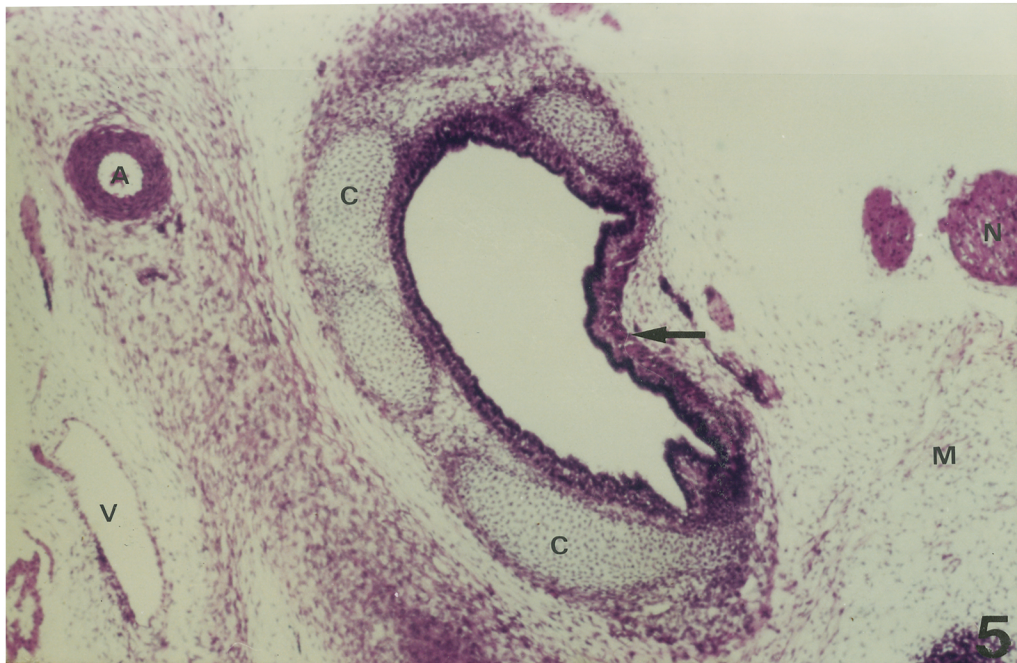


### Explanation of Figures

Fig. 5. Light photomicrograph of 50 day old bovine fetal lung (Pseudoglandular Stage), showing an intrapulmonary bronchus (arrow) surrounded by hyaline cartilage (C). Cross section of an artery (A), vein (V), nerve (N) and mesenchymal tissue (M).  
(Harrys' hematoxylin and eosin, X100)

Fig. 6. Light photomicrograph of 50 day old bovine fetal lung (Pseudoglandular Stage), showing different degrees of endodermal bud's development (arrows) with a pseudo-stratified columnar epithelium where the majority of the nuclei occupied the cell's apical poles. Mesenchymal cells surround the endodermal buds, forming a very compact layer (M). Elastic fibers are not apparent.  
(Verhoeff's method, X100)



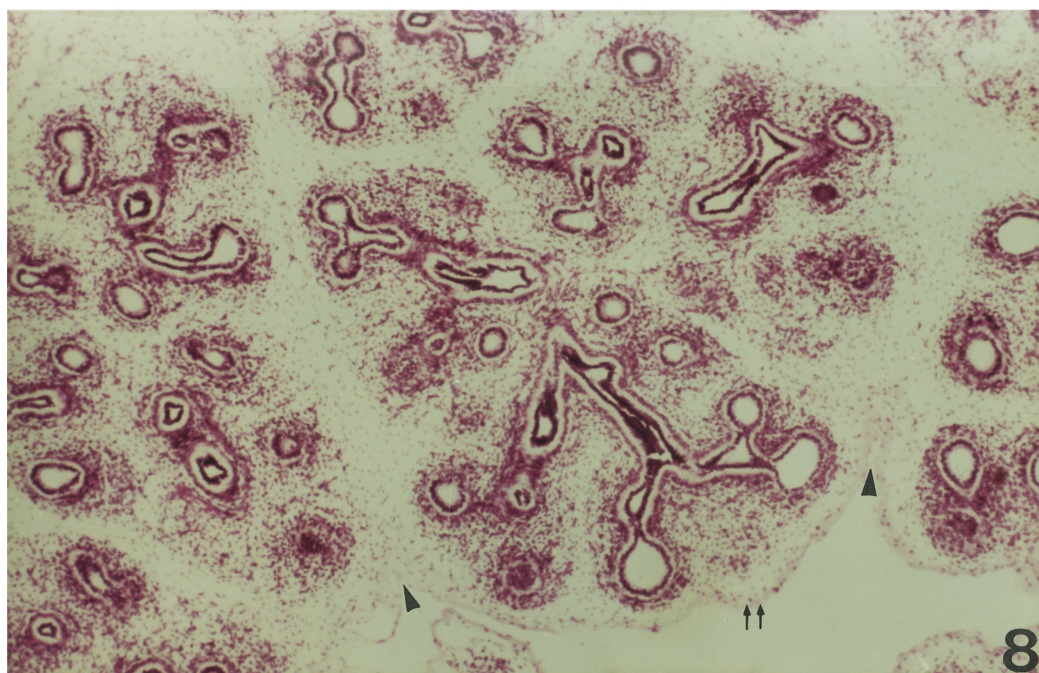
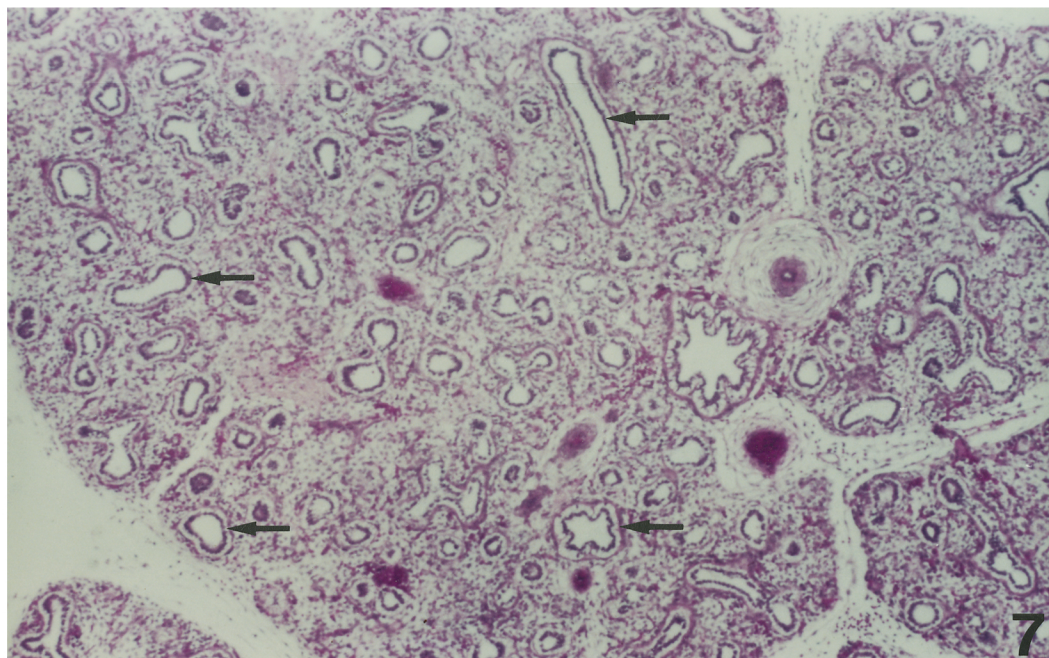


## Explanation of Figures

Fig. 7. Light photomicrograph of 70 day old bovine fetal lung, showing the gradual change of architecture which is going on during the Pseudoglandular Stage. The lumen of the air-conducting tubes is enlarged (arrows). The lining epithelium tends to be lower, vascularity is increased, and lobularity is present at this age.  
(Harrys' hematoxylin and eosin, X40)

Fig. 8. Light photomicrograph of 90 day old bovine fetal lung, showing a more advanced stage of the Pseudoglandular Stage. Lobulation (arrow) and pleura (double arrow) are present.  
(Harrys' hematoxylin and eosin, X40)



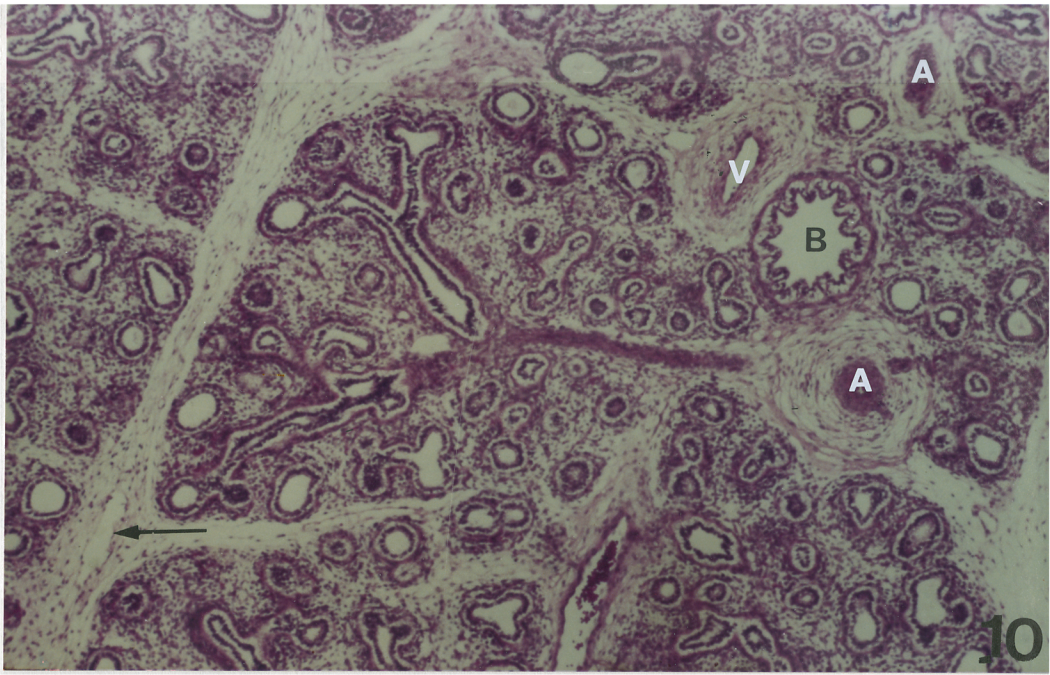
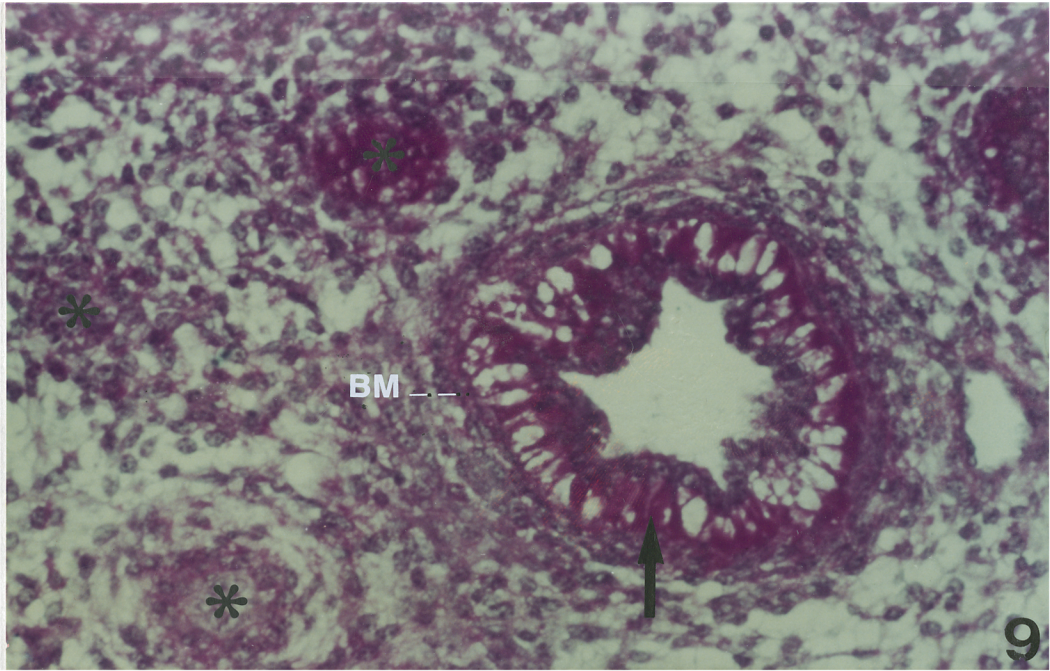


### Explanation of Figures

Fig. 9. Light photomicrograph of 90 day old bovine fetal lung, showing an air-conducting tube with a primitive basement membrane (BM). Glycogen is present in the cytoplasm of epithelial cells (arrow). Different degrees of bud endodermal development are also present (\*).  
(McManus' method, X200)

Fig. 10. Light photomicrograph of 120 day old bovine fetal lung, showing the general appearance of Pseudoglandular Stage. Bronchiole (B), vein (V), arteries (A), and lymphatic channel (arrow) are present between lobules.  
(Harrys' hematoxylin and eosin, X40)



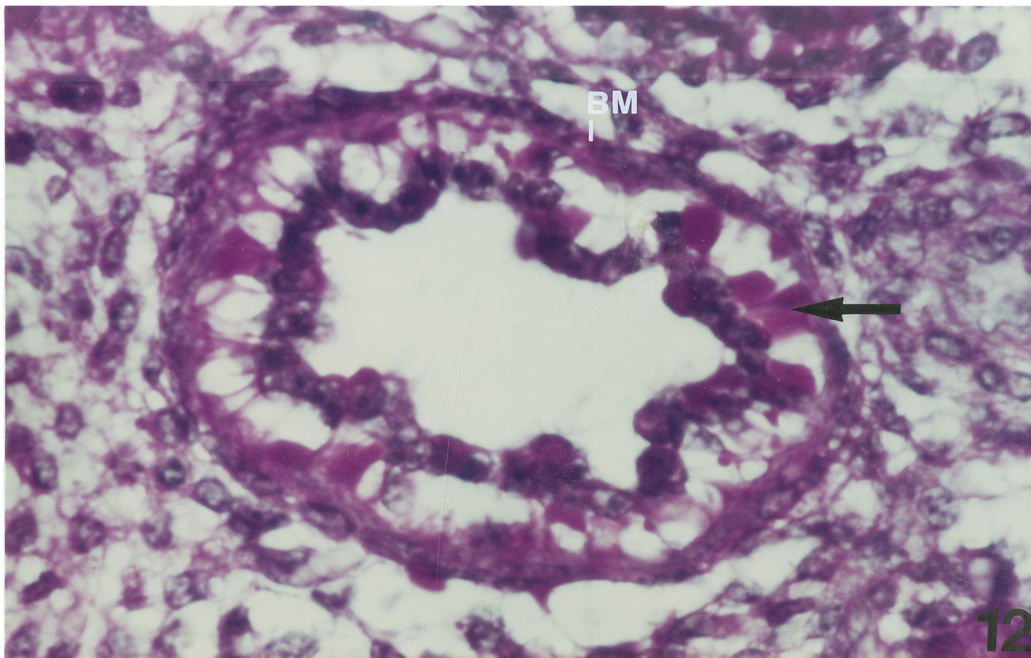
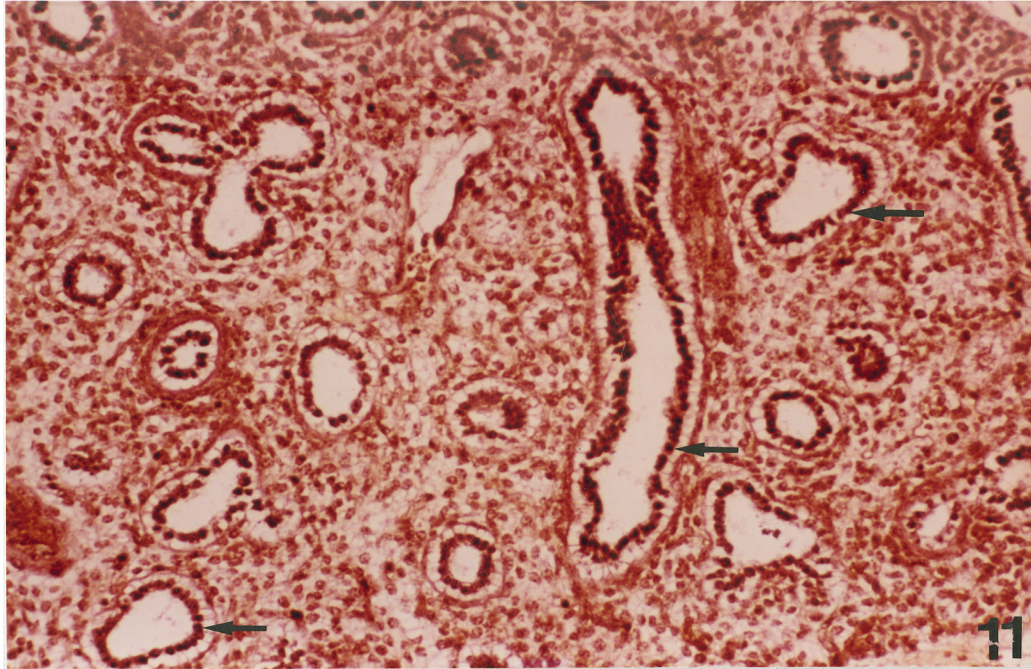


### Explanation of Figures

Fig. 11. Light photomicrograph of 120 day old bovine fetal lung (Pseudoglandular Stage), showing black stained nuclei of the air-conducting tube's columnar epithelium (arrow). No elastic fibers have appeared in the lung parenchyma. (Verhoeff's method, X100)

Fig. 12. Light photomicrograph of 120 day old bovine fetal lung (Pseudoglandular Stage), showing a cross section of an air-conducting tube. The epithelial cells contain less glycogen (arrow) than previous ages and the basement membrane is not well differentiated (BM). (McManus' method, X400)



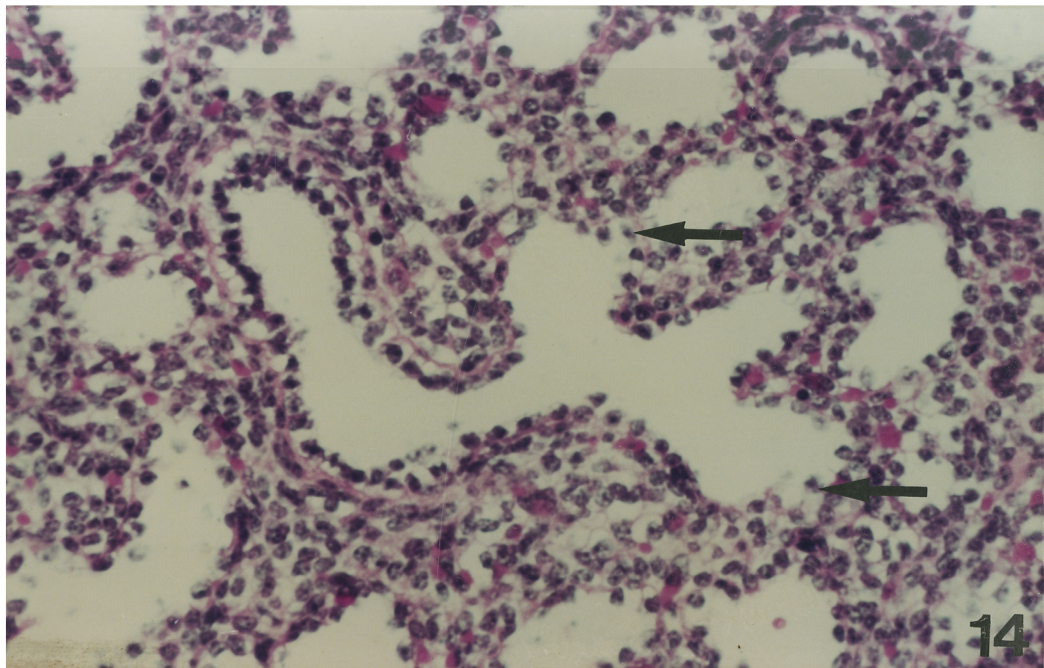
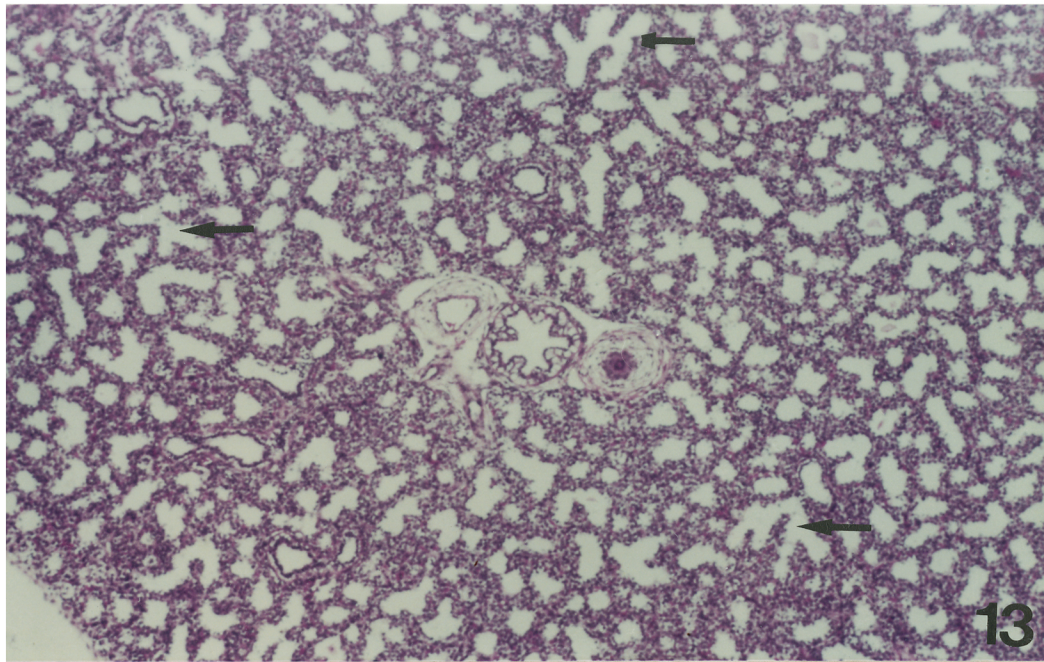


### Explanation of Figures

Fig. 13. Light photomicrograph of 150 day old bovine fetal lung, showing the general architecture of the Canalicular Stage. The lumens of the air-conducting system are enlarged (arrows). Connective tissue between the air-conducting tubes is very cellular.  
(Harrys' hematoxylin and eosin, X40)

Fig. 14. Light photomicrograph of 150 day old bovine fetal lung, showing the process of canalization. Some epithelium of the air-conducting tubes is irregular and has a degenerating appearance (arrows).  
(Harrys' hematoxylin and eosin, X200)





### Explanation of Figures

Fig. 15. Light photomicrograph of 165 day old bovine fetal lung.

No elastic fibers appear in the Canalicular Stage and the connective tissue shows hypercellularity (\*). An amniotic cell is present in the lumen of one air-conducting tube (arrow).

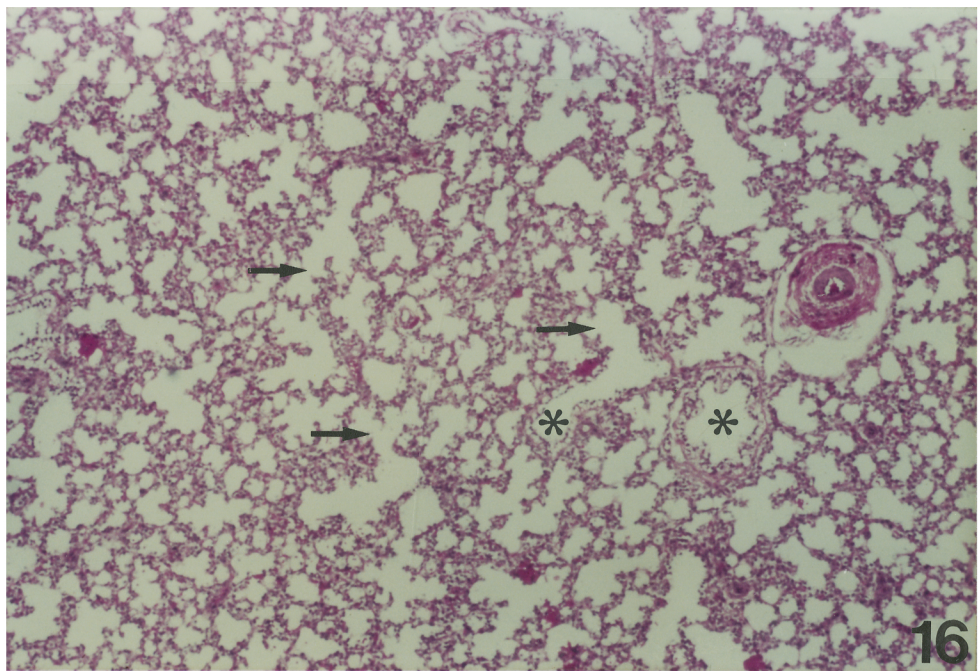
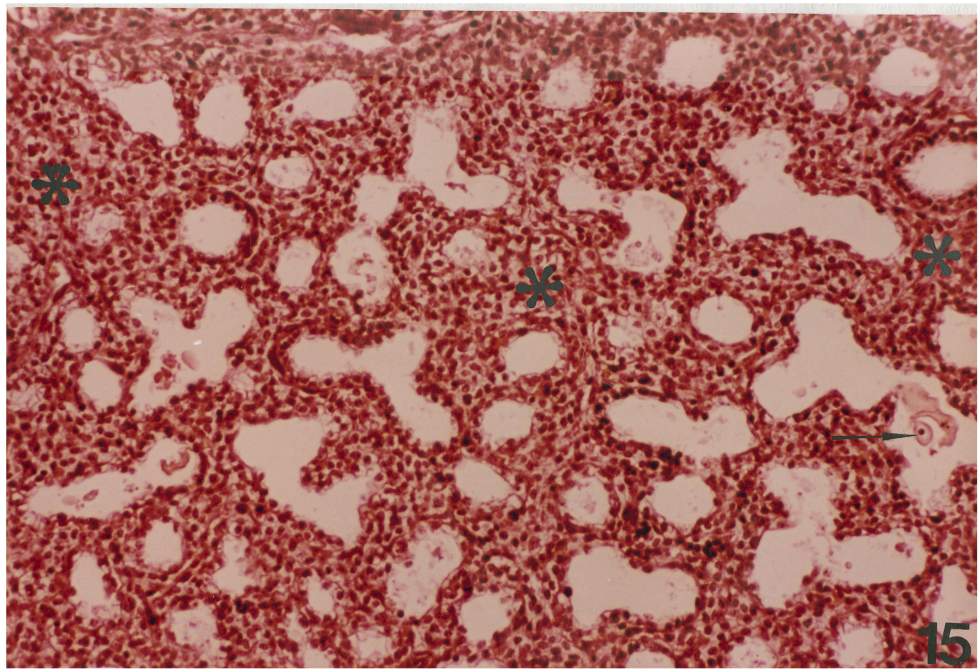
(Verhoeff's method, X100)

Fig. 16. Light photomicrograph of 220 day old bovine fetal lung,

showing the characteristic architecture of the Saccular Stage. In this stage airways (\*) and primitive respiratory portions of the lung (arrows) can be distinguished.

(Harrys' hematoxylin and eosin, X40)



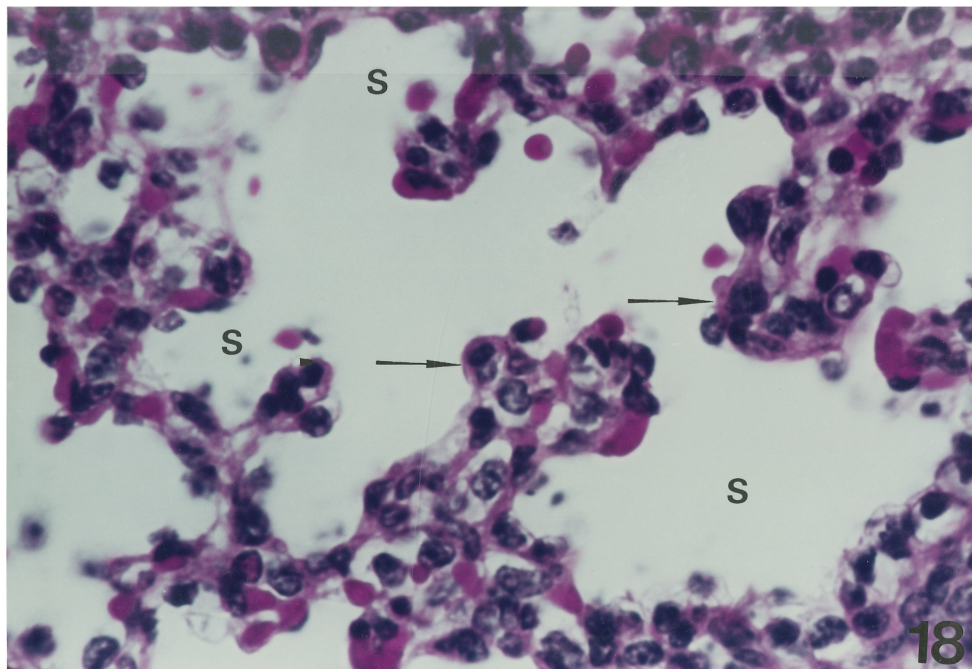
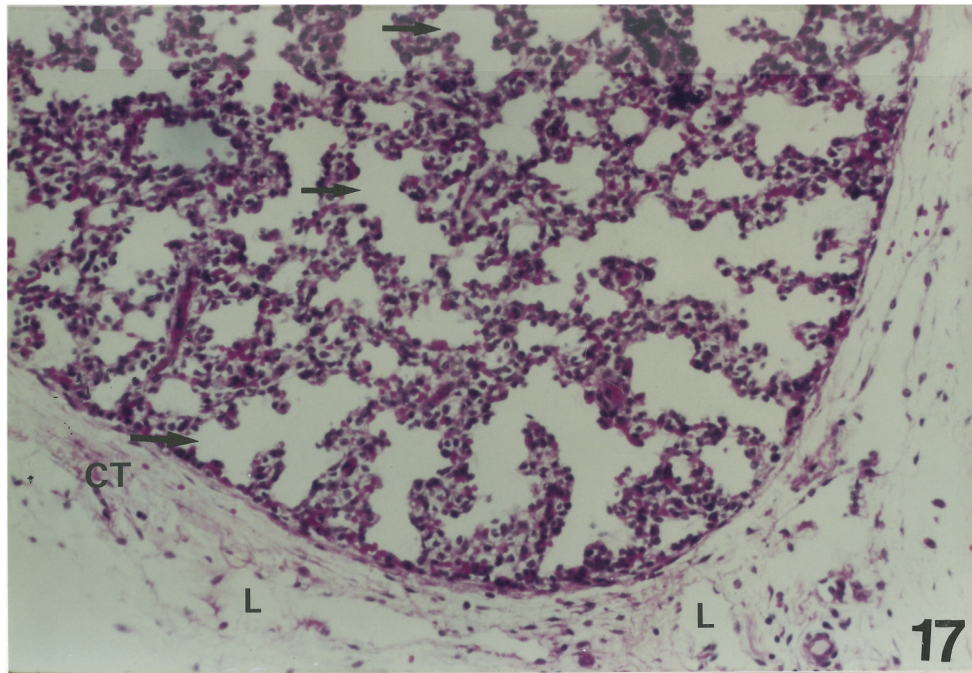


### Explanation of Figures

Fig. 17. Light photomicrograph of 220 day old bovine fetal lung (Saccular Stage), showing the presence of irregular shaped saccules (arrows) and increased vascularity around the future respiratory portions of the lung. Connective tissue (CT) separates lobules and lymphatic channels (L) are present.  
(Harrys' hematoxylin and eosin, X100)

Fig. 18. Light photomicrograph of 220 day old bovine fetal lung (Saccular Stage), showing saccularization (S) and thick septa (arrows) which induce the formation of saccules.  
(Harrys' hematoxylin and eosin, X400)

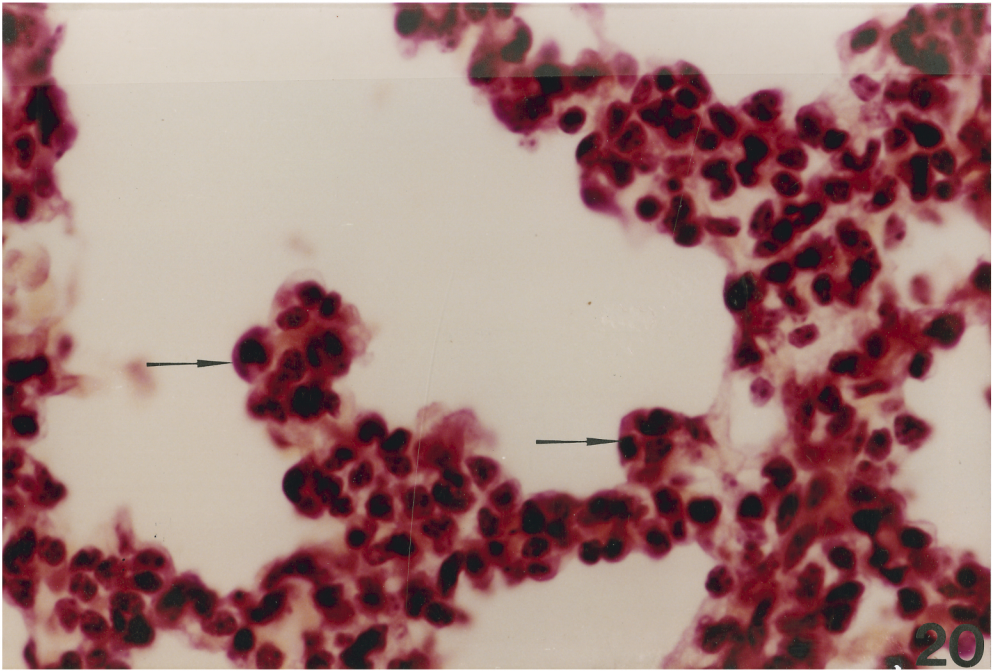
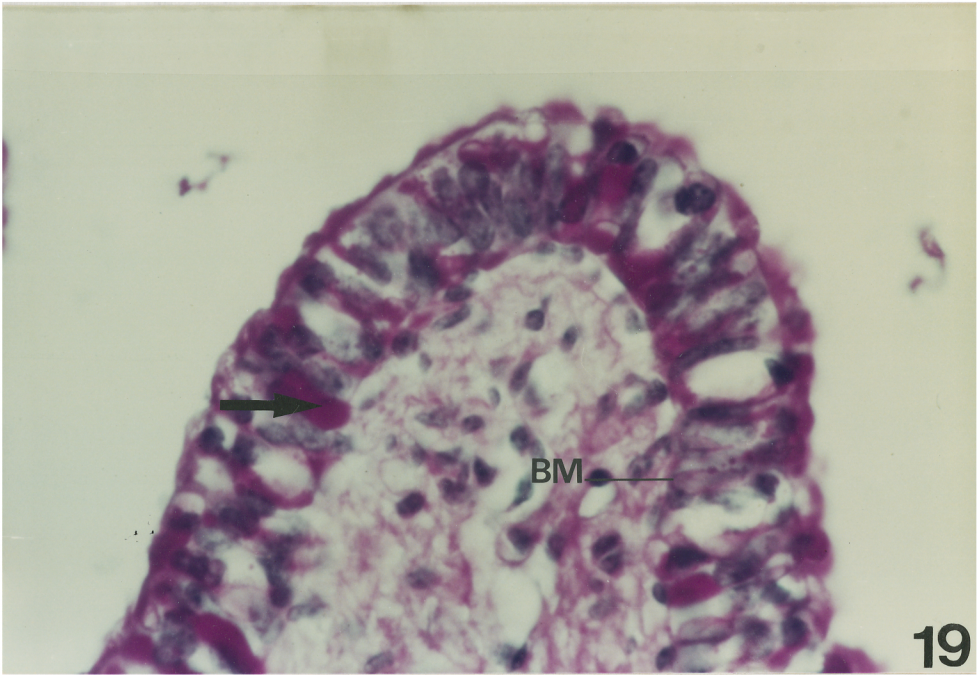




### Explanation of Figures

Fig. 19. Light photomicrograph of 220 day old bovine fetal lung (Saccular Stage), showing traces of glycogen in the epithelial cells (arrow) and a well defined basement membrane (BM).  
(McManus' method, X400)

Fig. 20. Light photomicrograph of 220 day old bovine fetal lung (Saccular Stage), showing the intersaccular septa (arrows) containing a large number of nuclei black stained and the absence of elastic fibers.  
(Verhoeff's method, X400).

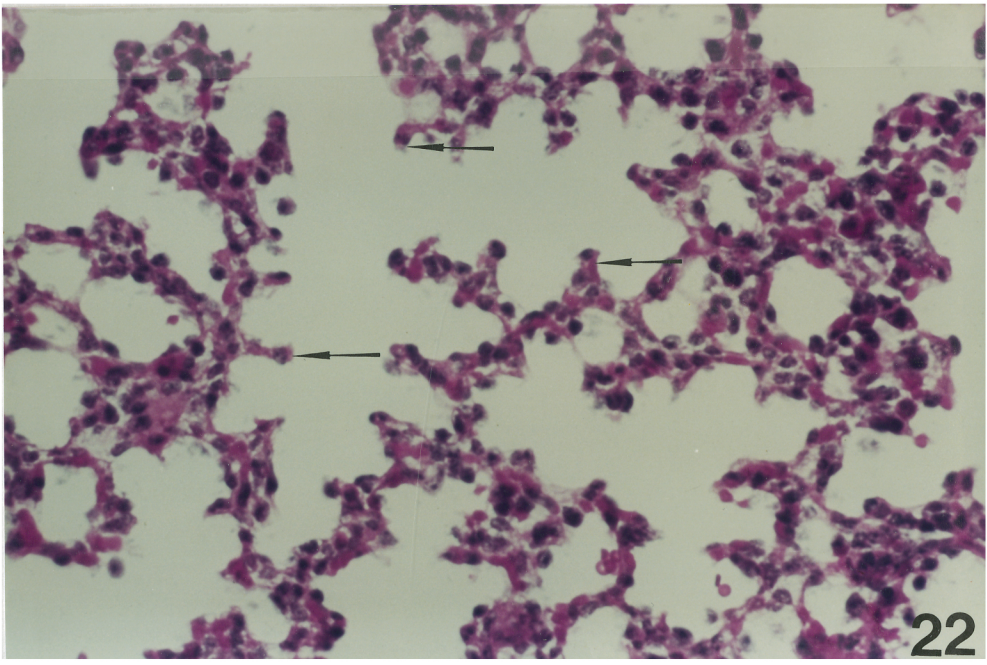
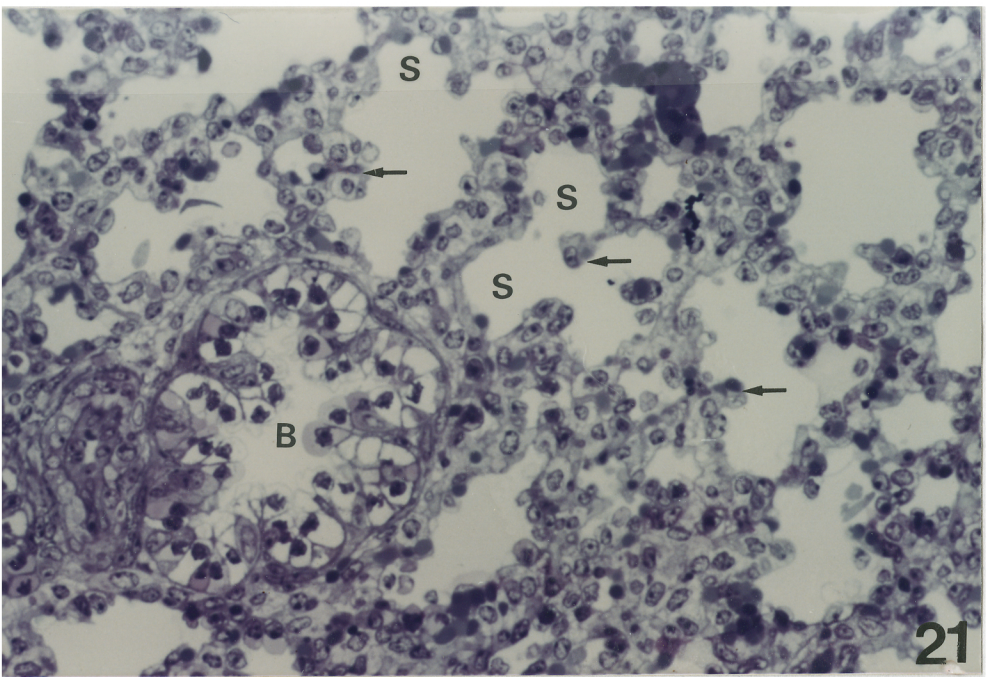


### Explanation of Figures

Fig. 21. Light photomicrograph of 220 day old bovine fetal lung (Saccular Stage), showing a bronchiole (B), saccules (S), and saccular septa (arrow). Type I and type II pneumocytes are difficult to distinguish at this stage. (Toluidine Blue's stain, X200)

Fig. 22. Light photomicrograph of 240 day old bovine fetal lung, showing the general appearance of the Alveolar Stage. A change in architecture from the saccular stage can be appreciated. Inter-alveolar septa (arrows) are thinner than those of previous stages. (Harrys' hematoxylin and eosin, X100)

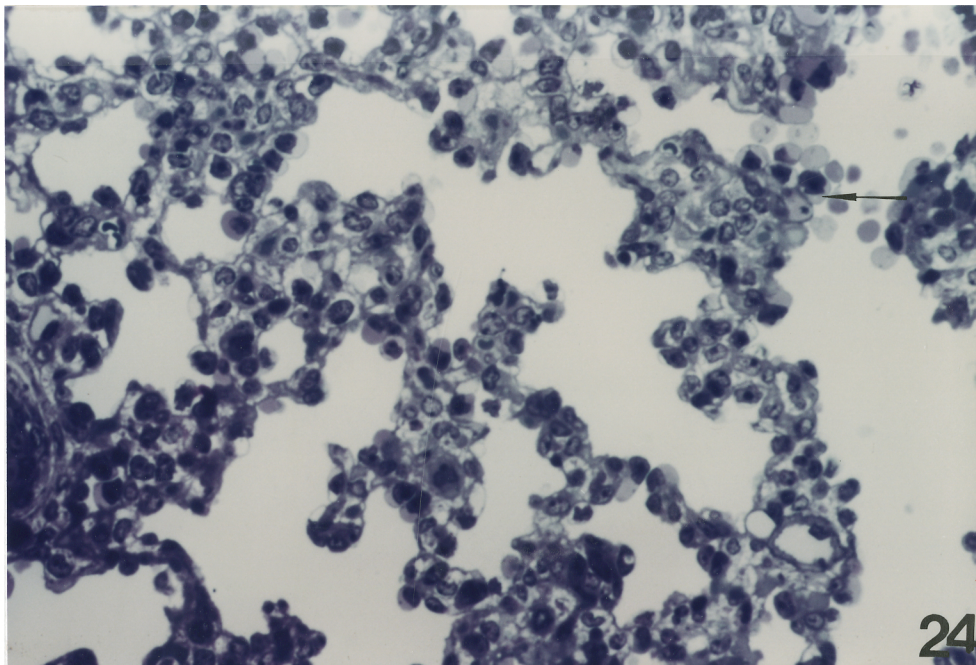
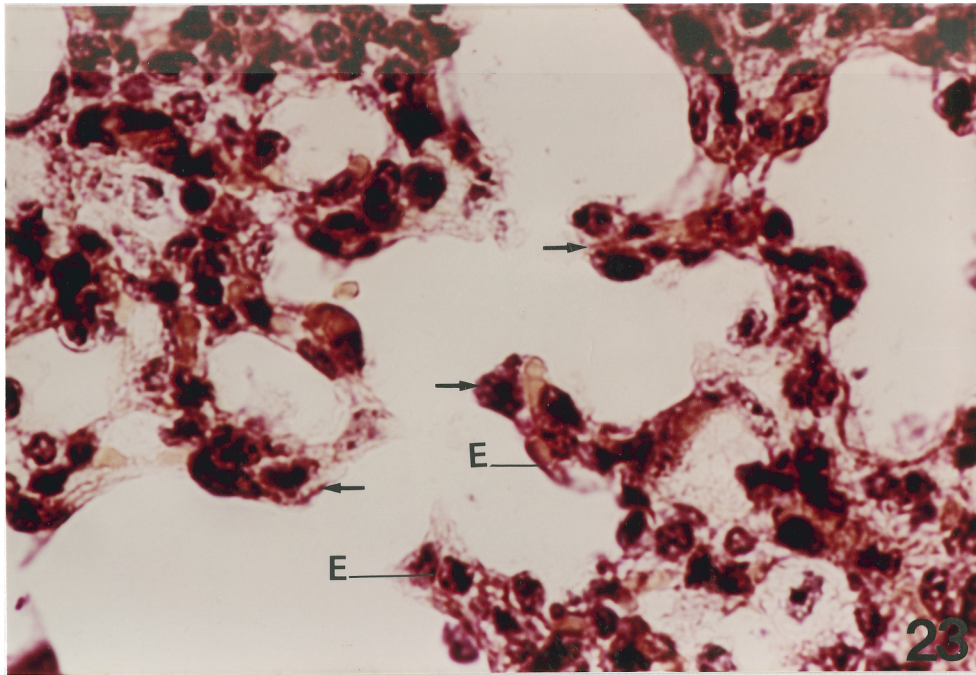




### Explanation of Figures

Fig. 23. Light photomicrograph of 240 day old bovine fetal lung (Alveolar Stage), showing the formation of primitive alveoli by interalveolar septa (arrows). Elastic fibers appear to be present in the septa (E).  
(Verhoeff's method, X400)

Fig. 24. Light photomicrograph of 240 day old bovine fetal lung (Alveolar Stage), showing the presence of type II pneumocyte (arrow) in the primitive alveolar wall.  
(Toluidine Blue's stain, X200)

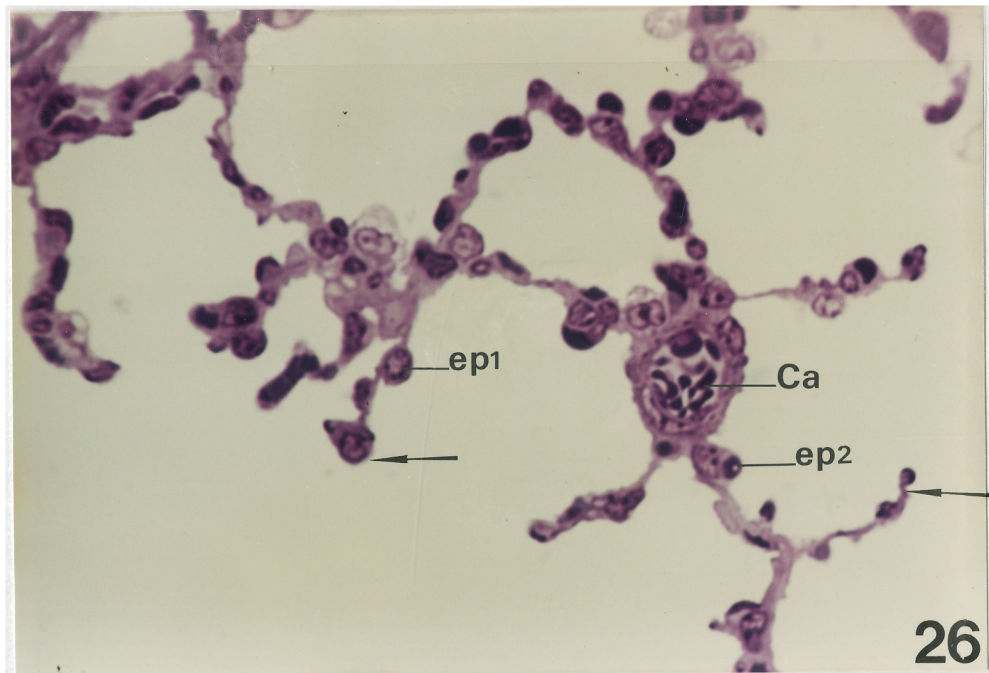
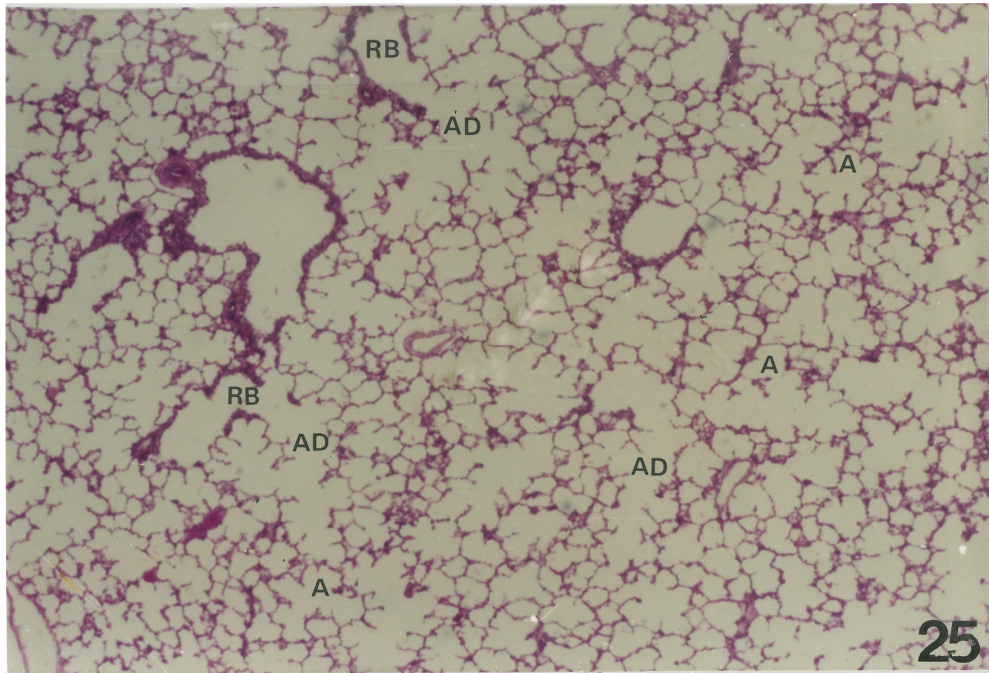




### Explanation of Figures

Fig. 25. Light photomicrograph of 260 day old bovine fetal lung, showing the characteristic architecture of the Alveolar Stage. Respiratory bronchioles (RB), alveolar ducts (AD), and alveoli (A) are well defined at this age. (Harrys' hematoxylin and eosin, X400)

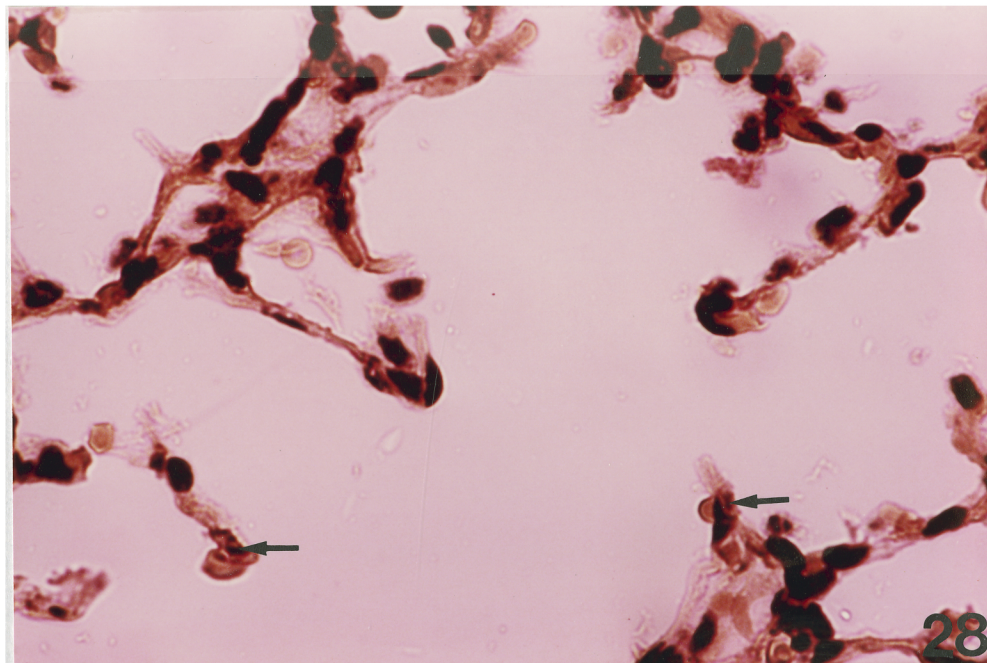
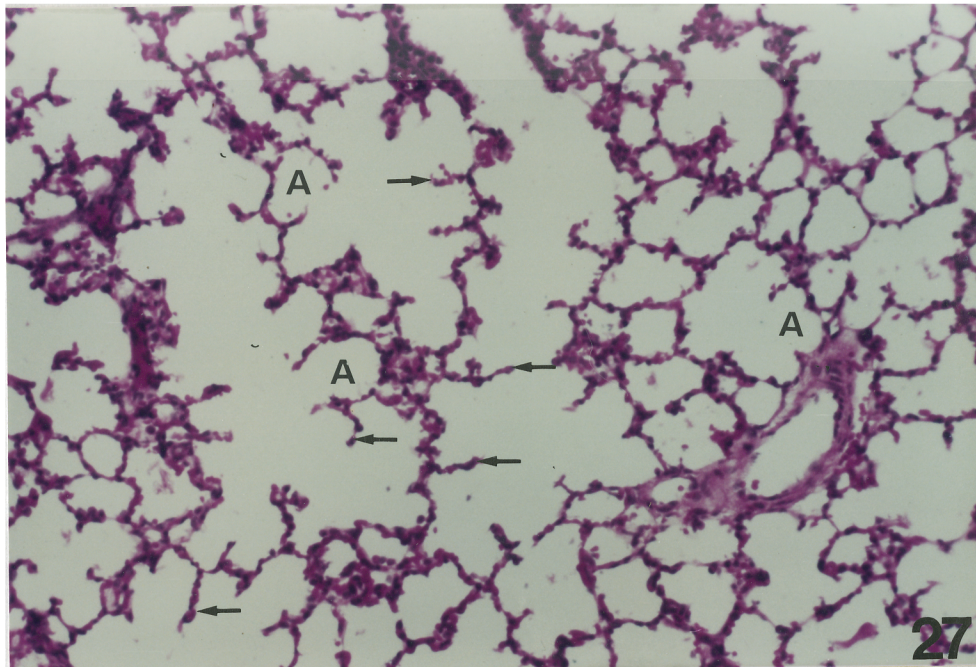
Fig. 26. Light photomicrograph of 260 day old bovine fetal lung (Alveolar Stage), showing interalveolar septa (arrow), a capillary (CA) in the interalveolar wall, plus type I (ep 1) and type II (ep 2) pneumocytes. (Toluidine Blue's stain, X200)



### Explanation of Figures

Fig. 27. Light photomicrograph of 260 day old bovine fetal lung (Alveolar Stage), showing the formation of primitive alveoli (A) and thinner septa than in 240 day old fetus (arrows).  
(Harrys' hematoxylin and eosin, X100)

Fig. 28. Light photomicrograph of 260 day old bovine fetal lung (Alveolar Stage), showing the possible presence of elastic fibers (black stain) in the tips of the interalveolar septa (arrows).  
(Verhoeff's method, X400)



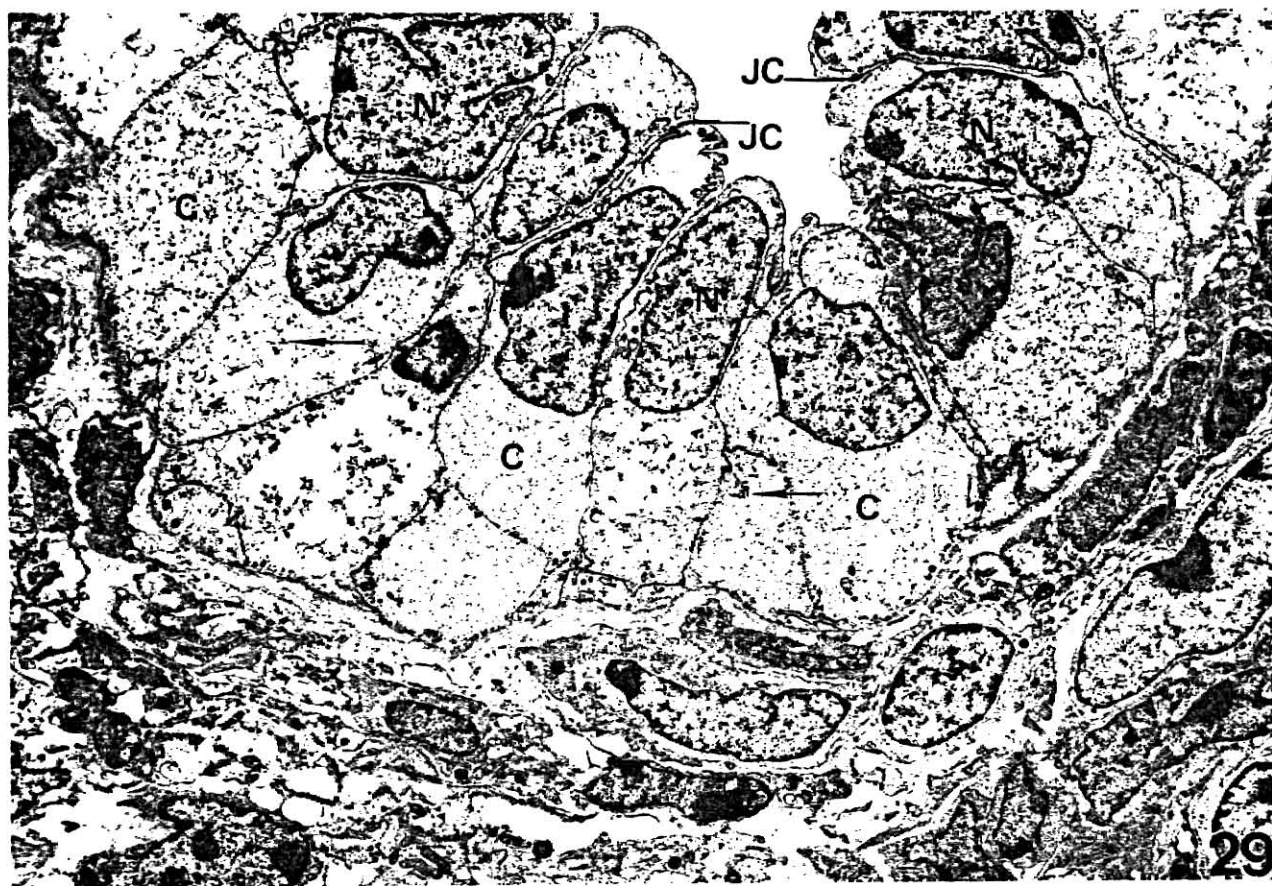


## Explanation of Figures

Fig. 29. Transmission electron micrograph of calf lung, 150 gestation days (Canalicular Stage), showing epithelial cells which cover the future air-conducting way. Note the apical position of nuclei (N), the appearance of cytoplasm (C), the presence of glycogen (arrows), and epithelial junctions (JC).

Total magnification: X3,554

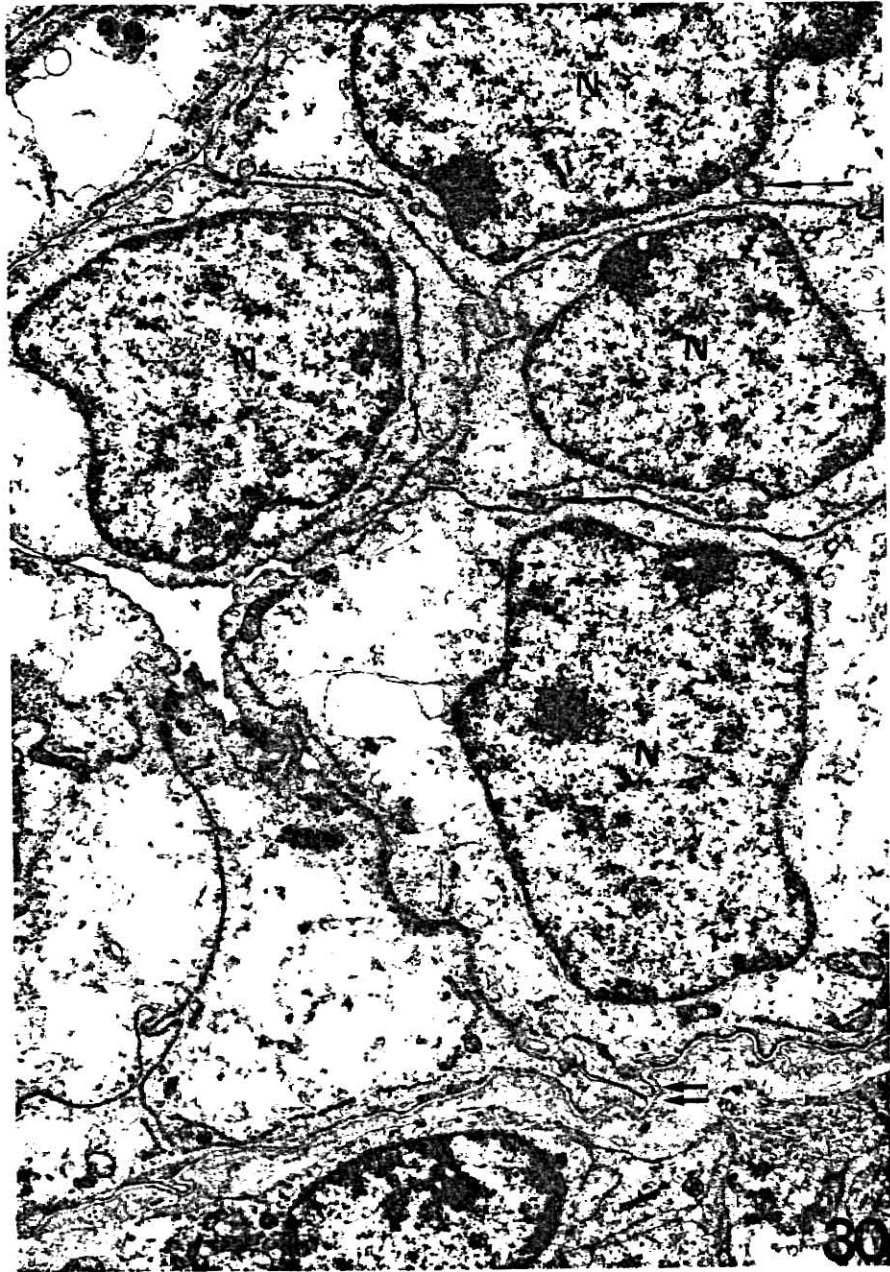




### Explanation of Figures

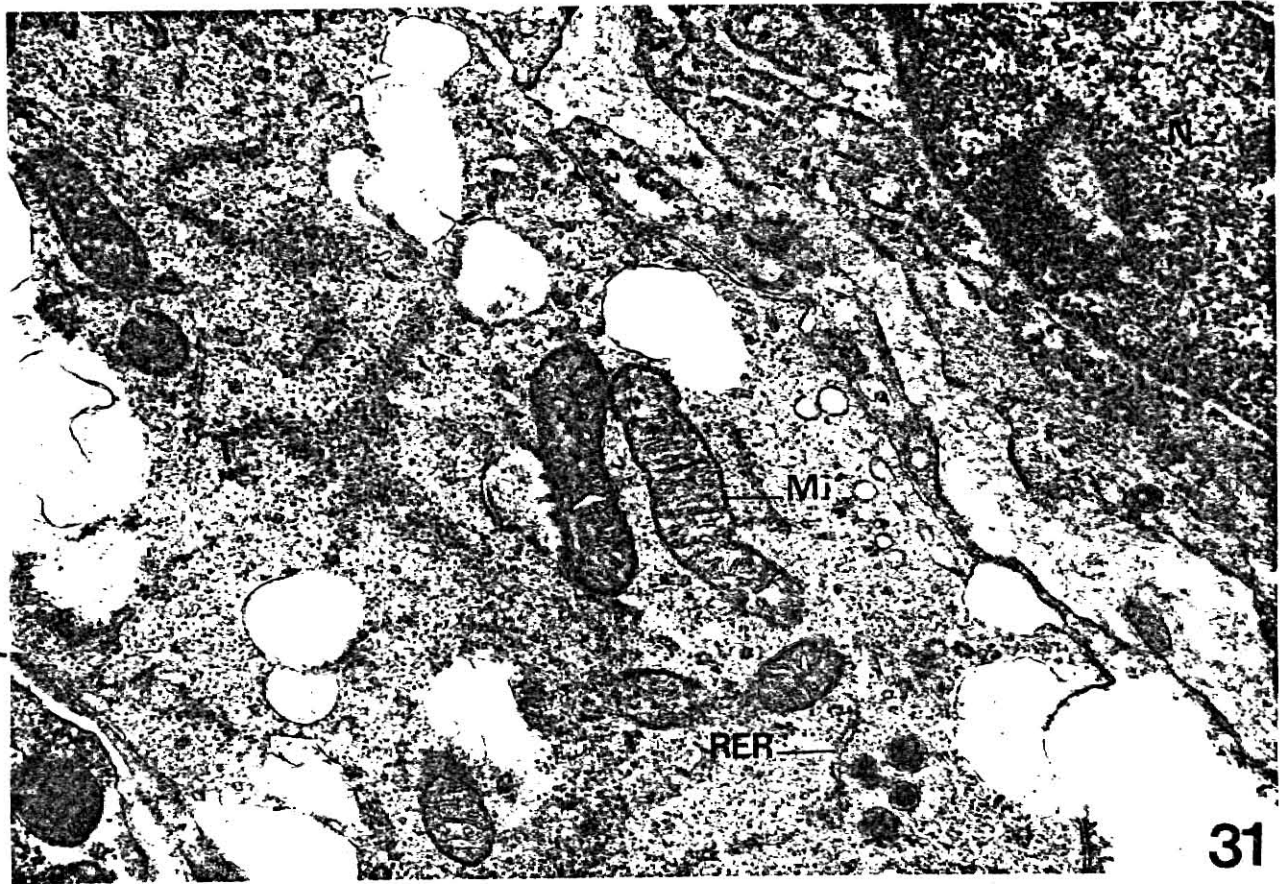
Fig. 30. Transmission electron micrograph of calf lung, 150 gestation days (Canalicular Stage), showing at high magnification the characteristics of the epithelial cells. Irregular nuclei (N) are surrounded by structureless cytoplasm. Organelles (arrows) are lacking or degenerating. The basal part of epithelial cells interdigitate with the basement membrane (double arrows).

Total magnification: X8,854



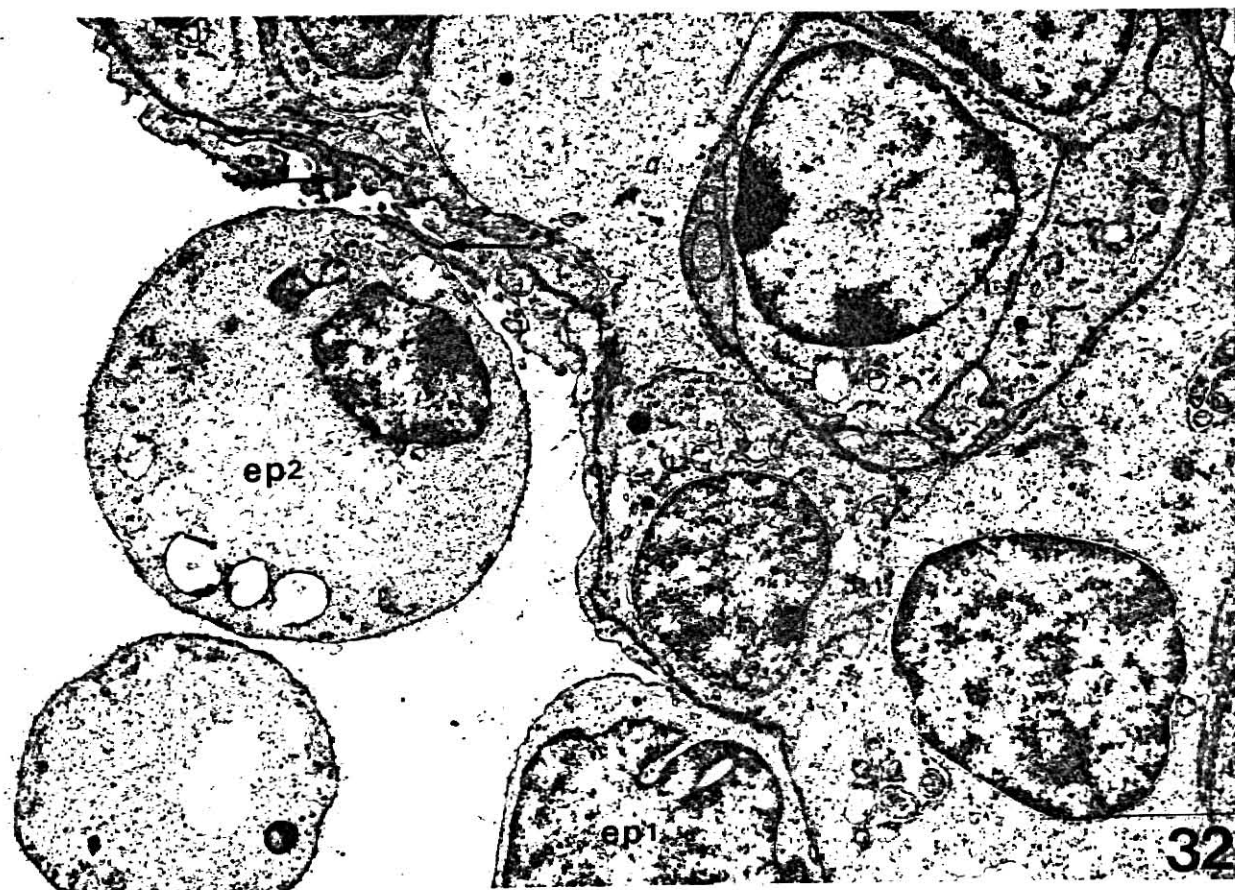
## Explanation of Figures

Fig. 31. Transmission electron micrograph of calf lung, 150 gestation days (Canalicular Stage), showing some of the organelles present in the cytoplasm of a cell located in the lung interstitium. Mitochondria (Mi), rough endoplasmic reticulum (RER), and nucleus (N) can be distinguished. Total magnification: X2,831



### Explanation of Figures

Fig. 32. Transmission electron micrograph of calf lung, 210 gestation days (Saccular Stage), showing the presence of type I (ep 1) and type II (ep 2) pneumocytes present in the saccular wall. Lamellar bodies have not yet developed in type II cell. Tight junction complexes between type I and type II epithelial pneumocytes are present (arrows).  
Total magnification: X7,560

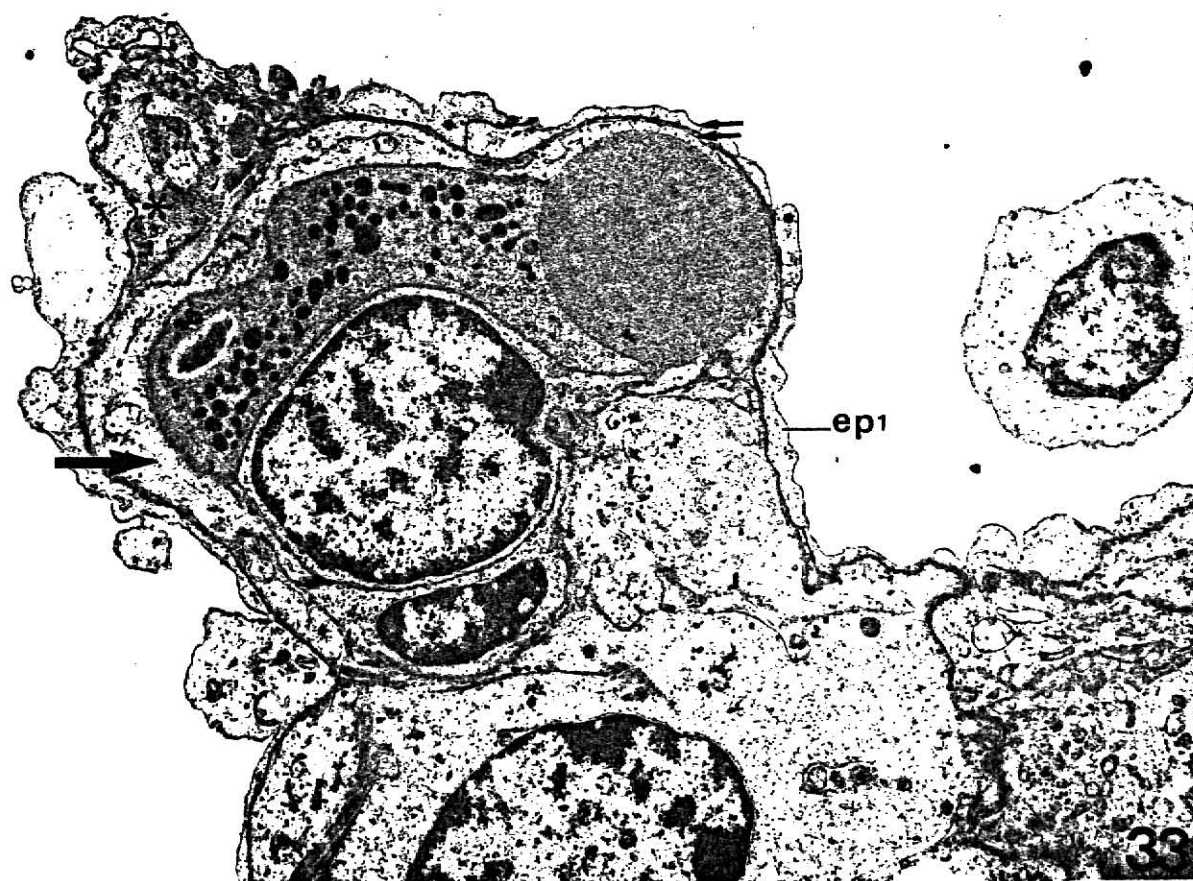


### Explanation of Figures

Fig. 33. Transmission electron micrograph of calf lung, 210 gestation days (Saccular Stage), showing a saccular septum composed of a capillary (arrow) covered by type I pneumocyte cytoplasmic extensions (ep 1). Note the presence of vesicles in both cells' cytoplasm (double arrow). Connective fibers are present in the tip of the septum (\*).

Total magnification: X6,836





# Explanation of Figures

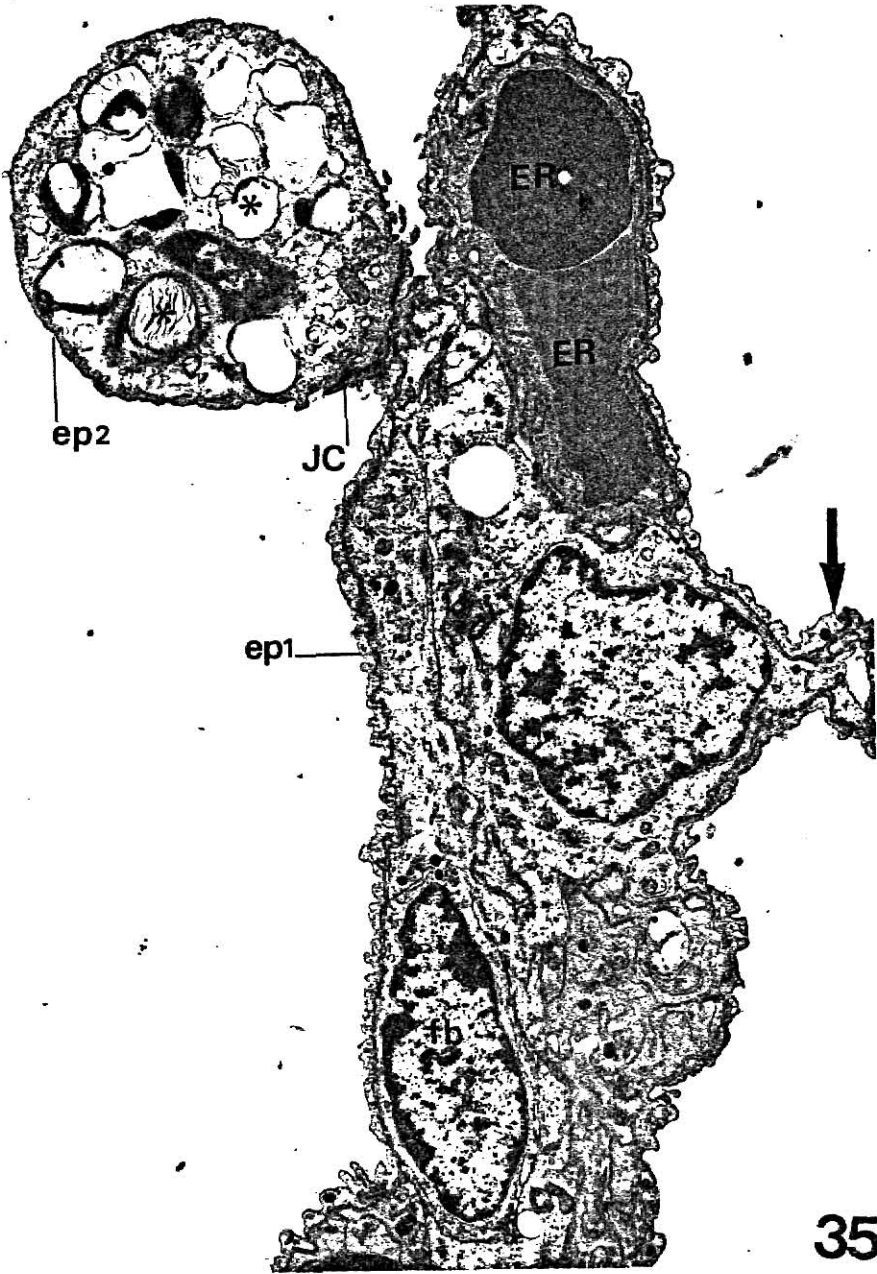
Fig. 34. Transmission electron micrograph of calf lung, 240 gestation days (Alveolar Stage), showing an interalveolar septum characterized by the presence of fibroblast (fb), capillaries (cap), vesicles (arrow), and elastic and collagen fibers (\*).  
Total magnification: X8,996



### Explanation of Figures

Fig. 35. Transmission electron micrograph of calf lung, 240 gestation days (Alveolar Stage), showing a portion of saccular septum with developing alveolar septal bud (arrow). Type II pneumocyte (ep 2) with lamellar bodies (\*), tight junction complexes (JC), erythrocytes (ER) in capillar, cytoplasmic extension of type I pneumocyte (ep 1) and a fibroblast (fb) are present.

Total magnification: X6,027

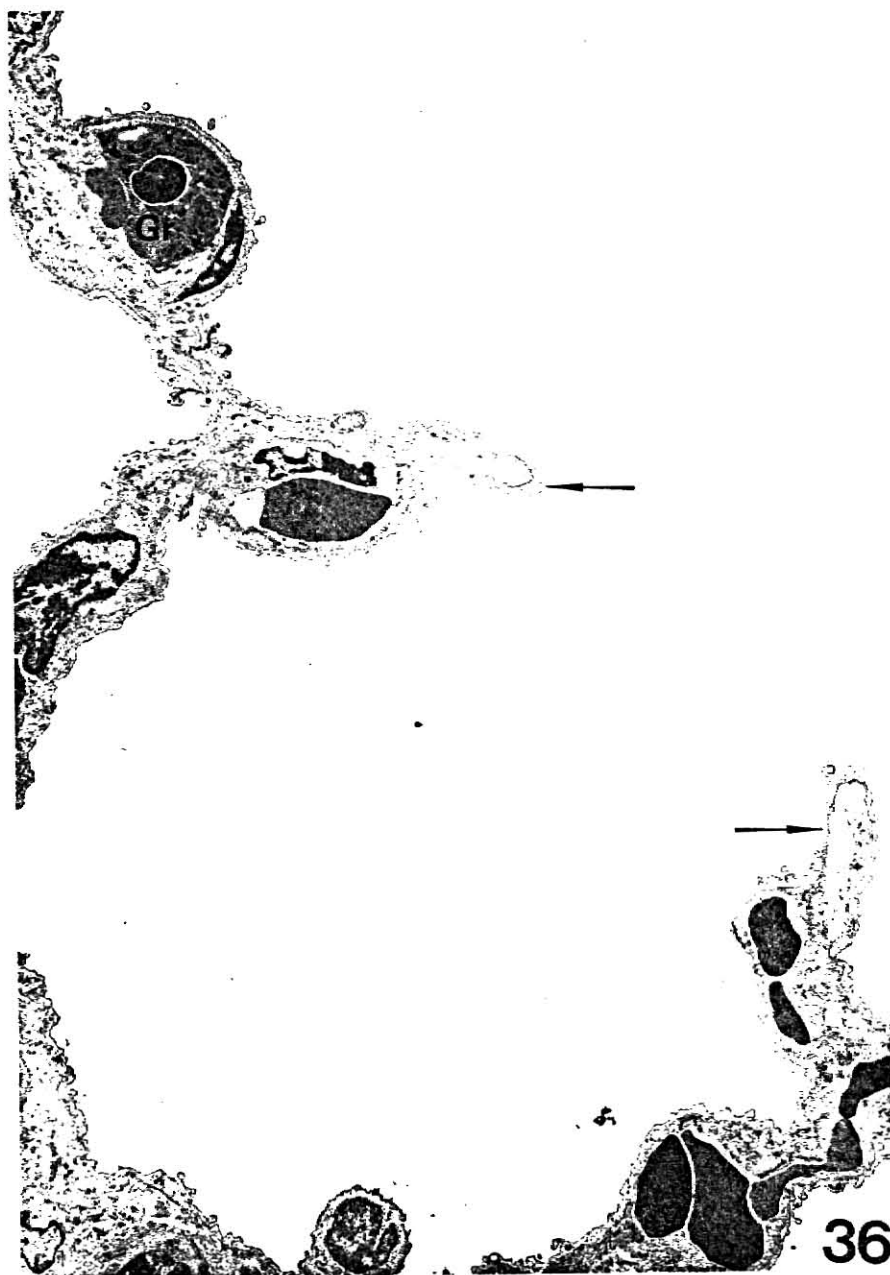


### Explanation of Figures

Fig. 36. Transmission electron micrograph of calf lung, 260 gestation days (Alveolar Stage), showing developing septal buds (arrows) and a granulocyte with a capillary (Gr).

Total magnification: X3,375

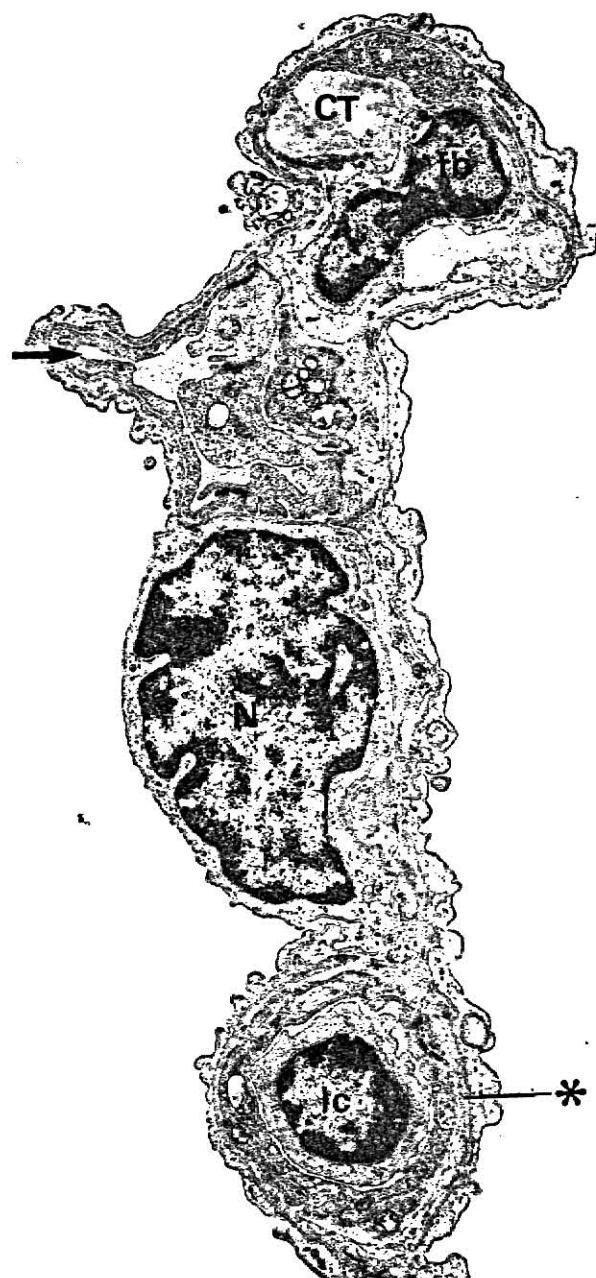




### Explanation of Figures

Fig. 37. Transmission electron micrograph of calf lung, 260 gestation days (Alveolar Stage), showing an interalveolar septa with capillaries (arrows), lymphocyte (lc), nucleus of type I pneumocyte (N), interstitial fibroblast (fb), epithelial basal lamina (\*), and connective tissue fibers in septal distal extremity (CT).

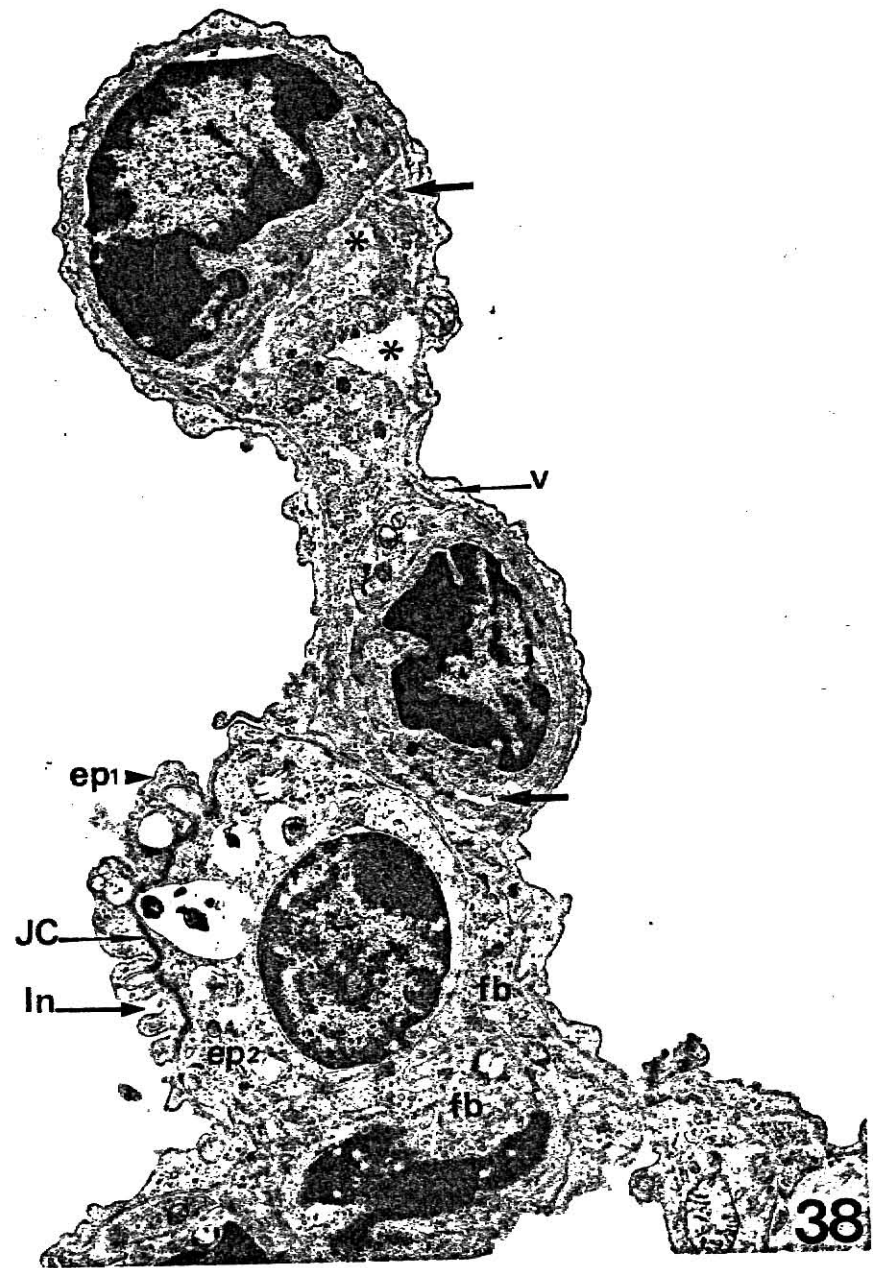
Total magnification: X11,790



### Explanation of Figures

Fig. 38. Transmission electron micrograph of calf lung, 260 gestation days (Alveolar Stage), showing an interalveolar septum with unopened capillaries (arrows), connective tissue fibers (\*), fibroblast (fb), type II pneumocyte (ep 2) still covered by type I pneumocyte (ep 1), invaginations of the plasma membrane of type I pneumocyte (In), vesicles (Ve), and tight junction complexes between type I and type II pneumocytes (JC).

Total magnification: X11,250

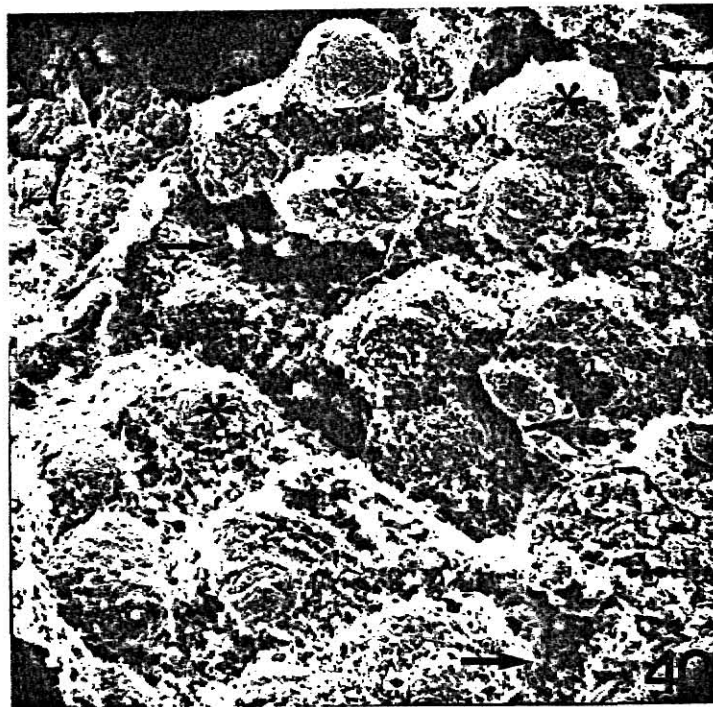
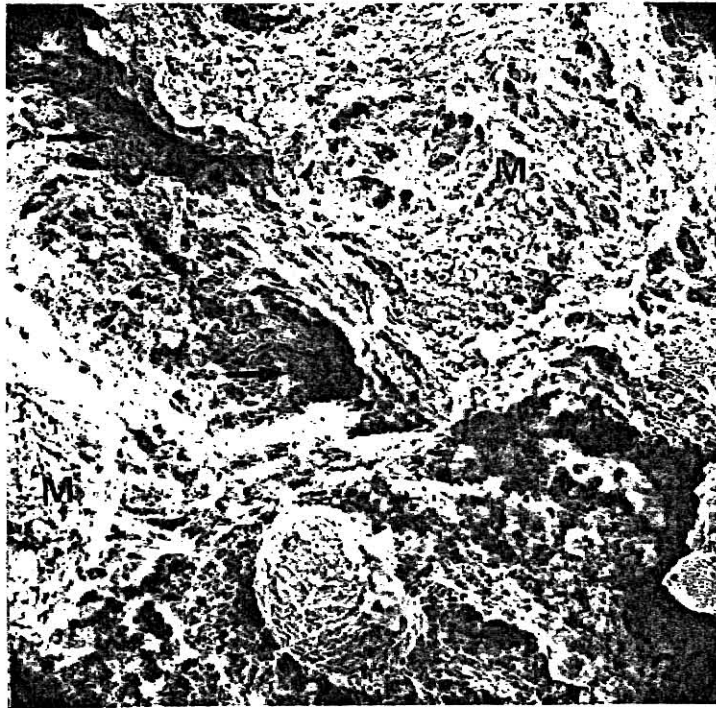


### Explanation of Figures

Fig. 39. Scanning electron micrograph of bovine fetal lung at 70 days of gestation (Pseudoglandular Stage), showing narrow lumens (arrows) surrounded by mesenchymal connective tissue (M).  
(10 KV, W.D. 24 mm, X200)

Fig. 40. Scanning electron micrograph of bovine fetal lung at 90 days of gestation (Pseudoglandular Stage), showing epithelial cells (\*) which cover the future air-way tubes. Few openings are seen at this age (arrows).  
(10 KV, W.D. 24 mm, X200)





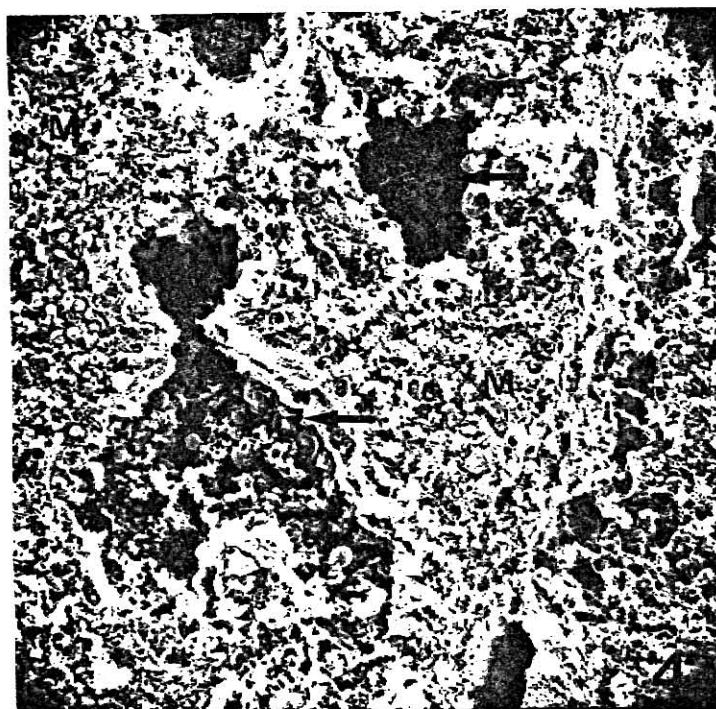
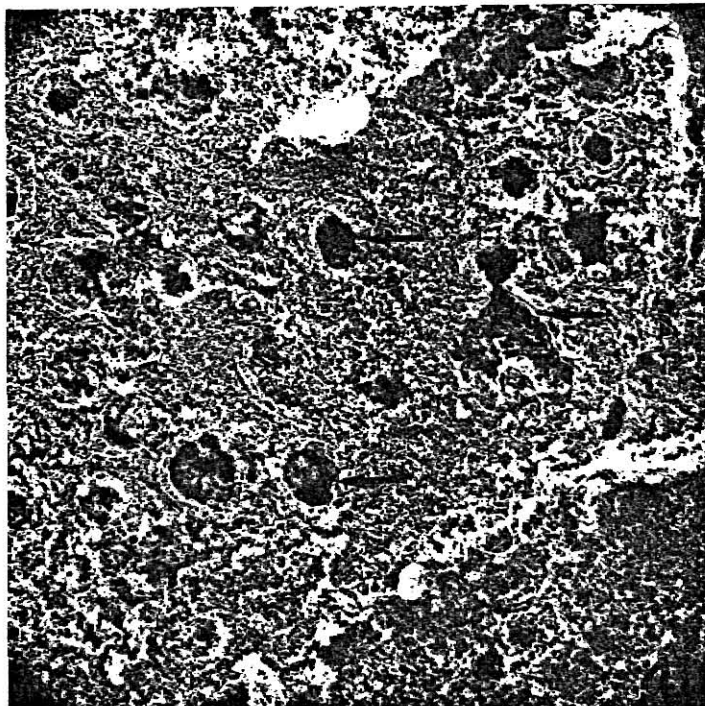
### Explanation of Figures

Fig. 41. Scanning electron micrograph of bovine fetal lung at 150 days of gestation, showing a change in architecture in the pulmonary parenchyma. The Canalicular Stage is characterized by the presence of increased numbers of openings of future air-passages (arrows).

(10 KV, W.D. 22 mm, X200)

Fig. 42. Scanning electron micrograph of bovine fetal lung at 150 days of gestation (Canalicular Stage), showing a high magnification of the pulmonary parenchyma with enlarged lumens (arrows) of the future air-conducting system, separated by mesenchymal connective tissue (M).

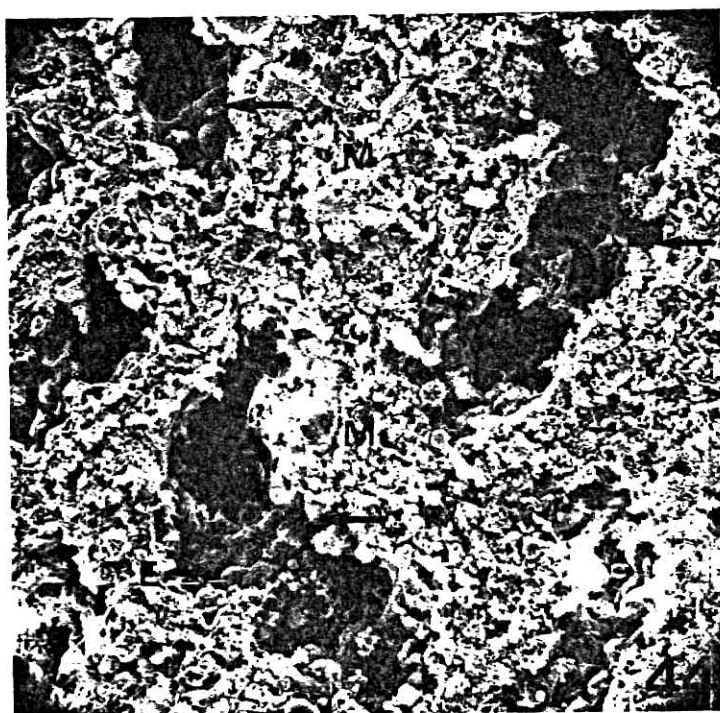
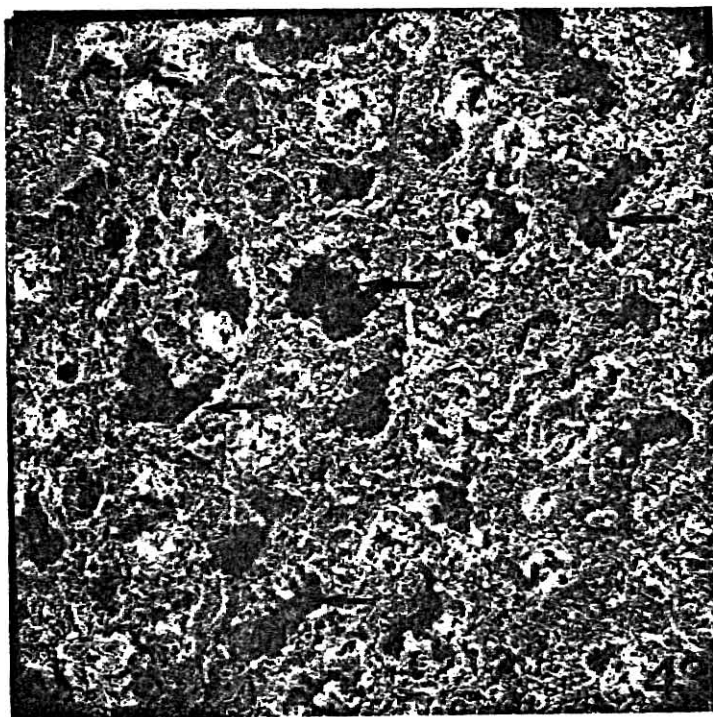
(10 KV, W.D. 22 mm, X600)



### Explanation of Figures

Fig. 43. Scanning electron micrograph of bovine fetal lung at 180 days of gestation (Canalicular Stage), showing the appearance of canalization process at a later stage. Increased numbers of air-ways appear in the pulmonary parenchyma (arrows).  
(10 KV, W.D. 19 mm, X200)

Fig. 44. Scanning electron micrograph of bovine fetal lung at 180 days of gestation (Canalicular Stage), showing at high magnification the presence of enlarged lumens (arrows) separated by a small amount of mesenchymal connective tissue (M). Epithelial cells are seen protruding into the lumen (E).  
(10 KV, W.D. 19 mm, X600)

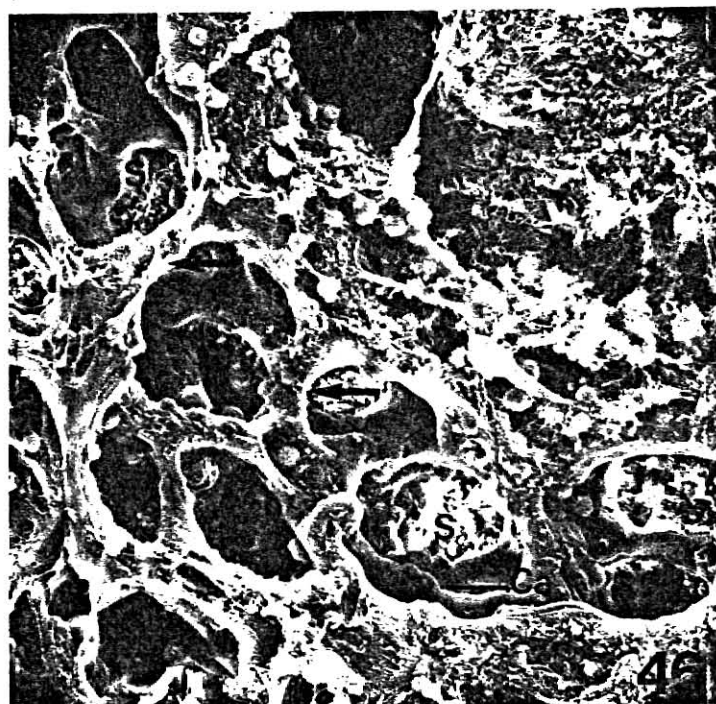
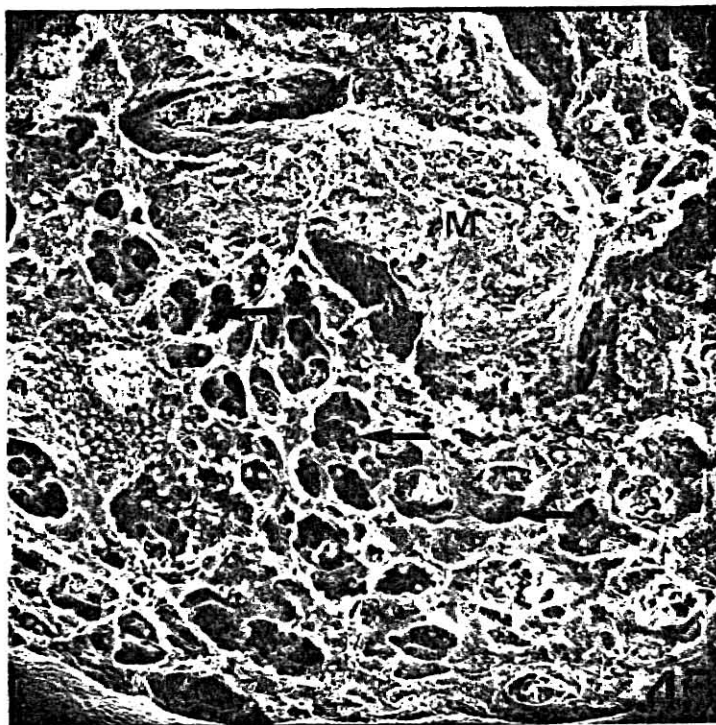


### Explanation of Figures

Fig. 45. Scanning electron micrograph of bovine fetal lung at 220 days of gestation, showing an architecture that differs from the previous stages. The pulmonary parenchyma shows the presence of primitive saccules characteristic of the Terminal Sac Stage (arrows). A small amount of mesenchymal tissue is present (M).  
(10 KV, W.D. 24 mm, X200)

Fig. 46. Scanning electron micrograph of bovine fetal lung at 220 days of gestation (Terminal Sac Stage), showing at high magnification the appearance of the saccules (S), characterized by thick intersaccular septa (arrows) and protrusions of the surface due to underlying capillaries (Ca).  
(10 KV, W.D. 24 mm, X600)

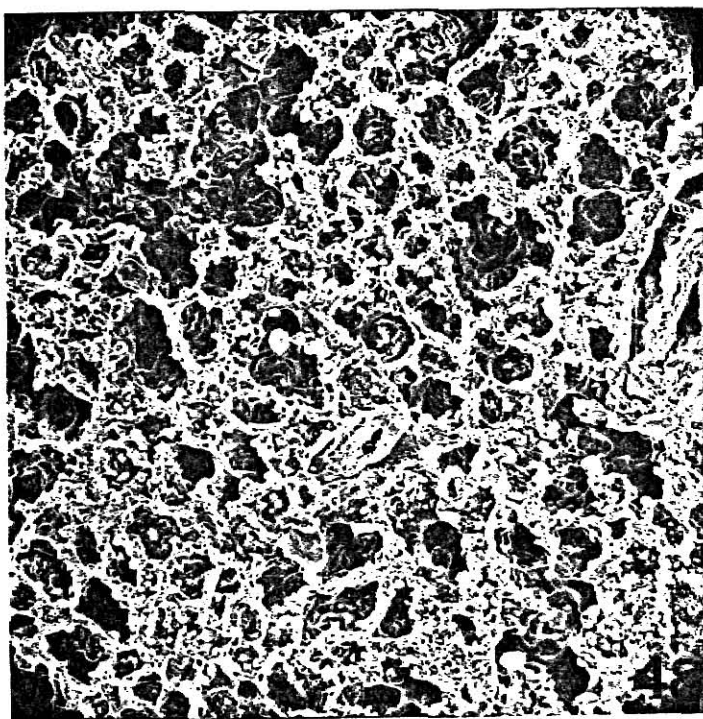
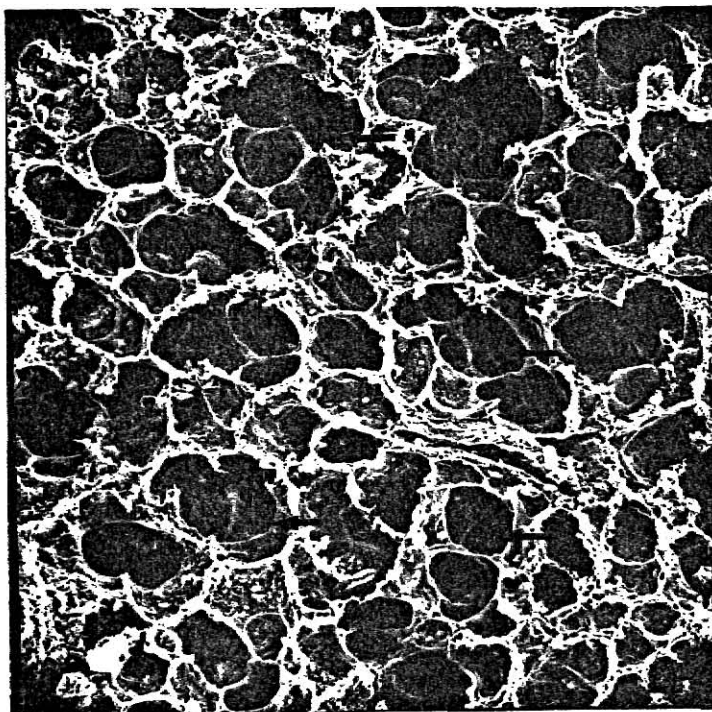




### Explanation of Figures

Fig. 47. Scanning electron micrograph of bovine fetal lung at 250 days of gestation, showing a different morphology than the previous stage. The Alveolar Stage is characterized by the presence of primitive alveoli (arrows).  
(10 KV, W.D. 24 mm, X200)

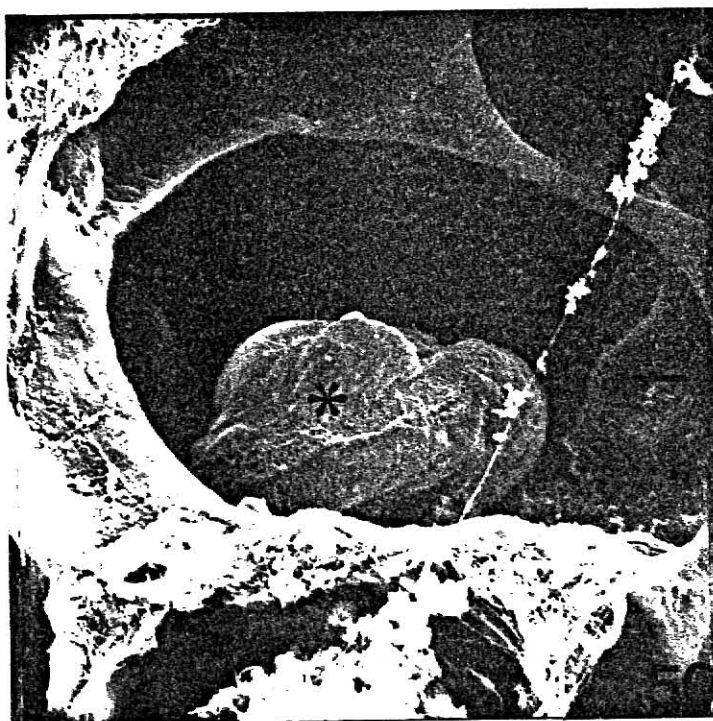
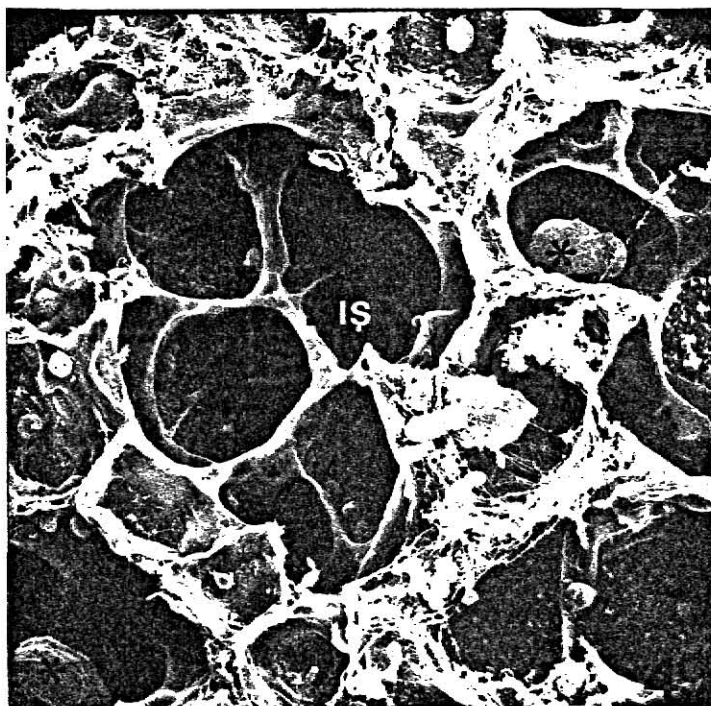
Fig. 48. Scanning electron micrograph of bovine fetal lung at 250 days of gestation (Alveolar Stage) at the same magnification but in different area (close to the pleura), showing the appearance of the pulmonary parenchyma where it resembles the Terminal Sac Stage. Note the differences in septa thickness.  
(10 KV, W.D. 24 mm, X200)



### Explanation of Figures

Fig. 49. Scanning electron micrograph of bovine fetal lung at 250 days of gestation (Alveolar Stage), showing the primitive alveolar appearance. Thin interalveolar septa (IS) are present in which epithelial cell margins arise above the surface (arrows). Free cells are present in the alveolar lumens (\*).  
(10 KV, W.D. 24 mm, X200)

Fig. 50. Scanning electron micrograph of bovine fetal lung at 250 days of gestation (Alveolar Stage), showing at high magnification one of the free cells in the alveolar lumen. It is characterized by an irregular shape without cytoplasmic extensions (\*). A capillary located under the epithelium protrudes into the alveolar space (arrow).  
(10 KV, W.D. 13 mm, X2,000)



## Explanation of Figures

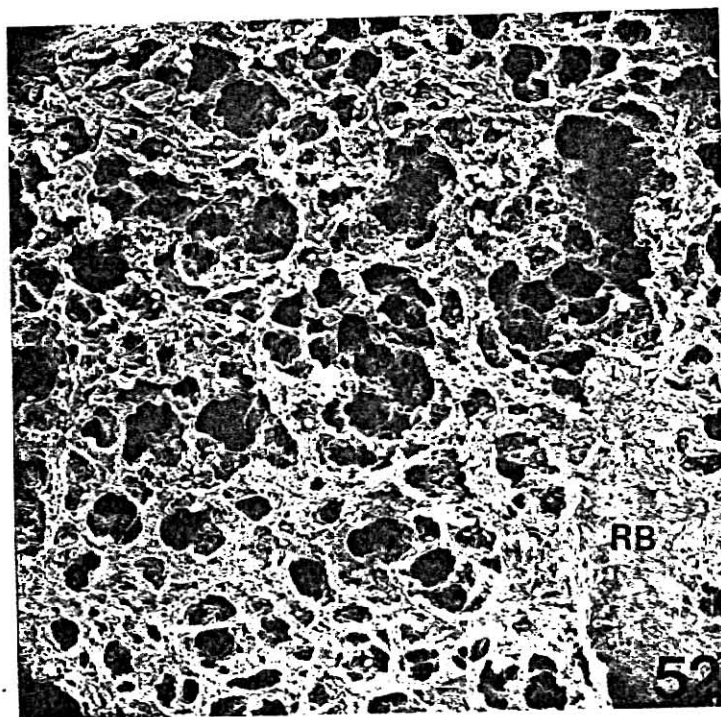
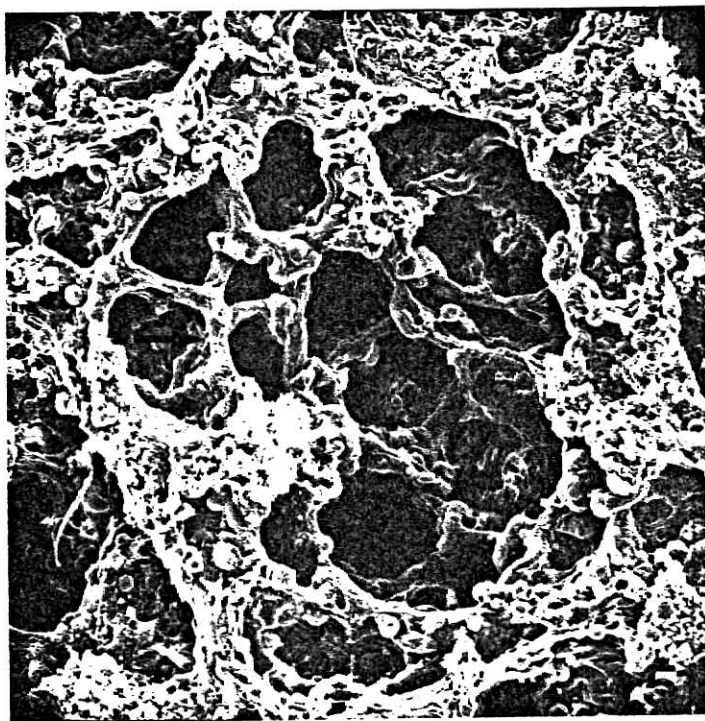
Fig. 51. Scanning electron micrograph of bovine fetal lung at 270 days of gestation (Alveolar Stage), showing the presence of thicker interalveolar walls (arrows) resembling the Saccular Stage.

(10 KV, W.D. 24 mm, X600)

Fig. 52. Scanning electron micrograph of bovine fetal lung at 270 days of gestation (Alveolar Stage), showing a general view of the primitive alveolarization process going on in the lung parenchyma. A portion of a respiratory bronchiole is seen (RB).

(10 KV, W.D. 24 mm, X200)





PRENATAL DEVELOPMENT OF BOVINE LUNG

by

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AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Anatomy and Physiology

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1981

Prenatal growth of the mammalian lung has been categorized into 5 periods: (1) Embryonic, (2) Pseudoglandular, (3) Canalicular, (4) Terminal Sac, and (5) Alveolar.

At birth only 8% of the total alveoli are present in man and most air exchange is accomplished via saccules. Gestational dates for onset of each lung development phase have been described for man, rabbit, sheep, and rat.

The present study used the right diaphragmatic pulmonary lobe from 60 bovine fetuses. Fetal age was estimated from measurements and other characteristics published in veterinary literature. Light, transmission, and scanning electron microscopes were used in this research. Histologic examinations indicated that the Embryologic Period was characterized by an endodermal outgrowth from the primitive foregut by 30th gestational day. The Pseudoglandular Period was in evidence from the 50th day until the 120th day of gestation and characterized by endodermal buds resembling glands surrounded by mesenchymal tissue. The Canalicular Period appeared from the 120th day until the 180th day of gestation. During this stage the lumen of the air-conducting system was enlarged by an epithelium degenerating process. The Terminal Sac Period was seen between the 180th to 240th day of gestation. Saccules were formed by appearance of thick intersaccular septa. Alveolar development began by the 240th gestation day. Primitive alveoli with thinner septa were evident during this period.

Pulmonary vascularization and elastic content increased from the earliest period of development to the Alveolar Period and glycogen content decreased. Transmission electron microscopy revealed epithelial

cytoplasm lacking organelles by the 150th gestation day. A degenerating process occurred during the Canalicular Period. Type I and type II pneumocytes were present by the 210th gestation day, but lamellar bodies were not well developed in the type II pneumocytes. Collagen and elastic fibers were seen in the intersaccular and interalveolar septa.