Diagnosis and management of alveolar proteinosis: the rôle of electron microscopy

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Costello, J. F., Moriarty, D. C., Branthwaite, M. A., Turner-Warwick, M., and Corrin, B. (1975). *Thorax*, **30**, 121–132. **Diagnosis and management of alveolar proteinosis: the rôle of electron microscopy.** The diagnosis and management of three cases of pulmonary alveolar proteinosis are described. The electron microscopic appearances of lung biopsy material, lung washings, and sputum and the value of this method of sputum examination in extremely ill patients are demonstrated. The practical details of controlled volume bronchial lavage are described and the good clinical and physiological response of patients reported. The findings have been compared with those of experimental pulmonary alveolar proteinosis, and the pathogenesis of the condition is discussed.

Alveolar proteinosis is a disease of uncertain aetiology characterized by the presence in pulmonary alveoli of deposits of periodic acid Schiff (PAS) positive, proteinaceous material rich in lipid (Rosen, Castleman, and Liebow, 1958). Early diagnosis of this rare condition is important because good therapeutic results can be obtained by the use of bronchial lavage (Ramirez-R., 1966). Most commonly, the diagnosis is established by lung biopsy although examination of the sputum by light microscopy occasionally reveals amorphous, eosinophilic material which may be suggestive but not pathognomonic (Vidone *et al.*, 1966). Electron microscopy of lung biopsies has demonstrated intra-alveolar macrophages containing characteristic annular inclusions and these were found in our first case. Annular inclusions were sought and found in the bronchial washings from a second patient, and this experience enabled the correct diagnosis to be made by electron microscopic examination of the sputum in a third patient, who was considered too ill for biopsy. The diagnostic features and the short-term results of bronchial lavage in these three patients are reported here.

CLINICAL FEATURES

CASE 1 A 44-year-old dairy worker developed progressive dyspnoea over two years, associated with intermittent left chest pain. An abnormal chest radiograph, showing generalized shadowing, had been noted before the onset of pulmonary symptoms, and a provisional diagnosis of sarcoidosis had been made. No treatment was given and his dyspnoea increased until at the time of referral, two years after the onset of symptoms, he was breathless climbing only six or seven stairs. At that time he had marked finger clubbing but no other abnormal physical signs.

A chest radiograph showed extensive shadowing in the mid-zones extending from the hila; the results of pulmonary function tests and measurements of arterial blood gas tensions are recorded in Table I. Small amounts of sputum were expectorated with difficulty and light microscopy showed only scanty leucocytes and no positive PAS material.

A lung biopsy, obtained at right thoracotomy, showed groups of alveoli filled with granular exudate which stained positively with PAS. This confirmed the diagnosis of alveolar proteinosis. The alveolar walls were normal on light microscopy. Treatment by bronchopulmonary lavage was initiated, the first

TABLE I
CASE 1

	Predicted	Pre-lavage	After 2nd Lavage
PaO$_2$ (mmHg)	90– 100	81	70
TLC (ml)	5530	4400	4920
FRC (ml)	3350	2700	3020
FVC (ml)	4180	2950	3750
TF(ml/min/mmHg)	26·5	14·4	13·7

procedure being undertaken three weeks after thoracotomy when the incision was well healed.

Lavage of the right lung was carried out on two occasions, separated by an interval of five days. After the first procedure, there was a temporary increase in dyspnoea associated with a fall in arterial oxygen tension which recovered to the pretreatment value within a few days. Following the second lavage, there was no obvious change in his condition. At the time of discharge, the chest radiograph showed some decrease in the density of shadowing in the right lung field and there was marginal improvement in lung volume measurements (Table I). Further lavage and follow-up have been undertaken elsewhere.

CASE 2   A 25-year-old army signalman noticed mild dyspnoea for three months before a routine chest radiograph showed diffuse shadowing in both lung fields. The dyspnoea progressed and he developed an early morning cough, productive of small amounts of mucoid sputum; the only abnormal physical signs were finger clubbing and some diminution in breath sounds. A number of investigations were undertaken, culminating in right lung biopsy obtained through a limited anterior thoracotomy. The sections showed dilated alveolar spaces with thin septa. The alveoli were filled with a homogeneous eosinophilic material in which there were crystal clefts.

Bilateral bronchopulmonary lavage was undertaken on four occasions, with an interval of three to five days between treatment of the two sides. There was considerable symptomatic improvement, with a noticeable increase in sputum volume and decrease in viscosity lasting for several weeks after each lavage. Radiologically each treatment was followed by some decrease in the density of shadowing (Figs 1 and 2) but complete clearing was not achieved and some indices of pulmonary function remained abnormal (Table II).

CASE 3   A 22-year-old shop assistant developed gradually increasing dyspnoea over a period of two years until she was breathless and fatigued walking on the level. The chest radiograph showed bilateral pulmonary infiltration. A provisional diagnosis of sarcoidosis had been made but prednisone (5 mg three times per day) for six months had not altered her downhill course. By the time of admission she was grossly cyanosed and breathless at rest; several respiratory tract infections caused further deterioration in her condition. Thoracotomy to obtain a lung biopsy was considered too hazardous and sputum was therefore sent for both light and electron microscopy. Light microscopy revealed finely granular PAS-positive material consistent with a diagnosis of alveolar proteinosis and this was supported by electron microscopy.

In spite of the obvious risks in this severely disabled girl with a Pao_2 of only 28 mmHg (while breathing air), lavage was undertaken as soon as the diagnosis

had been established. The right lung was lavaged on two occasions, separated by an interval of three days, and this sequence was repeated for the left lung during the following week. Treatment resulted in marked symptomatic improvement. This was confirmed radiologically and by physiological and biochemical measurement (Table III). Further lavage has been carried out at intervals and the patient is now only dyspnoeic when running but still has residual radiographic and physiological abnormalities (see Table III and Fig. 3).

ELECTRON MICROSCOPY

Ultrastructural studies were made on lung tissue from case 1, lung washings from case 2, and both sputum and lung washings from case 3. The appearances are of interest for two reasons. Not only did the experience obtained in the first two cases help in making the diagnosis on sputum examination in case 3, but certain observations in these three patients are in accord with previously expressed views on the pathogenesis of alveolar proteinosis in the experimental animal (Corrin and King, 1969, 1970; Vijeyaratnam and Corrin, 1973).

CASE 1   Epon-embedded sections of the lung tissue submitted for electron microscopy were first examined by light microscopy. This showed an appearance quite different from the classical features of alveolar proteinosis seen in the paraffin-embedded tissue. Instead of a finely granular acellular material, foamy macrophages filled the alveoli. Such cells are found scattered singly in the abundant acellular material of alveolar proteinosis, but in this tissue the cells preponderated and extracellular material was sparse. The appearances were those of endogenous lipid pneumonia rather than alveolar proteinosis, and presumably represented the changes at the edge of a proteinotic area. Electron microscopy confirmed that the free cells were macrophages and showed that they contained numerous complex inclusions (Fig. 4). The inclusions were membrane-bound and contained many densely osmiophilic granules, weakly osmiophilic amorphous droplets or moderately osmiophilic lamellar bodies. All these structures were considered to represent lipid of varying type, contained within phagolysosomal vacuoles. Also free in the alveolar lumen was a little debris similar to the material seen within macrophages, representing material which had yet to be ingested or which had been released from effete macrophages.
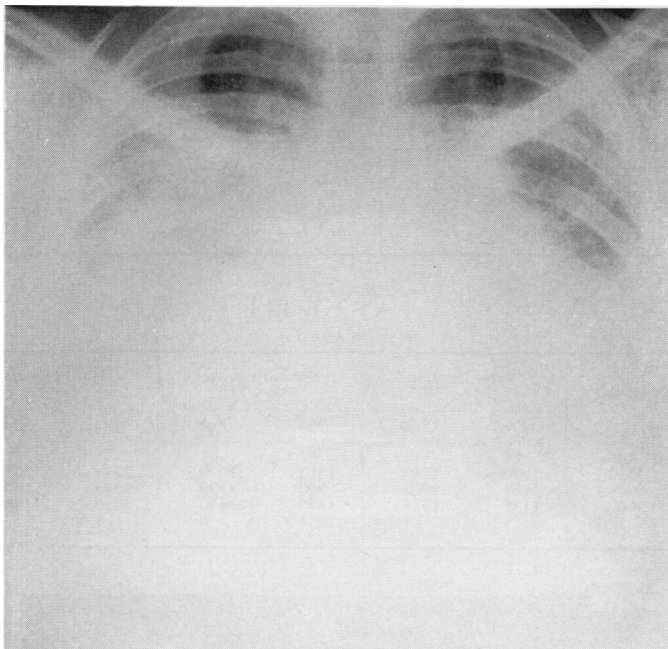
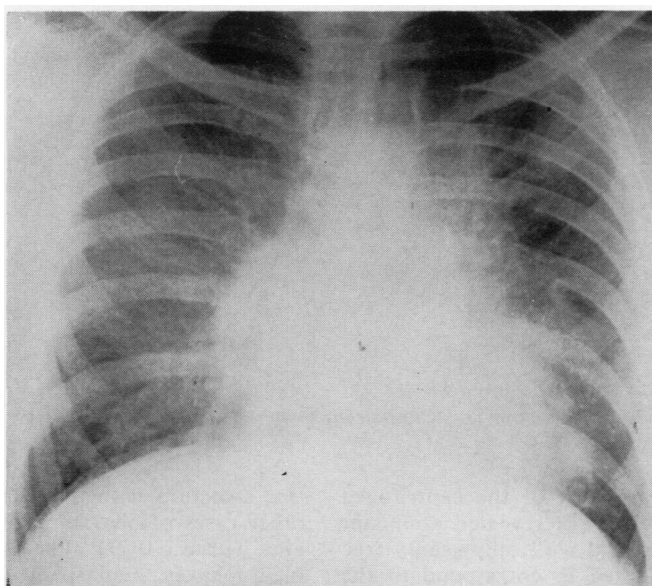FIG. 1. *Case 2. Chest radiograph before bronchial lavage.*

FIG. 2. *Case 2. Chest radiograph after bilateral bronchial lavage.*

TABLE II
CASE 2

	Predicted	First Lavage (July 1972)		Second Lavage (Aug. 1972)		Third Lavage (Feb. 1973)		Fourth Lavage (Sept. 1973)	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post
PaO$_2$ (mmHg)	90–100	68	85	—	66	55	62	70	76
TLC (ml)	6830	4500	4030	—	5528	5390	5790	4800	4990
FRC (ml)	3740	2200	2058	2130	2628	3390	3500	2265	2620
FVC (ml)	4960	3275	3400	3400	3625	3400	3960	3500	3800
TF (ml/min/mmHg)	31·7	12·5	15·5	12·9	17·4	15·4	17·9	13·9	16·8

TABLE III
CASE 3

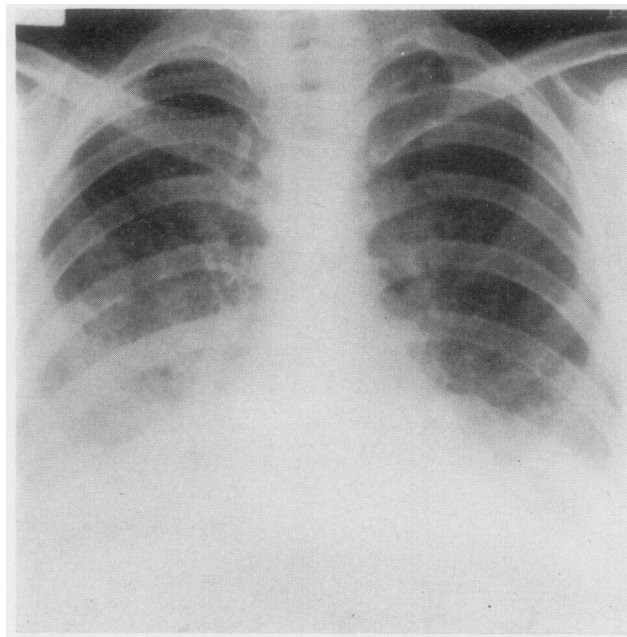|	Predicted	First Lavage (May 73)		Second Lavage (May 73)		Third Lavage (July 73)		Present Status (Sept. 73)
		Pre	Post	Pre	Post	Pre	Post	
PaO$_2$ (mmHg)	90–100	28	36	37	41	45	55	84
TLC (ml)	5520	1930	1710	1710	2670	2240	2650	3020
FRC (ml)	3020	1180	960	960	1770	1340	1600	1820
FVC (ml)	4000	1155	1375	1375	1600	1730	1980	2255
TF (ml/min/mmHg)	29·2	4·8	4·9	4·9	6·5	6·0	13·2	12·1

FIG. 3. *Case 3. Chest radiograph, September 1973 (see Table III).*

CASE 2 Electron microscopy of the centrifuged deposit of the lung washings revealed abundant acellular granular material with only scanty free cells. This was considered to correspond to the classical alveolar proteinosis material seen in the lung on light microscopy, proving identical in its fine structure with that described in the lung in other cases of alveolar proteinosis (Basset, Soler, and Turiaf, 1973). The sparse free cells were macrophages, remarkable as in case 1 for an abundance of phagolysosomal lipidic inclusions, notably of a lamellar variety (Fig. 5). The more
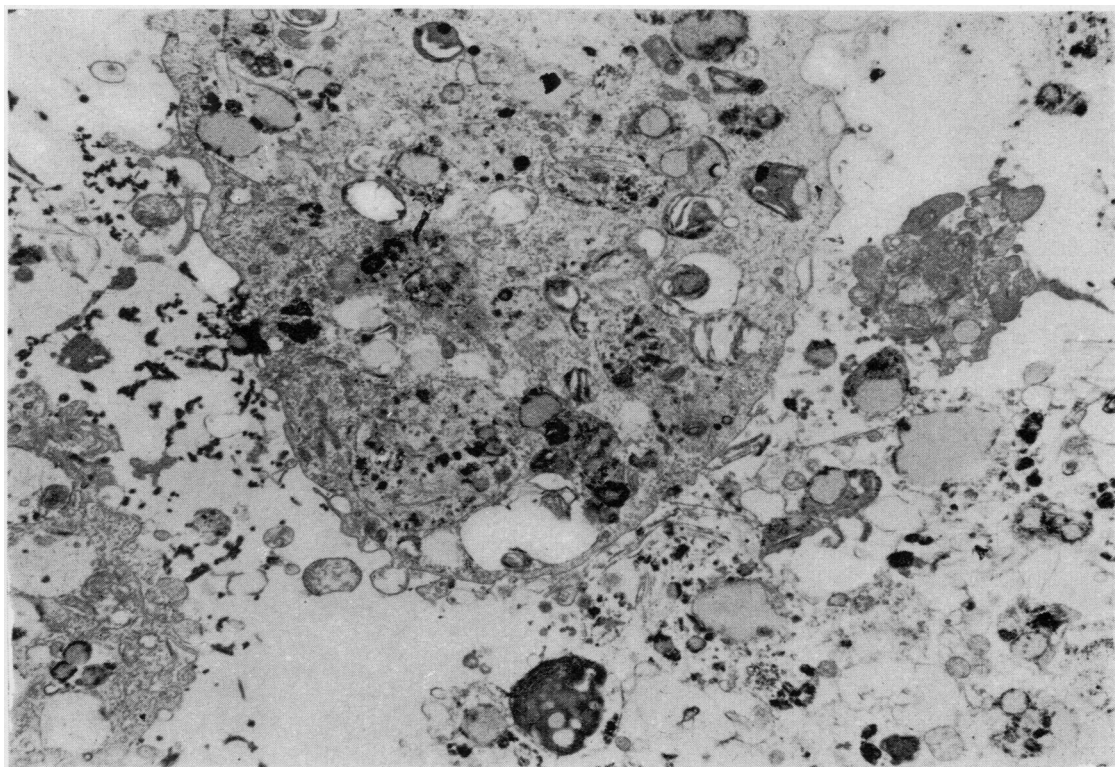
FIG. 4.   *Case 1.   Lung biopsy.   Alveolar macrophages containing complex lipidic phagolysosomes. Extracellular material of a similar nature is also seen free in the alveolar space (Electron micrograph (EM) ×7,500).*
   (All electron micrographs are of material fixed in methanol-free formaldehyde followed by osmium tetroxide, embedded in epon, and stained with lead citrate and uranium acetate.)

abundant free material consisted of osmiophilic rounded bodies which appeared either amorphous or had concentrically arranged lamellar structure. The lamellae were sometimes irregular and wavy (Fig. 6) but were more often smooth and regularly spaced (Figs 7 and 8). Evenly spaced lamellae had a unit membrane structure consisting of two electron dense lines separated by an electron lucent zone, total thickness 9 nm. The electron density of one unit membrane differed from the next but matched the next but one, and the centre to centre spacing of membranes of similar electron density was 30 nm, similar to that recorded by Basset *et al.* in biopsy material. The centre of the lamellar bodies was often amorphous or showed laminations devoid of a unit membrane structure and with 5 nm spacing (Fig. 8). Also present in the deposit were blunt-ended elongated

clefts corresponding to the cholesterol crystals consistently described in pulmonary alveolar proteinosis (Fig. 9).

CASE 3   Electron microscopy of the sputum showed poorer preservation of fine structure than the lung washings of case 2, but a marked similarity was nevertheless evident. The sputum contained masses of electron dense granular material and in particular lamellar bodies identical with those described in case 2 (Fig. 10). It was on the presence of these bodies that the electron microscopic diagnosis of alveolar proteinosis largely depended. Lung washings from this patient were later examined, and appearances similar to those in case 2 were again observed. Whilst intracellular lamellar bodies may be found
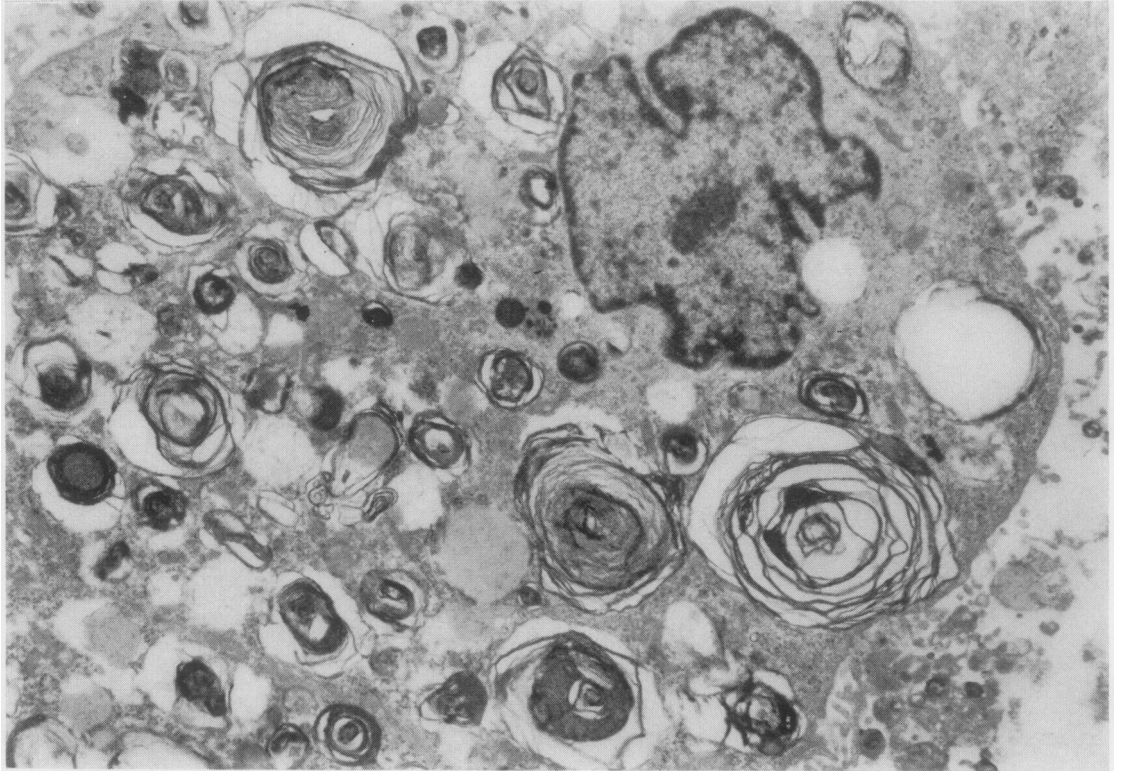
FIG. 5. *Case 2. Lung washings. A cell rich in lamellar inclusions. The inclusions are identical with those of the type II pneumocyte, but this cell is nevertheless considered to be a macrophage because it lacks microvilli and because identical cells in the experimental animal have been shown to contain abundant acid phosphatase and be avidly phagocytic. The inclusions represent ingested lipid, probably surfactant (EM ×9,000).*

in desquamative interstitial pneumonia, the presence of numerous extracellular lamellar bodies was considered characteristic of alveolar proteinosis.

BRONCHIAL LAVAGE

The technique employed for case 1 differed from that used for cases 2 and 3. In the first case, general anaesthesia was induced with thiopentone and pancuronium bromide and continued with halothane in 100% oxygen. A Robertshaw double-lumen tube was introduced with the endobronchial extension situated in the left main bronchus, and after denitrogenation for ten minutes, the lumen to the right lung was clamped off and the lung was allowed to collapse (by oxygen absorption) for a further ten minutes.

During this period the right limb of the tube was attached to a reservoir filled with normal saline buffered to a pH of approximately 7·4 and heated to 37°C. At the end of the ten-minute period of absorption collapse, the right limb of the tube was unclamped and the lung was allowed to re-expand, filling with saline at a hydrostatic pressure of 30 cm. After one litre had run in freely, the saline reservoir was disconnected and the fluid was allowed to drain passively into a measuring cylinder. Further increments of fluid were then run in and drained until a total of two litres had been used on the first occasion, and four litres on the second. In spite of vigorous physiotherapy at the end of each procedure, more than one litre of fluid was retained on both occasions. There were no changes in ECG, blood
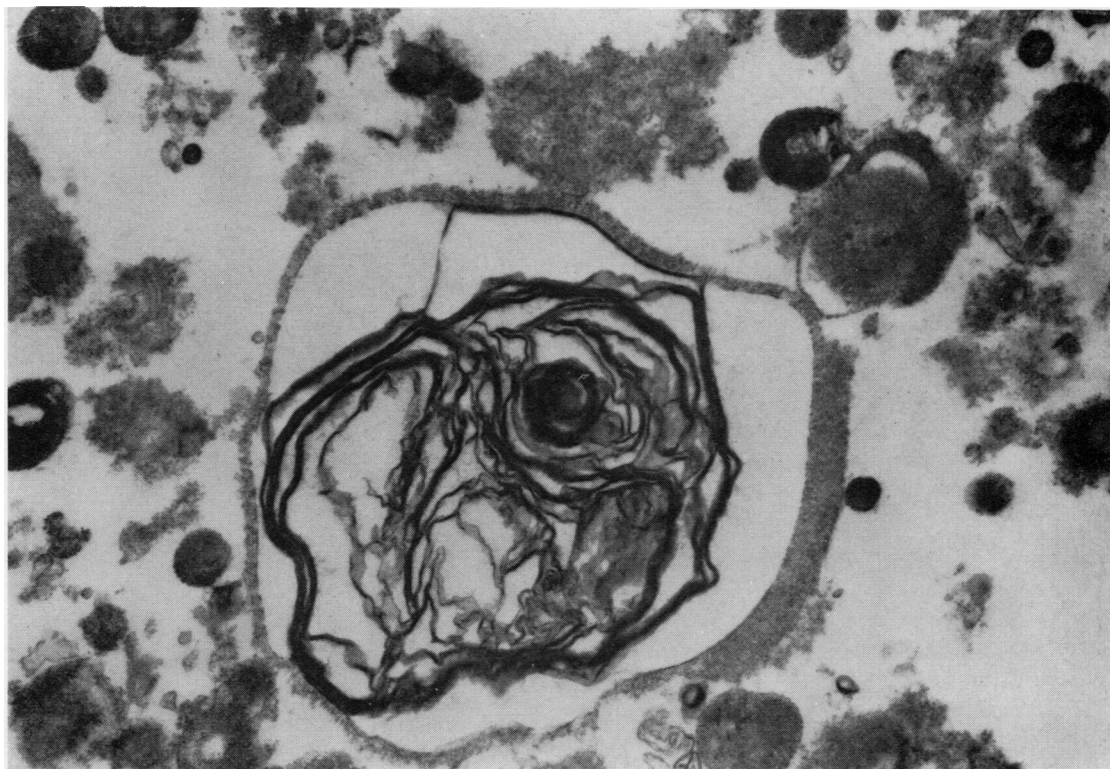
FIG. 6. *Case 2. Lung washings. A lamellar body with a wavy irregular structure (EM ×18,000).*

pressure or right atrial pressure during the procedure, and the serum electrolyte values remained within normal limits.

In view of the experience gained with case 1, it was considered preferable with cases 2 and 3 to employ the volume-controlled technique described by Kylstra *et al.* (1971) and Ramirez-R. (1966) and to use a larger volume of lavage solution. After induction of anaesthesia and intubation with a double lumen tube, as described above, the appropriate lumen of the tube was attached to the saline reservoir. Each minute, saline was allowed to enter the lung in a volume equal to the calculated basal oxygen uptake through that lung (assuming 55% of basal for the right and 45% of basal for the left lung). This was continued until a volume equal to the presumed functional residual capacity (FRC) had entered the lung (55% and 45% respectively for the right and left lungs of the measured pretreatment FRC). The procedure was carried out with the treated lung slightly dependent to encourage even distribution and minimize the tendency for fluid to flow back towards the trachea, but the full lateral position was avoided so that there would be little gravitational redistribution of pulmonary blood flow to the unventilated lung.

Once the lung had been filled to FRC, further increments of saline, 300 to 500 ml at a time, were run in and drained out until a total volume of up to 20 litres had been used. As the procedure continued, the fluid draining from the lung became much less opalescent, and lavage was discontinued when there was no further visible clearing. The initial volume equivalent to the FRC was then drained out, assisted by endobronchial suction and manual re-inflation of the lung. Only minimal fluid was retained when this technique was employed, and generally no more than 250–500 ml were 'lost' in a total lavage volume of 20 litres.

It is preferable to place the endobronchial

FIG. 7. *Case 2. Lung washings. Lamellar bodies with smooth regularly spaced membranes (EM ×27,000).*

extension of the double lumen tube in the lung which will remain ventilated so that suction to the other lung is facilitated. Confirmation of satisfactory placement of the tube by auscultation may be difficult because harsh breath sounds are often transmitted clearly from the trachea through the relatively airless lung, but visible asymmetry of chest wall movement is readily apparent. If satisfactory placement of the tube has not been achieved, a bubble appears in the tubing joining the saline reservoir to the double lumen tube after only one or two increments of saline have been run in. This appearance precedes any loss of saline into the ventilated lung but if it is ignored, fluid will be audible in the ventilator circuit by the time the treated lung has been filled to functional residual capacity. It is wise therefore to regard the appearance of this bubble as an indication for removing as much fluid as possible from the treated lung before withdrawing the tube and re-positioning it.

DISCUSSION

The diagnosis of alveolar proteinosis may prove difficult. In all our cases there was a delay ranging from six months to more than two years between the onset of symptoms and the establishment of a firm diagnosis. The symptoms of alveolar proteinosis are non-specific and there are often few physical signs. Tests of pulmonary function, arterial blood gas tension measurements, and radiological examination are abnormal but not diagnostic, and it may be difficult to distinguish the condition from pulmonary oedema, fibrosing alveolitis, sarcoidosis or, occasionally, chronic pulmonary infection. Sputum examination however has proved a useful diagnostic aid (Vidone *et al.*, 1966). The advantages of light microscopy to demonstrate PAS positive material in the sputum are that it is both quick and simple and may be undertaken safely as soon as the condition is suspected. Electron microscopy is a more complex procedure but appears to be a useful
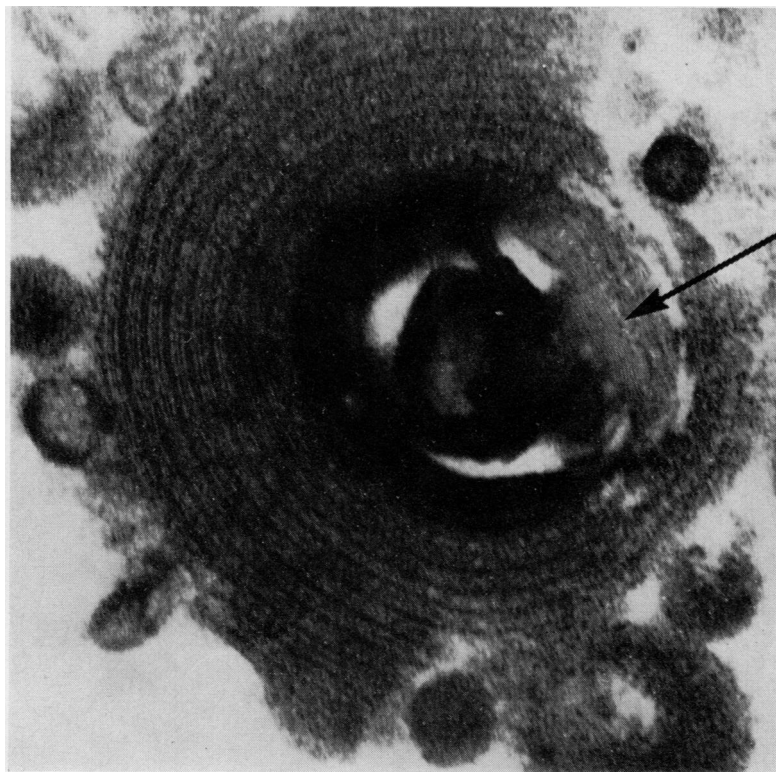
FIG. 8.   *Case 2.   Lung washings.   An osmiophilic body with a dense central core and outer lamellae which have a unit membrane structure and alternately varying electron density. The arrow indicates laminations with shorter periodicity (EM ×100,000).*

nfirmatory method of examining the sputum. though open lung biopsy is almost always equivocal, the fact that this procedure entails th hazard and discomfort for the patient means it it is often deferred until many other tests ve been carried out. Moreover, a further in-val for recovery from thoracotomy is desirable fore bronchopulmonary lavage is undertaken. ill biopsy of the lung is a possible alternative, t the hazards of pneumothorax may be pro- bitive in patients with severe restrictive lung ease.

The electron microscopic study of the three tients reported here has not only contributed the diagnosis but has enabled us to make a mparison with animals showing a similar con-ion. The experimental condition was first commented upon in rats exposed to silica dust (Corrin and King, 1966) and subsequently amplified by Gough (1967), Heppleston (1967), Gross and deTreville (1968), and Corrin and King (1969, 1970). Corrin and King (1970) emphasized the development in animals through a stage of endogenous lipid pneumonia, and such appearances in our first patient were therefore of great interest. In this patient different portions of the same needle biopsy showed lipid pneumonia and alveolar proteinosis respectively, and the experimental studies suggest that the former condition represents a stage in the development of alveolar proteinosis. The electron microscopic appearances of both the human and murine alveolar proteinosis material suggest excessive surfactant accumulation while the distribution of the dust
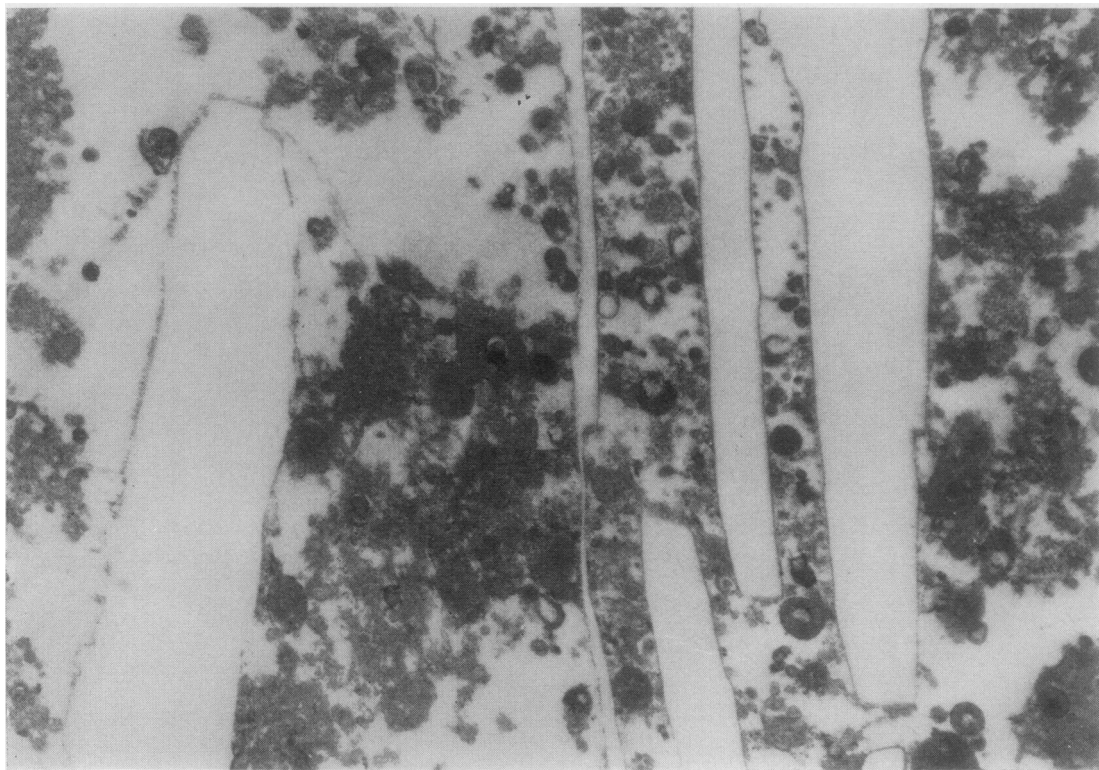
FIG. 9. *Case 2. Lung washings. Cholesterol crystal clefts amid osmiophilic granules* (*EM* ×12,000).

marker in the experimental condition supports the suggestion that the normal pulmonary clearance mechanism becomes overloaded by the excess surfactant (Corrin and King, 1970). Diverse dusts may produce the condition experimentally, and Davidson and Macleod (1969) noted that many of the human cases had been exposed to a dusty environment. More recently, alveolar proteinosis has been described in rats given a chemical agent (iprindole) by mouth (Vijeyaratnam and Corrin, 1973) while Gray (1973) has noted an increased frequency of immunological abnormalities in childhood alveolar proteinosis. These diverse features suggest that alveolar proteinosis may represent a characteristic but non-specific pattern of pulmonary response to various injurious agents.

Once a firm diagnosis has been established, the choice of therapy lies between bronchopulmonary lavage and the inhalation of a trypsin aerosol. The

latter technique has been reviewed recently (Riker and Wolinsky, 1973), and good results have been reported, but daily inhalations of the aerosol from a Bird ventilator are needed for three months, and there is generally no detectable benefit for up to three weeks. Considerable co-operation is required from the patient if treatment is to be carried out successfully, and the provision of a domiciliary ventilator and a supply of compressed air or oxygen may be expensive and inconvenient. Inhaled trypsin can cause emphysema in experimental animals, and there is therefore a possibility that prolonged use in high dosage could be detrimental.

Bronchopulmonary lavage is an effective form of therapy and may be undertaken with only minimal risk if a one-lung anaesthetic technique is used, but particular care is needed to ensure correct positioning of the tube.

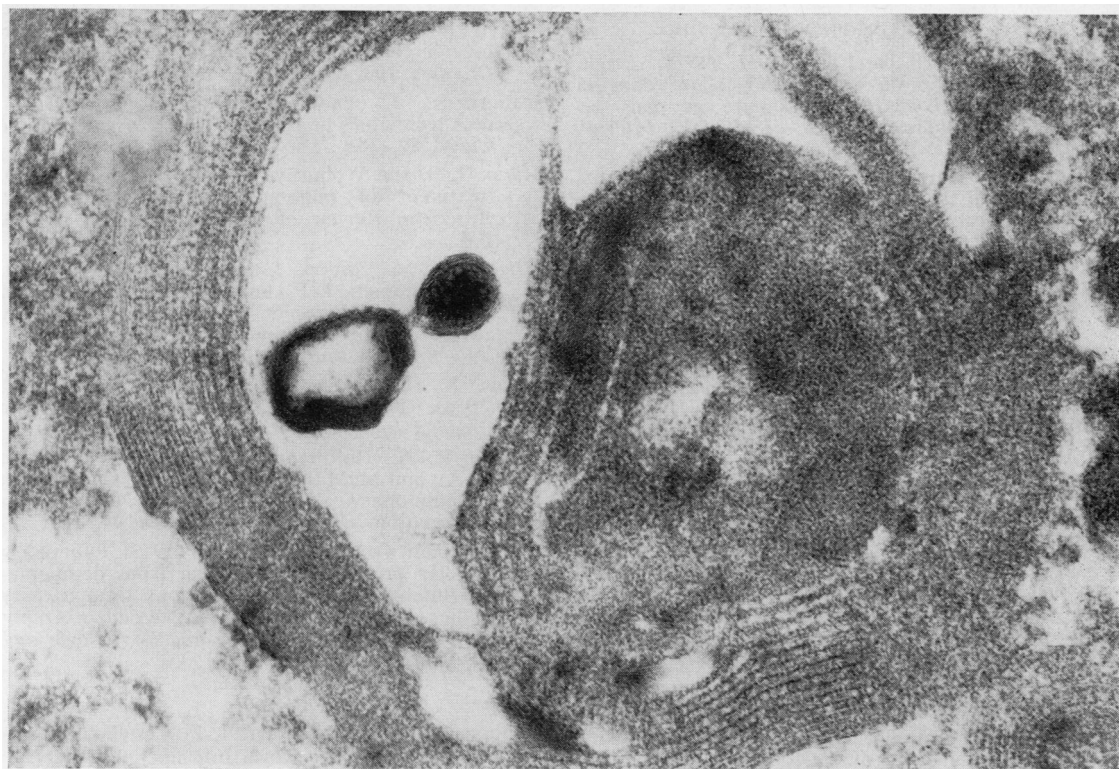Repeated lavage using large volumes of saline

FIG. 10.   *Case 3.   Sputum.   A lamellar body similar to those seen in case 2 (EM ×58,500).*

is generally necessary, but, using the volume-controlled technique, there is little absorption of fluid, and perfusion of the airless lung is minimized by the influence of the hydrostatic pressure within it (Rogers *et al.*, 1972). Gradual filling of the lung over a period of ten to 20 minutes (depending on calculated basal oxygen uptake and FRC) encourages uniform distribution of the fluid and may minimize mechanical damage to delicate alveolar tissue. If the collapsed lung is allowed to expand rapidly under the weight of the saline-filled reservoir, mechanical damage and uneven distribution may occur more readily; this may account for the temporary deterioration in case 1 after the first procedure. Even when the volume-controlled technique is employed, the full benefit may not be apparent for a day or two. This delay could represent the deleterious effect of residual fluid retained within the lung, or could be related to the loss of surfactant from previously normal alveoli.

Using this technique, there has been no deterioration in arterial blood gas tensions or pulmonary function tests following the 16 treatments which have been undertaken on cases 2 and 3; on several occasions the arterial oxygen tension has risen immediately afterwards. Both these patients are now almost symptom-free and when lavage is undertaken, the fluid returned is only slightly opalescent. In spite of this, both show persistent radiological and functional abnormalities. This could be due to residual proteinaceous material which cannot be dislodged by lavage or, alternatively, may represent a fibrotic reaction provoked by the disease. Although the nature of this residual abnormality remains uncertain, it seems probable that incomplete resolution could be minimized by earlier diagnosis and prompt treatment and that electron microscopy may help to achieve this.

REFERENCES

Basset, F., Soler, P., and Turiaf, J. (1973). Étude ultrastructurale du contenu alvéolaire dans la protéinose alvéolaire pulmonaire et dans les pseudo-protéinases. *Annales de Médicine Interne*, **124**, 279.

Corrin, B. and King E. (1966). Inhalation experiments comparing the action of quartz and cristobalite on the rat lung. Proc. 113th meeting of the Pathological Society, p. 14. *Journal of Pathology and Bacteriology*, **92**.

—— and —— (1969). Experimental endogenous lipid pneumonia and silicosis. *Journal of Pathology*, **97**, 325.

—— and —— (1970). Pathogenesis of experimental pulmonary alveolar proteinosis. *Thorax*, **25**, 230.

Davidson, J. M. and Macleod, W. M. (1969). Pulmonary alveolar proteinosis. *British Journal of Diseases of the Chest*, **63**, 13.

Gough, J. (1967). Silicosis and alveolar proteinosis. *British Medical Journal*, **1**, 629.

Gray, E. S. (1973). Autoimmunity in childhood pulmonary alveolar proteinosis. *British Medical Journal*, **4**, 296.

Gross, P. and deTreville, R. T. P. (1968). Alveolar proteinosis. Its experimental production in rodents. *Archives of Pathology*, **86**, 255.

Heppleston, A. G. (1967). Atypical reaction to inhaled silica. *Nature*, **213**, 199.

Kylstra, J. A., Rausch, D. C., Hall, K. D., and Spock, A. (1971). Volume-controlled lung lavage in the treatment of asthma, bronchiectasis, and mucoviscidosis. *American Review of Respiratory Diseases*, **103**, 651.

Ramirez-R., J. (1966). Bronchopulmonary lavage: new techniques and observations. *Diseases of the Chest*, **50**, 581.

Riker, J. B. and Wolinsky, H. (1973). Trypsin aerosol treatment of pulmonary alveolar proteinosis. *American Review of Respiratory Diseases*, **108**, 108.

Rogers, R. M., Szidon, J. P., Shelburne, J., Neigh, J. L., Shuman, J. F., and Tantum, K. R. (1972). Hemodynamic response of the pulmonary circulation to bronchopulmonary lavage in man. *New England Journal of Medicine*, **286**, 1230.

Rosen, S. H., Castleman, B., and Liebow, A. A. (1958). Pulmonary alveolar proteinosis. *New England Journal of Medicine*, **258**, 1123.

Vidone, R. A., Hoffman, L., Hukill, P. B., Nesbitt, K. A., and McMahon, F. J. (1966). The diagnosis of pulmonary alveolar proteinosis by sputum examination. *Diseases of the Chest*, **49**, 326.

Vijeyaratnam, G. S. and Corrin, B. (1973). Pulmonary alveolar proteinosis developing from desquamative interstitial pneumonia in long term toxicity studies of iprindole in the rat. *Virchows Archiv für pathologische Anatomie und Physiologie und für klinische Medizin*, **358**, 1.