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Pulmonary Alveolar Septal Amyloidosis

A Scanning- and Transmission Electron Microscopy Study

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Summary. A case of primary amyloidosis is presented which diffusely involved the alveolar septa. The lung was studied by light microscopy and by transmission and scanning electron microscopy. The fine structure of the amyloid material showed it to be porous, homogeneous, and an acellular substance consisting of interwoven bundles of amyloid fibrils.

The fine structure of the amyloid material was considered to explain the normal gas diffusion across the alveolar respiratory membrane.

The diagnosis of amyloidosis was first made from a uterine cervical biopsy specimen.

Introduction

Amyloid deposition in the lower respiratory tracts is classified into four types, (1) vascular, (2) diffuse alveolar septal, (3) amyloid tumors in the bronchi, and (4) amyloid tumors in the lung substance (Bachmann, 1967).

Diffuse alveolar septal amyloidosis in primary amyloidosis is a rare lesion and only a few cases have been described (Burümcekci, 1938; Sappington et al., 1942; Meessen, 1962; Bachmann, 1967; Rajan et al., 1970; and Beck, 1970).

In the present case, postmortem lung material was examined not only by light microscopy but also by scanning and transmission electron microscopy. Although autolytic changes had obscured some of the cellular detail, the amyloid material and its relationship to the alveolar basement membranes could still be observed. The lung function changes, which were minimal, were subsequently explained by the structural changes observed.

Clinical History

A 56-years-old woman complained of tiredness, paresthesia of the fingers, and increasing dyspnea for 4 years. There was no previous history of illness. Four months previously she had developed a foul-smelling green vaginal discharge which was found to contain *Trichomonas vaginalis*. A biopsy of the uterine cervix 1 month before admission showed vascular amyloidosis and chronic cervicitis. Following a suspicious chest X-ray which showed a diffuse bilateral multinodular lung infiltration, she was admitted to Odense University Hospital for further investigation.

The patient, a thin woman of 57 kg, was mildly cyanosed, dyspneic at rest, and had a pale and waxy appearance. The right thenar muscles were atrophied and she complained of paresthesia in her right first, second, and third fingers. Her mouth and tongue were dry. The thyroid gland was diffusely enlarged. A systolic murmur could be heard all over the precordium and X-ray showed enlargement of the cardiac shadow. An ECG showed low

voltage in the limb leads and right deviation and negative T in I, II, V_5, and V_6. There were some basal crepitations in the lungs. The liver and spleen were palpable. The urine contained albumin and hyaline and granular casts.

Biopsies taken from the upper respiratory tract, stomach, liver, rectum, skin, and skeletal muscle showed widespread amyloidosis. Laboratory tests showed the following:—Hb 12.4 g per 100 ml. r.b.c 3.6×10^6, w.b.c. 0.5×10^8 cells/per μl. Differential white blood count normal e.s.r. 43 mm per h. Blood pCO_2 and pO_2 were normal. O_2 saturation of Hb 91%, HCO_3 + 19 mmol per liter (normal 21.26 mmol/l). Base excess 6.1 mmol per liter (normal −2.2 to +2.3 mmol/l). The blood creatinine, sugar, and cholesterol levels were normal. Protein electrophoresis showed decreased total albumin. IgM 0.5 g/l, IgG 4.8 g/l, IgA 7.5 g/l. Total alkaline phosphatase 1950 units per litre (normal 8–280 units/l). Most of the alkaline phosphatase was of liver origin. Lung function tests showed a reduced total capacity.—The patient deteriorated steadily and died 1 month after admission.

Postmortem examination showed the body of an emaciated woman with slight ankle edema. The heart showed moderate hypertrophy of both ventricles and the myocardium was firm. The pleurae were thickened and fibrous with adhesions. Both pleural cavities contained about 800 ml of clear thin fluid. The lungs remained expanded both before and after removal from the chest, and the cut surfaces showed a finely honeycomb appearance which resembled a fine sponge. There were firm nodules present in all lobes up to 5 mm diameter. The upper respiratory air passages were normal. The tongue was firm in texture. The liver was very enlarged (2,765 g), pale, and firm, and the spleen was large 450 g and pale and waxy in appearance. The large intestine contained plaquelike lesions. Kidneys enlarged (280 g) and pale with some surface pitting and there was an infarct in the lower pole at the left kidney. Apart from a subserous fibroid in the uterus the genital tract was normal.

Lugol's iodine solution applied to tissue slices from many organs produced a mahogany-brown color change which on the subsequent application of 1% sulphuric acid turned a deep blue color.

Light-Microscopic Examination

Amyloid change was detected by the use of thioflavin T which produced an intense yellow-green fluorescence, by Congo red stain, and a green birefringence when seen under polarized light, by orthochromatic staining with toluidine blue and by metachromatic staining with azure A. Positive results with these stains indicated the presence of the glycosaminoglycans and the glycosaminoglycuronoglycans (acid mucopolysaccharides) in the amyloid substance.

Hematoxylin and eosin-stained lung sections showed diffuse infiltration with amyloid in the alveolar septal walls especially in relation to the basement membrane and within the walls of blood vessels (Figs. 1 and 2). The pulmonary arterioles were surrounded by amyloid which extended into the alveolar septa. Cellular debris containing red blood cells and macrophages were present. Scattered foreign-body giant cells and many of the macrophages contained amyloid material in their cytoplasm. In many instances the arteriolar lumens appeared to be obliterated by the mural amyloid deposition. Amyloid material was present in the larynx, trachea, bronchi, and bronchioles and the thickened pleura was diffusely infiltrated with amyloid. Amyloid was also present in the walls of the pleural blood vessels.

Electron-Microscopic Examination by Scanning Electron Microscopy (SEM) and by Transmission Electron Microscopy (TEM)

All the examinations were done on postmortem material. The right lung was fixed with 10% formalin and kept for 10 days. Specimens for SEM were further fixed in buffered glutaraldehyde for 15 min and postfixed in OsO_4 1%, dehydrated in acetone, and finally dried by a critical point drying method using an E 3000 polason apparatus. Finally the specimen was coated with carbon and gold. The specimen was examined in a Jeol JSM-U2 scanning electron microscope.

Specimens for TEM were fixed in buffered 5% glutaraldehyde, postfixed in phosphate-buffered OsO_4 1%, dehydrated in alcohol and propylenoxid, and finally embedded in Epon. Thin sections were cut in a LKB ultramicrotome and examined in a Hitachi MS8 electron microscope.

SEM Observations

Amyloid material was seen within the basement membranes underlying the pneumocytes, in the alveolar interstitial space, and in the walls of the blood vessels. Owing to the large masses of amyloid in the walls of the arterioles and basement membranes these sites were chosen for scanning electron-microscopic examination. Low magnification showed amyloid as an amorphous acellular homogeneous material present both in the vessel walls and in the alveolar basement membrane (Figs. 3 and 4). Figure 4 shows amyloid present in cementlike form between collagen fibers. High magnification (Fig. 5) showed amyloid material forming intertwined fibers made up of bundles of amyloid fibrils giving the material a porous texture. No cell structures could be seen in the affected parts of the arteriolar walls.

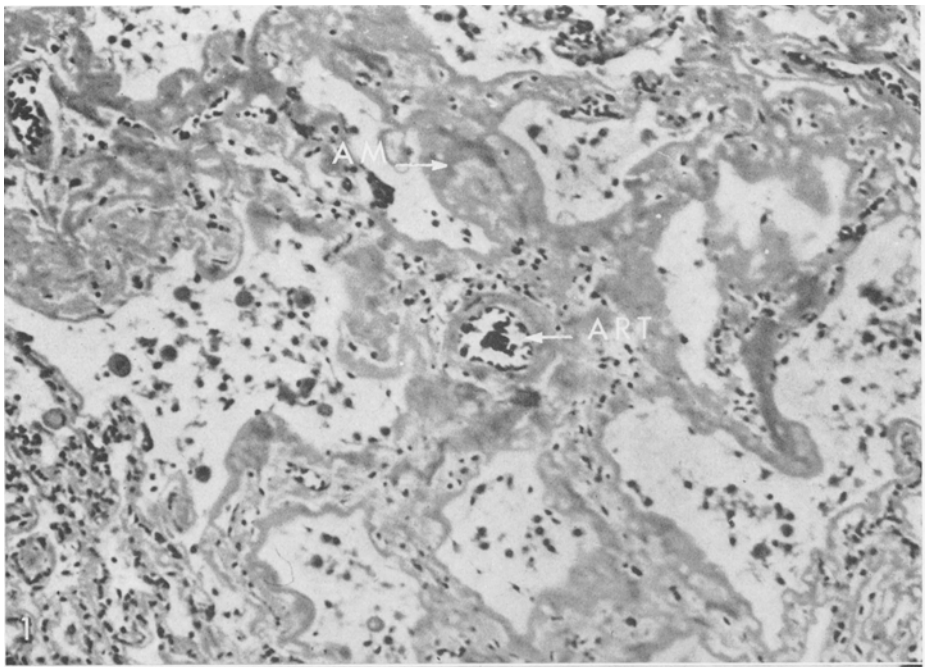
TEM Observations

TEM examination confirmed the localization of amyloid as seen by light microscopy. Amyloid material was present in the interstitial space of the septa and was deposited as a thick layer (AM) in the basement membrane (BM) which can no longer be recognized (Figs. 6 and 7). Amyloid material lay in close apposition to interstitial fibroblasts, elastic, and collagen fibers.

Discussion

Deposition of amyloid material in the respiratory tract is not uncommon. It may occur both in the upper and lower parts of the respiratory system and in the lower part, four varieties were described by Bachmann. Diffuse septal amyloidosis in the lung in primary amyloidosis is rare and six cases have been published previously. The diffuse alveolar septal form of lung amyloidosis not only occurs in primary amyloidosis but may occur in multiple myelomatosis complicated by amyloidosis as described by Bachmann.

Reintoft and Christensen (1972) reported minute amyloid deposits in the alveolar septa following the discovery of cardiac amyloidosis in a series of consecutive postmortems on patients over 60 years of age.



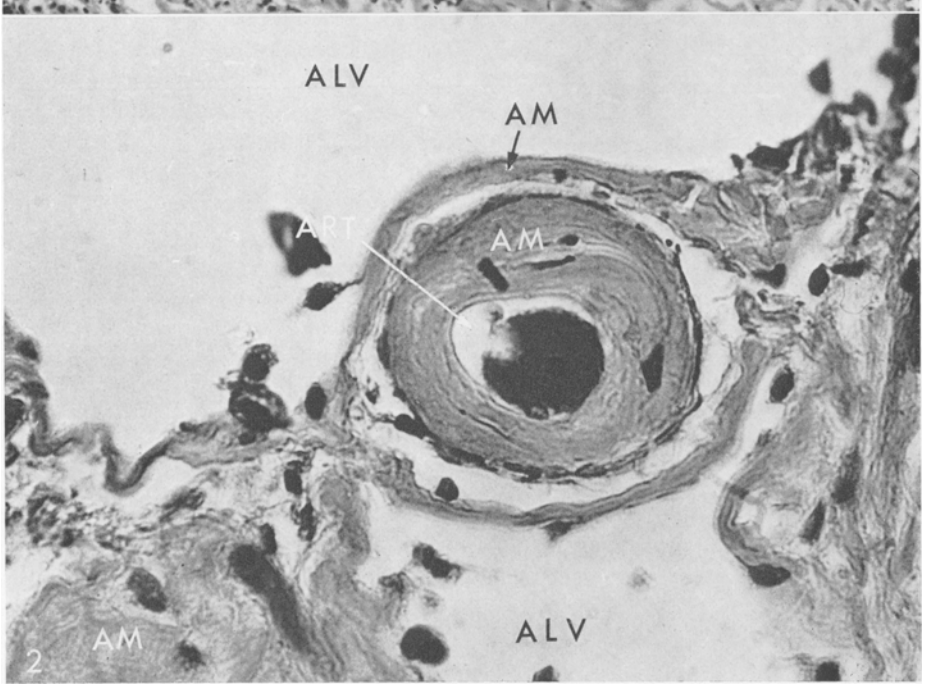


Fig. 1. Light micrograph of lung tissue showing amyloid (*AM*) in alveolar septal wall and within blood vessel (*ART*). H & E. $\times 250$

Fig. 2. Light micrograph of amyloid (*AM*) in alveolar septal wall especially in relation to basement membrane of pneumocytes and within wall of arteriole (*ART*). Alveolus (*ALV*). H & E. $\times 400$

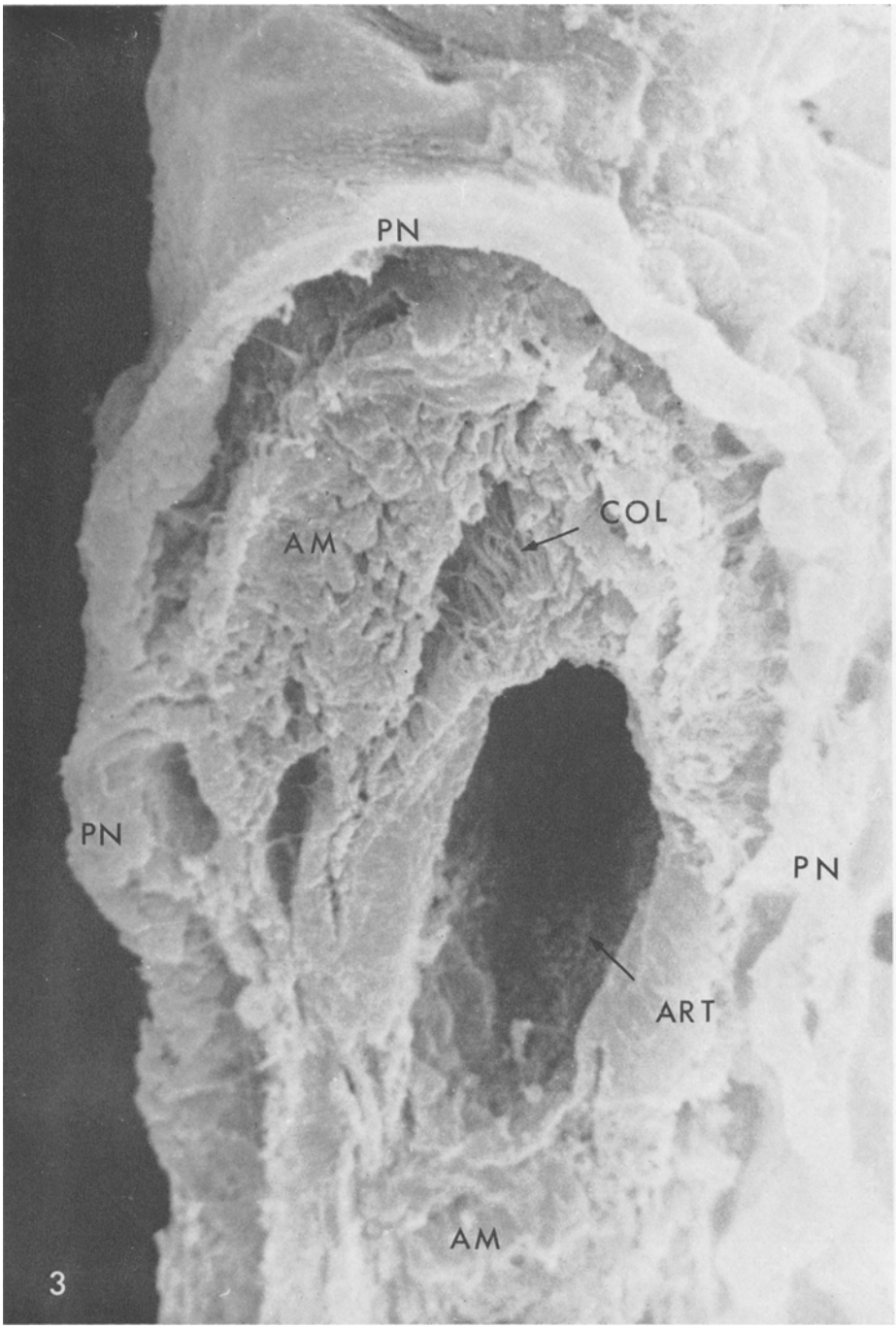


Fig. 3. Scanning electron micrograph showing alveolar septa with pneumocytes (*PN*) and presence of amyloid (*AM*) between collagen fibers (*COL*) of interstitium. Arteriole (*ART*). S.E.M. $\times 3{,}200$

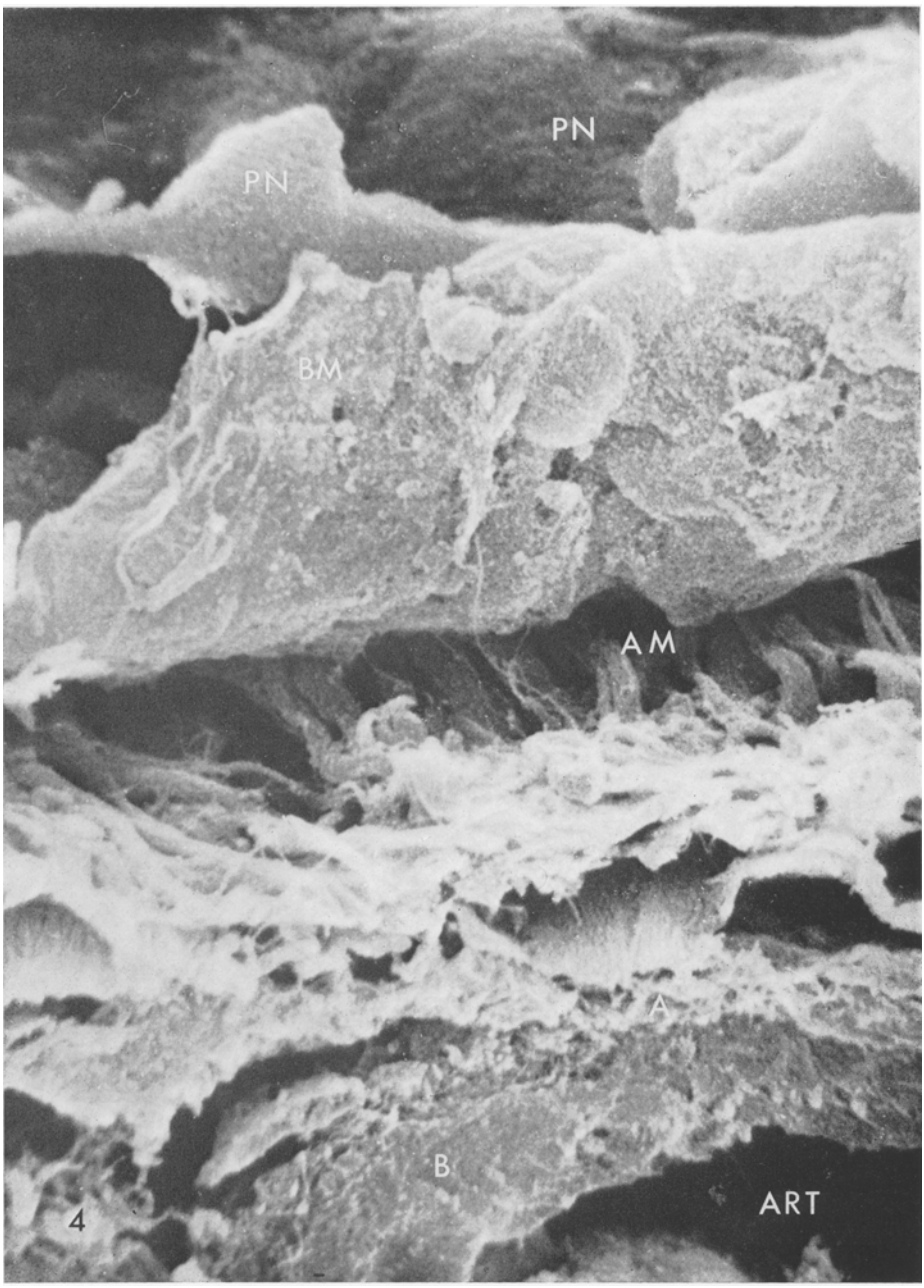


Fig. 4. Scanning electron micrograph depicting edge of alveolar septum with pneumocyte (*PN*) and basement membrane (*BM*) in elevated position. Amyloid material and collagen fibers present in interstitium (*AM*). Amyloid material is also present in tunica adventitia marked (*A*) and in tunica media marked (*B*). Arteriole (*ART*). S.E.M. × 4,800

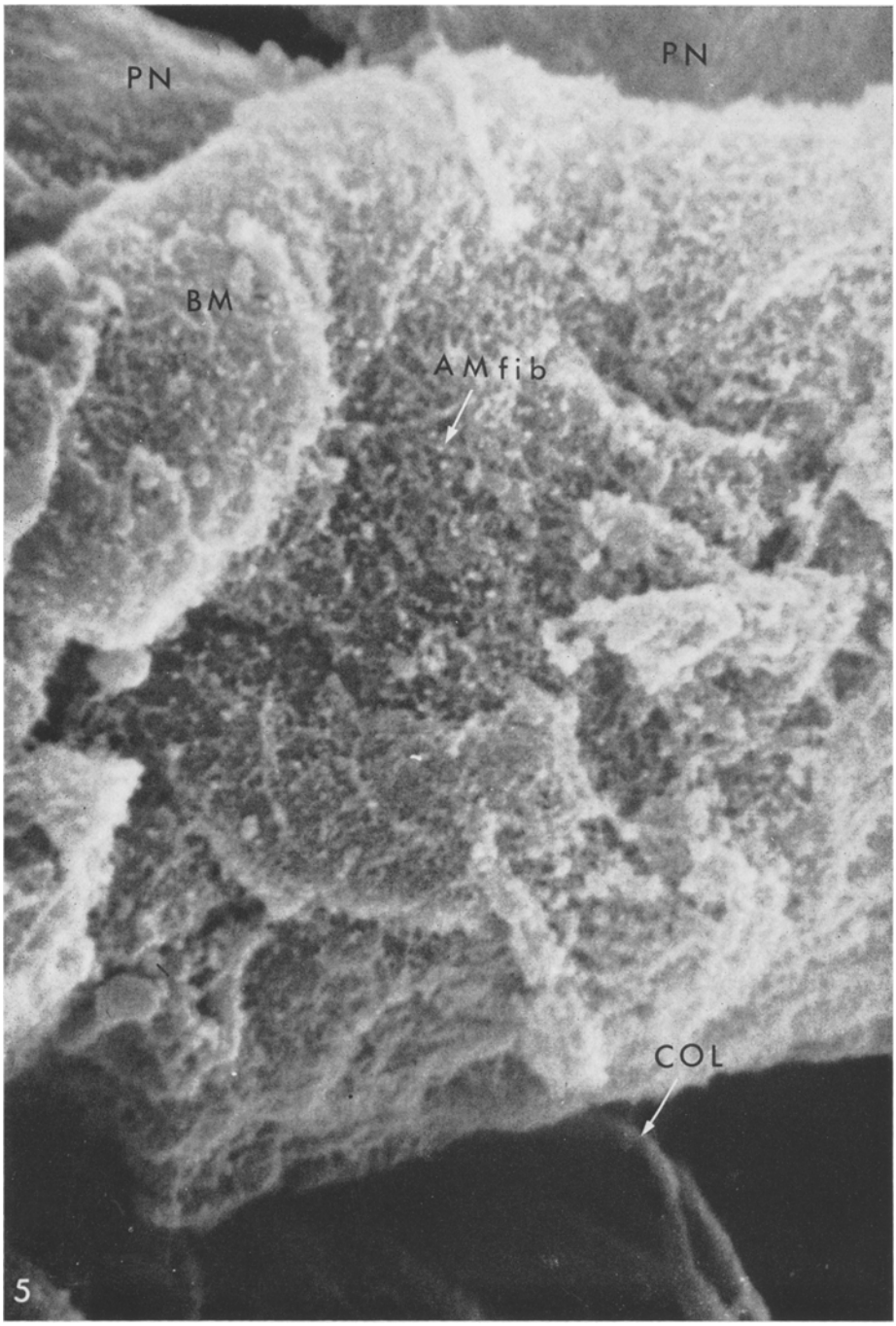


Fig. 5. Scanning electron micrograph showing a higher magnification of same area as in Figure 4; amyloid material seen as interwoven bundles of amyloid fibrils (*AM-fib*), forming porous structure. Note size and arrangement of the collagen fibers (*COL*). S.E.M. × 1,600

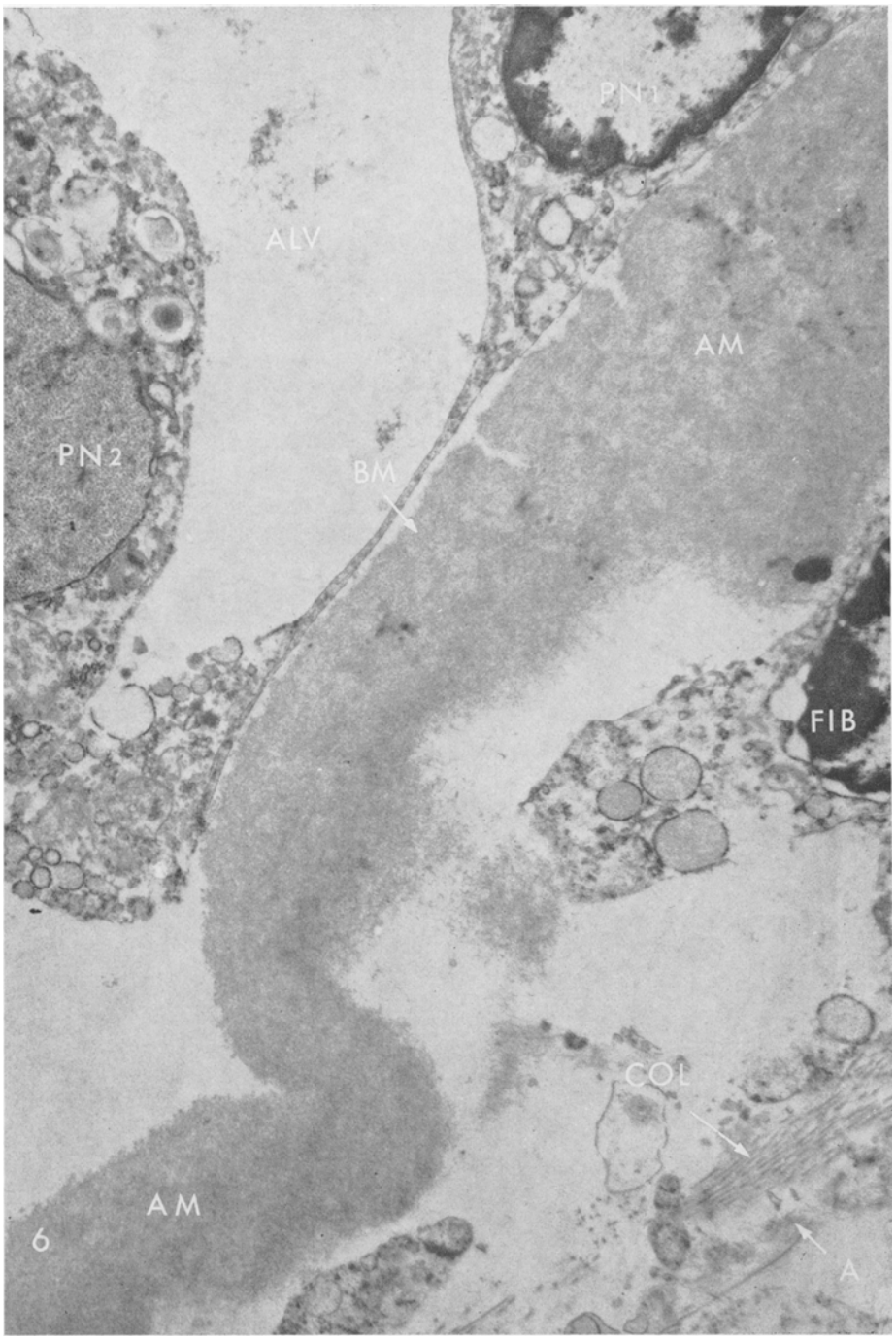


Fig. 6. Transmission electron micrograph showing wall of alveolus with pneumocyte type 1 (PN_1), pneumocyte type 2 (PN_2), and place of basement membrane (BM), which has completely disappeared. Amyloid material (AM), fibroblasts (FIB), collagen fibers (COL), alveolus (ALV). (A) marks amyloid material within the interstitium. T.E.M. $\times 4{,}200$

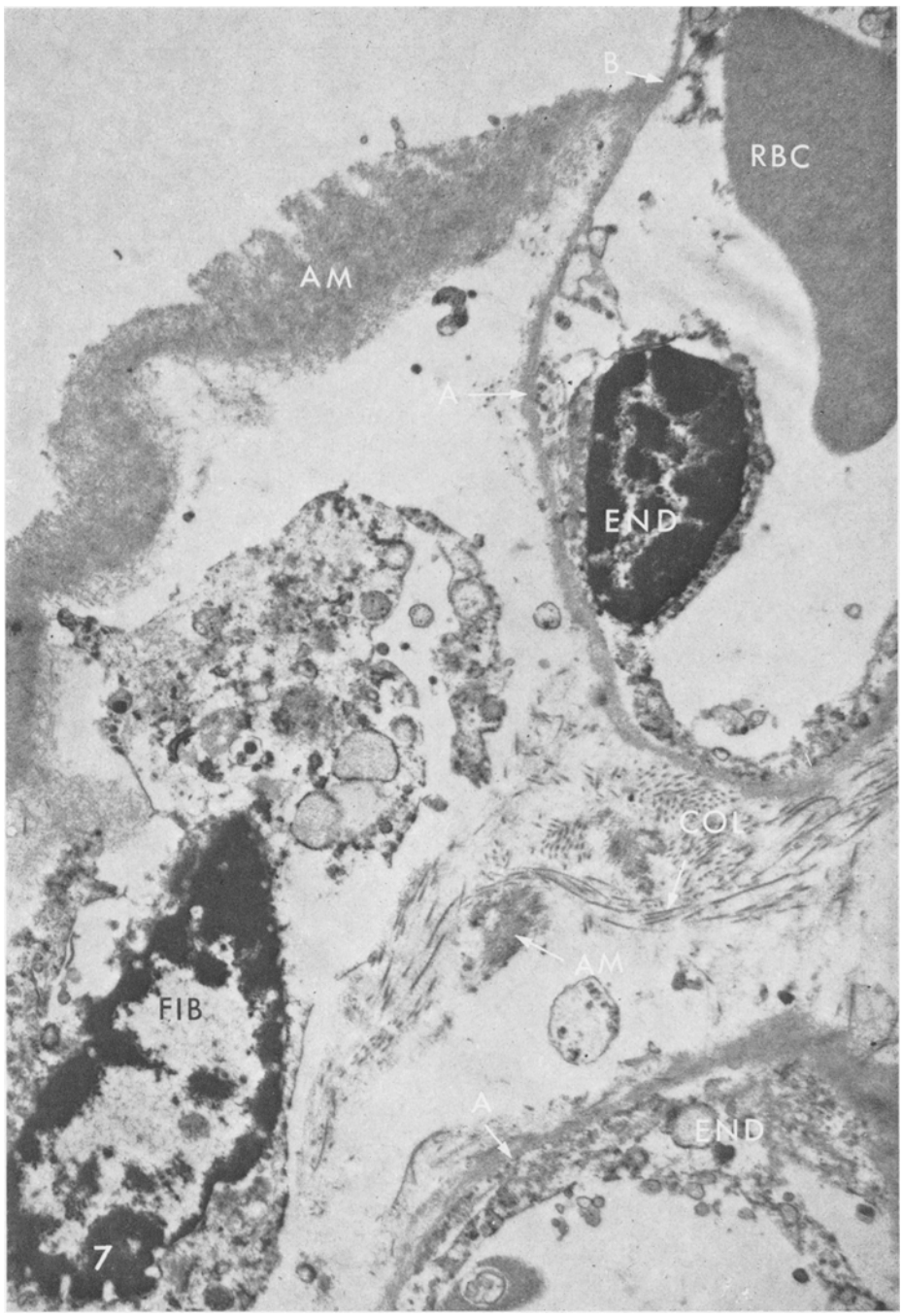


Fig. 7. Transmission electron micrograph showing amyloid material (*AM*) within basement membranes of pneumocytes and endothelial cells (*A*) which have completely disappeared. Point marked (*B*) depicts direct alveolocapillary junction with amyloid material. T.E.M. × 4,200

The present case does not differ from previously reported cases except the one described by Rajan et al. These authors demonstrated deposits in the alveolar septa and in the arteries of the lungs and hearts and within smooth and skeletal muscles. In the lung these workers found the amyloid material only in a nodular form within the interstitium, and most of the alveolar capillary walls failed to show any amyloid. The basement membranes underlying both the pneumocytes and the alveolar capillary endothelium were nearly intact.

In the present case amyloid was found within the vessel walls and in the basement membranes beneath pneumocytes and endothelial cells as well as within the interstitial space and in many other organs. The SEM demonstrated the characteristic porous structure of the lung amyloid (Fig. 5).

The orientation of the bundles of amyloid fibrils is a good demonstration of the fibrillar ultrastructure of amyloid as reported by Shirahama and Cohen (1967). The fibrillar and porous ultrastructure of amyloid may explain how despite the finding of large amounts of amyloid diffusely deposited in the septal walls and in some areas replacing these structures there was no barrier to the diffusion of gases.

In the present case despite the severe involvement of the alveolar septa gaseous diffusion was only slightly affected. The O_2 saturation was 91% and the diffusion capacity was within the lower level of normal values both at rest and during exercise. Although amyloid is an inert substance the deposition of which in the tissues is accompanied by little or no cellular reaction, pressure atrophy and later fibrosis may occur in the lung, however, (Hartung, 1964), which then leads to restrictive ventilatory insufficiency as in the present case.

This case confirms the fibrillar ultrastructure of amyloid as previously described by Frühling et al. (1960). The SEM has shown that not only is the amyloid fibrillar in character but it is arranged in a meshwork of interwoven fibrils giving it a porous texture through which gases might easily diffuse.

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