

PULMONARY LYMPHANGIOMYOMATOSIS

Three New Cases Studied With Electron Microscopy

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Three cases of pulmonary lymphangiomyomatosis are described, with emphasis on the ultrastructural changes. The clinicopathologic features corresponded to those previously described. Each patient was a female in the reproductive years; breathlessness and recurrent pneumothoraces were the predominant clinical features. Histologically, the lungs showed a focal interstitial infiltrate of short, spindle-shaped mononuclear cells compatible with primitive smooth muscle, which was associated with irregular emphysema and hemosiderosis. Electron microscopy confirmed the smooth muscle nature of the pulmonary infiltrate and showed the presence of cells intermediate between smooth muscle and fibroblasts. Abnormalities were also noted in the pulmonary connective tissue that are possibly related to the fragility of the lung in this condition.

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PULMONARY LYMPHANGIOMYOMATOSIS IS A DISTINCTIVE disease marked by widespread proliferation of muscle throughout the lungs. This may form part of a more extensive syndrome in which extrapulmonary lymphatics show a similar muscular proliferation. Alternatively, the proliferative process may be confined to extrapulmonary lymphatics.^{6,25} All these features may in turn be found in tuberous sclerosis (epiloia, Bourneville's disease),^{2,8} but there are notable differences in the sex distribution and family history of pulmonary lymphangiomyomatosis and tuberous sclerosis.

Corrin, Liebow, and Friedman⁷ described 28 cases of pulmonary lymphangiomyomatosis, which brought the total reported to 57, but the syndrome is still poorly recognized. We have recently seen three further cases and in each of these we were able to study the ultrastructure of the lung, a feature that has not been previously reported.⁸ Electron microscopy confirms the smooth muscle nature of the cellular infiltrate and demonstrates alterations in the accompanying interstitial collagen that may be of im-

portance in the considerable revision of the pulmonary architecture found in this condition.

CASE REPORTS

Patient No. 1

A 46-year-old white woman complained of shortness of breath of increasing severity following an acute flu-like illness almost 3 years ago. Chest roentgenogram showed a diffuse reticular micronodulation, maximal at the bases. Respiratory function tests demonstrated an obstructive syndrome with hypoxia ($\text{PaO}_2 = 56$ Torr) and normocapnia. Recurrent pneumothoraces necessitated surgery when many small bullae were noted on the surface of the lung. Biopsy of the lung established the diagnosis of lymphangiomyomatosis.

A series of investigations was then carried out. Pulmonary angiography showed inhomogeneity of distribution with areas of hypovascularization in the right upper and both left lobes. Cardiac catheterization demonstrated pulmonary arterial hypertension (44/20 mm Hg with a capillary pressure of 10 mm Hg). At intravenous urography, a tumor of the right kidney was found, and arteriography suggested an angiomatous proliferation. Lymphography showed dilatation of the terminal part of the thoracic duct, and lacunae in the lumbar lymph nodes. Antismooth-muscle antibodies were detected in significant titer on two occasions. Endocrinologic investigations were normal, except for an unexplained increase of growth hormone on insulin stimulation.¹⁶

There were no features of Bourneville's tuberous sclerosis and, especially, no familial involvement. The patient had had two normal pregnancies, no gynecological abnormalities, and no hormonal treatment. Two years before the onset of the present disease she had been treated for 8 months with reserpin and chlorphentermine for obesity and systemic hypertension (BP 160/90 mm Hg.).

Three months after biopsy, functional respiratory tests showed a predominantly obstructive syndrome

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⁸ While this paper was in press, an ultrastructural study on histogenesis of the lung lesions was published in *Cancer* by J. J. Vasquez *et al.* (Vol. 37:2321-2328, 1976.)

with a 60% decrease of vital capacity, normal pulmonary compliance, a 75% decrease of carbon monoxide transfer, and a considerable imbalance of the blood-air distribution. Six months later, her breathlessness had severely increased and she had a generalized honeycomb pattern on chest roentgenogram. The hypoxia was 50 Torr, with hypercapnia 60-70 Torr. Her condition deteriorated rapidly and she died approximately 3 years after the onset of the disease.

Postmortem examination showed honeycomb lungs, no gross abnormalities of the pulmonary blood vessels, lymphatics or lymph nodes, a tumor attached to the outer border of the right kidney, uterine fibroids, and a normal brain.

Patient No. 2

A white woman, aged 43, who had suffered from transitory thoracic pains and dyspnea on exertion for the past 3 months, was admitted to hospital with bilateral pneumothorax. Pleurectomy was done when it was found that the surface of both lungs were scattered with small translucent bullae. Samples of the lung showed the histologic features of lymphangiomyomatosis and smooth muscle proliferation was also present in the pleura and mediastinal lymph nodes.

After surgery the chest roentgenogram showed a reticular micronodulation of both fields, slightly worse at the bases. Respiratory function tests showed an obstructive syndrome, a low pulmonary compliance, a decrease of DLCO, and an imbalance of blood-air distribution. Pulmonary angiography demonstrated hypervascularization with angiomatous features in the left costo-phrenic angle. Pulmonary arterial and capillary pressures were normal. Renal arteriography showed three angiomatous foci in the left kidney. Lymphography demonstrated uneven filling of irregularly dilated lymph channels and enlarged lymph nodes, but no abnormality of the thoracic duct. Genetic, immunologic, and endocrinologic investigations were all normal, apart from a high level of growth hormone.¹⁵

Nine years before 17 myomas had been removed from the uterus but no hormonal treatment had been given and she had never been pregnant. One sister had a hysterectomy at age 49 years. Another sister had a bilateral hip dislocation operated on at age 33 years; she had always been small (1.39 m) and was sexually immature until hormonal treatment was given when she was 23. There was no family history of tuberous sclerosis.

The patient returned home and, despite her radiologic and functional pulmonary impairment, leads a relatively normal life.

Patient No. 3

A 26-year-old white woman was operated on for pneumothorax, her fourth in 3 years, two on each side. She had a serous, but not chylous, pleural effusion at the time of her third pneumothorax, when a chest roentgenogram showed a fine basal retic-

ulomiconodulation. There was no significant family history or evidence of tuberous sclerosis. Gynecologic enquiries revealed no menstrual irregularities and she had never been pregnant. Oral contraceptives had been used for a few weeks prior to the first pneumothorax. Examination showed no evidence of uterine or ovarian disease. At thoracotomy the surface of the lung presented an unusual microbullous appearance and a biopsy showed lymphangiomyomatosis.

LIGHT MICROSCOPY

Lung Biopsies

All three cases showed focal thickening of alveolar and bronchiolar walls by glomangioma-like groups of cells characteristic of pulmonary lymphangiomatosis.^{7,16,23} These cells were slightly elongated and eosinophilic with a relatively high nuclear-cytoplasmic ratio, appearances compatible with primitive smooth muscle (Fig. 1). A similar cellular infiltrate was seen in the parietal pleura in patients 1 and 2. In places, the airways were distorted by this infiltrate, the lumen being narrowed at some points and dilated at others. There was focal breakdown of alveolar walls, responsible for the cystic airspaces noted at thoracotomy. Infiltration of blood vessel walls, especially small veins, was evident in patient 1 particularly, where calcium and iron deposits on disrupted elastic sheaths were also seen with appropriate stains. Hemosiderin-laden alveolar and interstitial macrophages were found in all three patients and were very prominent in some areas.

In summary the light microscopic features were similar to those described previously^{7,16,23} and were characteristic of pulmonary lymphangiomyomatosis.

Postmortem (Patient 1)

The pulmonary changes were similar to those seen in the biopsy but were more advanced; in particular the alveolar wall destruction and consequent emphysema were more severe. The tumour attached to the outer border of the right kidney was a lymphangiomyoma. Tissue taken from above the left kidney showed an angiomolipoma. The pituitary (Professor Racadot) showed no adenoma but a slight increase in number and size of acidophil cells.

ELECTRON MICROSCOPY

The cellular infiltrate consisted of elongated elements generally rich in microfilaments, with focal electron-dense condensations, pinocytotic vesicles and prominent basal laminae (Fig. 2),

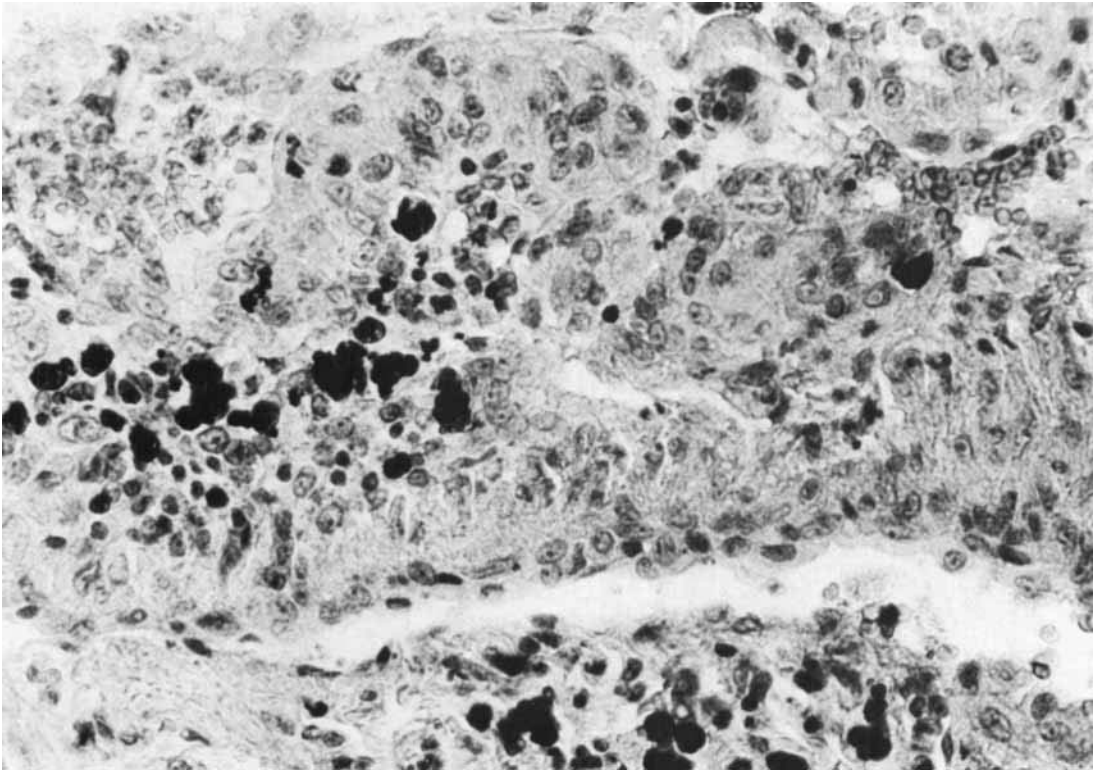


FIG. 1. Clumps of pale, slightly elongated cells are seen near a lymphatic, whose wall is thickened by an infiltration of similar cells. The dark deposits are hemosiderin (Hematoxylin and eosin, $\times 230$).

the features of smooth muscle. Much of this smooth muscle was of normal configuration but the following abnormalities were noted. The size of the cells sometimes varied, many assuming giant proportions, and glycogen, normally present in small amounts, occasionally formed large pools in the cytoplasm. The orientation of the smooth muscle cells was frequently quite irregular and both longitudinally and transversely sectioned cells were found together. Microfilaments were sometimes not readily identifiable and the basement membrane was occasionally discontinuous. Unusual cytoplasmic inclusions were occasionally evident. These were membrane-bound, electron-dense and sometimes cross-striated (Fig. 3). Their identity was uncertain, but they may be lysosomal in nature. Certain cells, otherwise recognizable as smooth muscle, contained an abundant rough endoplasmic reticulum, a feature more characteristic of fibroblasts and suggesting the appearance of intermediate cell forms (Fig. 4). Cells clearly recognizable as fibroblasts were also seen mixed with these intermediate cell forms and smooth muscle.

The interstitial connective tissue was also fre-

quently abnormal. The collagen fibers varied in thickness and shape and in longitudinal section separation or twisting of the fibers was seen. In transverse section the collagen fibers were often jagged or stellate in outline (Fig. 5) or separated by excessive amounts of Thiery-positive²² mucopolysaccharide ground substance. The elastin fibers were unusually straight in longitudinal profile, suggesting excessive rigidity (Fig. 6), and often showed increased electron density, partly due to an increased microfibrillar content. Basement membranes of the alveolar epithelial and endothelial cells were generally thickened, homogenous and electron-dense (Fig. 7). Capillary basement membranes were sometimes reduplicated, and extremely electron-dense deposits were found in some areas in the elastin fibers (Fig. 7), the basement membranes (Fig. 8) and ground substance, corresponding to the hemosiderin and calcium seen with the light microscope. Hemosiderin deposits were also seen within interstitial and intra-alveolar macrophages and occasionally in type II pneumocytes.

From the examination of many 1- μ m thick sections, it was evident that the cellular infiltrate

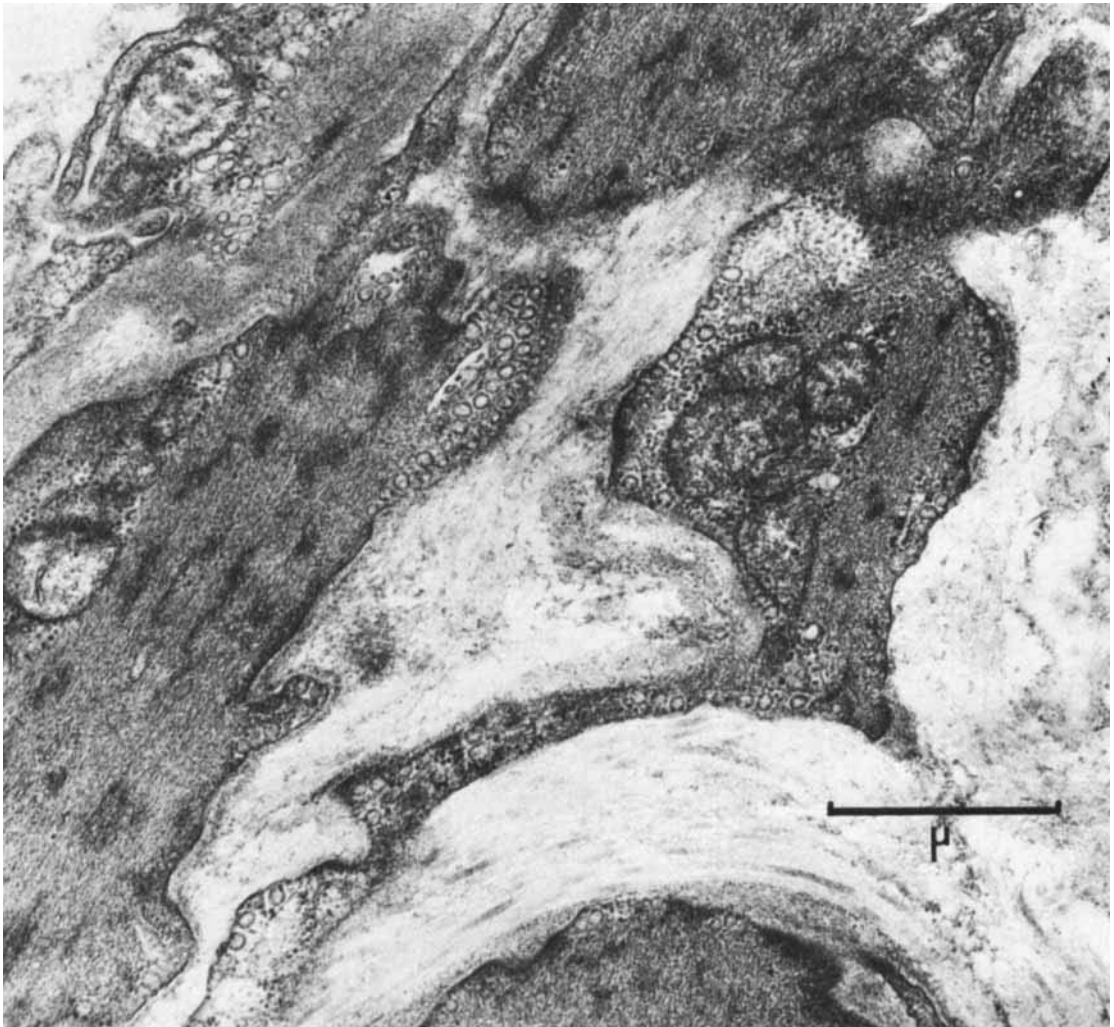


FIG. 2. The pulmonary infiltrate consists of smooth muscle cells recognizable by their microfilaments with focal condensations, pinocytotic vesicles, and basement membranes (Electron micrograph (EM), $\times 31,875$).

was often related to lymphatic channels (Fig. 1); within the areas of cellular proliferation, thin sections confirmed that there were often prominent lymphatics, recognizable by their luminal and abluminal endothelial projections and discontinuous basement membrane.^{10,12} No abnormalities were evident in these lymphatics apart from dilatation and an increase in their number. Some blood capillaries showed gross thickening of the endothelium, increased numbers of pericytes, and reduplication and thickening of the basement membrane, whereas others appeared to be dilated unduly with gaps between the endothelial cells. Occasionally, the endothelial cells of small blood vessels displayed prominent bundles of cytoplasmic microfilaments, as described by Bensch, Gordon and Miller.¹

Type 1 epithelial cells were often thickened and displayed short irregular microvilli on their surface. Typical type II cells were not increased in number but cells intermediate between I and II with microvilli, scanty lamellar inclusions, many free ribosomes, electronlucent cytoplasm, and a flattened squamous configuration were evident. Unusually large numbers of nerve fibers, otherwise normal in appearance, were found in some areas.

DISCUSSION

As is the rule in pulmonary lymphangiomyomatosis, our patients were all women in their reproductive years and were the only members of their families affected. This contrasts with tu-

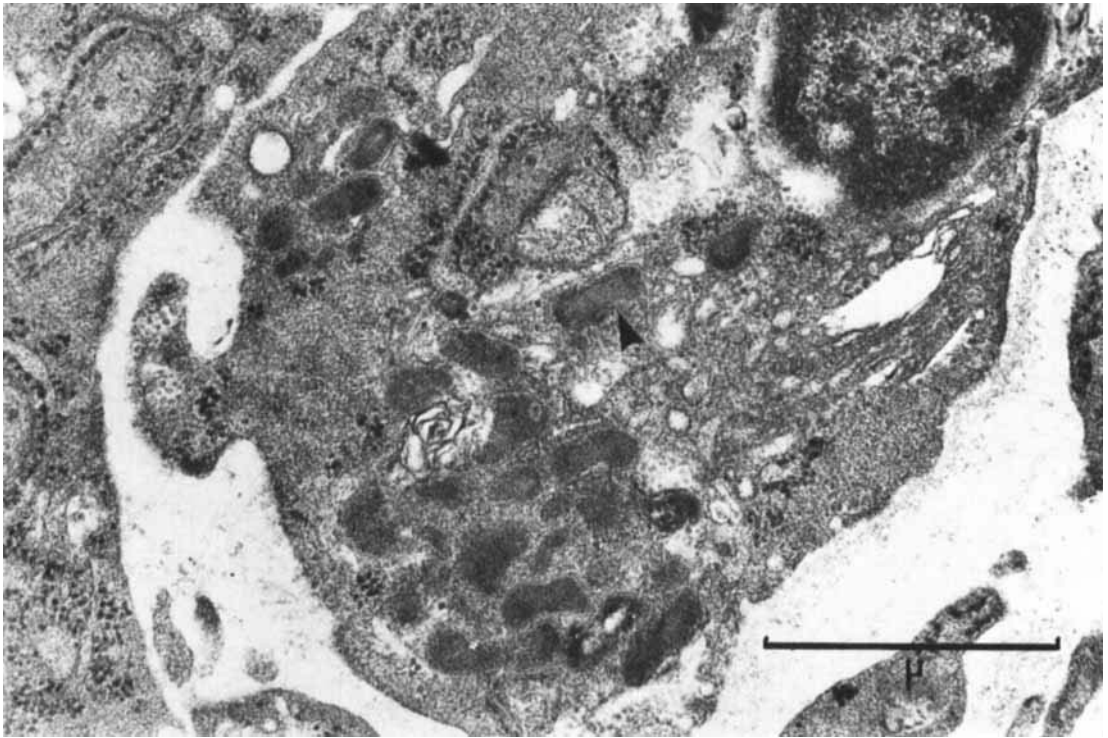


FIG. 3. A smooth muscle cell containing many membrane-bound dense bodies, some of which are cross-striated (EM, $\times 38,000$, reduced from $\times 45,000$).

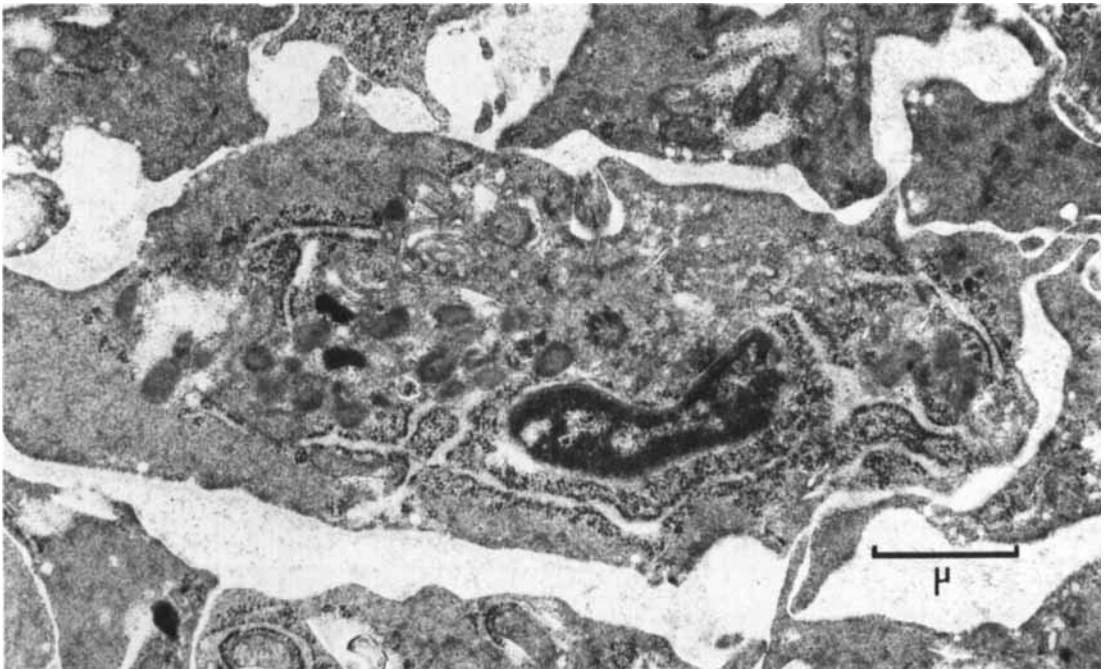


FIG. 4. Elongated cells rich in both microfilaments and rough endoplasmic reticulum, intermediate in appearance between smooth muscle and fibroblasts (EM, $\times 18,500$ reduced from $\times 25,000$).

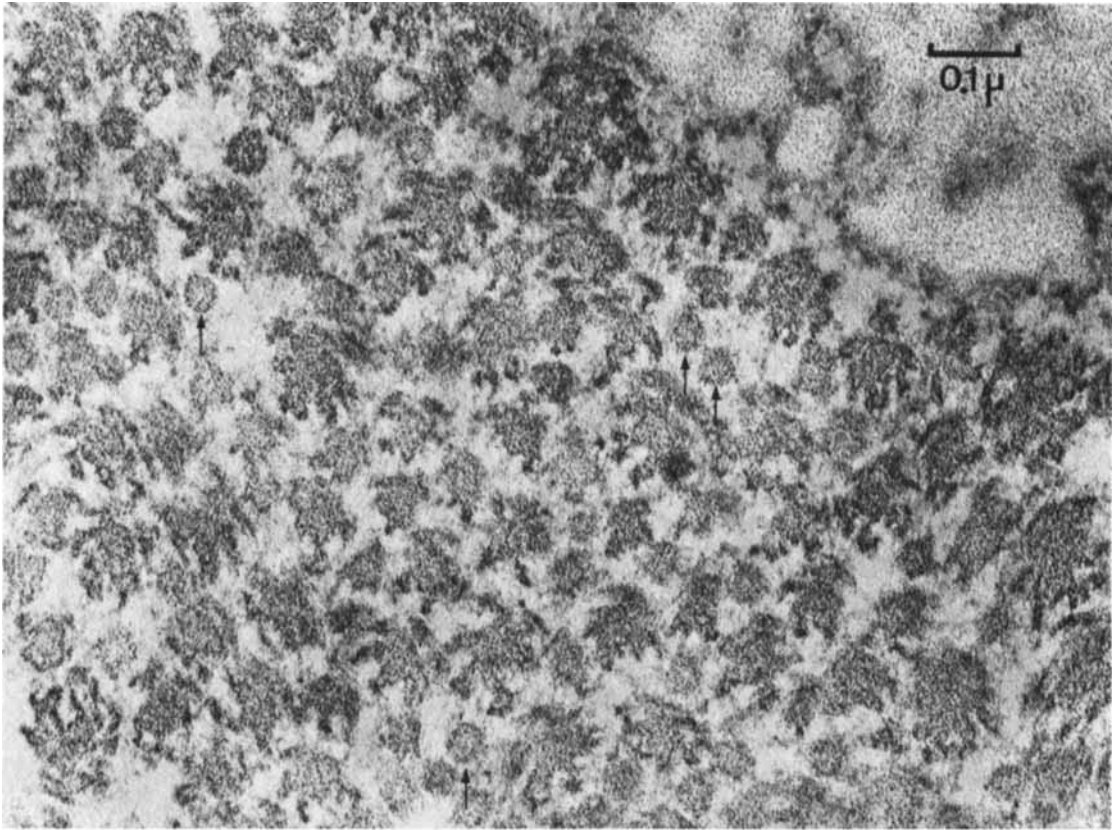


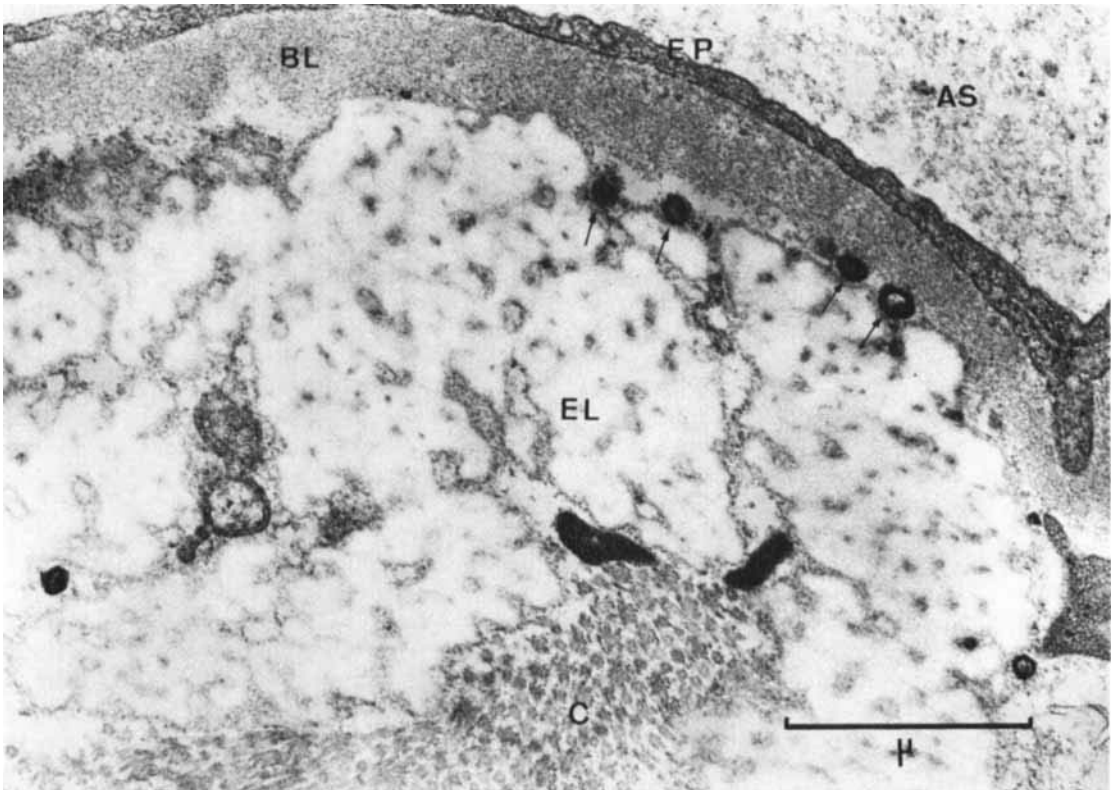
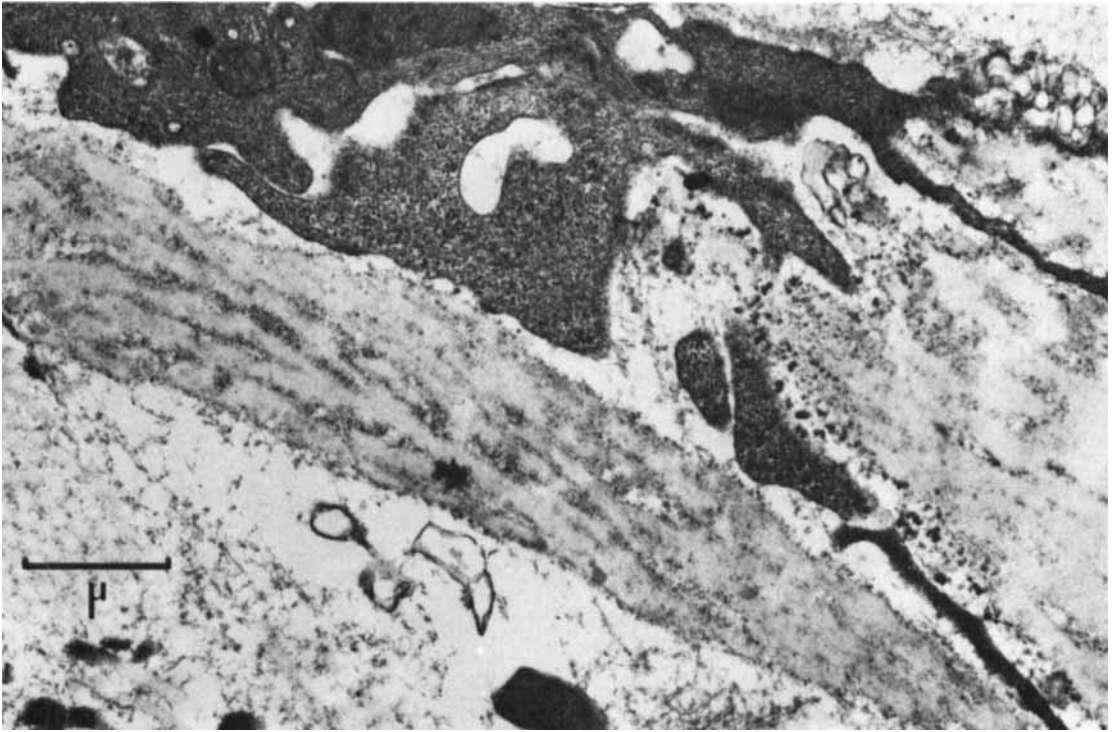
FIG. 5. In cross section many collagen fibers are stellate in outline. Arrows indicate normal fibers (EM, $\times 117,000$ reduced from $\times 137,000$).

berous sclerosis, which has a familial pattern, is often present at birth, and affects both sexes. This seemingly clear distinction becomes somewhat blurred, however, when it is realized that patients with a family history of tuberous sclerosis who show pulmonary involvement are almost exclusively females and their clinical status is dominated by their lung disease, the neurologic stigmata of tuberous sclerosis seldom being prominent.^{8,23} The pathology of the lung in these two conditions appears to be identical^{2,7,8,23} and features such as renal angiomyolipomas and extrapulmonary lymphangiomyomas may be found in either. It has, therefore, been suggested that pulmonary lymphangiomyomatosis represents a "forme fruste" of tuberous sclerosis.^{16,23,25} However, the exclusively female sex distribution suggests that hormonal factors may

also be operating. The experimental induction of fibromyomas in guinea pigs with estradiol¹³ supports this possibility, but only a few patients with lymphangiomyomatosis have had hormonal abnormalities or prior treatment with hormones such as gonadotrophin²⁵ or progesterone.⁷ Whether the condition responds to hormone therapy is unclear, androgens having only been tried terminally³ without obvious effect. The abnormal levels of growth hormones in two of our patients, coupled with pituitary hyperplasia and ovarian thecal sclerosis in one of them, are of uncertain relevance to the etiology; they may be coincidental or secondary to the disease, but are recorded in the hope that similar measurements will be made in other cases and their significance clarified. Chlorphentermine has marked pulmonary effects in the ex-

FIG. 6. (Top) The centrally situated elastin fibers are unusually straight, suggesting excessive rigidity (EM $\times 18,750$, reduced from 21,850).

FIG. 7. (Bottom) Capillary basement membrane (top) is thickened. It is separated from abnormal collagen fibers (bottom) by abundant elastin containing a few dense hemosiderin deposits (EM, $\times 33,500$, reduced from $\times 45,000$).



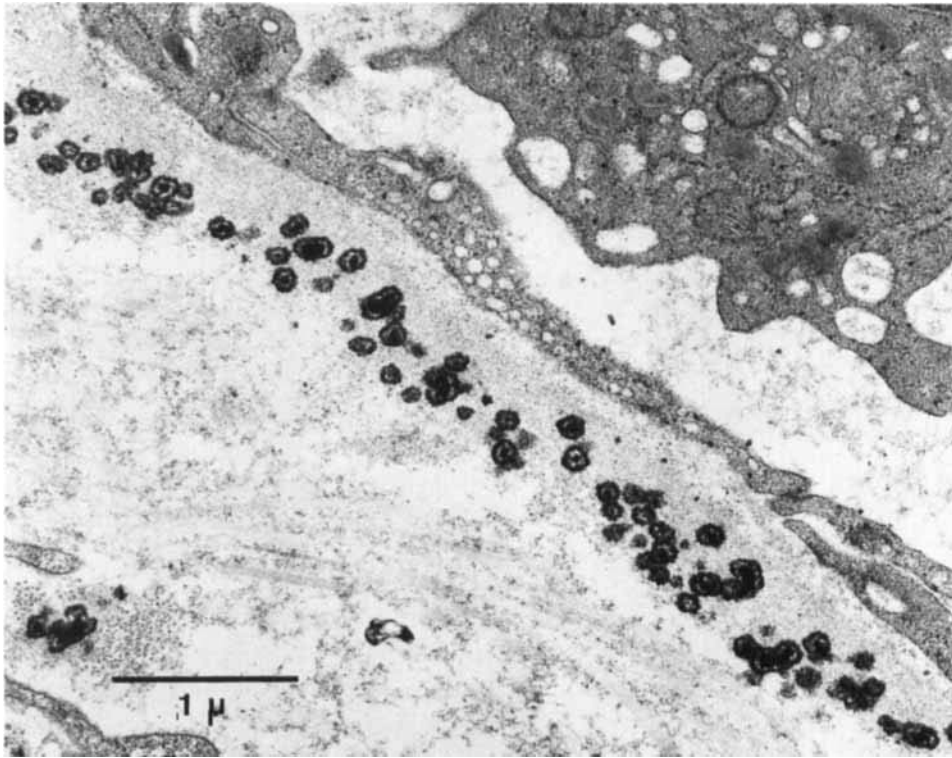


FIG. 8. Numerous hemosiderin deposits are seen along the deeper aspect of the alveolar epithelial basement membrane (EM, $\times 26,000$).

perimental animals^{10,14,20} but these differ profoundly from those of lymphangiomyomatosis and chlorphentermine was only administered to one of our patients.

Clinically, pulmonary lymphangiomyomatosis is marked by breathlessness of slow onset and insidious progression, accompanied by any of three complications: pneumothorax, chylous effusions, and lung hemorrhage. Corrin, Liebow and Friedman⁷ have explained the pathologic basis of all these features on the basis of their light microscopic studies. The proliferative process, sometimes accompanied by fibrosis, both thickens and disrupts the alveolar wall, while involvement of the bronchioles leads to a valve-like obstruction, dilatation of surviving alveoli, and eventually a cystic "honeycomb" lung. Pneumothoraces frequently follow from rupture of the cysts. Increased fragility of the pulmonary interstitium may also be expected from the alterations in collagen identified by our ultrastructural studies. The proliferative process also infiltrates and occludes venules in the lung and hemosiderosis is often evident histologically; less often, pulmonary hemorrhage is manifest clinically as hemoptysis. The smooth muscle pro-

liferation similarly occludes lymphatic channels and many tortuous collaterals may be found at operation or necropsy. Rupture of these leads to chylous effusions and such leakage may be initiated by operative interference or drill biopsy. Flooding of the alveoli with a protein-rich exudate and chyluria have also been described.

None of our patients has developed chylous effusions, but they resemble the previously recorded cases in every other respect. Our first patient presented in an advanced stage, whereas the others have been diagnosed earlier in the course of the disease. It is apparent from these three patients that there is a good correlation between the severity of the clinical features, radiologic appearances, respiratory function tests and pathology. The outlook for our two surviving patients must be regarded as extremely poor. Spontaneous arrest of the process is exceptional and the disease usually pursues a relentlessly downhill course, leading to death from pulmonary insufficiency within 1 to 10 years of the onset of symptoms.⁷

The smooth muscle nature of the proliferating cells is suggested by their light microscopic appearances; Wolff²⁵ confirmed this in an extra-

pulmonary lymphangioma by electron microscopy. Our electron microscopic studies of the lung conform to those of Wolff in this respect and the condition may therefore be more fully termed pulmonary lymphangioliomyomatosis. The presumed lymphatic origin of the smooth muscle is based on its association with lymphatics in the lung and with angiomyomas in extrapulmonary lymphatics and lymph nodes. Nevertheless, within the lungs the smooth muscle proliferation is not confined to lymphatics and future studies may indicate a need for further nosologic modification.

Electron microscopy is of further interest in studying the interstitial fibrosis that accompanies the smooth muscle proliferation. The connective tissues, both collagenous and elastic, are abnormal. Similar abnormalities of collagen have been described in dermatosparaxis,* an hereditary disease of calves and sheep resulting from procollagen peptidase deficiency that leads to disruption of connective tissue fibers in the

skin.^{9,19} These changes are probably nonspecific, as they also have been reported in varicose veins²⁶ and rat lung exposed to nitrogen dioxide.²¹ It may be expected that, as in dermatosparaxis, such collagen abnormalities may be accompanied by an increase in connective tissue fragility, corresponding to the breakdown of the alveolar architecture that is such a prominent feature of lymphangiomyomatosis.

A further feature noted on electron microscopy was the presence of cells intermediate between smooth muscle and fibroblasts. Such cells have previously been described in various organs, including the normal lung,^{5,11,18} and may explain the apparent contractility of alveolar walls in response to histamine.⁴ Collet and des Biens⁵ suggested that these myofibroblasts represent a common progenitor for both fibroblasts and myoblasts, but others^{18,24} regard them as activated smooth muscle cells. Their presence may also explain both the proliferation of smooth muscle in alveolar walls distant from lymphatics in our patients and the interstitial fibrosis noted in other cases of pulmonary lymphangiomyomatosis.

* C. M. Lapière: Personal communication.

ADDENDUM

While this paper was submitted for publication, the authors were aware of an article by Stephen Ray Gray, Charles B. Carrington, and John L. Cornog: "Lymphangiomyomatosis—Report of a case with ureteral involvement and chyluria," *Cancer* 35:490–498, 1975.

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