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Pulmonary Veno-Occlusion—An Immune Complex Disease?

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Summary. A case of pulmonary hypertension due to veno-occlusive disease is described. The patient was a 33 year old woman who died 11 months after first complaining of breathlessness. Veno-occlusive disease was diagnosed on lung biopsy and confirmed post-mortem. Electron microscopy of the lung biopsy showed electron-dense deposits in the capillary basement membranes, and immunoglobulin and complement were demonstrated in a corresponding position by immunofluorescent microscopy. It is suggested that immune complexes may have initiated thrombotic occlusion of the small pulmonary veins.

Pulmonary veno-occlusion is now recognised as a rare cause of pulmonary hypertension. Sometimes the onset of symptoms is sudden and resembles influenza, suggesting infection as a possible initiating mechanism, but apart from this possibility practically nothing is known of the cause. We describe here a case of pulmonary vein occlusion investigated by immunofluorescent and electron microscopy, both of which provide evidence of an immune reaction centered on the basement membrane of small pulmonary blood vessels, suggesting that thrombosis may have been initiated by immune complexes.

Case Report

A 33 year old woman who had previously led an active life, first became aware of dyspnoea whilst on holiday in the Canary Islands. There was no wheeze or chest pain and only a slight unproductive cough. Her dyspnoea progressed over 4 months with decreasing tolerance and she was admitted to hospital for investigation. There was no preceding history of chest illness. She had never been on oral contraceptives or appetite suppressing drugs, and her pregnancies had been uneventful. There was no history suggestive of deep vein thrombosis in the legs. She had no systemic symptoms such as weight loss, fever or arthopathy. Clinically she was a slim, breathless, cyanosed young woman with clubbing of the fingers. There were no added sounds in the chest, and no evidence of cardiovascular disease. Chest X-ray showed bilateral patchy shadowing with linear opacities and slight prominence of the main pulmonary artery. Pulmonary function studies showed a restrictive pattern with impaired gas transfer but no evidence of airways obstruction. Pulmonary hypertension was confirmed by right heart catheterisation, the pulmonary artery pressure being 90/40 mm. The Mantoux test was negative, as were a Kveim test and liver biopsy (serum bilrubin varied between 1.2–2.5 mg-%, with the other liver function tests normal). Considerable attention was paid to the possibility of harmful environmental factors, as she lived and worked on a horticultural small-holding and until a year previously had tended poultry. She was in contact with numerous chemical fertilisers, insecticides, fungicides and herbicides including paraquat, but there was no suggestion that these had been misused. A considerable amount of damp and possibly mouldy straw was in use, mainly for the strawberry beds, but serological and skin tests to a wide variety of allergens were negative. Fibrosing alveolitis was suspected and on open lung biopsy performed.

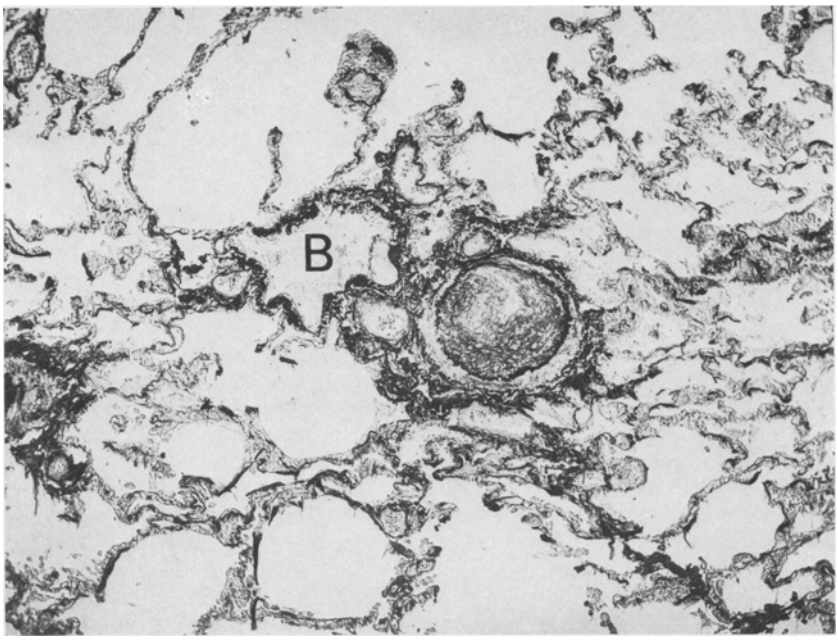

Fig. 1. Lung biopsy. A pulmonary artery, recognisable by its double elastic laminae and position next to an airway (*B*), shows almost total obliteration of its lumen by fibro-elastotic intimal proliferation. Elastic van Giesen (EVG) × 95

Histological examination of the lung biopsy showed prominent vascular changes, exceeding those to be expected from the parenchymal damage which amounted to only mild interstitial fibrosis largely confined to the interlobular septa. The small pulmonary arteries showed medial hypertrophy and very marked cellular intimal proliferation which almost totally occluded the lumen (Fig. 1). There was only slight haemosiderosis and the pulmonary veins were at first reported to be normal. However, review of the sections showed pronounced occlusion of pulmonary veins by a cellular intimal proliferation (Fig. 2). The sections were referred to Professor D. Heath who also considered that the brunt of the disease had fallen on the venous side of the pulmonary circulation. Professor Heath commented also on the development of a distinct muscular media in some small veins so that they mimicked arteries, a factor which could clearly confuse the distinction of venous from arterial disease.

Immunofluorescent Microscopy of the Lung Biopsy

Fluorescein conjugated monospecific anti IgG, IgM, IgA, complement (β_{1A} and β_{1C}) and fibronogen were applied to frozen unfixed sections. An indirect method using the unconjugated mono-specific goat anti-human immunoglobulins followed by conjugated rabbit anti-goat antisera was also undertaken. Specificity of the positive results was checked in the direct method by prior absorption of the antisera by specific immunoglobulins and by blocking with an additional middle layer of appropriate and inappropriate unconjugated antisera.

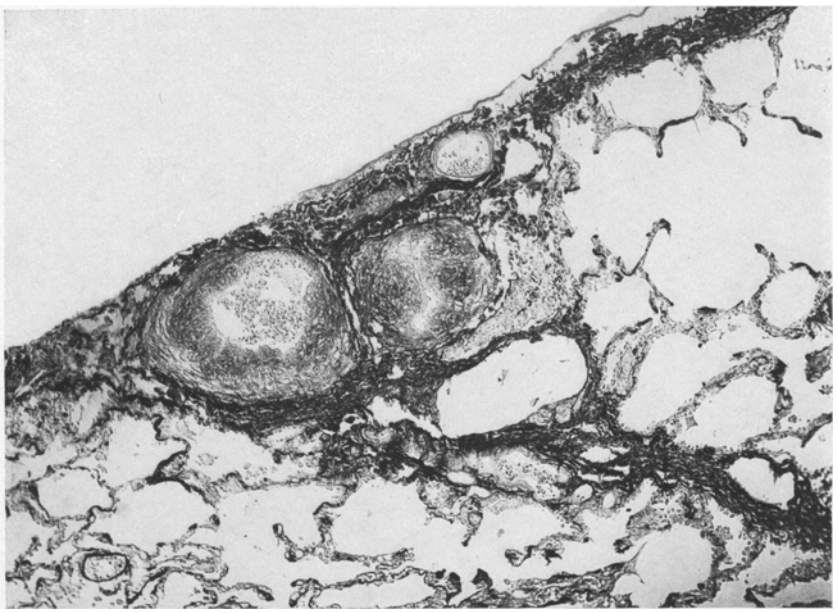

Fig. 2. Lung biopsy. Two pulmonary veins, recognisable by their elastin pattern and position at the junction of an interlobular septum with the pleura, show intimal proliferation and marked reduction of their lumens. EVG × 95

The results using the direct and indirect methods were similar. Deposits of IgG and complement were found scattered irregularly along the alveolar walls (Fig. 3). No fluorescence was obtained using anti IgA, anti IgM or anti-fibrinogen; neither was fluorescence seen within alveolar macrophages nor in any other structure of the lung.

Electron Microscopy of the Lung Biopsy

The alveolar structure of the lung could be readily recognised but there was an increase of alveolar macrophages and thickening of the air/blood barrier, principally by changes in the interstitial tissues and capillary walls. The alveolar epithelium showed only minor alterations amounting to no more than mild oedematous swelling of the cytoplasm of the type I pneumocytes. There was interstitial fibrosis and an increased number of undifferentiated interstitial septal cells. The endothelium was markedly thickened so that the capillary lumen was often reduced to a thin slit. There was also pronounced thickening of the endothelial basement membrane; this is normally equal in thickness to that of the alveolar epithelium (30–40 nm) but in this case measured up to 700 nm. Many capillaries showed reduplication of the basement membrane, the various layers of which were separated by cell processes thought to represent portions of either pericytes or proliferating endothelial cells. All these changes we have noted in a variety of pulmonary diseases and consider non-specific. Of particular interest, however, was the frequent finding of electron-dense deposits within the capillary

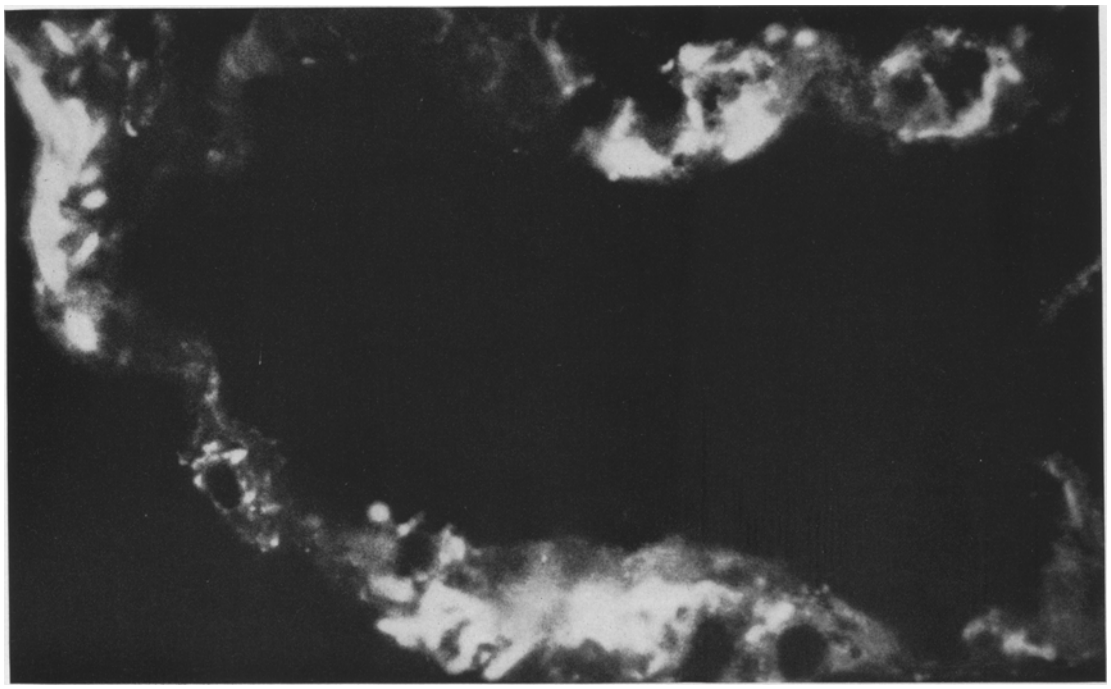

Fig. 3. Lung biopsy. Immunofluorescence demonstrates deposits of IgG in the alveolar walls × 74

basement membrane or in a subendothelial position (Fig. 4–7). These were amorphous or finely granular and measured from 0.2 to 0.9 μm in diameter. They generally had a slightly irregular outline and were not membrane-bound.

Further Course and Post-mortem Examination

The further course was marked by an initial response to corticosteroids, but this was not maintained. Azathioprine was added to her therapy and when she developed a productive cough antibiotics were given but these made no difference to her symptoms. Despite increasing prednisone therapy to a maximum of 60 mg daily she became increasingly short of breath and deeply cyanosed. Severe systemic disturbances developed with gross proximal muscle wasting, considered to be more than steroid myopathy, increasing jaundice, purpura and ulceration of the tongue with no evidence of thrombocytopenia and sterile blood cultures. The ESR rose to 147 mm/hr (previously 35–55 mm/hr), and the white cell count to 14000 with 95% neutrophils. The Hb dropped to 8.8 g-% and right ventricular failure developed. There was no response to steroids, topical and systemic antibiotics, digitalis or diuretics and she died 13 months after the onset of the first symptoms.

Post-mortem examination showed marked wasting with petechial haemorrhages in the skin over the thighs and abdomen. The heart weighed 265 gms. The right ventricle was very dilated and grossly hypertrophied. Differential weighing of the ventricles by the method of Fulton, Hutchinson and Jones (1952) gave a weight of 77 gms for the free part of the right ventricle and a weight of 94 gms for the combined weight of the interventricular septum and left ventricle. The heart valves were normal and there was no evidence of a septal defect. The left atrium was normal and the orifice of each pulmonary vein showed no evidence of obstruction.

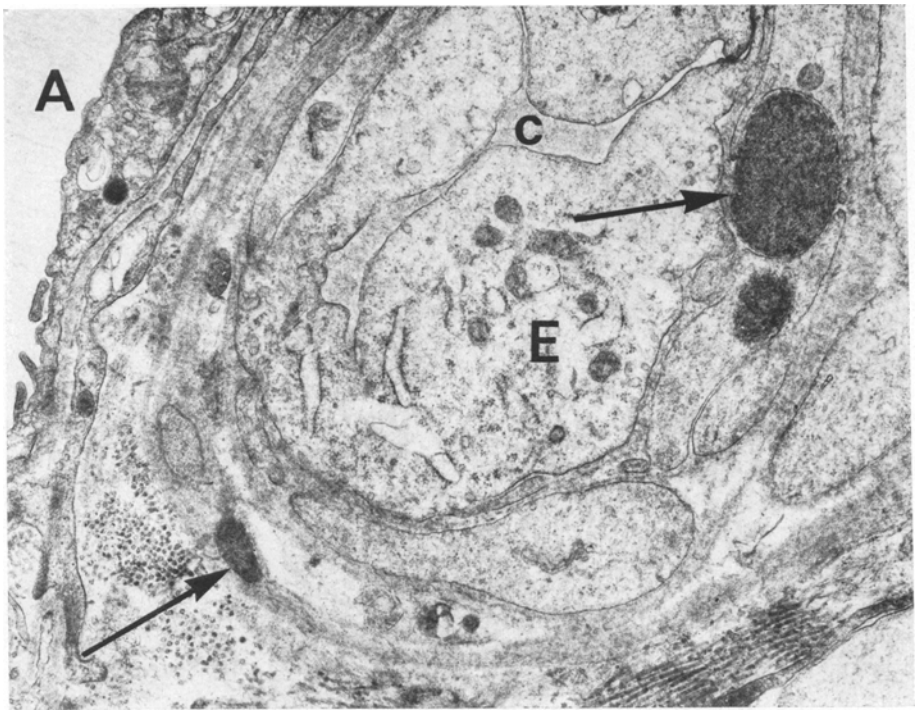

Fig. 4. Lung biopsy. Electron dense deposits (arrows) are seen in a reduplicated capillary basement membrane. The endothelium (*E*) is thickened, reducing the capillary lumen (*C*) to a slit. *A* Alveolar lumen. Glutaraldehyde/osmic acid/uranyl acetate/lead citrate (GOUL) × 15000

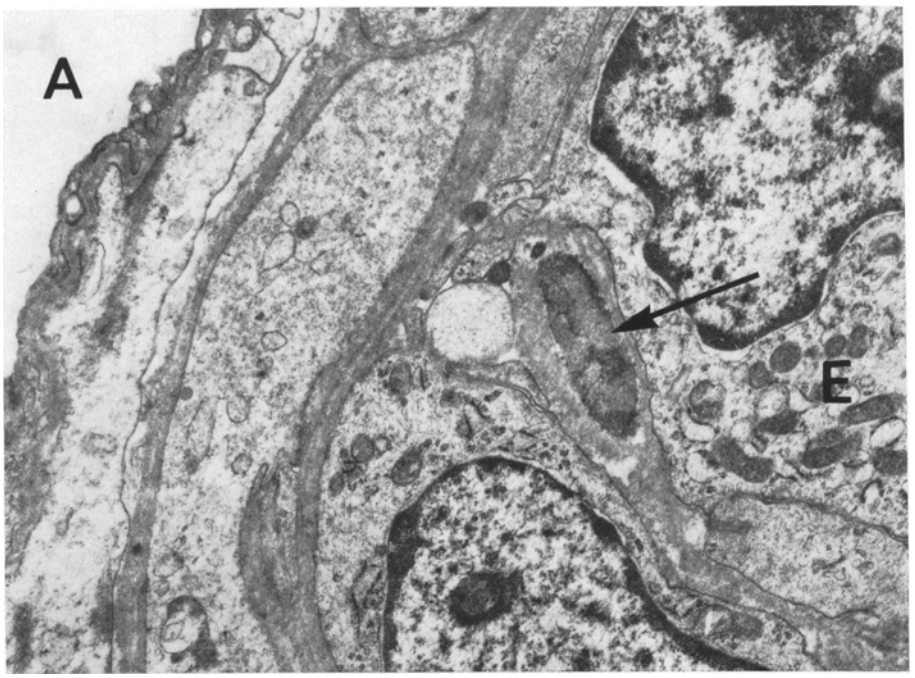

Fig. 5. Lung biopsy. The arrow indicates material of varying electron density situated within the capillary basement membrane. *E* endothelium, *A* alveolus, GOUL × 15000

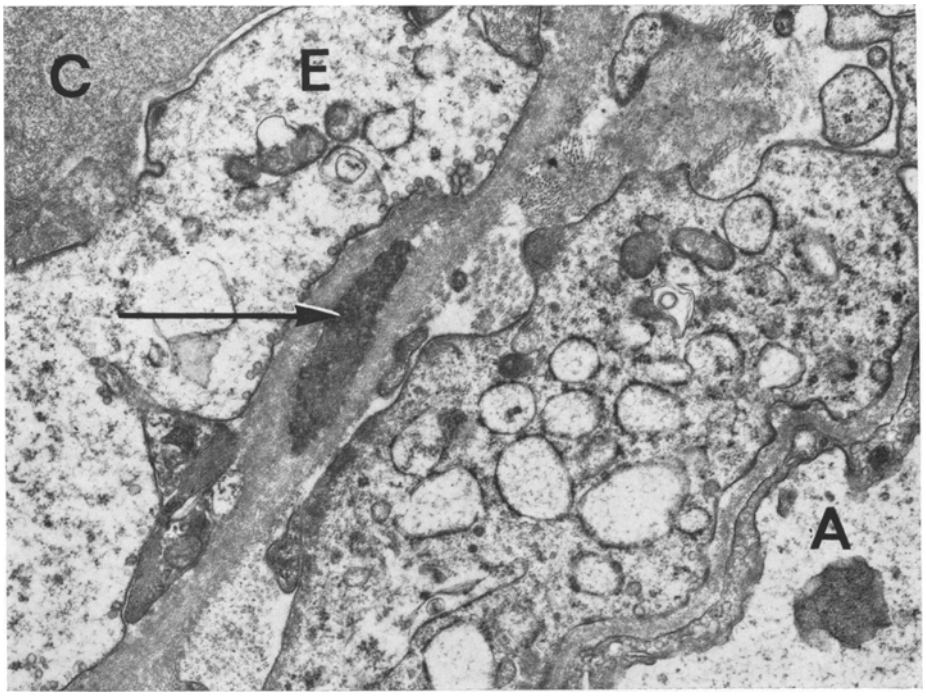

Fig. 6. Lung biopsy. There is marked thickening of the endothelium (*E*) of an alveolar capillary (*C*) and its underlying basement membrane; the latter encloses an electron dense deposit (arrow). The alveolar lumen (*A*) is bordered by normal epithelium resting on a normal basement membrane. GOUL × 22000

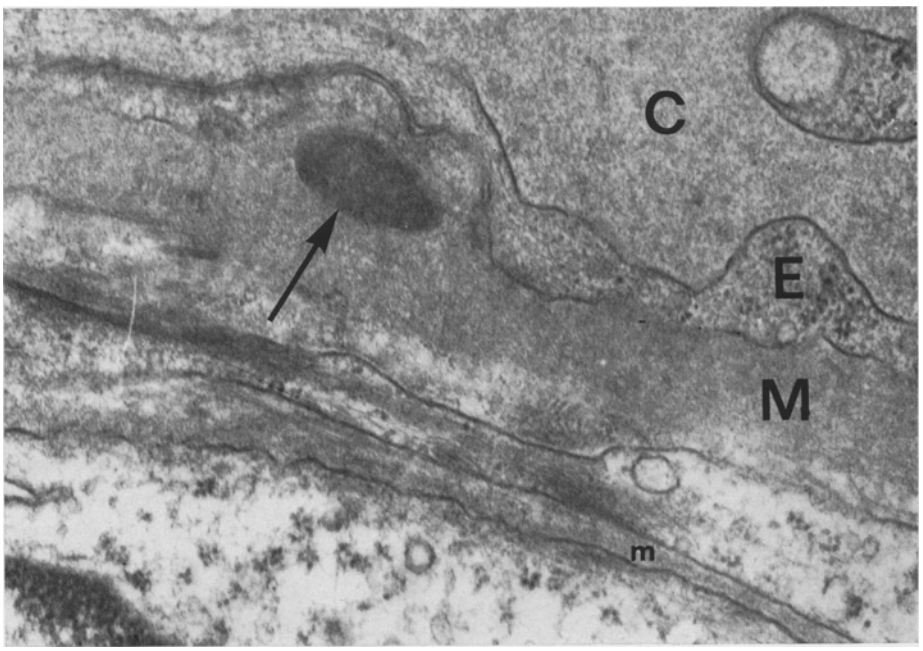

Fig. 7. Lung biopsy. There is a subendothelial deposit (arrow), and the capillary basement membrane (*M*) is greatly thickened compared with that of the epithelium (*m*). *C* capillary, *E* endothelium. GOUL × 44000

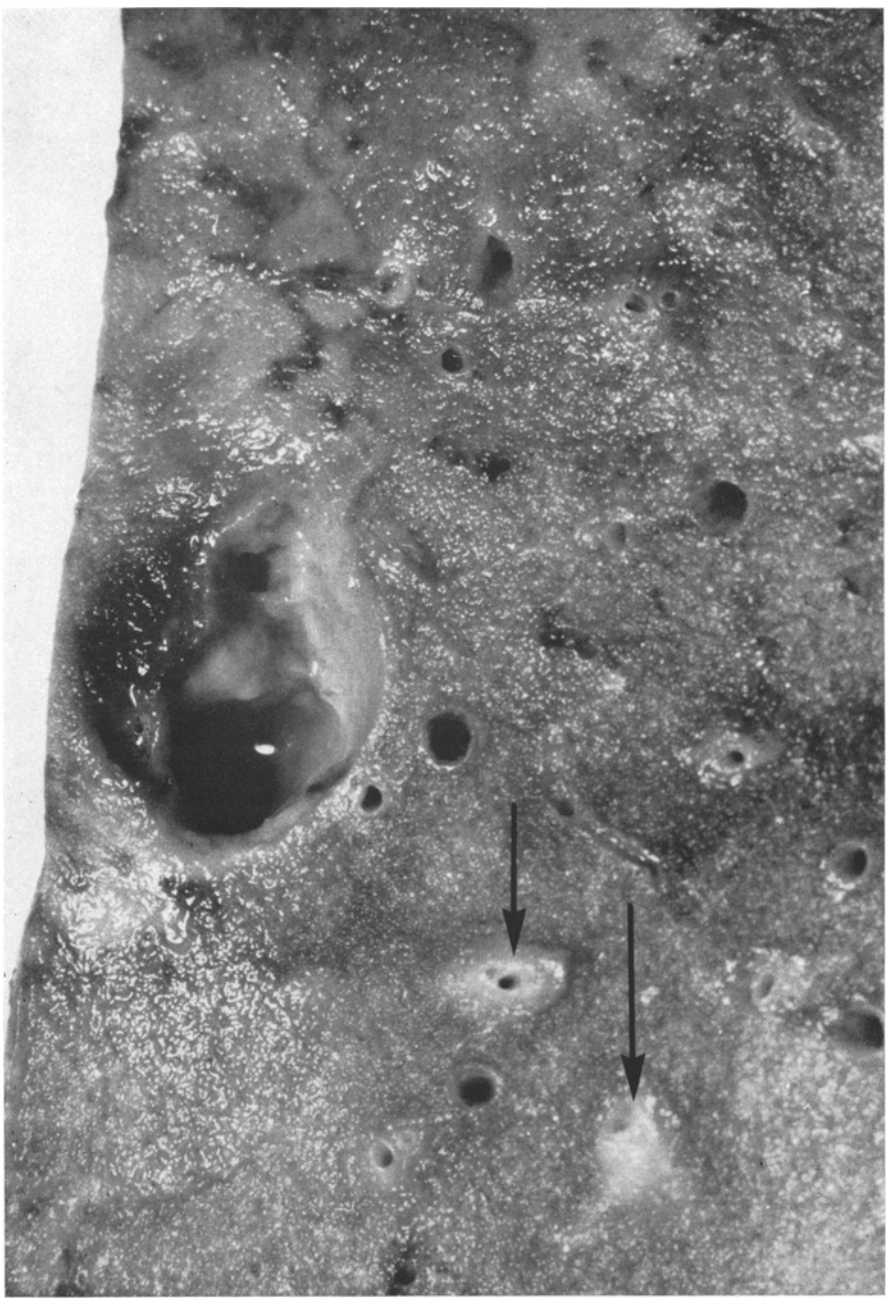

Fig. 8. Lung at post mortem. Pale infarcts with a congested border are seen along the pleural surface, and in one area there is cavitation. Arrows indicate narrowed or occluded pulmonary veins with a surrounding cuff of pale fibrous tissue. × 2

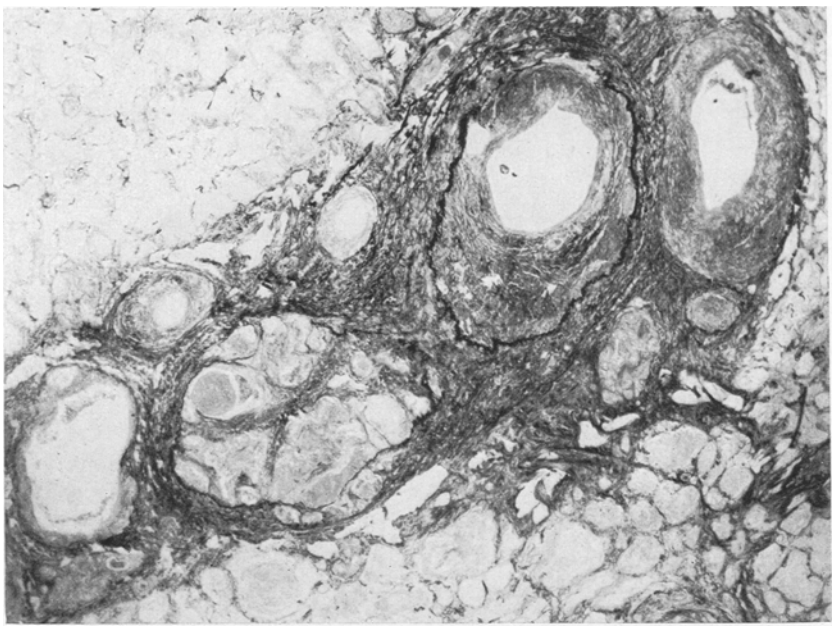

Fig. 9. Post mortem lung. Pulmonary veins in a interlobular septum show intimal thickening or complete occlusion with recanalisation. EVG × 36

The air passages contained a small amount of mucus. The right lung weighed 650 gms and the left lung weighed 600 gms. The visceral pleura of each lung showed dilated veins and lymphatics. The right lung was examined immediately and the left lung after perfusing Wentworth's fluid into the main bronchus and fixing for 48 hours in the inflated position. The lungs showed numerous solid, yellowish-white areas scattered throughout all lobes, the largest measuring 4.5 × 4 × 3 cms. Some of these areas showed necrosis with the formation of ragged cystic spaces, up to 1.5 cm in diameter (Fig. 8). While many of these areas were adjacent to the pleural surface, others were situated deeply within the lobe. In many places there was a brown tinge suggesting haemosiderosis. The large pulmonary arteries showed a few streaks of atheroma and the walls of the medium-sized arteries were thickened. There was no evidence of embolism or thrombosis in the large pulmonary arteries. The large pulmonary veins appeared normal but a few veins measuring 5 mms in diameter situated in the septa were occluded. Occlusion was much more widespread in veins measuring 2.5 mms in diameter, and there was fairly sharp demarcation between occluded and non-occluded veins at a point where small tributaries entered vessels of much larger calibre. Many of the occluded veins were surrounded by a halo of firm fibrotic tissue measuring 3 to 5 mms in diameter (Fig. 8). There was no evidence of fresh thrombus in the pulmonary veins.

The liver and spleen showed chronic venous congestion. A little red marrow was present in the shaft of the right femur. All other organs were normal.

Microscopical examination showed that the yellowish-white areas were old infarcts and that one necrotic, cystic space contained fungus, probably Aspergillus. The most striking changes were seen in the pulmonary veins and venules which were occluded by acellular, dense, fibrous tissue. The elastic laminae of the veins appeared to be thickened and there were often several small lumens which were thought to represent recanalisation (Fig. 9). In the collagen surrounding the veins, there were numerous thin-walled blood vessels (Fig. 10) and on serial section some of these communicated with the recanalised lumens of occluded veins. These vessels presumably represented an attempt to form a collateral circulation. Several veins were examined by serial sections and the first sign of occlusion was an eccentric intimal thickening by collagen with no evidence of recent or organising thrombus. The muscular pulmonary arteries showed hypertensive changes with marked medial hypertrophy and intimal

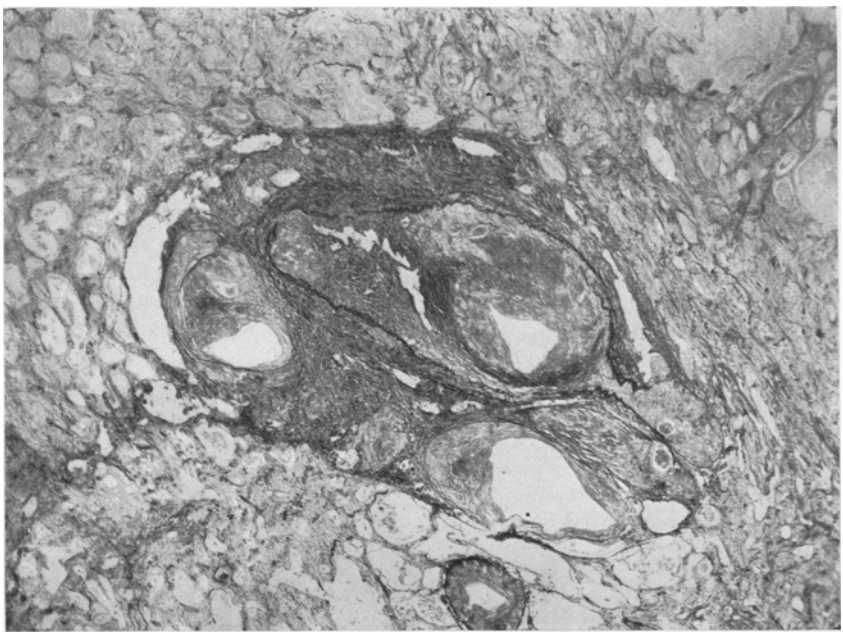

Fig. 10. Post mortem lung. A pulmonary vein shows marked fibrous proliferation of its intima and is surrounded by dense collagen in which there are several other vascular channels. EVG × 36

proliferation. Some of the small pulmonary arteries contained recent thrombus while others showed intimal fibrosis and evidence of recanalisation. These arterial changes were considered to be secondary to venous occlusion. The alveoli surrounding the occluded veins showed interstitial fibrosis and were lined by cuboidal and, occasionally, columnar epithelium producing a honeycomb lung in an early stage. Perl's stain confirmed the haemosiderosis. Microscopical examination of muscle from the arm, thigh and calves showed no evidence of polyarteritis nodosa. Sections from the thyroid, pankreas, skin, adrenal, spleen, kidneys and vertebra showed no significant abnormality, whilst the liver showed chronic venous congestion.

Discussion

Pulmonary veno-occlusion appears to be one of the rarest causes of pulmonary hypertension. The 13 cases published to date were reviewed by the British Medical Journal in 1972 since when three more have been reported (Braun *et al.*, 1973; Rosenthal *et al.*, 1973; Liebow *et al.*, 1973). Liebow, however, mentions almost incidentally a personal collection of 27 unreported cases, and it is likely that the condition is commoner than supposed, many cases going unrecognised because the venous nature of thickened blood vessels is not appreciated. Furthermore in the later stages of veno-occlusive disease considerable scarring often develops and the distinction from idiopathic pulmonary fibrosis may be very difficult. Congestion and oedema are not always prominent and haemosiderosis may not be a apparent. On the other hand, haemosiderosis may sometimes overshadow the vascular changes leading to a wrongful diagnosis of primary pulmonary haemosiderosis (Carrington and Liebow, 1970). The factors most likely to lead to a correct histological diagnosis are an awareness of the condition

coupled with knowledge of the correct anatomical localisation of the pulmonary veins and the use of stains to demonstrate elastin and haemosiderin. Another helpful but inconsistent feature is the so-called "endogenous pneumoconiosis" (Walford and Kaplan, 1957) resulting from fragmentation and encrustation with iron and calcium of the elastic laminae of occluded vessels and a subsequent giant cell reaction mimicking a dust disease. Liebow (1967) has ascribed some such cases to chronic venous occlusion. Iron encrustation of vessel walls alone, early in pulmonary hypertension, also suggests venous involvement (Walton and Heath, 1960). In the present case proper recognition came from identifying diseased blood vessels as veins from their positions in the interlobular septa and pleura, distant from the airways, and confirmation of their venous nature by the use of elastin stains. Even so, controversy over the histological diagnosis existed until the necropsy findings dispelled all doubt.

The aetiology of this rare disease is uncertain. Although thrombus has been recognised in only a minority of the recorded cases the occlusive process is generally assumed to be thrombotic in origin (Liebow *et al.*, 1967; Wagenvoort, 1972). Several cases have suggested an infective aetiology (Crane and Grimes, 1960; Brewer and Humphreys, 1960), particularly the infant studied by Wagenvoort and his colleagues (1971) in whom there was both myocarditis and pneumonitis. A viral aetiology has been particularly implicated (Liebow *et al.*, 1967) and in one case (Stovin and Mitchinson, 1965) toxoplasmosis may have played a causal role. An immune basis has not been previously suggested but immune complex disease might well stem from a viral infection. In the present case a fungus was found at necropsy but this is almost certainly an example of opportunist infection resulting from immunosuppressive therapy. There was no evidence of a previous infection which may have precipitated the original illness, but we believe that the evidence for immune deposits in vessel walls is strong, and in this situation immune complexes might lead to thrombotic occlusion by activation of the "contact" clotting factors or platelets.

Whilst our immunofluorescent and electron microscopic findings may each have been only suggestive by themselves, taken together they represent substantial evidence for the presence of immune material in the walls of small pulmonary vessels. The almost exact correlation between these two investigative procedures assumes even greater significance when it is realised that of some 30 lung biopsies from patients with a variety of lung diseases specifically examined by both techniques for evidence of immune complexes, this case was the only one to show positive results by both methods. Further confirmation of circulating immune complexes was obtained by C_{1q} studies on the patients serum.

Several other lung diseases are at present suspected of having an immune basis but so far as we know the evidence for an immune reaction in the lung rests solely upon immunofluorescent microscopy, as in idiopathic fibrosis (Nagaya *et al.*, 1973), rheumatoid lung (de Horatias *et al.*, 1972) and Goodpasture's syndrome (Koffler *et al.*, 1969); solely upon electron microscopy as in systemic lupus erythematous (Kuhn, 1972), or is circumstantial, as in Behçets disease (Davies, 1973), Wegeners granulomatosis and extrinsic allergic alveolitis. Studies combining the specificity of immune reactions with the fine structural detail provided by electron microscopy would be of great value in all these diseases. To date our combined studies in cryptogenic fibrosing alveolitis have proved

negative by both techniques, although before electron microscopic studies were commenced positive results were obtained by immunofluorescence in a few patients. The findings in the present case therefore constitute evidence for immune complexes in the lung at least as strong as that adduced for several more likely contenders, such as allergic alveolitis. Confirmatory evidence is required and it is recommended that new cases of pulmonary veno-occlusive disease be examined by immunohistochemical and ultrastructural methods in addition to the exhaustive microbiological investigations previously advocated.

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