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ULTRASTRUCTURE OF THE CAMEL'S PULMONARY ALVEOLI WITH SPECIAL REFERENCE TO THE AIR-BLOOD BARRIER

(With 7 Figures)

By

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التركيب المجهرى الدقيق للحويصلات الهوائية فى الجمل وحيد السنام

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اجريت هذة الدراسة لتوضيح التركيب النسيجى الالكترونى للحويصلات الهوائية وقد اجريت هذة الدراسة على ست عينات من رئة الجمال (وحيد السنام) التى تظهر عليها علامات الصحة. وقد اخذت أجزاء مختلفة من الرئة لعمل قطاعات بالميكروسكوب الالكترونى. وقد اظهرت النتائج ان جدار الحويصلة يتكون من نوعين من الخلايا: خلايا حرشفية رقيقة جدا وخلايا مكعبة تتميز بوجود خميلات على سطحها ويحتوى السيتوبلازم على حويصلات صفائحية. وقد وجد شكلين من الخلايا الليفية التى تقطن فى الحاجز بين الحويصلات الشكل الاول يتميز بنواة كبيرة وكمية قليلة من السيتو بلازم والشكل الثانى يتميز بنواة نشطة وكمية وفيرة ومتطورة من الشبكة الاندوبلازمية المحببة. ولقد ميزت منطقتان على طول الغشاء المبطن للشعيرات الدموية، منطقة رفيعة السمك ونادرا ما يوجد بها حويصلات الاحتساء والأخرى سمكها عريض وتحتوى على كمية اكبر من حويصلات الاحتساء.

SUMMARY

The present study was conducted to characterize the cellular population lining the alveoli of the camel's lung. It focused on the ultrastructural findings and their reflection on the physiological role in gas exchange and transcytosis through the air-blood barrier (ABB). It was carried on adult camel using electron microscope. Ultrastructural examination revealed that the pulmonary alveoli were lined with a continuous epithelium comprising two major cell types; the predominant, attenuated pneumocyte type I and the less popular, irregularly cuboidal pneumocyte type II. Two forms of fibroblasts were distinguished; the most remarkable feature of the first form was its well-developed and abundant rough endoplasmic reticulum. The second form was characterized by

large, irregular, dark stained nucleus and little amount of cytoplasm. The most obvious feature of endothelial cells was the concentration of small vesicles (*pinocytotic vesicles*) adjacent to the endothelial cell membranes. They were circumscribed by a continuous basal lamina. Along the same endothelial cell, two cytoplasmic zones were existing; a thin cytoplasmic area containing few or no plasmalemmal vesicles (a-vesicular area) and another thicker cytoplasmic area with numerous plasmalemmal vesicles and endocytotic pits (vesicular area).

Key Words: *Camel, lung, pneumocytes, ultrastructure, air-blood barrier*

INTRODUCTION

The lung is a complex organ of pronounced vital functions that interrelate and influence each other. It is perfectly adapted to fulfill the exchange of gases. The latter function takes place at the level of the alveolar capillary unit that composed of the endothelial cells lining the capillary and the epithelial cells lining the alveoli.

Many ultrastructural studies on the pulmonary parenchyma have been reported in horse (Kaup. *et al.*, 1990), sheep (Bouljihad and Leipold, 1994) and goat (Kahwa *et al.*, 1997). There were no available literature on the cellular components of the air-blood barrier in camel.

The present study aims to provide a basic understanding of the micro-morphological features of the cell population of the alveolar epithelial lining in the camel's lung with special reference to the characterization of the air-blood barrier (ABB). Such understanding would be of key importance to correlate between the ultrastructural findings and certain physiological adaptations to life in the desert harsh conditions.

MATERIALS and METHODS

The lungs of 6adult, apparently healthy camels were collected from a local abattoir. The routine clinical health assessment has certified that these lungs were free of congestion and any gross lesions. Small pieces were dissected from the representative respiratory parts of the lungs and were cut into about $3mm^3$. They were immediately fixed at room temperature in 4% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 24 hours. The tissues were washed several times in phosphate buffer and postfixed in 1% osmium tetroxide. Dehydration was done in ascending grades of ethanol followed by embedding in epoxy resin.

Semithin sections were prepared and stained with 1% toluidine blue for light microscopic examination. Ultrathin sections were cut with a diamond knife, double stained with uranyl acetate and lead citrate (Drury and Wallington, 1980) and were examined with Philips transmission electron microscopy unit at Ain Shams University Hospital.

RESULTS

The pulmonary alveoli were lined with a continuous epithelium comprising two major cell types; the predominant, attenuated pneumocytes type I and the less popular, irregularly cuboidal pneumocytes typeII). The pneumocytes type I was demonstrated by an oval or flattened nucleus that occupied the mid-portion of the cell (Fig.1A&B). Thin cytoplasmic extensions extended from the perinuclear region and lined the major part of the alveolar surface and the interalveolar septa. Cytoplasmic organelles, mainly vesicular structures and mitochondria, were confined to the perinuclear region. The free surfaces of these cells were usually undulant, meanwhile, their basal surfaces were resting on a relatively dense basal lamina (Fig.1A&B). The adjacent pneumocytes were attached to each other by tight junctions (Fig. 2A&B).

Pneumocytes types II were lesser in number and comparatively larger in size than type I. The cell was irregularly cuboidal in outlines and sporadically insinuated amongst the predominant attenuated pneumocytes. The cell was characteristically bulging into the alveolar space and was easily identified by its numerous short microvilli projecting from the free surfaces. Some of these microvilli were covered by a thin, electron-dense fuzzy film representing the amorphous surfactant (Fig. 2B). The cytoplasm contained rough endoplasmic reticulum, small-sized mitochondria, lipid vacuoles and lamellar bodies (Fig. 2A&B). The basal lamina underneath type II cells was particularly in close proximity with fibroblasts and blood capillaries that lying in the interalveolar septa (Fig. 3A&B).

The interstitum of the interalveolar septa was characterized by its unique vascular and fibrocellular components. The most common cellular element of the interstitum was the fibroblasts. Two forms of these cells were distinguished according to their cytoplasmic features. The most remarkable feature of the first form was its well-developed and abundant rough endoplasmic reticulum (Fig. 3A&B). They were located in the area underneath pneumocyte type II. The second form was

characterized by large, irregular, darkly stained nucleus and little amount of cytoplasm (Fig. 4A&B). Their cytoplasmic extensions were ramifying in close apposition to the basal laminae underneath both pneumocyte and endotheliocytes (Fig. 1A). The interstitial fibroblasts reside together with cross and longitudinal sections of collagen fibrils.

The cytoplasm of the capillary endothelium was relatively scanty with few organelles, mostly concentrated in the perinuclear zone. The most obvious feature was the concentration of small vesicles (*pinocytotic vesicles*) adjacent to the endothelial cell membranes. Endothelial cells were circumscribed by a continuous basal lamina (Fig. 5A&B). Their nuclei bulged into the capillary lumen. Along the same endothelial cell, two cytoplasmic zones were existing; a thin cytoplasmic area containing few or no plasmalemmal vesicles (a-vesicular area), mainly in close association with pneumocyte type I, and another thicker cytoplasmic area with numerous plasmalemmal vesicles and endocytotic pits (vesicular area) (Fig. 2A) & (Fig. 7A&B). The thin area of the endothelial cell, adjoining pneumocyte type I and their fused basal laminae constitute the thinnest portion of the air-blood barrier (Fig. 6A).

There were considerable differences in the morphology of the air-blood barrier. In some areas, the afore-mentioned thin portion of the air-blood barrier was made up of smooth endothelial and epithelial cells (pneumocyte I) together with their fused, common basal lamina (Fig. 6A). In other areas, luminal folding of the endothelial cells with an increase of the pinocytotic vesicles was observed and a moderate increase in the thickness of the basal lamina with an accumulation of extracellular fibrillar matrix (Fig. 6B).

The presence of pulmonary macrophages was a consistent feature in most of the alveolar capillaries and alveolar lumen. Some macrophages were observed in the interalveolar septa. They were large in size, their cytoplasm contained phagosomes, multivesicular bodies and few small mitochondria.

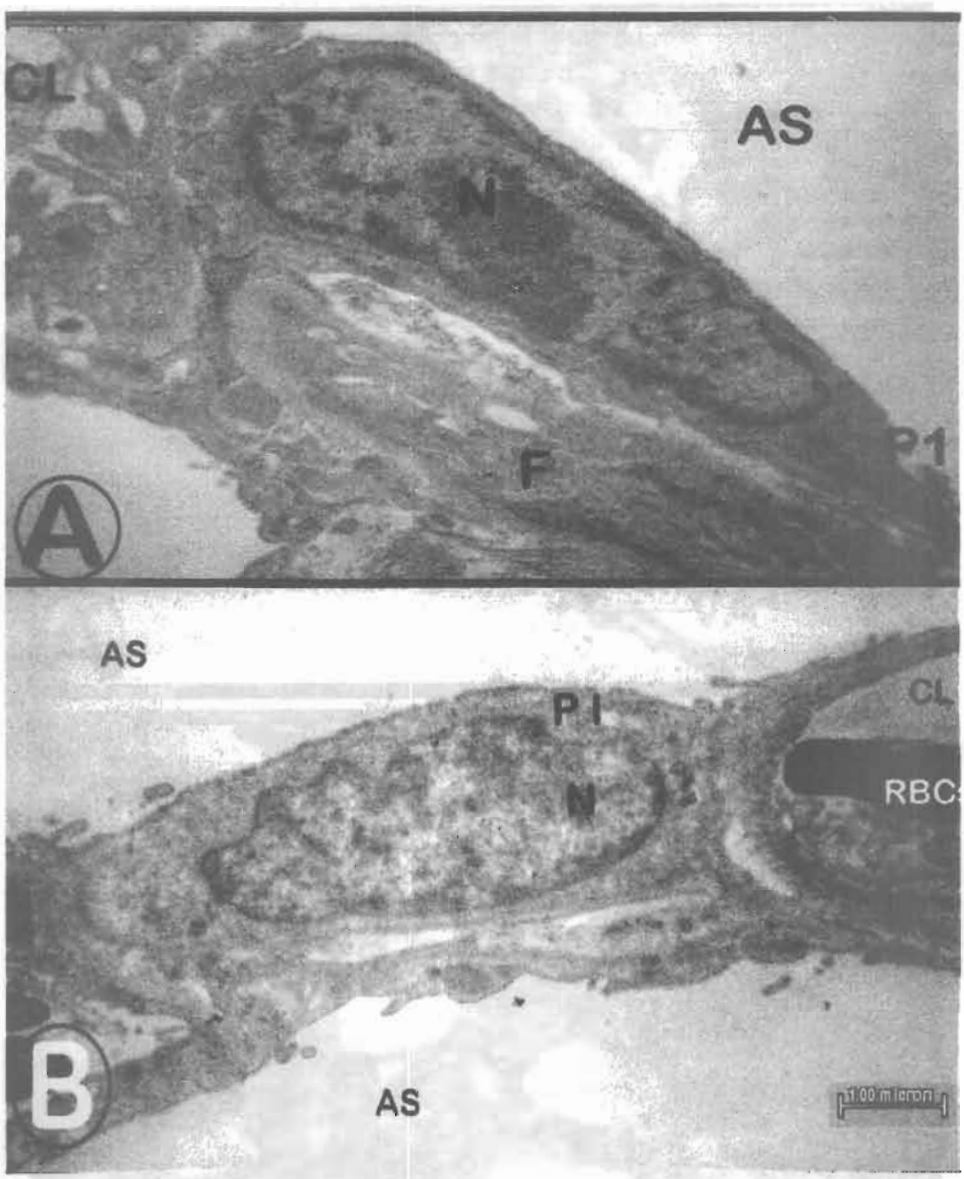
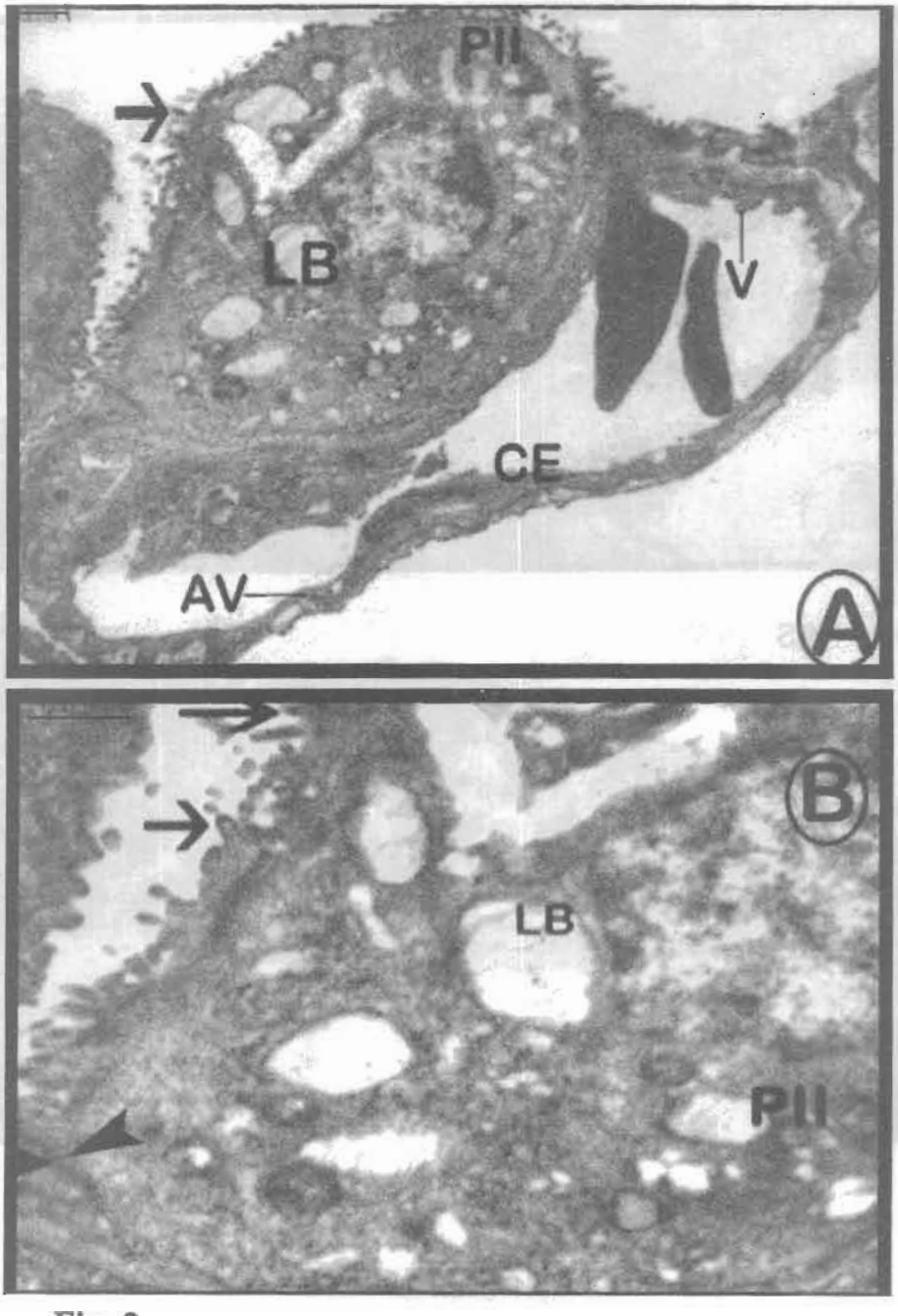


Fig. 1



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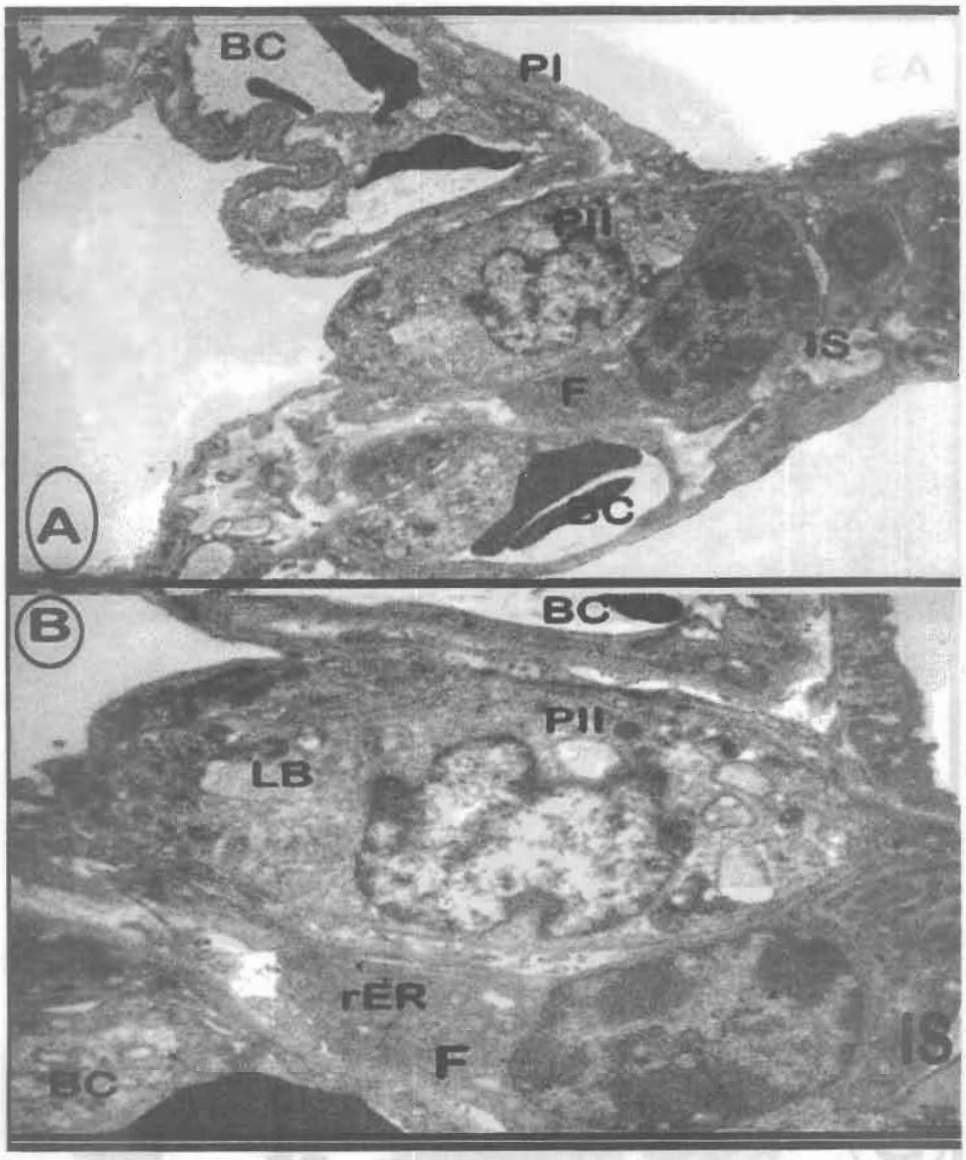

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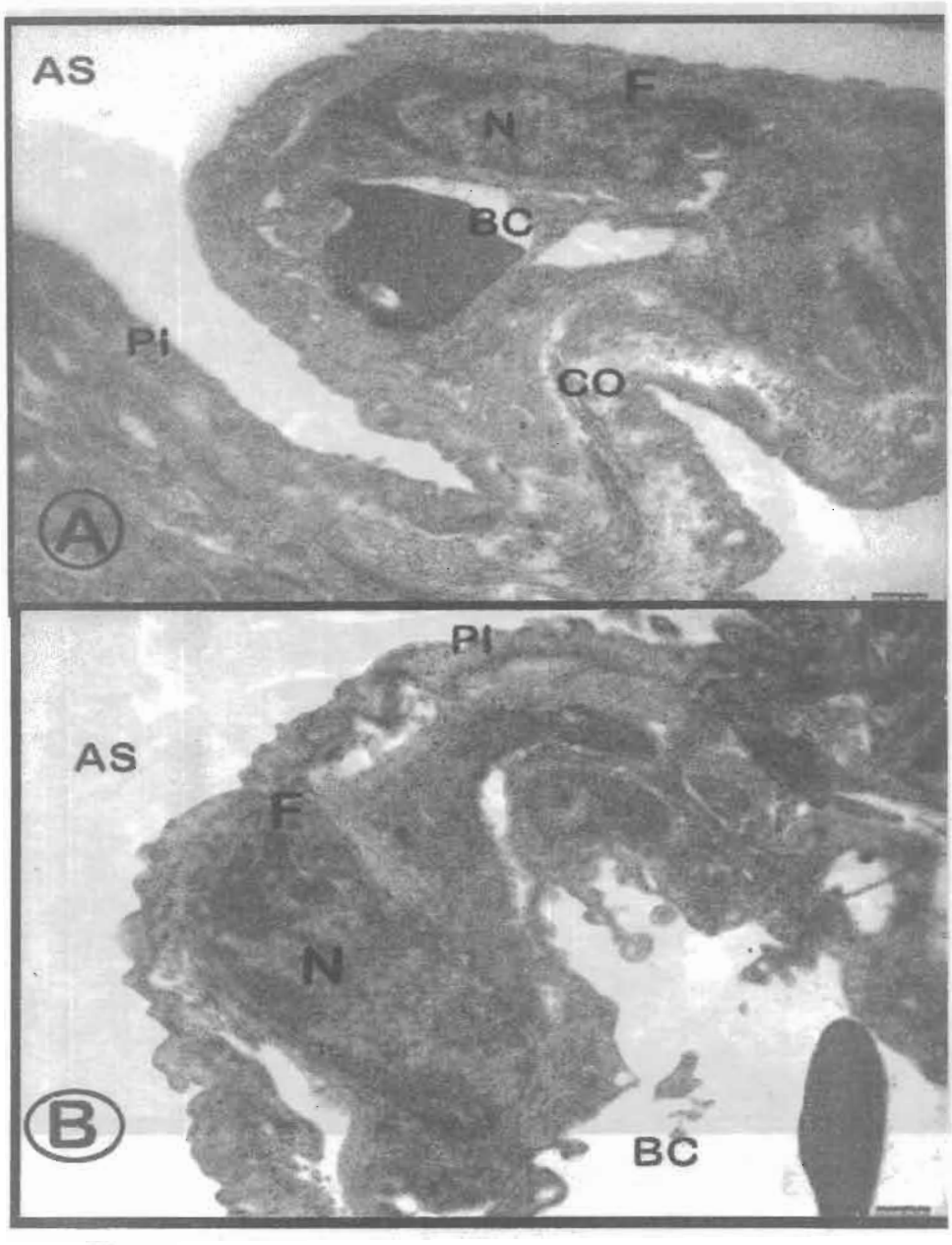

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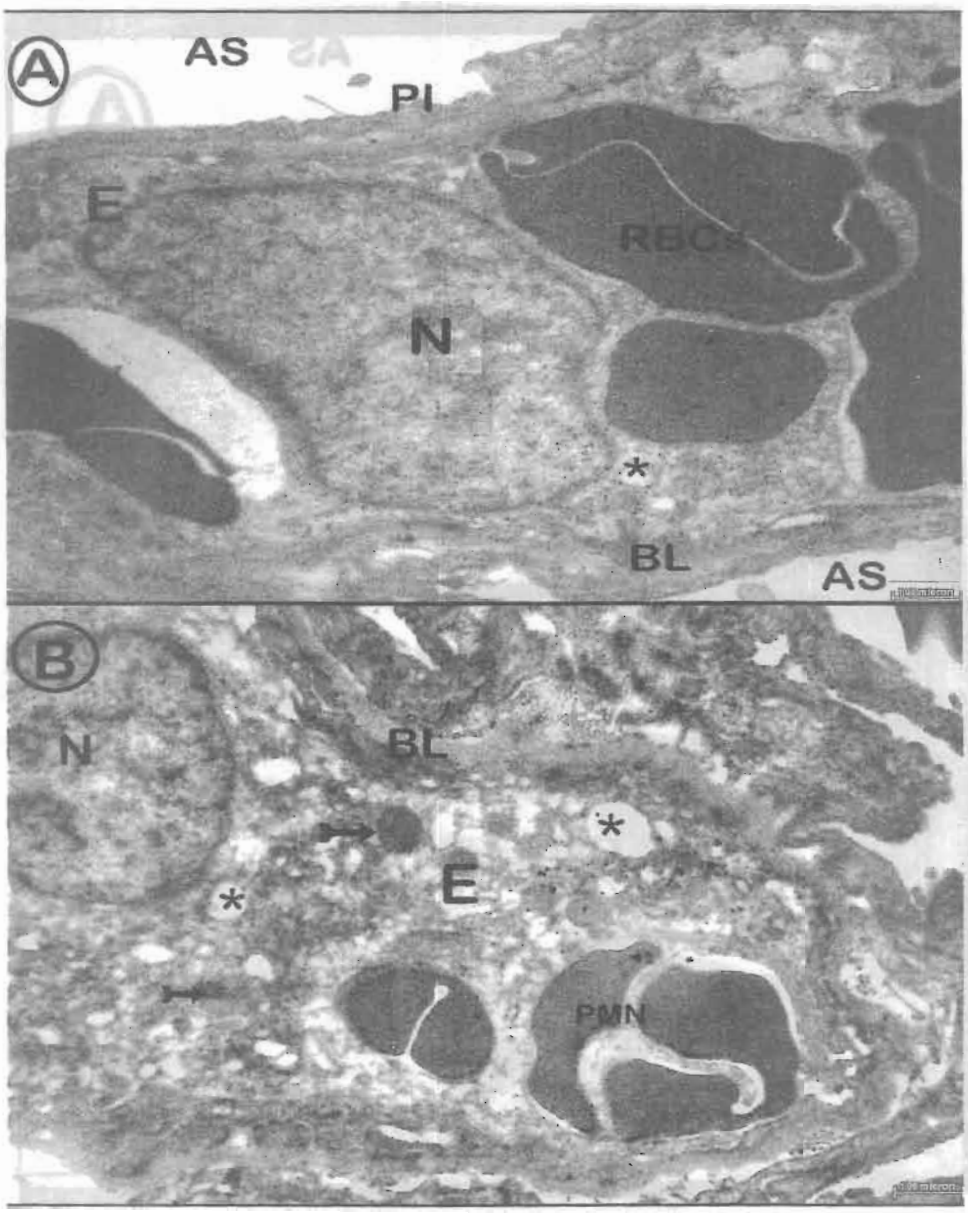

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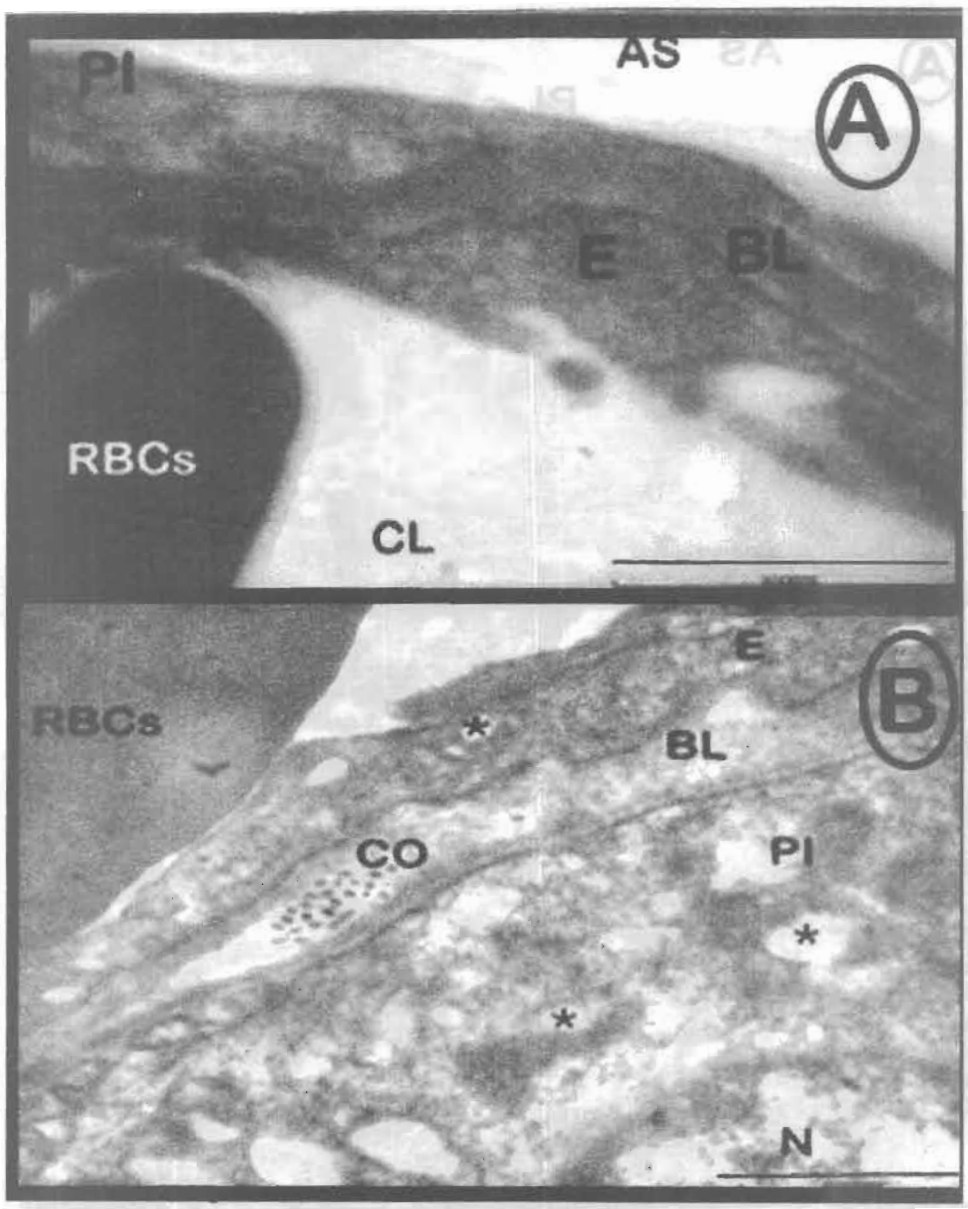

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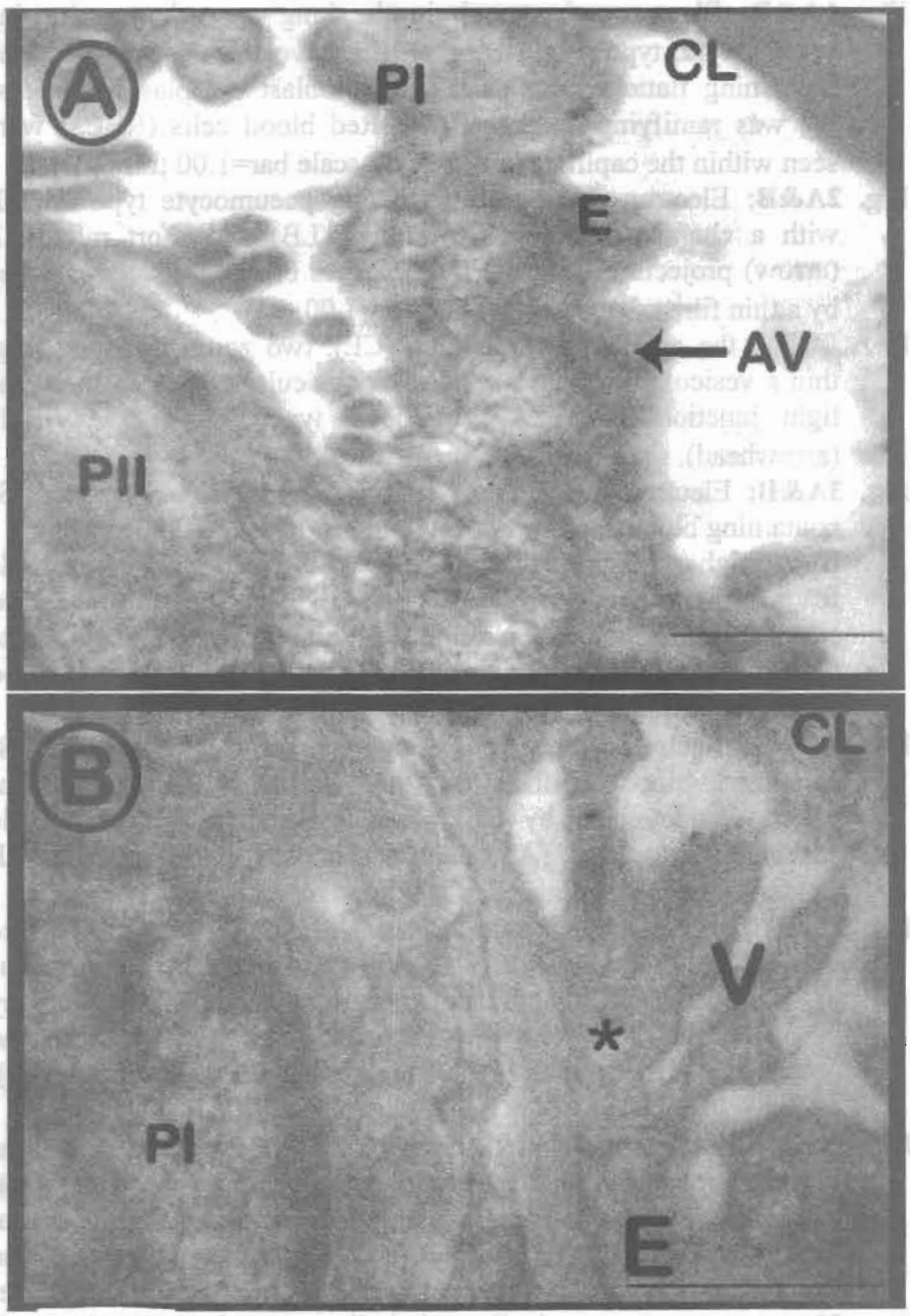

Fig. 7

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Fig. 1A&B: Electron micrograph in the lung parenchyma showing pneumocyte type I (PI) lining the alveolar space (AS) and containing flattened nucleus (N). Fibroblast cytoplasmic process (F) was ramifying underneath PI. Red blood cells (RBCs) were seen within the capillary lumen (CL). scale bar=1.00 μm

Fig. 2A&B: Electron micrographs showing pneumocyte type II (PII) with a characteristic lamellar bodies (LB) and short microvilli (arrow) projecting from the free surfaces of the cells and covered by a thin film of surfactant. scale bar=1.00 μm

In A. Along the capillary endothelium (CE), two zones were existing: thin a vesicular zone (AV) and thick vesicular zone (V). In B The tight junctions between pneumocyte type I & II are visible (arrowhead). scale bar=1.00 μm

Fig. 3A&B: Electron micrographs showing interalveolar septum (IS) containing blood capillaries (BC) and fibroblast F (first form).
B. A higher magnification of A showing the most remarkable feature of the first form of fibroblast (F) was its well-developed and abundant rough endoplasmic reticulum (rRE). (PI, pneumocyte type I; PII, pneumocyte type II; LB, lamellar body and BC, blood capillary) scale bar=1.00 μm

Fig. 4A&B: Electron micrographs showing interalveolar septum (IS) containing blood capillary (BC) and fibroblast F (second form) which characterized by darkly stained nucleus (N) and little amount of cytoplasm. (AS, alveolar space; PI, pneumocyte type I; CO, collagen fibers scale bar=1.00 μm

Fig. 5A&B: Electron micrograph of alveolar capillary showing surface view of the endotheliocyte (E).It is circumscribed by a continuous basal lamina (BL) and contained large nucleus (N), many mitochondria (arrow) and pinocytotic vesicles (asterisk). RBCs and polymorph nuclear cells (PMN) are visible. (AS, alveolar space). scale bar=1.00 μm

Fig. 6A&B: Electron micrographs of air-blood barriers at different areas of the alveolar wall. In A. the basement membrane of the air-blood barrier (BL) was of even thickness with continuous lamina densa. In B, the air-blood barrier of an uneven thickness because of the accumulation of extracellular material (CO). (As, alveolar space; PI, pneumocyte type I; E, endotheliocyte and (asterisk), small vesicles). scale bar=1.00 μm

Fig. 7A&B: Electron micrograph of endotheliocytes (E) lining the alveolar capillary. In A. endotheliocyte shows thin cytoplasmic area containing few or no plasmalemmal vesicles (avesicular area AV) In B, It shows thicker cytoplasmic area with numerous plasmalemmal vesicles (Asterisk) and endocytotic pits (Vesicular area V). PI, pneumocyte type I; PII, pneumocyte type II; CL, capillary lumen) scale bar=1.00 μm.

DISCUSSION

The most important part of the lung is the alveoli. Their number in the human lung is estimated to be 300 million, representing a total surface area of approximately 80 m^2, which is available for gaseous exchange between the inspired air and the blood (Cormack, 1987). The majority of the alveolar surfaces were lined by pneumocytes type I. It was demonstrated by an oval or flattened nucleus that occupied the mid-portion of the cell. Cytoplasmic organelles, mainly vesicular structures and mitochondria, were confined to the perinuclear region. The free surfaces of these cells were usually undulant; meanwhile, their basal surfaces were resting on a relatively dense basal lamina. These Ultrastructural findings were in accordance with Bouljihad & Leipold (1994) in sheep, Maya Simionescu (2001) in human and Doaa Zaghloul (2004) in camel. Various studies have reported the important role of transcytosis in alveolar epithelial cells. In human lung, it is reported that pneumocyte type I and the associated endothelial cells contain numerous vesicles and membrane invaginations (including caveoli and clathrin-coated pits) that are expected to play important roles of internalization of proteins and transcellular movement of cargo proteins (Kwang & Asrar, 2003). Receptor-mediated endocytosis can occur by at least two different vesicle systems, one coated with clathrin and the other with caveolin (Apodaca, 2001). The adjacent pneumocytes were attached to each other by tight junctions. Pneumocyte type II was partially covered by the adjoining type I. Tightness of the alveolar epithelial cells as regulated by zonulae occulentes help to keep the air space relatively dry for efficient gas exchange (Kwang & Asrar, 2003). In human lung, interdigitation between TI&TII pneumocytes were confined to the basal surface around the perimeter of the TII pneumocyte whereas in rabbit lung the interdigitations were confined to the lateral surfaces of the TII pneumocytes (Faye *et al.*, 2003) allowing adequate surface area for tight junctions. the difference in junctional complexes between the various alveolar epithelial cells perhaps signify a different pattern of intercellular

transport, thus influencing the pathogenesis and resolution of alveolar pulmonary edema (Kahwa *et al.*, 1997).

Pneumocytes type II were identified by their numerous short microvilli projecting from the free surfaces in addition to the characteristic lamellar bodies in their cytoplasm. The recently released surfactant from such cells spreads to form a film over the alveolar space (Scorokin, 1967). Once released from type II cells, the phospholipids component of the surfactant becomes a part of lipoprotein complex which is called tubular myelin because it forms highly distinctive tubular lattice (Gil & Reis, 1973). Doaa Zaghloul (2004) suggested that the need of a thick layer of surfactant on the alveolar cells is to decrease the loss of water through evaporation in the hot dry weather and to protect the alveolar cells against the injury from the sharp edges of the inhaled sand particles. Weibel (1974) reported that Type II pneumocyte retain the capacity for mitosis and are believed to serve as progenitor cell for type I as well as for type II cells.

Regarding the blood capillaries lying in close proximity to the alveolar pneumocyte, it is observed that along the same endothelial cell, two cytoplasmic zones were existing; a thin cytoplasmic area containing few or no plasmalemmal vesicles, mainly in close association with pneumocyte type I, and another thicker cytoplasmic area with numerous plasmalemmal vesicles and endocytotic pits. This result is in accordance with Maya Simionescu (2001) who classified the Air-blood barrier into two zones; a vesicular zone and vesicular one and he reported that the major exchanges of O_2/CO_2 take place at the avesicular zone. The concentrations of small vesicles (*pinocytotic vesicles*) adjacent to the endothelial cell membranes have been demonstrated by many authors. Clathrin-coated pits are present in alveolar type I and type II cells whereas caveolin-coated plasmalemmal vesicles (caveola) are present in both pulmonary vessel endothelial cells and alveolar type I epithelial cells, suggesting possible transcytosis of protein via these structural features (Camphell, *et al.*, 1999 and Newman, *et al.*, 1999). Alveolar epithelial protein transport appears to be protein specific in that some proteins are translocated across the air-blood barrier via receptor-mediated transcytosis (IgG), whereas other proteins may opt for non-specific endocytosis (Xiu Fen Sui *et al.*, 2005). In the present study the endothelial cells were circumscribed by a continuous basal lamina.

The pulmonary vascular endothelium presents a selective barrier that actively regulates paracellular movement of circulating macromolecules and cells into extravascular tissues and compartments

(Dudek and Garcia, 2001). Zonula adherens /peripheral actin band forms a continuous belt around the apical rim of the cell where it is strategically localized to modulate Ec-Ec interactions and paracellular pathway (Wong and Gotlieb 1986). Under physiological conditions, protein tyrosine phosphatase appears to maintain basal endothelial barrier function through the restraint of tyrosine phosphorylation within the Zonula adherens multiprotein complex. Schnittler, H. J. *et al.* (1990) suggested that actin and myosin in endothelial cells played a central role in regulating the width of the intercellular clefts, thereby controlling the paracellular pathway of vascular permeability. The pulmonary epithelium is the primary barrier to solutes compared with the pulmonary capillary endothelium. The alveolar epithelial barrier offers significant resistance to the diffusion of electrolytes and small hydrophilic solutes compared with endothelial lining of the lung vasculature (Taylor & Gaar 1970).

The presence of pulmonary macrophages was a consistent feature in most of the alveolar capillaries and alveolar lumen. Some macrophages were observed in the interalveolar septa. Chitko-McKown *et al.,* (1991) reported that the pulmonary alveolar macrophages were more phagocytic than the pulmonary intravascular macrophages. They were large in size, their cytoplasm contained phagosomes, multivesicular bodies and few small mitochondria. This was in accordance with Kwang and Asrar, (2003) who stated that Granulocyte/ macrophage colony-stimulating factor (Gm-Csf) regulates the clearance and catabolism of surfactant apoproteins and that endocytosis and phagocytosis by alveolar macrophages are essential for these processes. Some studies suggested that defective macrophage function leads to accumulation of surfactant apoproteins and lipids in the alveolar air space (Dranoff *et al.,* 1994 and Dranoff and Mulligan, 1994). Cell degradation of human and rat lung surfactant protein A by alveolar macrophages was three times greater than that of bovine surfactant protein (Bats and Fisher 1996).

Two forms of interstitial fibroblasts were distinguished according to their cytoplasmic features. It is possibly indicating a sequence of functional phases of these cells. At least two cell populations of fibroblasts have been described in mammalian lungs. One form is associated with the fibrillar elements of the extracellular matrix and another form, myofibroblasts, is described as having bundles of microfilaments believed to have contractile properties (Kapanci *et al.,* 1979 and Kapanci *et al.,* 1992). By performing a 3 dimensional reconstruction of human alveolar wall fibroblasts using electron

microscope, Faye *et al.,* (2003) determined that there is a population of fibroblasts that directly connect the endothelium to epithelial T2 pneumocytes through apertures in the basal laminae. This effectively creates a continuous cellular substrate from the capillary to the alveolar lumen along which leukocytes may migrate.

The thin area of the endothelial cell, adjoining pneumocyte type I, and their fused basal laminae constitute the thinnest portion of the air-blood barrier. This is in accordance with Maya Simionescu (2001) and Rossela (2004). A continuous lamina densa was observed in the thin area of the ABB. Furuyama and Mochitate (2000) stated that laminin and entactin are required for the synthesis of a continuous lamina densa in the basement membrane. Entactin, is produced by the alveolar epithelial cells, is an integral basement membrane component that binds laminin and type IV collagen (Senior *et al.,* 1996).

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