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Degenerative Processes in the Pathogenesis of Pulmonary Alveolar Lipoproteinosis

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Summary. Electron microscopy in an infant of 4 months with pulmonary alveolar lipoproteinosis showed filling of the alveoli with osmiophilic lamellar bodies. Similar structures were present in the cytoplasm of type I alveolar epithelial cells and to a lesser extent in the capillary endothelium and interstitium. These changes represent widespread degenerative processes in the lung caused by an unidentified cytotoxic agent. In this patient the disease is comparable to the drug-induced cytotoxic animal model and differs from the dust-induced hypersecretory animal model.

Key words: Lung – Electron microscopy – Pulmonary alveolar proteinosis – Degeneration.

Introduction

Pulmonary alveolar proteinosis (Rosen et al., 1958) is a condition in which the air spaces are filled with an acellular finely granular deposit which stains well with eosin and the periodic acid-Schiff reaction. Lipoproteinosis is possibly a better name because in addition to proteins derived from the blood (Hawkins et al., 1967) the alveolar material contains much complex lipid including palmitoyl lecithin, the principal surface active agent of the lung (Ramirez-R. and Harlan, 1968). Surface tension-lowering activity is lacking however, but is restored by ethanol (McClenahan and Mussenden, 1974) and the alveolar material may therefore be considered to contain altered lung surfactant. Electron microscopy is in accordance with this, showing osmiophilic lamellar and lattice structures (Basset et al., 1973; Costello et al., 1975) similar to the surfactant secretion vacuoles of type II pneumocytes and to bodies believed to be altered surfactant found in small numbers in the normal alveolus (Mendenhall and Sun, 1964; Gil and Weibel, 1969).

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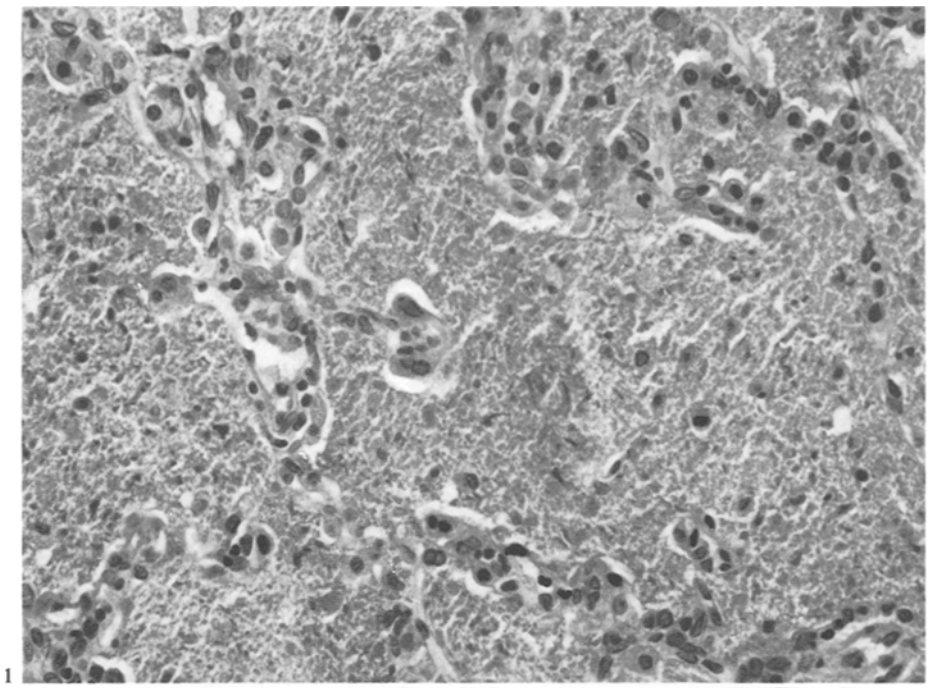
The cause of alveolar proteinosis is seldom identified, but many patients have dusty occupations (Davidson and McCleod, 1969), and occasionally heavy exposure to silica dust appears to have been responsible (Buechner and Ansari, 1969; Hoffmann et al., 1973; Xipell et al., 1977). An animal model employing silica and other dusts is available (Corrin, 1962; Corrin and King, 1966, 1969, 1970; Gross and deTreville, 1968; Heppleston, 1967; Heppleston et al., 1970; Heppleston and Young, 1971) and the pathogenesis of this experimental condition is well understood. It develops through a phase of endogenous lipid pneumonia characterised by foamy fat-laden macrophages filling the alveoli and apparently incapable of effective alveolar clearance. These cells eventually disintegrate and the alveolar lumen becomes filled with their released cytoplasmic contents which compact into the characteristic alveolar proteinosis material. In man, areas of lung adjacent to alveolar proteinosis often contain many foamy macrophages and the pathogenesis of the human disease is probably similar (Schober et al., 1974; Costello et al., 1975). Heppleston et al. (1974) employed tracer substances in the experimental model and showed a threefold increase in surfactant synthesis. Surfactant clearance also increases but fails to keep pace with the secretory process and there is a relative clearance failure. There are therefore good grounds for regarding alveolar proteinosis as a hypersecretory response to non-specific irritants.

Another animal model suggests that a quite different mechanism may also result in pathological features very similar if not identical to those of alveolar proteinosis. Vijeyaratnam and Corrin (1973), studying the effect of high doses of the anti-depressive drug iprindole found widespread degenerative changes in the rat lung. Autophagocytic vacuoles and myelinic figures (residual bodies) were found in the flattened type I alveolar epithelial cells, capillary endothelium and bronchiolar Clara cells. These degenerative structures were extruded from the epithelial cells and taken up by alveolar macrophages, only to be released again when these cells subsequently died. Gradual compaction of this cellular detritus led to the appearences of alveolar proteinosis. The early stages of this degenerative process have also been reported in rats receiving the anorexic drug chlorphentermine (Lüllmann-Rauch et al., 1972; Heath et al., 1973; Smith et al., 1973). The final stages of the drug ingestion model are similar to those of the dust inhalation model but the source of the lipid differs: in the latter it represents a hypersecretory process and in the former widespread cellular degeneration.

We have occasionally seen alveolar proteinosis in patients who have been treated with cytotoxic drugs for conditions such as leukaemia, and similar cases have been reviewed by Lakshminarayan et al. (1976). Whilst it is possible that in these patients, the leukaemia, the chemotherapy or an unidentified opportunistic infection may have acted as a non-specific stimulus to surfactant secretion by type II cells, as in the dust exposed rat, degenerative processes in type I cells, as in the iprindole treated rat, may provide an alternative explanation. The possibility of this alternative pathogenetic mechanism operating in man is supported by our electron microscopic studies of a child with alveolar proteinosis, and our findings in this patient form the basis of this report.



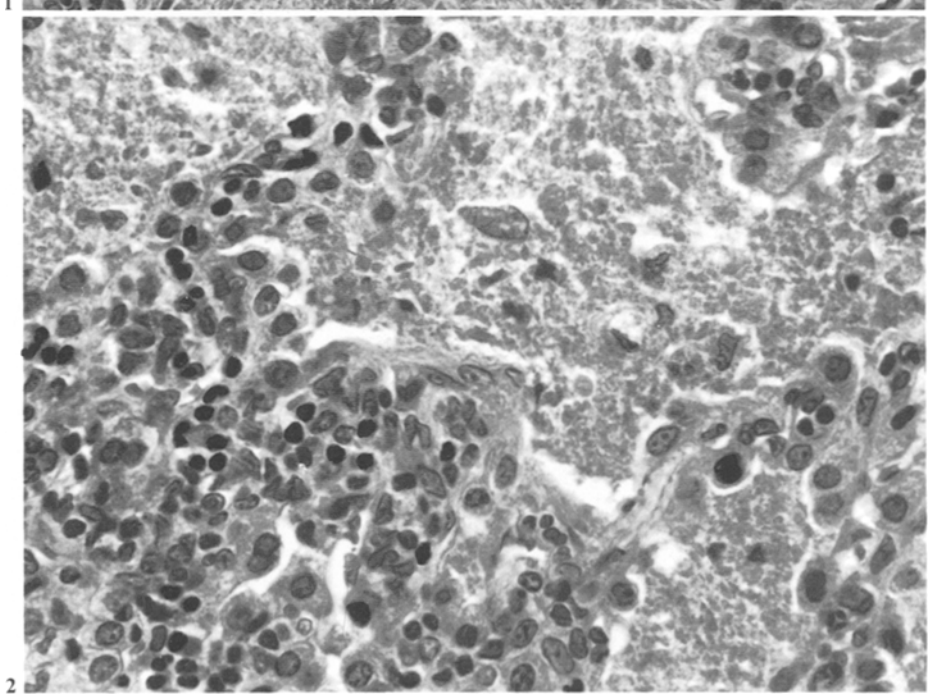


Fig. 1. The alveoli are filled with a finely granular deposit and there are no obvious alterations to the alveolar walls. Haematoxylin and eosin (H & E) $\times 125$

Fig. 2. The alveolar lumen is filled with the finely granular deposit of alveolar lipoproteinosis but there is also an interstitial lymphocytic infiltrate. H & E $\times 500$

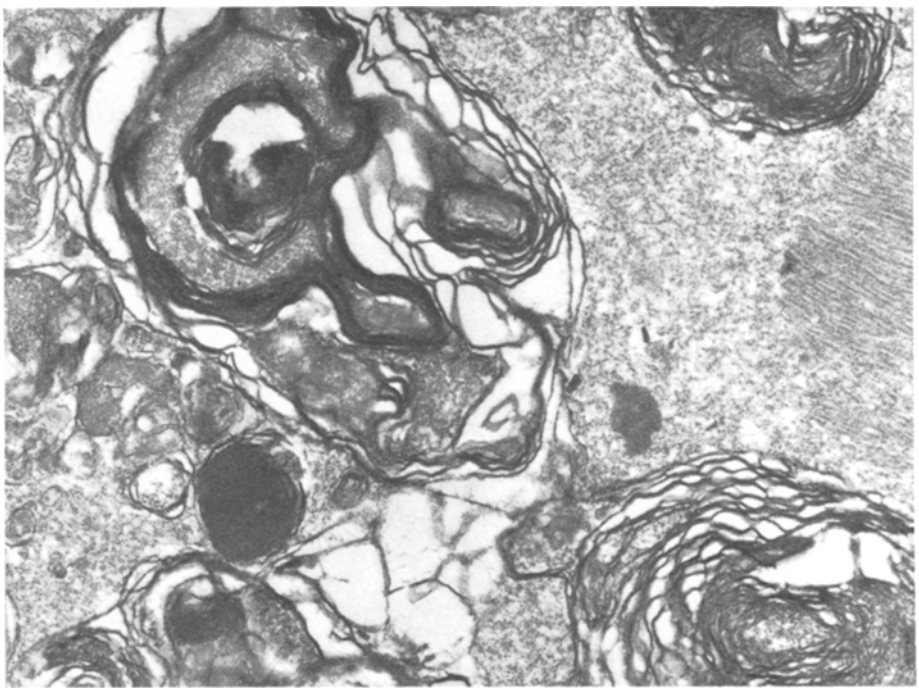


Fig. 3. The alveolar material consists of osmiophilic lamellar bodies. Electron micrograph (EM) × 34,200

Case Report

A male Moroccan baby born prematurely (7 months 3 weeks gestation, birth weight 2.8 kg) of consanguinous parents (cousins) was admitted to the University St Pierre Hospital, Brussels, at the age of 4 months for anaemia and hepatomegaly. On admission he weighed 4 kg, haemoglobin 8 g%, WBC 15,800 with 56% neutrophils and 43% lymphocytes, later rising to 24,000. A diagnosis of galactosaemia was supported by failure of the patient's erythrocytes to metabolise galactose. A small intestinal biopsy showed no abnormality. Chest X-ray showed micronodular shadowing throughout both lungs. He was treated with Bactrim (trimethoprim and sulphamethoxazole) but his paO_2 deteriorated to 39 mm Hg on air and he was given 40% oxygen. An open lung biopsy (details below) showed alveolar proteinosis and a lymphocytic infiltrate, but no evidence of pneumocystis, cytomegalovirus or fungal infection. Cultures for viruses, mycoplasma, bacteria including tubercle bacilli, and fungi were negative. For three weeks he was treated with steroids and 40% oxygen, and then briefly connected to a respirator (60–70% oxygen) before being given a right lower lobe washout. He died of a pneumothorax 30 h later, 10 weeks after the onset of symptoms, having steadily deteriorated over this period. Needle samples taken after death showed a fatty liver and pulmonary changes similar to those seen in the biopsy. No further post mortem examination was permitted.

Pathological Studies

For light microscopy lung tissue was fixed in Bouin's solution and 5 μ paraffin sections were stained with haematoxylin and eosin, by the periodic acid-Schiff (PAS) reaction, with Masson's

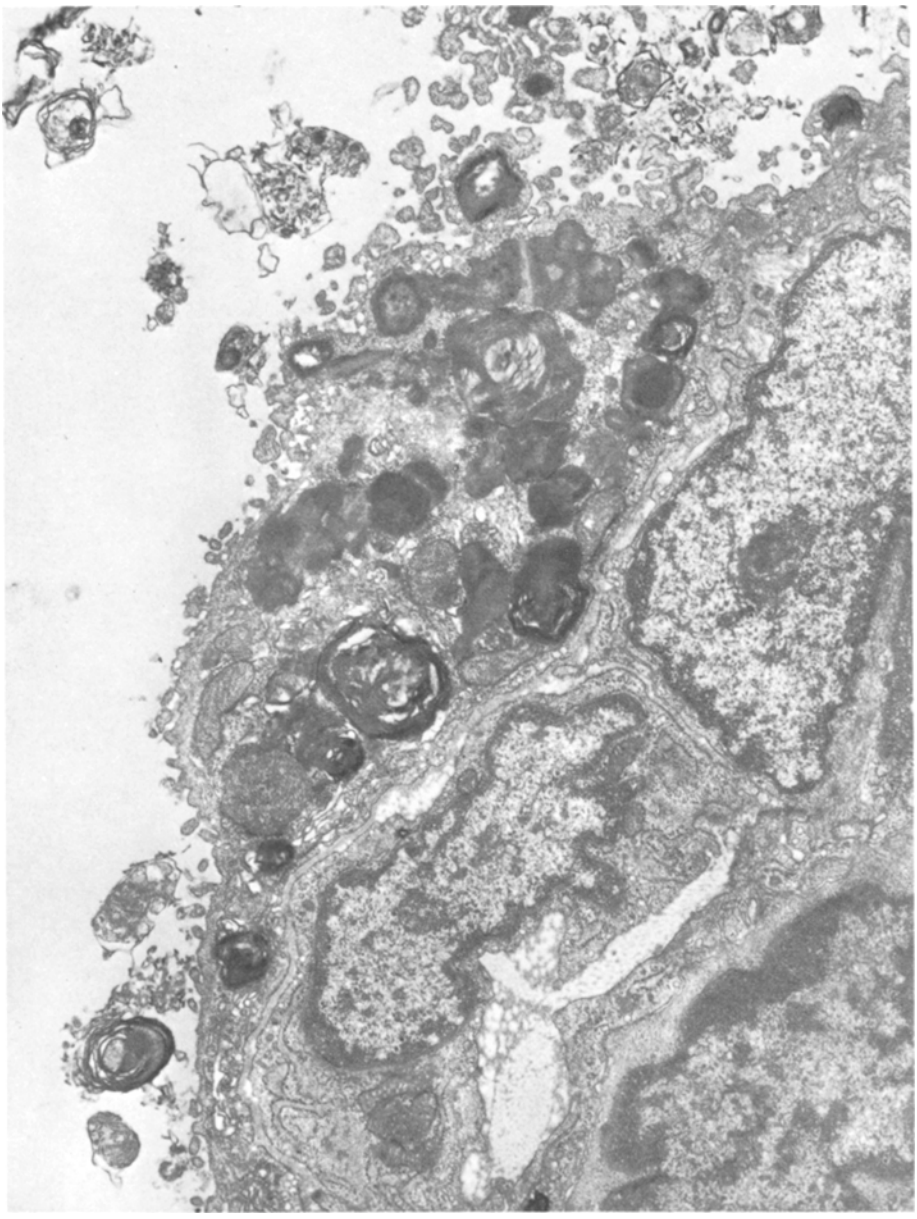


Fig. 4. A type I alveolar epithelial cell containing numerous osmiophilic lamellar structures (myelin figures, the residual bodies of autophagocytic cytoplasmic degradation). Similar structures are seen free in the alveolar lumen. EM $\times 1200$

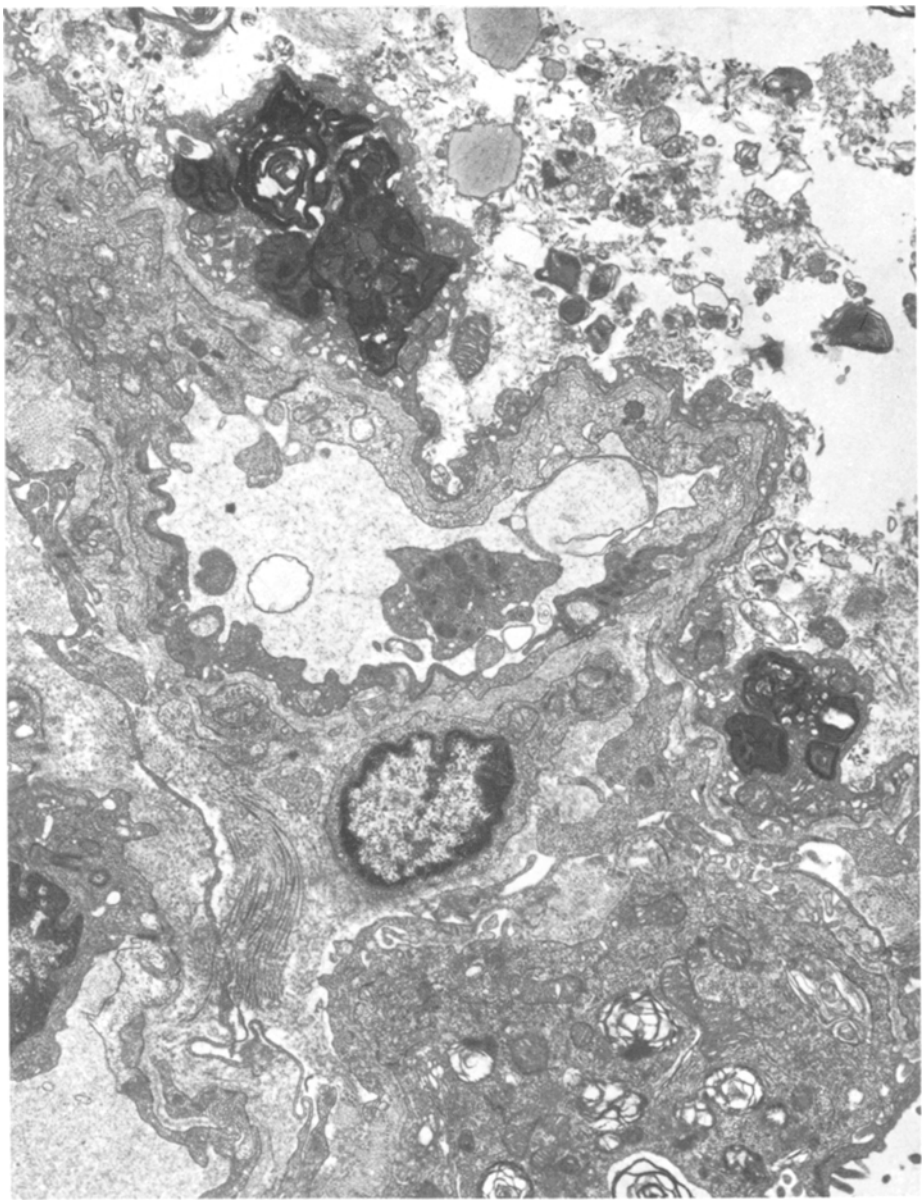


Fig. 5. A type I alveolar epithelial cell (top left to centre right) contains two large collections of densely osmiophilic lamellar material which is also seen free in the alveolus. A capillary (centre) shows endothelial blebbing but a type II epithelial cell (bottom right) appears normal. Osmiophilic lamellar inclusions are a normal feature of these cells: they represent surfactant secretion vacuoles and are structurally different from the myelin figures seen in type I cells. EM ×8100

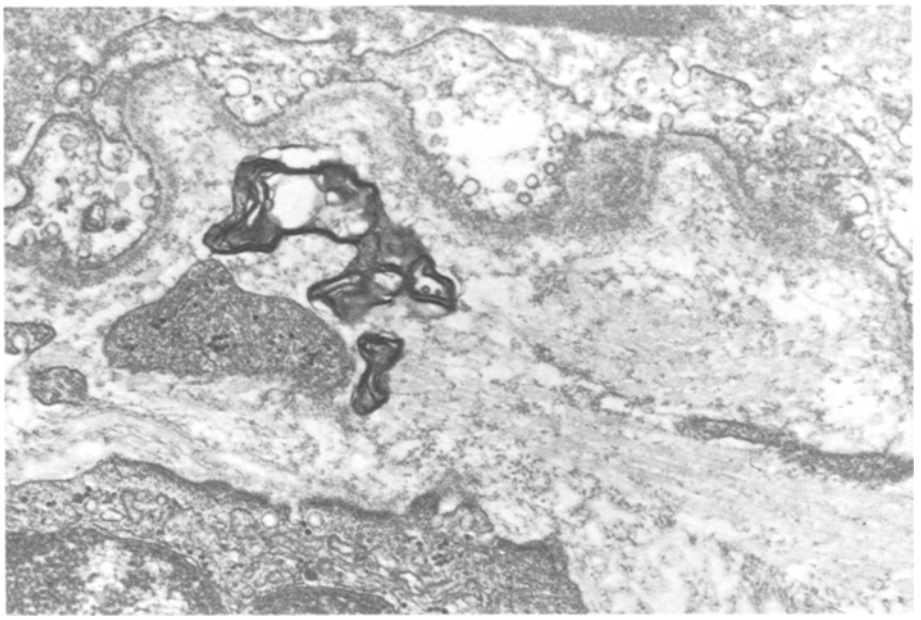


Fig. 6. A myelin figure in the alveolar interstitium. EM ×24,000

trichrome and methenamine silver. For electron microscopy, small pieces of the lung biopsy were fixed for 90 min in phosphate buffered 4% glutaraldehyde (pH 7.4), rinsed overnight in glucose-enriched (5.4 g per litre) phosphate buffer and post-fixed in 2% osmium tetroxide for 90 min. Semi-thin Epon sections stained with toluidine blue were examined by light microscopy, and thin sections of selected areas were stained with uranyl acetate and lead citrate for examination in a Siemens Elmiskop 101 at 80 kV.

On *light microscopy* patchy alterations were found in the lung, Some alveoli appeared normal, some contained aspirated mucus whilst others were filled by a finely granular, densely eosinophilic and strongly PAS positive deposit in which there were occasional mononuclear cells and a rare acicular crystal cleft, the characteristic appearences of alveolar proteinosis (Figs. 1 and 2). There was also a mild interstitial lymphocytic infiltrate occasionally forming small focal collections (Fig. 2). There was no consistent spatial relationship between the alveolar proteinosis and the lymphocytic infiltration. No viral inclusions, fungal elements or Pneumocystis carinii organisms were identified. Semi-thin Epon sections confirmed the granular nature of the intra-alveolar deposit and showed that it was osmiophilic. In a few alveoli vacuolated mural cells taken to be type II pneumocytes appeared to be increased in number but this was not generally evident even in the areas of alveolar proteinosis.

Electron microscopy showed that the alveolar deposit contained many lamellar bodies 0.25–2.75 μm diameter made up of irregularly whorled densely osmiophilic membranes (Fig. 3), together with many small membranous fragments, amorphous material and occasional lipid droplets. Pronounced changes were seen in type I pneumocytes whose thin lateral extensions contained many membrane bound vacuoles filled with complex osmiophilic lamellar bodies similar to those observed free in the alveolar lumen and presumable the source of the free bodies (Figs. 4 and 5). Occasional lamellar bodies were seen in capillary endothelial cells and free in the interstitium (Fig. 6). The capillary endothelial cells also showed intracytoplasmic fluid blebbing and subendothelial fluid collections (Fig. 7). Free in the alveolar lumen there were occasionally found disintegrating cells laden with osmiophilic lamellar bodies (Fig. 8). Type II pneumocytes contained numerous lamellar inclusions but apart from a possible increase in the number of these organelles appeared normal.

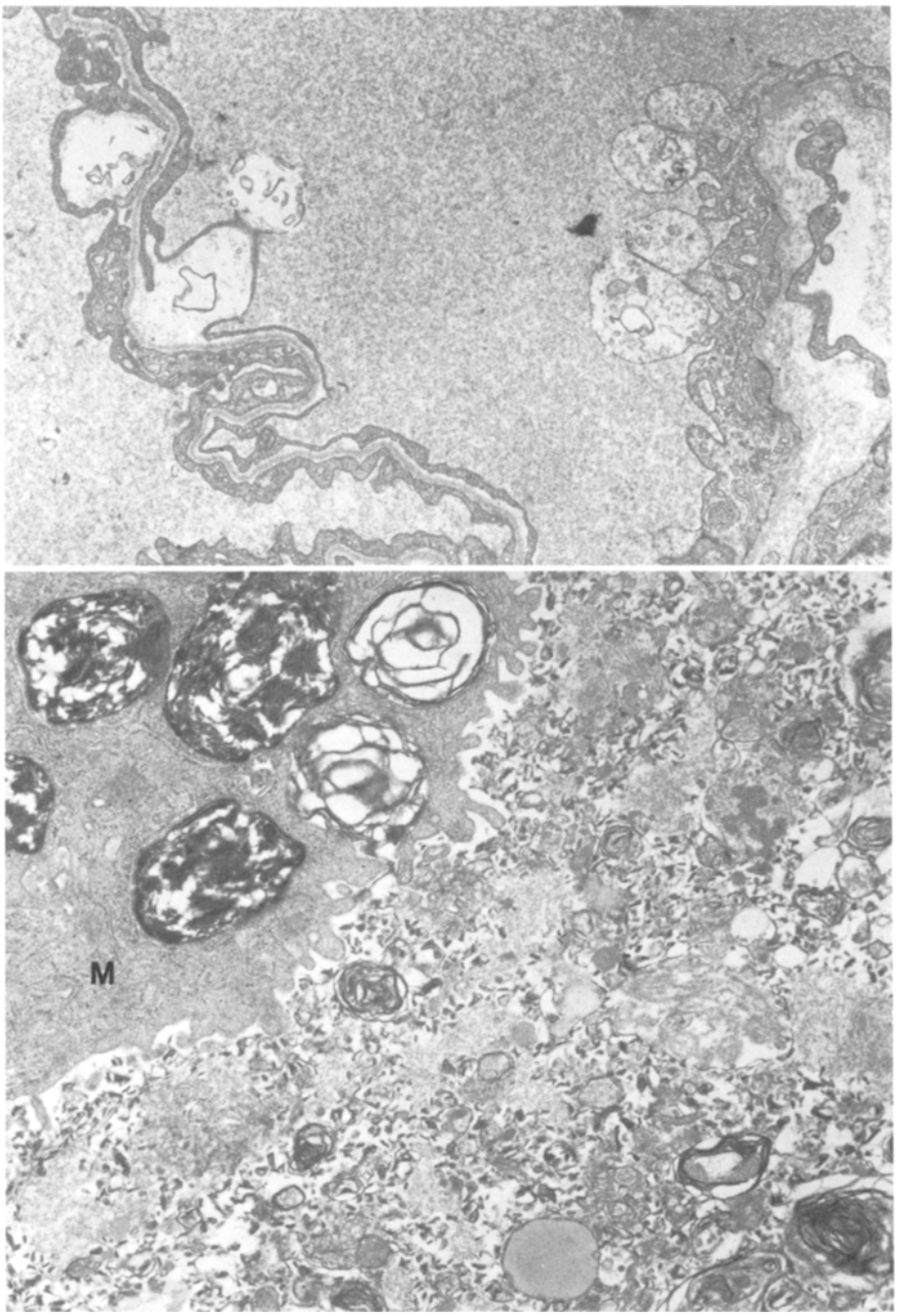


Fig. 7. An alveolar capillary showing endothelial blebbing. To the left there is also subendothelial and subepithelial oedema. EM ×12,000.

Fig. 8. Besides cellular debris and osmiophilic lamellar bodies the alveoli contain macrophages with similar inclusions (*M*). EM ×16,000

Discussion

The diagnosis of pulmonary alveolar proteinosis in this infant rests on our morphological observations: the changes observed correspond to the original light microscopic description (Rosen et al., 1958) and subsequent reports of ultrastructural studies in this disease (Basset et al., 1973; Costello et al., 1975). The principal point of interest is the similarity of the changes to those observed in the iprindole-treated rat (Vijeyaratnam and Corrin, 1973) indicating that in this patient the disease represents widespread degenerative processes in the lung leading to the accummulation of lysosomal degradation products in the lumen of the alveoli. The presence of foamy alveolar macrophages suggest that the subsequent treatment of this material is similar to that previously outlined in the pathogenesis of alveolar proteinosis, namely ingestion but not digestion of epithelial material by alveolar macrophages and its later release and compaction into a characteristically staining alveolar deposit (Corrin and King, 1970; Schober et al., 1974; Costello et al., 1975). A profound difference from the dust-induced experimental model is that there the intra-alveolar accummulation represents excessive pulmonary surfactant (Corrin and King, 1970; Heppleston et al., 1974). Most examples of the disease in man probably correspond to the dust-induced model in that lung washings are rich in dipalmitoyl lecithin (Ramirez-R. and Harlan, 1968; McClenahan and Mussenden, 1974). However when it is seen in leukaemic patients (Lakshminarayan et al., 1976; Carnovale et al., 1977) cytotoxic drug effects may be important.

The cause of the cytotoxicity in our patient was not identified. The interstitial lymphocytic infiltrate suggested a viral infection but none was identified by culture or electron microscopy. A further possibility is that the lipidic cytoplasmic inclusions may represent a lipid storage disorder but our data provide little support for this, going no further than identifying galactosaemia in life and a fatty liver post mortem. Many children with this disease have an immunological defect (Gray, 1973) but on this aspect our investigative data are again incomplete, clinical attention having to be concentrated upon immediate supportive measures. The cause of the cytotoxicity therefore remains unknown but there can be little doubt that the alveolar lipoproteinosis represents a cytotoxic effect.

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