Osmiophilic inclusions in the Type-II pneumonocyte in the lung of the dromedary camel

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Abstract: Small stripes of the camel's lung were examined with the TEM. Type-II pneumonocytes appeared as large rounded cells occupying the niches and the corners of the alveolar walls. They showed microvilli on their free surface and adjoining the Type-1 pneumocytes through desmosomes. Their cytoplasm was rich with organoids. The most peculiar feature of this cell was the osmiophilic inclusion bodies which were thought to contain surfactant material or its precursor. Some of these inclusions appeared vacuolated or possessed homogenous density. Others showed lighter osmiophilic material accumulated around a heavily osmiophilic core or they appeared lamellated. It seems possible that this morphological difference may well be related to the difference in the chemical constituents of these inclusion bodies.
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Key words: Type-II cell, lamellar bodies, lamellar bodies, inclusion bodies

1. Introduction

The epithelium which lines the alveoli in mature mammalian lung is composed of two types of cells Type-1 and Type-II cells (Karrer, 1956) or membranous and granular pneumonocytes (Macklin, 1954). In a wide range of mammalian species the Type-II cells contain large number of inclusion bodies (Schulz, 1959 and Campiche, 1960). These inclusions are sites of acid phosphatase activity (Corrin, et al., 1969 and Etherton and Botham, 1970). Balis and Conen (1964) demonstrated acid phosphatase reaction in the smaller inclusions of rats. Hatasa and Nakamura (1965) showed similar activity in mouse inclusion bodies.

A considerable body of evidence has been accumulated which suggests that the contents of the inclusion bodies of Type-II cells are secreted into the alveolar lumen and they are probably a source of pulmonary surfactant (Sorokin, 1967; Kuhn, 1968 and Meban, 1972). This surface active material confers stability on lung tissue and has been characterized as phospholipid (Klauset al., 1961). Spencer (1985) mentioned that the surfactant material is normally absent in those animal species which possess no inclusion bodies in the Type-II cells and disappear when their alveolar epithelium is damaged.

Morphologic characteristics of the Type-II cells and their inclusion bodies have thus far been poorly defined in domestic animals. This report describes the EM appearance of Type-II pneumonocytes and their normal inclusion bodies in the lung of the one-humped camel.

2. Material and Methods

Small strips of lung tissue from 9 apparently healthy adult camels were used. The tissue was immediately fixed in cold 2.5 % glutaraldehyde with 0.05M sodium cacodylate buffer (pH 7.4) for 2 hours. Then they were post fixed in 1 % sodium cacodylate buffered osmium tetroxide for another 1 hour. The samples were dehydrated and embedded in Epon/Araldite. Thin sections were cut using a diamond knife on a Sorvall MT-2 ultramicrotome. They were stained in 5 % uranyl acetate in 70 % ethanol, followed by lead stain (Sato, 1968), then carbon coated and examined in a Philips TEM 300 at 60 Kv.

3. Results

The Type-II pneumonocytes appeared as rounded cells attached to the alveolar epithelial basement membrane and occupy the niches and corners of the alveolar walls. They were in the relatively thick portions of the interalveolar septum, frequently at the angles formed by 2 or 3 interalveolar septa (Fig.l). These cells were comparatively extensive in distribution. The size of the individual cell was large when compared with the adjacent Type - I cell or the alveolar capillaries. Their free surface facing into the alveoli carried many microvilli (Fig. 2). The lateral wall surfaces were partially covered by thin cytoplasmic extensions of the adjoining Type-I pneumonocyte. Along their boundaries, typical tight junctional complexes were seen (Fig.3).

The Type-II cells had oval or spherical nuclear profiles oriented parallel to the surface. The nucleus showed irregularities in its contour and occupied a great part of the cell volume. It also possessed a distinct relative amounts and distribution of hetero and euchromatin. Well-defined aggregations of heterochromatin was identified along the inner margin of the nuclear membrane. Each nucleus possessed one or two well-developed, eccentric nucleoli. The cytoplasm contained numerous mitochondria with variable shapes and sizes. They showed closely packed cristae and a dense matrix (Fig. 2). The Golgi complex was loosely ordered and consisted of extensive and widely dispersed cisternae. The rough-surfaced ER is extensive and consisted of relatively short dilated cisternae located throughout the cytoplasm. Free ribosomes were also found. Parallel bundles

and aggregates of intermediate filaments which had been identified as cytoskeleton were regularly observed in the cytoplasm.

Inclusion bodies:

They are a constant finding in Type-II cells as they are believed to be the site in which pulmonary surfactant is stored. Surfactant in Type-II pneumocytes was contained in a limiting membranous bodies or droplets. With gluteraldehyde fixation many inclusion bodies showed regular whorls of electron dense laminae (Fig. 4). These unique osmiophilic membrane-bound inclusions were significantly more numerous and more complexly structured in normal Type-II pneumonocytes. However, some of the examined cells had only a few of them. Inclusion bodies were randomly distributed within the cytoplasm. However, they were mostly found on one side of the cytoplasm and had no strict spatial relationship with mitochondria and other organelles. Some of them were in close apposition to the surface of the plasma membrane or even fused with it so that the contents were exposed to the alveolar space (Fig. 2). Different forms of these inclusions were observed. The most pronounced were the lamellar bodies (Fig. 5). They appeared spherical or ovoid bodies formed by numerous parallel membranes disposed in a concentric arrangement. These membranes were smooth and displayed a wavy course around a core of homogeneous electron-dense material. In general, the inclusion bodies showed both lamellae and homogenous density.

Occasionally, a few inclusions showed an almost complete absence of non-lamellar material and had a vacuolated appearance with only a thin peripheral rim of heavily osmiophilic lamellae (Fig. 6). Other inclusions within the cytoplasm either showed a lamellar appearance (Fig. 7) or were composed of both lamellae and homogenous density (Fig. 8). In addition, there were a large number of inclusions that had a uniformly heavily osmiophilic nature (Fig. 9). Also some inclusions showed lighter osmiophilic material accumulated around a heavily osmiophilic central or peripheral core (Figs.10&11). Other inclusions possessed dark osmiophilic lamellae around a moderate dense core (Fig.12).

In some Type-II pneumonocytes some inclusions had a heterogenous content or containing concentric osmiophilic lamellae in an electron lucent matrix (Fig. 13). Other inclusions had multivesicles set in an electron lucent or moderate electron dense matrix (Fig. 14). multivesicular inclusion may have light osmiophilic background density surrounding the contained multivesicles (Fig.15).

Another type appeared peppered with dense, irregular, granular deposits of maximum osmiophilia (Fig. 16). It has been found that prolonged gluteraldehyde fixation prior to osmication may result in droplets or inclusions of lesser electron density, lucent halos or apparently empty spaces.

Also some inclusions often appeared as a small locule either appeared in contact or projecting outwards onto the cell surface. These bodies appeared with electron-lucid content (Fig. 17). Few inclusions appeared as a shell-like osmiophilic body with light or moderate osmiophilic background (Fig. 18).

Few ultrathin sections revealed the presence of some osmiophilic bodies free in some alveolar lumens, particularly in close proximity to the surfaces of Type-II pneumonocyte. These bodies either appeared with electron-lucid content or showed a lamellated appearance identical with those of Type-II cells (Figs. 19 & 20).

A complex lattice of membrane-like lamellar structures was observed freely in many alveolar spaces (Fig. 21). They appeared to be unattached to any other mass. however some, however, showed an apparent contiguity to the adjacent alveolar cell plasma membrane (Figs. 22).

In the majority of samples, the lattice and the lamellar forms appeared as one structure; a portion of the structure being lattice-like and a portion was lamellar. At high resolution the continuity of the lattice membranes with those of the lamella ted forms were clear (Fig. 22). some of these structures, however, appeared distinctly as either lamellar or lattice without such combination.

Associated with such osmiophilic structures in the alveolar lumens were occasional cell debris or a layer of osmiophilic homogenous material.

The pattern of the osmiophilic membranes in the lamellar masses varied greatly but usually was in the form of parallel trilaminar membranes arranged in the form of straight lines and concentric curves. The lattice-like structure appeared as collections of osmiophilic membranes arranged in irregular or square grid-like patterns.

Each of these osmiophilic membranes was approximately 60 to 80 A^0 thick. The distance between membranes varied in each structure with a range of 100 to 700 A^0. In the lattice structures, thickening occurred at the junctions of membranes (Fig. 22).

4. Discussion

According to the above observations, the most characteristic feature of the Type-II pneumonocytes was the various-shaped osmiophilic inclusion bodies. Some of these inclusions were vacuolated or appeared with homogenous density. Others showed lighter osmiophilic material accumulated around a heavily osmiophilic core or may have, concentric osmiophilic lamellae in an electron lucent matrix.

Sorokin (1967) has demonstrated that the osmiophilia of these inclusions can be abolished by a prior extraction of the tissues with a chloroform-methanol mixture. This finding is consistent with his hypothesis that the contents of the inclusion bodies are primarily phospholipid.

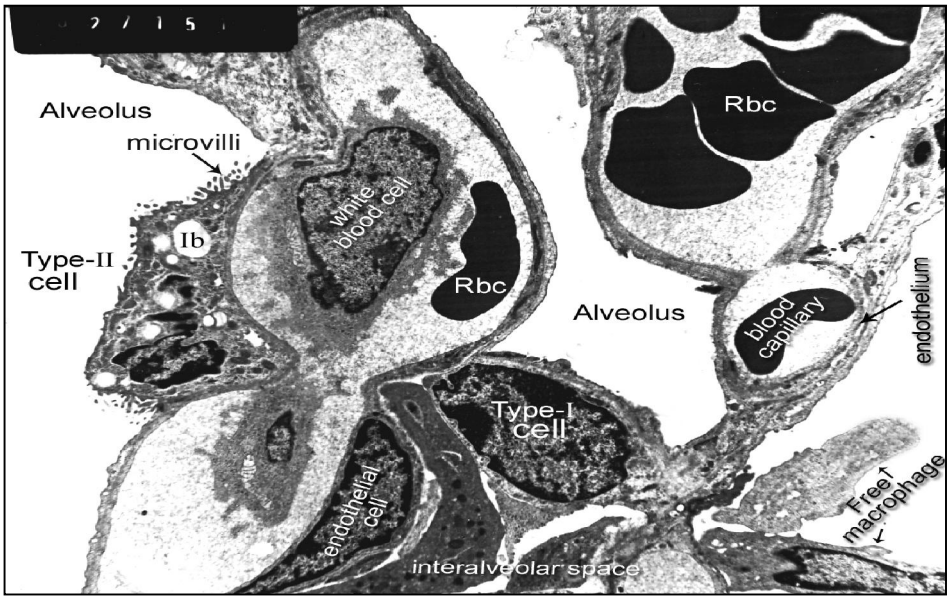

Fig. 1: Type-II pneumonocyte occupying a relatively thick portion of the interalveolar wall. Uranyl acetate-lead citrate, X 2 600

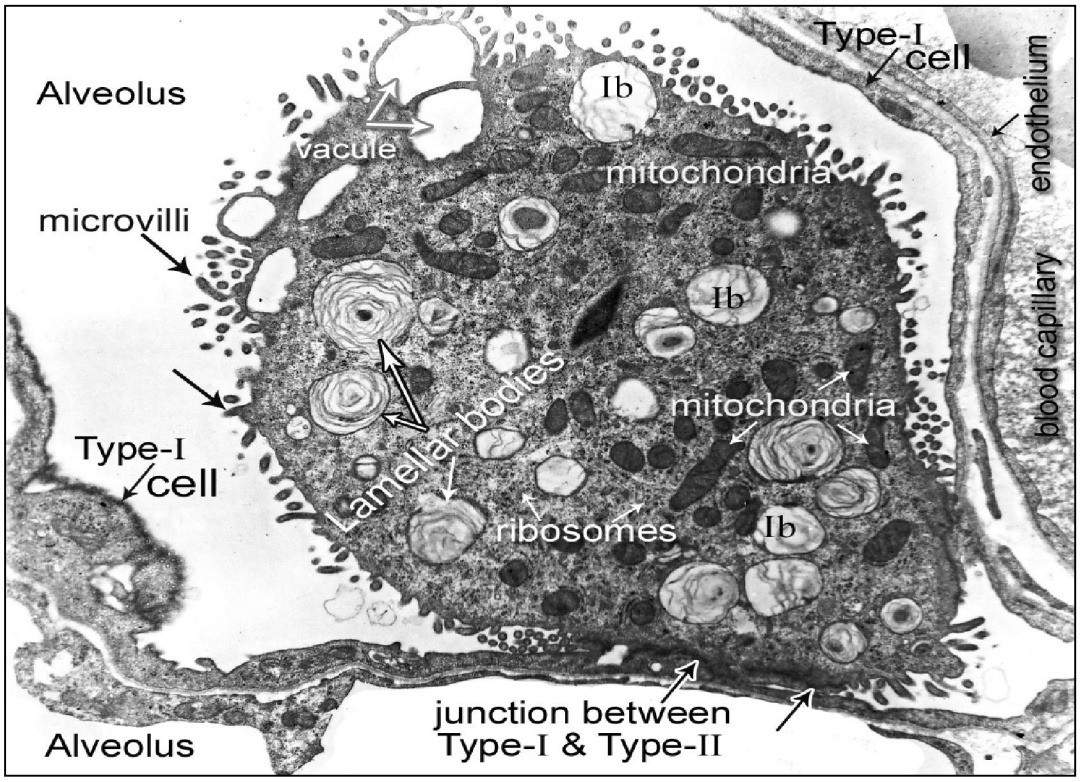

Fig. 2: Type-II pneumonocyte. Uranyl acetate-lead citrate, X 12 000

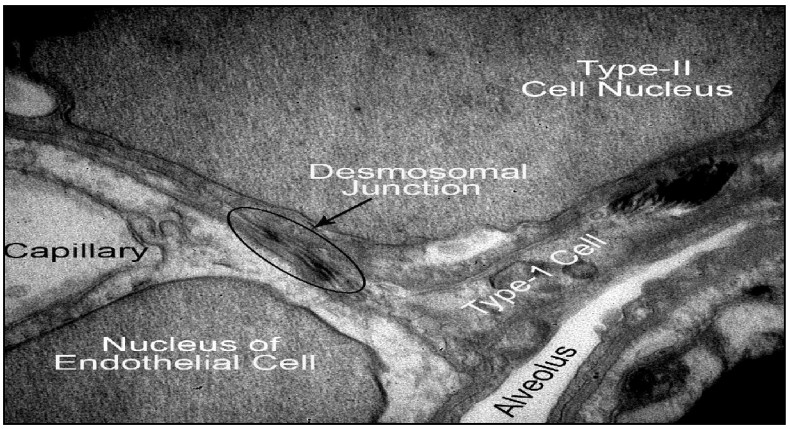

Fig. 3 : Junction between Type-I and Type-II cells (arrows). Uranyl acetate-lead citrate, X 22000

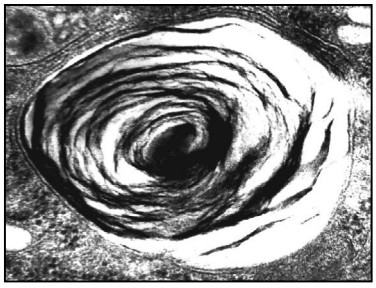

Fig.4: Inclusion bodies showing regular whorls of osmiophilic laminae , X 28 600

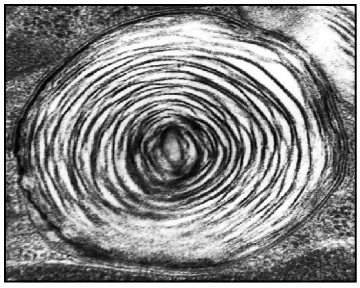

Fig.5: Inclusion body containing concentric osmiophilic lamellae in an electron lucent matrix, X 28600

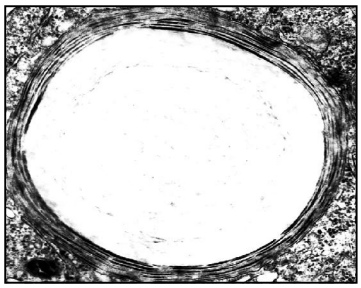

Fig.6: Inclusion body with very few peripheral osmiophilic lamellae, X 27000

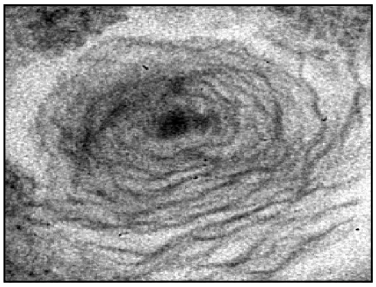

Fig.8: Inclusion body with heavily osmiophilic nature , X 22 300

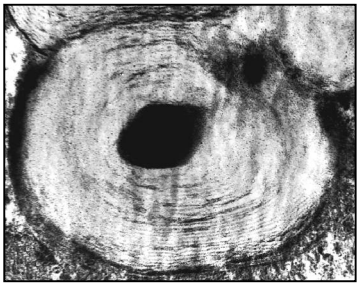

Fig.10: Lighter osmiophilic lamellae around a peripheral heavily osmiophilic core , X 17 000

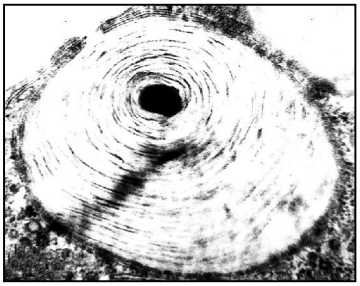

Fig.11: Lighter osmiophilic lamellae around a peripheral heavily osmiophilic core, X 17 000

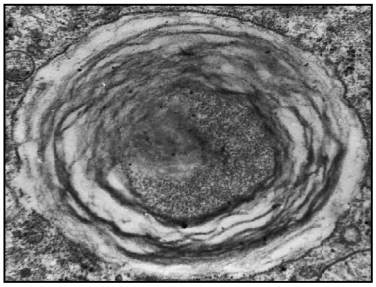

Fig.12: Inclusion body with heavily osmiophilic background, X 22 300

Fig.4: Inclusion bodies showing regular whorls of osmiophilic laminae , X 28 600

Fig.5: Inclusion body containing concentric osmiophilic lamellae in an electron lucent matrix, X 28600

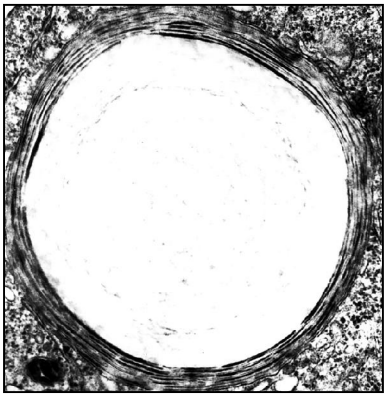

Fig.6: Inclusion body with very few peripheral osmiophilic lamellae, X 27000

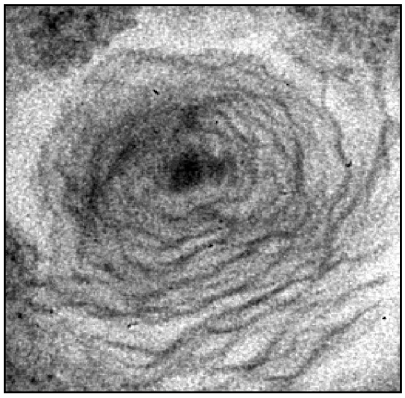

Fig.8: Inclusion body with heavily osmiophilic nature , X 22 300

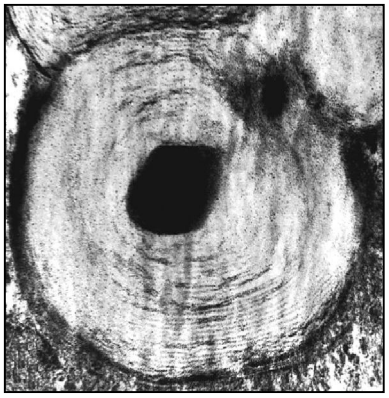

Fig.10: Lighter osmiophilic lamellae around a peripheral heavily osmiophilic core , X 17 000

Fig.11: Lighter osmiophilic lamellae around a peripheral heavily osmiophilic core, X 17 000

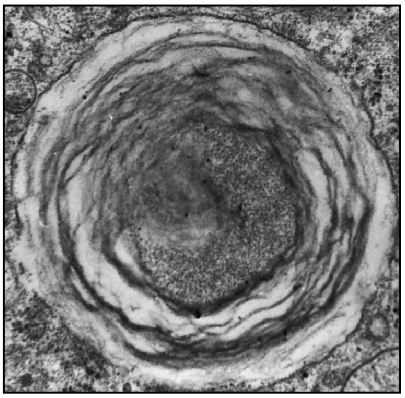

Fig.12: Inclusion body with heavily osmiophilic background, X 22 300

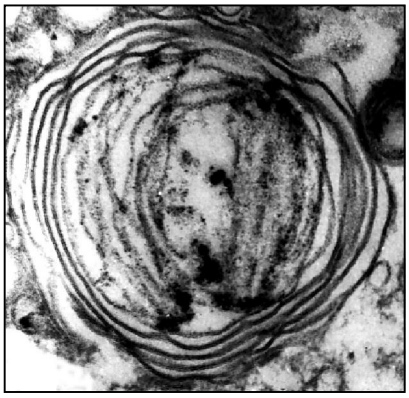

Fig.13: Lamellated inclusion body, X 28 600

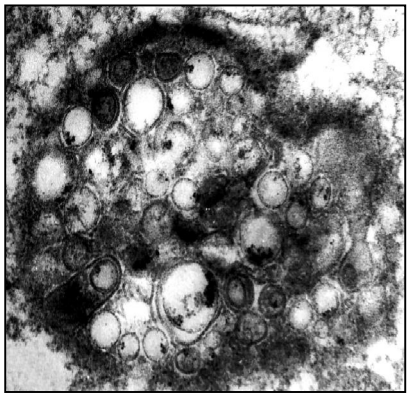

Fig.14: Multivesicular inclusion body with osmiophilic backround, X 22 000

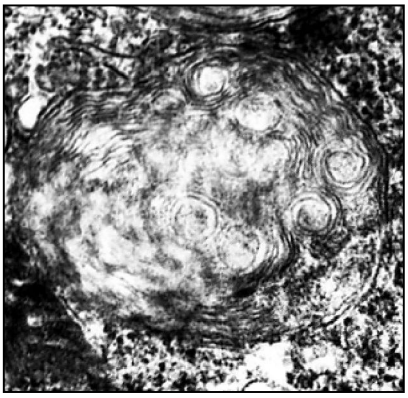

Fig.15: Multivesicular inclusion body, X 22 000

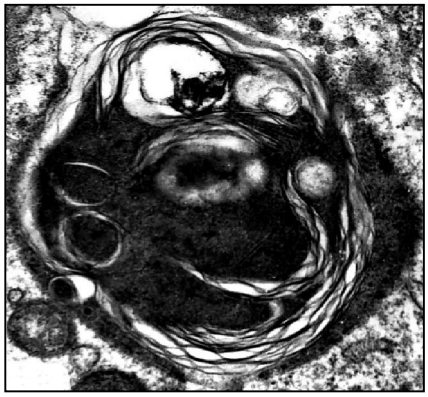

Fig.16: Multivesicular inclusion body with heavily osmiophilic nature, X 22 300

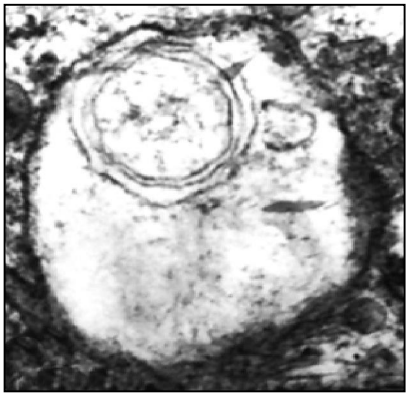

Fig.17: Inclusion body with small locule, X 17 000

Fig.18: Shell-like inclusion body, X 17 000

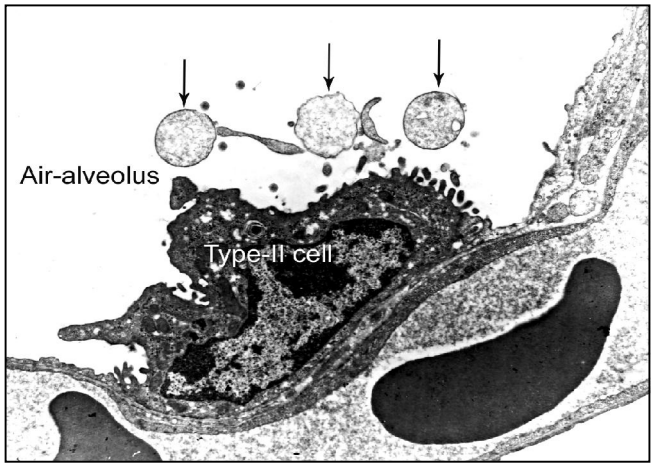

Fig. 19: Osmiophilic bodies (arrow) are shown free in the lumen of an alveolus in close relation to the surfaces of Type-II pneumocyte . Uranyl acetate-lead citrate, X 9 700.

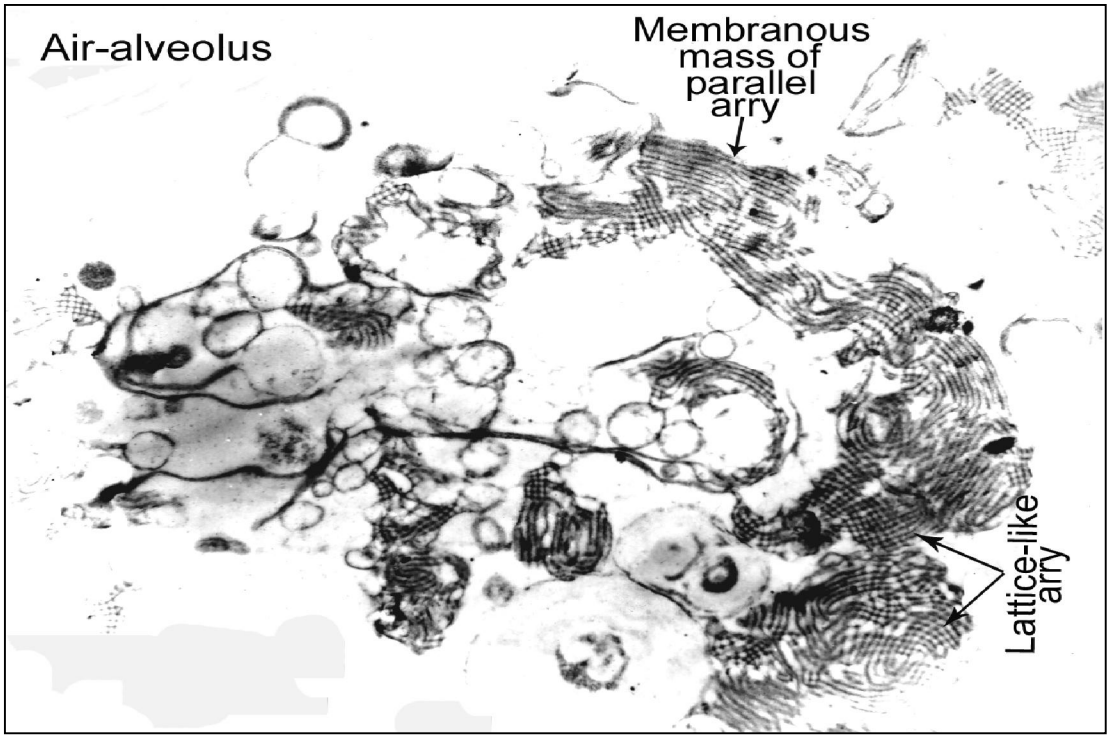

Fig. 20: A mass of membranous material in parallel array and lattice - like aril present free in the lumen of air-alveoli of camel's lung. Uranyl acetate - lead citrate, X 6 300.

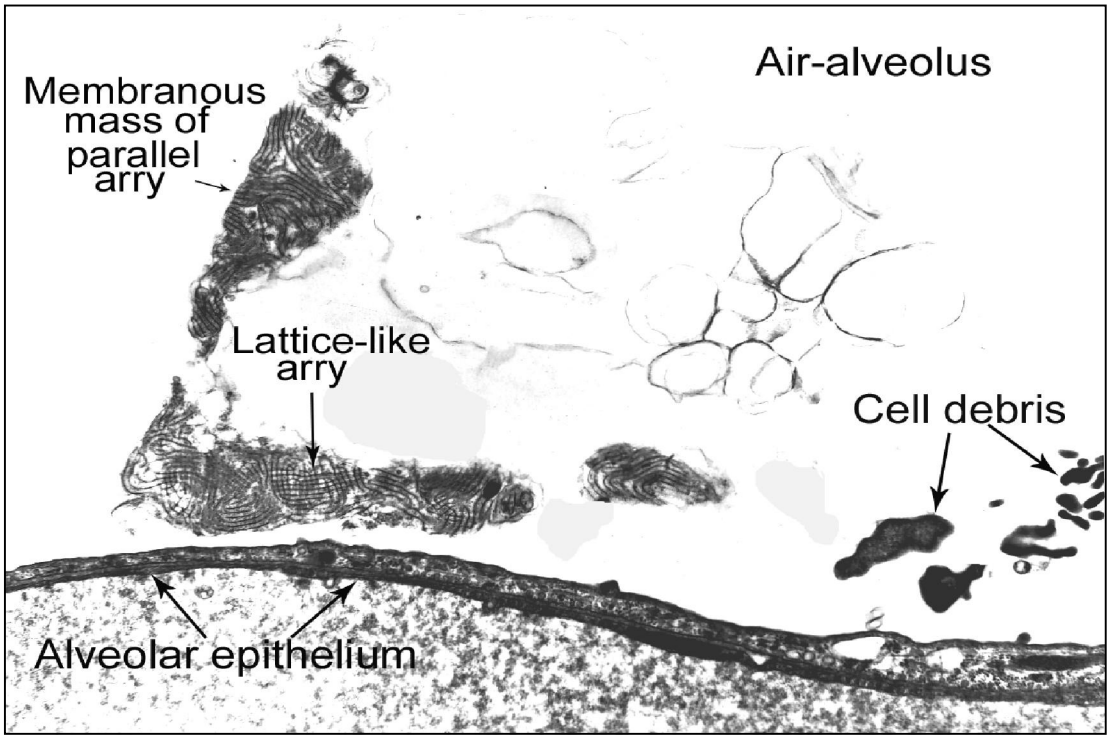

Fig.. 21 : Osmiophilic membranes in an alveolar lumen showing apparent contiguity to the adjacent alveolar epithelium. Notice the Cell debris. Uranyl acetate - lead citrate, X 6000.

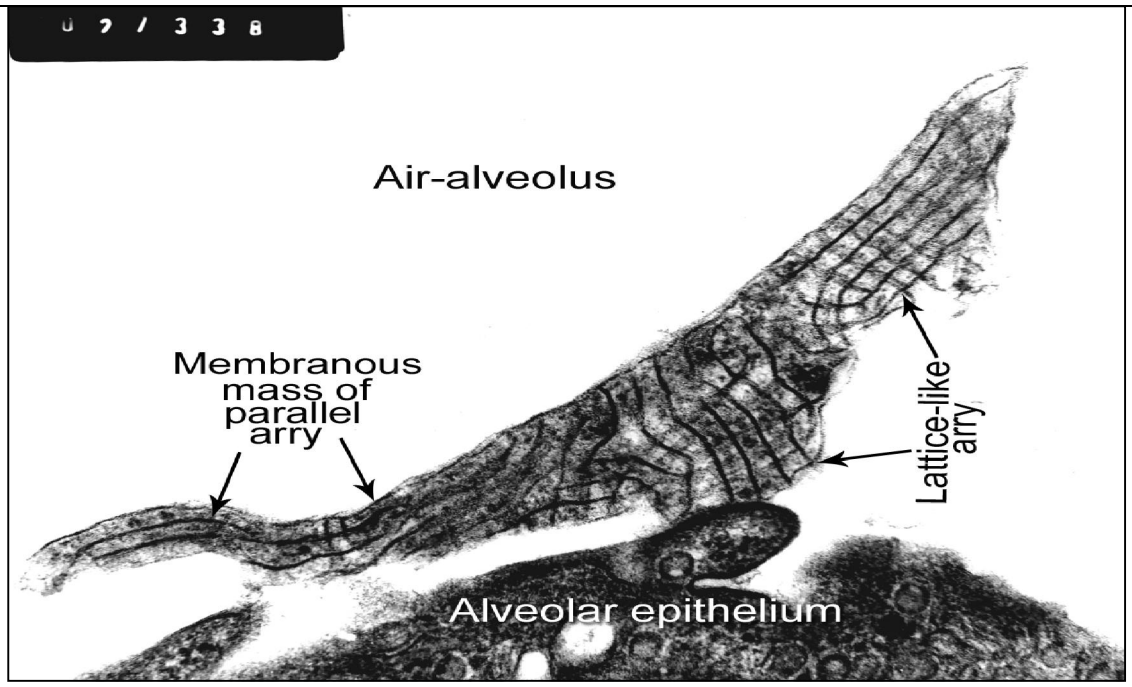

Fig. 22: Free osmiophilic membranes and lattice - like structures in the lumen of an air- alveolus. They are showing an apparent contiguity to the adjacent alveolar epithelium. Uranyl acetate - lead citrate, X 17 500.

Kikkawa and Spitzer (1969) postulated that while bound by a unit membrane the phospholipid exists in an anhydrous form and that when free within the alveoli it exists in a hydrous form. Klaus *et al.*(1962) considered that mitochondria in the Type-II cells were transformed into lamellar or inclusion bodies. According to Spencer (1985) EM studies have shown that mitochondria are not transformed into lamellar bodies.

The inclusions of Type-II cells are thought by some to be phospholipid material in the process of phagocytosis (Suzuki *et al.,* 1972). The opposing view, and still the majority view, is that these inclusion bodies represent surfactant material, phospholipid dipalmitoyl-Lecithin, synthesized within the Type-II cells (Clements and King, 1976; Collet and Chevalier, 1977 and Weibel and Gil, 1977). In an autoradiographic study, Niden (1967) and Petrick and Collet (1974) have shown that radioactive tritiated palmitate, a specific precursor of dipalmitoyl lecithin, given intraperitoneally in mice is soon incorporated in the inclusion bodies in Type-II cells and in Clara cells. Chevalier and Collet (1972) and Kikkawa and Kaibara (1979) found that the nonciliated Clara cells do produce a phospholipid, although it was not dipalmitoyl-Lecithin.

De Duve (1963) mentioned that acid phosphatase is commonly associated with the lysosomes of phagocytic cells. The presence of this enzyme in the inclusion bodies of Type-II cells (Goldfischer *et al.,* 1972) is therefore, unusual in view of the fact that these structures are generally regarded as being secretory vesicles. Nevertheless, the presence of such enzyme in secretory organelles is not restricted to the inclusion bodies of Type-II cells, since acid phosphatase has been demonstrated in secretory vesicles of Paneth cells (Coldfischer *et al.,* 1964) and anterior pituitary cells (Smith and Farquhar, 1966). Based on the above, the acid phosphatase probably performs an essential role in the ultimate synthesis of surfactant and is not fulfilling its usual function of lysosomal enzyme destruction of foreign materials. Furthermore, the inclusion bodies of Type-II pneumonocytes would appear to provide a further example of lysosomal structures assuming a secretory role. This is supported by the biochemical study of Buckingham, Heinemann, Sommers and McNary (1966). They indicated that surfactant (dipalmitoyl Lecithin) is synthesized from glycerol. The substitution of the -0H groups by one phosphate and two palmitic acid molecules results in the formation of dipalmitoyl phosphatidic acid which is then split by the acid phosphatase enzyme to form dipalmitoyl lecithin and dipalmitoyl phosphatidyl ethanolamine.

In agreement with Tyler and Pearse (1965); Said et al., (1968), the presence of a large number of mitochondria in the examined Type-II cells reflect a high level of oxidative metabolic activity in their cytoplasm. Moreover, the glycolytic and the hexos monophosphate pathway enzymes, described by the same authors, may be another important source of energy and may play a role in the synthesis of pulmonary phospholipids and Fatty acids.

Alkaline phosphatase (Kuhn, 1968) and adenosine triphosphatase (Meban, 1972) activities were demonstrated in the apical plasma membrane and microvilli of the Type-II cells. These two enzymes play a fundamental role in the transport of ions across the cell membranes of a number of tissues (Dunham and Glynn, 1961 and Auditore, 1962).

Judging from other localization, alkaline phosphatase and adenosine triphosphatase have previously been demonstrated on the microvilli of intestinal epithelium (Ashworthet al., 1963), the endothelium of capillaries (Marchesi and Barnett, 1963), moreover, the same opinion was mentioned by Sparter *et al.* (1958) in the basal infolding of the cell membranes in the kidney tubules and by Essener *et al.* (1958) in the microvilli of the bile canaliculi. In this view, it appears likely, therefore, that these two enzymes are involved in active transport mechanisms in Type-II pneumonocytes, and in particular with the extrusion of surface active agents from these cells.

As seen in this study, lamellar substance may sometimes be found within surface depressions of Type-II cells and although this has usually been regarded as evidence supporting its extrusion from the cell (Benschet al., 1964). Others have interpreted this finding as evidence for its phagocytosis by Type-II cells (Corrin *et al.,* 1969). Considerable debate has focused around the problem of whether Type-II cells possessed phagocytic activity. Although it is generally accepted that the great majority of alveolar macrophages, as elsewhere in the body, are derived from circulating cells of bone marrow origin, nevertheless Type-II pneumonocytes undoubtedly possessed limited phagocytic activity. There is general agreement in the dimensions and the morphologic detail of the lattice and the lamellar structures reported in camel's lung with those described in cattle by Schulz (1959) and Epling (1964). However, one major difference is evident, none of the previously cited literature has observed lamellar and lattice forms free in the lumens of pulmonary alveoli in apparently normal animal. The present study suggested that these forms were believed to represent an extracellular storage form or a breakdown product of surfactant. This is supported by the findings of Gil and Reiss (1973) and Kistler et al (1967) in rat.

Some examined sections are suggestive of possible continuity of the lattice-like material with the membranes of the lamellated forms. The same finding was described in cattle's lung by Epling (1964). Buckingham and Avery (1962) and Tyler and Pangborn (1964) have proposed that the inclusion bodies of Type-11cells may be the source of the surface active agent. They were thought to be primarily a lipoprotein, perhaps containing dipalmitoyl lecithin (Clements, 1962). The relation of the lattice and lamellar forms to this surface-active agent is of significance. Leeson and Leeson (1966) mentioned that the surface active agent has an important role to play in aeration of the lung. According to these authors, failure of digestion of the lattice and lamellar forms by lecithinases would indicate that their membranous material does not consist only of a lecithin. In 1966, leeson and Leeson mentioned that this material was not found in foetuses before birth. Finley and Ladman (1972) mentioned that surfactant is crucial to the respiratory function of the lungs, and derangements in surfactant production or degradation are important in several pathologic conditions including neonatal-respiratory distress syndrome, oxygen toxicity, atelectasis, radiation pneumonitis and pulmonary proteinosis.

Conclusion:

Based on the above it could be suggested that the inclusion bodies of Type-II pneumonocytes contain either surfactant or its precursor. It seems possible that differences in the morphological appearance of the inclusion bodies may well be related to difference in their chemical constituents.

It is possible to suggest a chain of events whereby the osmiophilic, lamellated and lattice-like forms in the alveolar lumens perhaps derived from the inclusion bodies of the pulmonary surface epithelium, discharge a lipoprotein into the alveolar spaces where it acts as a surface-active agent in the fluid film lining the alveoli. Moreover, these forms might protect pulmonary alveoli form irritation that might be caused by the dry and sandy surroundings of the desert. Also they might be a result of death and degeneration of alveolar epithelial cells in their normal attrition and replenishment.

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