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The pneumonocytes in the lung of *Xenopus laevis*

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INTRODUCTION

Electron microscope studies have shown that the alveoli of mammalian lung are lined by a continuous layer of epithelium which is composed of two different cell types (Schulz, 1959; Policard, Collet & Pregermain, 1959; Campiche, 1960). The membranous pneumonocytes (type I alveolar cells) are characterized by large cytoplasmic processes which extend out to cover most of the alveolar wall. In this way they form the outer layer of the air-blood barrier. In contrast, the granular pneumonocytes (type II alveolar cells) are cuboidal in shape and possess large lamellated inclusion bodies. These inclusions have recently been the subject of much investigation and it is now generally believed that they are involved in the storage and secretion of pulmonary surface-active agents (Kuhn, 1968; Williams, Vail & Valdivia, 1970; Meban, 1972*a*, *b*).

In comparison with mammals, relatively little is known about the respiratory epithelium in the lower vertebrate classes. Only two brief reports have been published on the ultrastructure of amphibian lung (Okada, Ishiko, Daido, Kim & Ikeda, 1962; Nagaishi, Okada, Ishiko & Daido, 1964). The present paper describes the fine structure of the respiratory epithelium in the lung of the clawed toad (*Xenopus laevis*).

MATERIALS AND METHODS

Adult male toads (*Xenopus laevis*) were decapitated and small blocks of lung tissue were excised and fixed for 3 hours at 4 °C in one of the following solutions:

(1) 4% glutaraldehyde in 0·1 M phosphate buffer (pH 7·3).
(2) 3% glutaraldehyde in 0·1 M cacodylate buffer (pH 7·3).
(3) glutaraldehyde-hydrogen peroxide fixative (Peracchia & Mittler, 1972).

The specimens were then washed for 18 hours in cacodylate buffer containing 0·25 M sucrose, post-fixed for 1 hour in 2% osmium tetroxide, rapidly dehydrated in ethanol, and embedded in Durcupan. Sections (silver-grey) were cut on a Reichert ultramicrotome, stained with alcoholic uranyl acetate (Stempak & Ward, 1964) and lead citrate (Reynolds, 1963), and examined in an AEI 801 electron microscope.

RESULTS

The respiratory portion of *Xenopus* lung consists of relatively large air sacs (150–300 μm in diameter) which contain a close network of capillaries in their walls (Fig. 1). In this paper the epithelial cells which line these air spaces are referred to as 'pneumonocytes'.

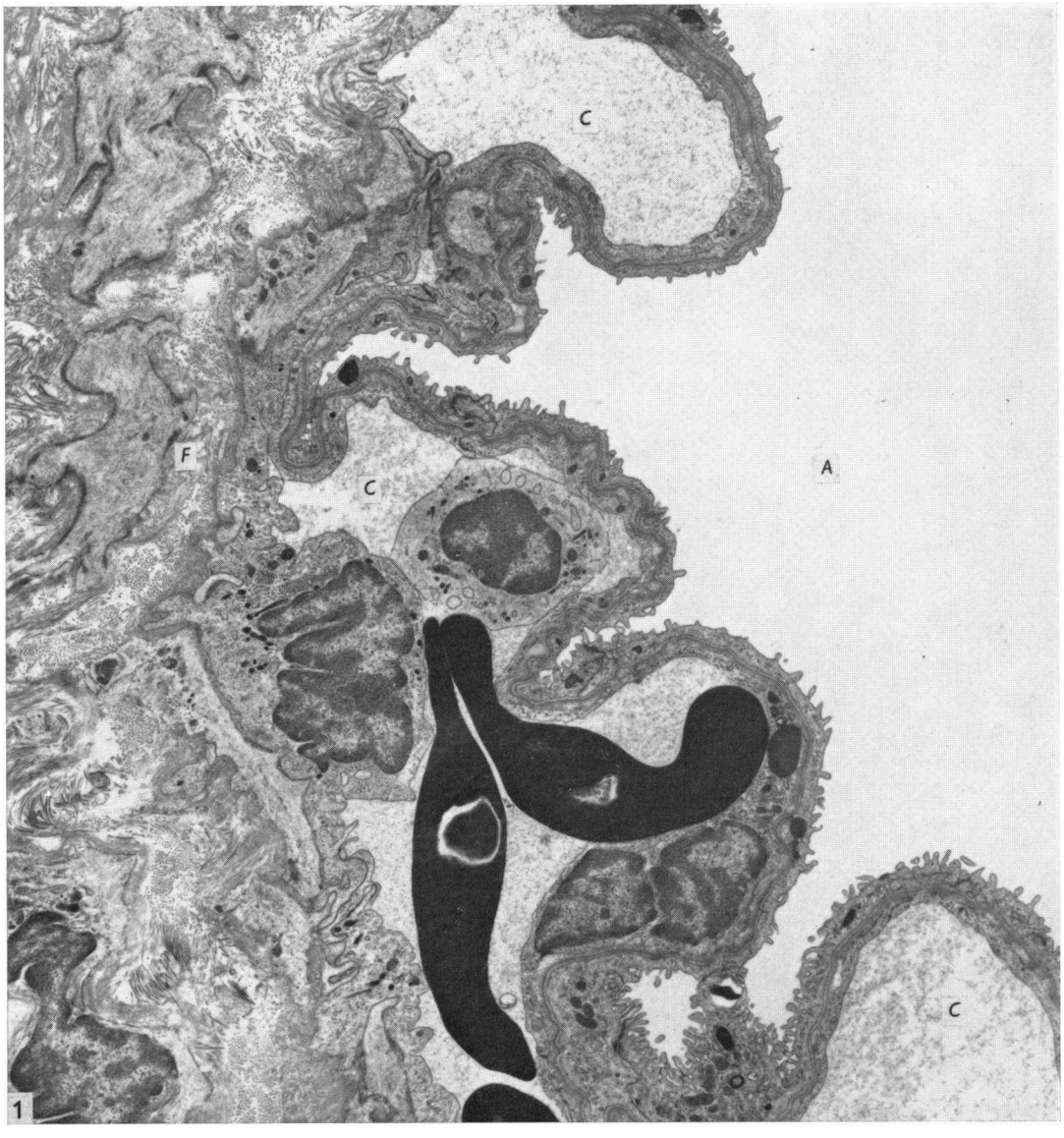


Fig. 1. Low power electron micrograph of an air sac wall. *A*, air space; *C*, lumen of pulmonary capillary; *F*, fibrous septum. ×5500.

The pneumonocytes are squamous in form. Each cell consists of a thick portion, which contains the nucleus, and a peripheral sheet of cytoplasm (Fig. 2). These cytoplasmic sheets are generally very attenuated (250–500 nm in thickness) and they spread out over the pulmonary capillaries to form the outer layer of the air-blood barrier (Figs. 3, 4). The lateral extent of the cytoplasmic sheets may be as much as 25 μm. The nucleus is not always situated at the centre of the squama; in some cases it occupies an asymmetrical position near the lateral cell border. In many cells the

nuclei are countersunk into recesses between adjacent capillary loops (Fig. 2). Not infrequently the pneumonocytes send cytoplasmic processes deep into the air sac wall (Fig. 3). Examination of serial sections shows that these processes do not necessarily contain the cell nucleus.

The free surfaces of the pneumonocytes bear numerous microvilli (Fig. 4). These are short cylindrical processes measuring 0·25–0·35 μm in length and 0·12–0·20 μm in diameter. Although present in all regions of the cell surface, the microvilli appear to be more plentiful on the membrane covering the thicker regions of the cell (Fig. 2). No filaments are observed within the interior of the microvilli.

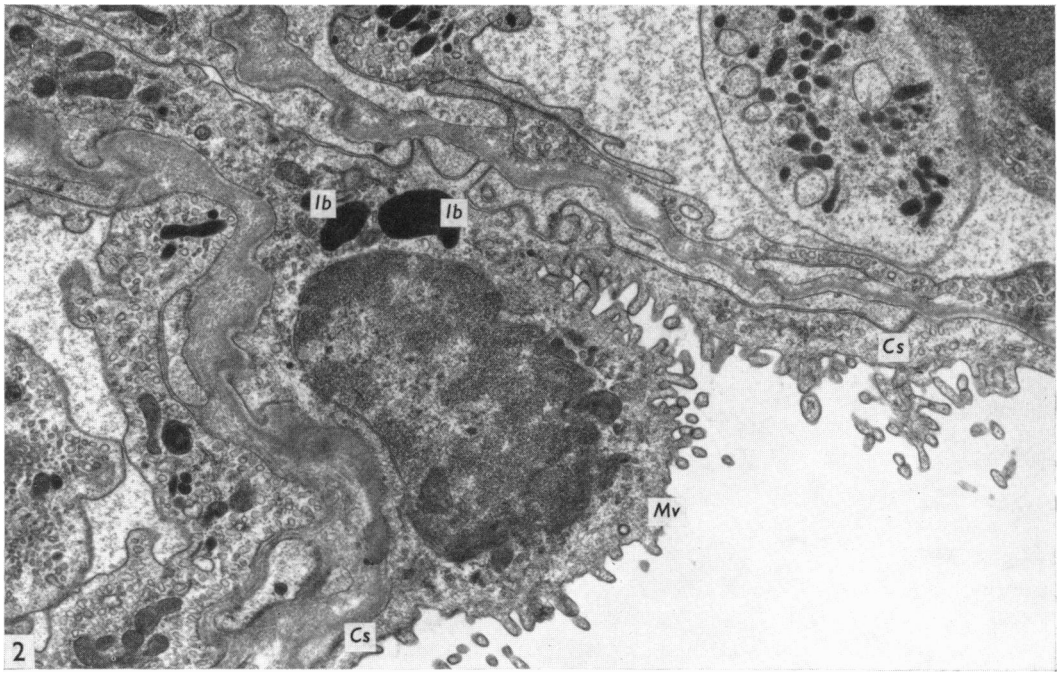
The lateral cell walls are separated along most of their length by a gap of about 40 nm. Towards the free surface the lateral plasma membranes of adjacent cells approximate and from a junctional complex which consists of a superficial tight junction, a short intermediate junction, and a large desmosome (Figs. 5, 6). Fine filaments radiate out from the desmosome plaque into the surrounding cytoplasm (Fig. 6). Additional desmosomes and interdigitating cytoplasmic processes are present deeper on the lateral walls of cells which make contact over a wide area (Fig. 5).

The cell nuclei are ovoid in shape and rarely show indentation of the nuclear membrane (Figs. 2, 7). Chromatin is evenly distributed within the nucleus and a well-differentiated nucleolus is often present (Fig. 7). The association of ribonucleoprotein granules with the outer nuclear membrane is seen infrequently.

The cytoplasm contains numerous free ribosomes and particles of β-glycogen (Fig. 7). Profiles of smooth endoplasmic reticulum are common, but only short lengths of the rough-surfaced reticulum are present. Mitochondria, usually few in number, are ovoid or rod-shaped, and possess an electron-dense matrix; the cristae are mainly of the transverse plate variety (Fig. 5). Mitochondria are sparse in the attenuated cytoplasmic sheets. The Golgi apparatus, which consists of several stacks of flattened cisternae and associated small agranular vesicles, does not appear to have a constant location within the cell. Multivesicular bodies are common in the supranuclear region of the cytoplasm (Fig. 10). Most cells contain a few lipid droplets; these droplets are only lightly stained by heavy metals and do not appear to be limited by a membrane (Fig. 9).

Every pneumonocyte contains several inclusion bodies. Although these organelles predominate in the supranuclear region, they also occur singly or in small groups in the subnuclear cytoplasm or in the attenuated cytoplasmic sheets (Figs. 2, 3). The inclusion contents are intensely osmiophilic and are either in the form of dense masses (Fig. 10) or irregular concentric lamellae (Fig. 7). Each inclusion is limited by a single membrane about 8 nm in thickness. A small proportion of the inclusions in any cell reach a relatively great size (up to 3 μm in maximum diameter). In such cases the contents are invariably in the form of several closely packed whorls of lamellae. Some inclusion bodies open at the free surface of the cell, and their contents are obviously discharged in this way (Fig. 8).

The pneumonocytes are separated from the connective tissues of the air sac wall by a dense basal lamina (Figs. 4, 7). In most specimens a thin electron-transparent space intervenes between the basal lamina and the cell membrane (Fig. 4). This space appears to be continuous with the general intercellular space. Micropinocytotic pits are particularly abundant in the basal plasma membranes of the pneumonocytes (Fig. 4).



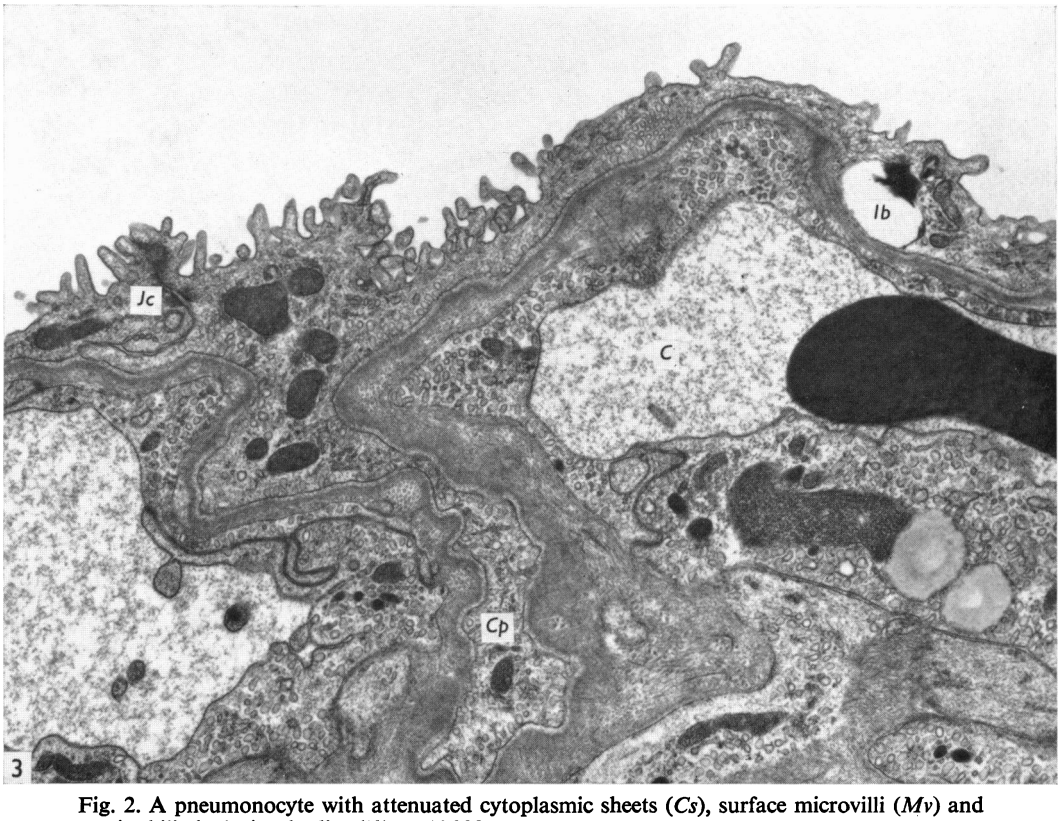
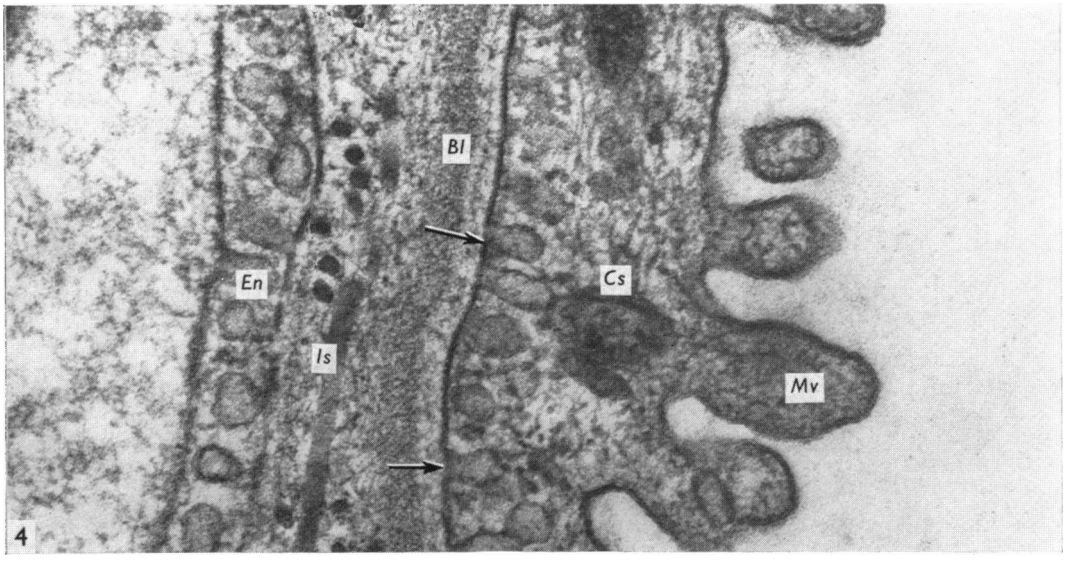


Fig. 2. A pneumonocyte with attenuated cytoplasmic sheets (*Cs*), surface microvilli (*Mv*) and osmiophilic inclusion bodies (*Ib*). ×13000.

Fig. 3. A cytoplasmic process (*Cp*) extending deep into the air sac wall. *Ib*, inclusion body; *Jc*, junctional complex; *C*, lumen of pulmonary capillary. ×13500.



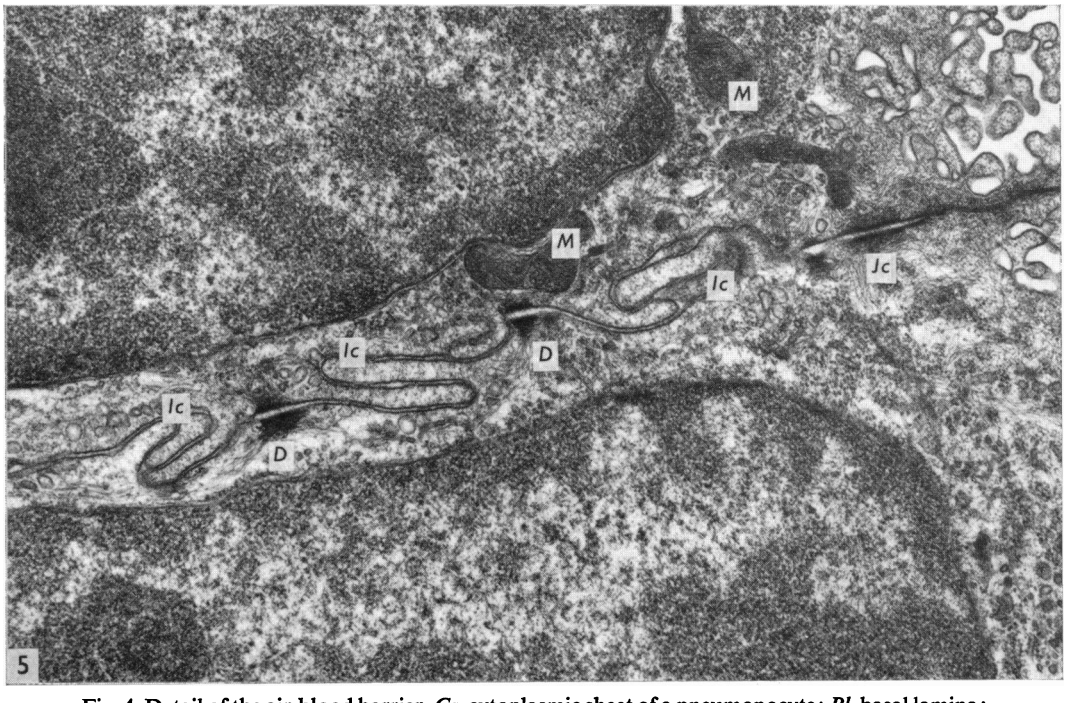


Fig. 4. Detail of the air-blood barrier. *Cs*, cytoplasmic sheet of a pneumonocyte; *Bl*, basal lamina; *Is*, interstitial space containing collagen fibres; *En*, cytoplasm of a capillary endothelial cell; *Mv*, microvillus; arrows, micropinocytotic pits. ×80000.

Fig. 5. The boundary between two pneumonocytes. *Ic*, interdigitating cytoplasmic processes; *D*, desmosome; *Jc*, junctional complex; *M*, mitochondrion. ×18000.

Deposits of membranous material are often seen in the air spaces. The membranes are thin (8–10 nm) and show a strong affinity for osmium stain (Fig. 11). They are generally arranged in whorls or loose tangles, although in some specimens short lengths of membrane are seen to be attached to the outer surface of the pneumonocytes. Dense amorphous material is often associated with the membranous profiles. Macrophages are not observed in the air spaces.

DISCUSSION

This study has shown that the air sacs of *Xenopus* lung are lined by a continuous layer of epithelium. The cells forming this epithelium are termed 'pneumonocytes' as they appear to be homologues of the alveolar cells in mammalian lung.

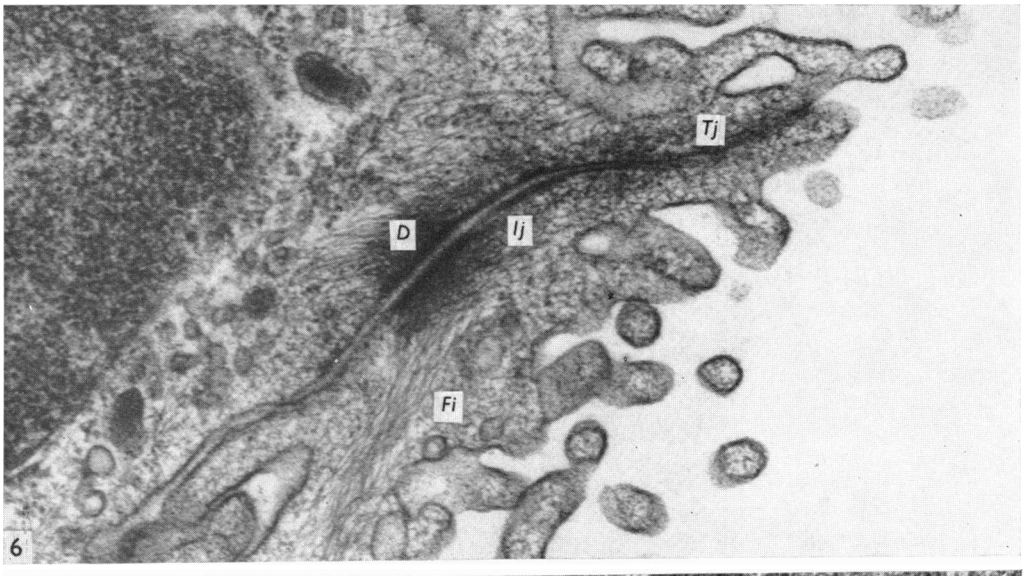
Only one variety of pneumonocyte is found in the lung of *Xenopus*. This cell has extensive cytoplasmic sheets, surface microvilli, and numerous osmiophilic inclusion bodies. These cytological features are reminiscent of both the membranous pneumonocyte (type I alveolar cell) and the granular pneumonocyte (type II alveolar cell) of mammalian lung. Differentiation of the respiratory epithelium into two distinct cell types, each having a different function, appears only to occur in the higher vertebrate classes.

The ultrastructural features of the respiratory epithelium in the lungs of frogs (*Rana nigromaculata*) and newts (*Triturus pyrrhogaster*) have been described by Okada *et al.* (1962). These workers have suggested that certain cells which are located deep in the clefts between adjacent capillaries may differ in function from those situated in more exposed regions of the air sac wall. However, there is no reason to believe that such functional heterogeneity exists among the pneumonocytes of *Xenopus* lung.

The significance of the microvilli on the surface of the pneumonocytes is not known. While it is possible that the large surface area provided by the microvilli could be related to absorption, the available experimental evidence suggests that little fluid movement takes place across the respiratory epithelium of adult amphibians (Kilburn, 1967). Alternatively, the microvilli may provide a reserve of plasma membrane which allows the cells to become distended with secretion products. It is interesting that Woodhouse & Rhodin (1963) have suggested a similar function for the microvilli on the secretory cells of Harderian glands.

In amphibians air is forced into the lungs by contraction of the muscles in the floor of the mouth. During this process a high positive pressure is generated and the air sac walls are put under considerable tension (Winchester & Lovell, 1961). However, the pneumonocytes in *Xenopus* lung appear to be particularly well adapted to withstand such tension. There is a rich supply of desmosomes and interdigitating cytoplasmic processes on the lateral cell walls. In addition, the cytoplasmic pegs which extend deep into the air sac walls may help to anchor the epithelium.

Despite an extensive search, macrophages have not been observed in the air spaces. Furthermore, there is little morphological evidence to suggest that the pneumonocytes have any significant phagocytic ability. Obviously further investigation is needed to determine how foreign particulate matter is removed from the respiratory portion of amphibian lung.



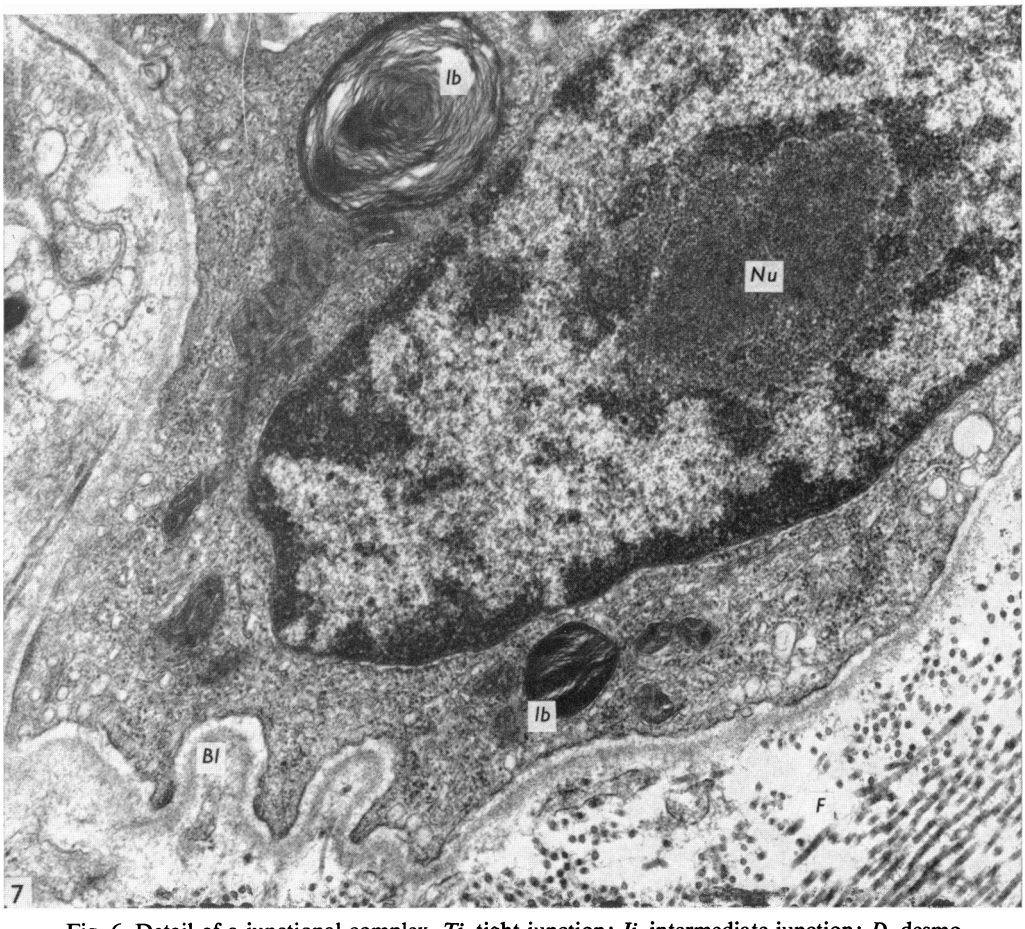
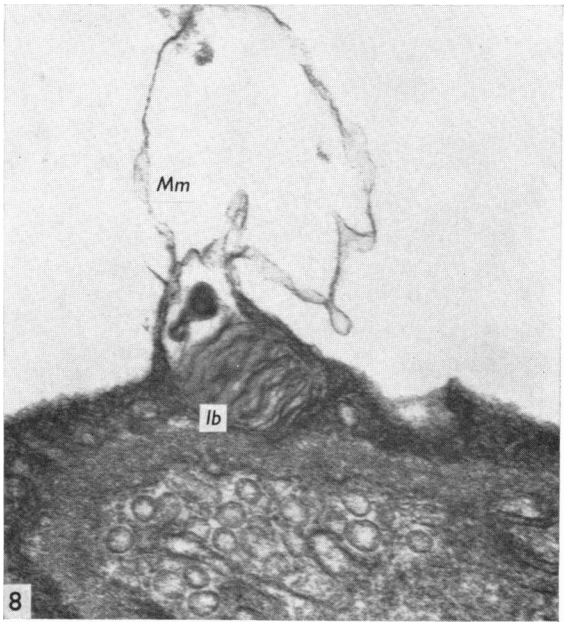
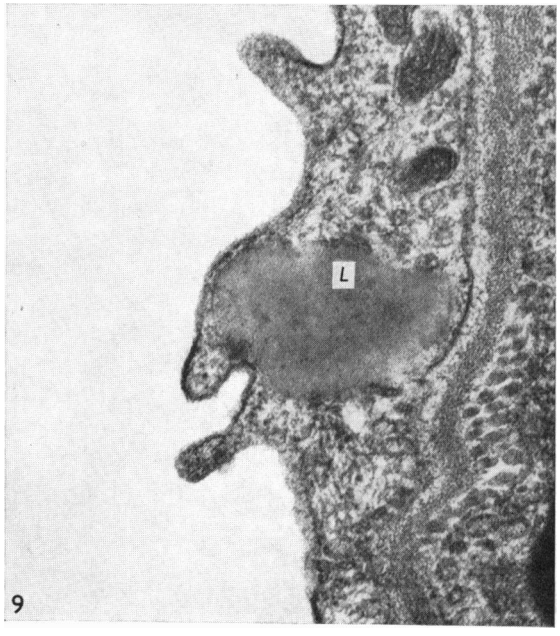
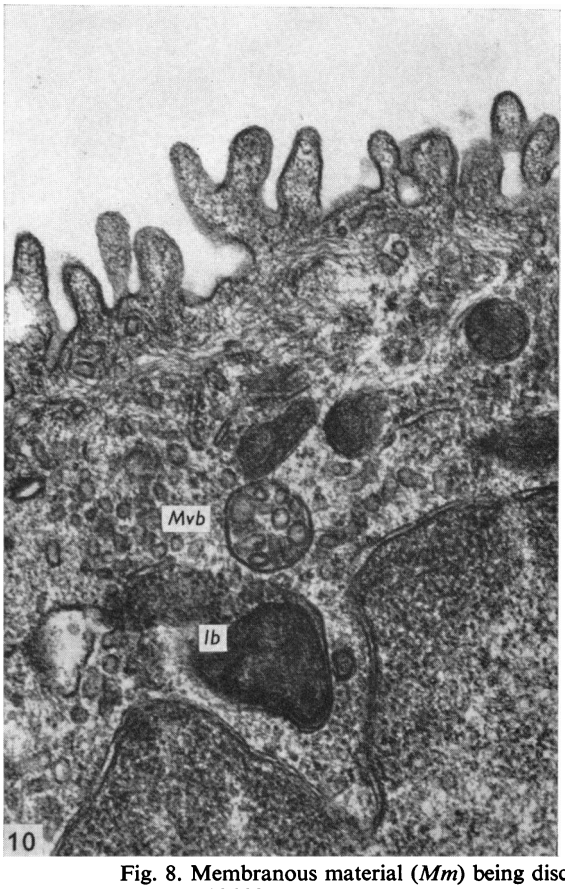


Fig. 6. Detail of a junctional complex. *Tj*, tight junction; *Ij*, intermediate junction; *D*, desmosome; *Fi*, fibrils extending out from desmosome plaque. ×40000.

Fig. 7. The basal surface of a pneumonocyte. *Ib*, lamellated inclusion body; *Nu*, nucleolus; *Bl*, basal lamina; *F*, fibrous tissue of air sac wall. ×34000.







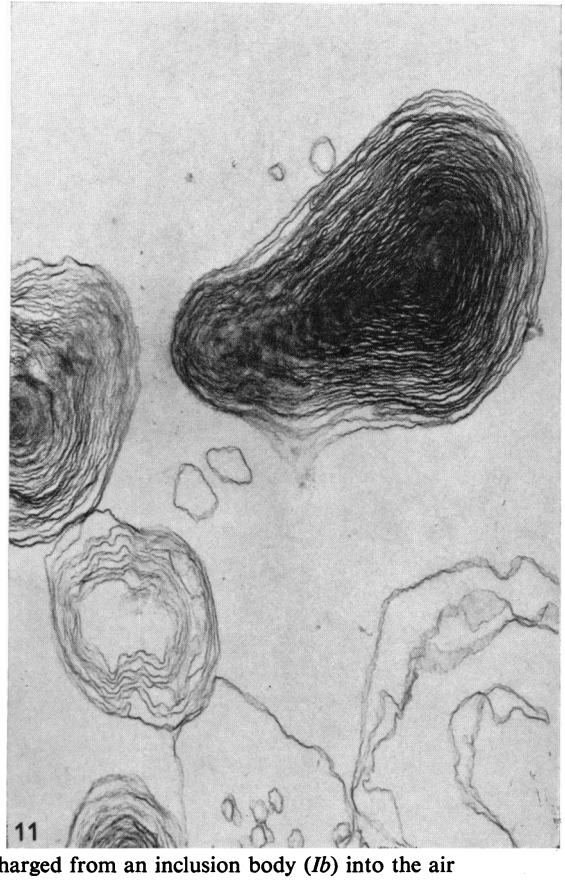


Fig. 8. Membranous material (*Mm*) being discharged from an inclusion body (*Ib*) into the air space. × 32000.

Fig. 9. Lipid droplet (*L*) within the cytoplasm of a pneumonocyte. The droplet does not possess a limiting membrane. × 50000.

Fig. 10. A multivesicular body (*Mvb*) and an osmiophilic inclusion body (*Ib*) in the supranuclear cytoplasm. × 38000.

Fig. 11. Whorls of osmiophilic membranes in the air space. × 19000.

The present study has shown that the inclusion bodies of pneumonocytes are intensely osmiophilic and that they often display a multilamellar structure. In addition, micrographs have been produced which apparently show inclusion bodies in the process of discharging their contents into the air space. These findings suggest that the inclusion bodies are secretory organelles and that they give origin to the osmiophilic lamellae which lie free in the air spaces.

In respect of its form and staining reactions, the membranous material in the air spaces of *Xenopus* lung closely resembles that observed in the respiratory portion of avian (Petrik & Riedel, 1968) and mammalian (Leeson & Leeson, 1966; Weibel & Gil, 1968) lungs. Moreover, Finley, Pratt, Ladman, Brewer & McKay (1968) have shown that the surface-active fractions of pulmonary washings contain a rich supply of myelin figures. The fact that the membranes are mainly composed of unsaturated phospholipid explains their great affinity for osmium stain. It is likely, therefore, that the air sacs of *Xenopus* lung are lined by a thin film of surface-active lipid and that the collections of membranes observed in the present study represent displaced fragments of this material.

SUMMARY

The pneumonocytes which line the air spaces of the lung in *Xenopus laevis* have been studied by electron microscopy. The pneumonocytes are squamous in form and possess attenuated sheets of cytoplasm which extend over the pulmonary capillaries. Microvilli are plentiful in the free cell surface and osmiophilic inclusion bodies are prominent in the cytoplasm. The lateral cell walls have numerous desmosomes and interdigitating cytoplasmic processes. The results of the study suggest that the pneumonocytes act not only as a selective barrier for gaseous diffusion but that they are also responsible for the secretion of surface-active agents.

I am grateful to Mr G. R. Dickson for his skilled technical assistance.

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