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The Ultrastructure of the Various Forms of Pulmonary Arterial Intimal Fibrosis

A.G. Balk, K.P. Dingemans, and C.A. Wagenvoort

Laboratory of Pathological Anatomy, University of Amsterdam, Wilhelmina Gasthuis, Amsterdam, The Netherlands

Summary. Intimal fibrosis of muscular pulmonary arteries may present in various forms and in varying degrees of severity according to the underlying condition. In patients with pulmonary hypertension, the type of intimal fibrosis is often significant with regard to prognosis and reversibility. For these reasons we have studied the ultrastructure of the thickened intimal layer in aged individuals, where intimal fibrosis occurs as a normal age change, and in patients with pulmonary hypertension associated with fibrosis of the lungs, mitral stenosis, chronic pulmonary thromboembolism and plexogenic pulmonary arteriopathy (either primary or secondary to congenital cardiac defects). In all these forms of intimal fibrosis, the cellular component of the subendothelial intimal layer was apparently almost exclusively the smooth muscle cell.

These cells usually had a haphazard arrangement. In primary and secondary plexogenic pulmonary arteriopathy, however, there was a more regular circumferential arrangement. The ultrastructural evidence suggested that the intimal cells were derived from medial smooth muscle cells.

Key words: Intimal fibrosis of pulmonary arteries – Ultrastructure of intima – Intimal smooth muscle cells.

Introduction

Intimal fibrosis in human muscular pulmonary arteries may develop in various conditions (Wagenvoort and Wagenvoort, 1977). It is a common age change, rarely absent in individuals over 40 years of age. Usually patchy and irregular in form, it rarely causes marked narrowing of the vascular lumen.

More severe intimal fibrosis is found as a result of thromboembolism, when thrombi are incorporated into the vascular wall. This form, which tends to

Send offprint requests to: A.G. Balk

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narrow or obstruct pulmonary arteries over short lengths, may in some instances cause pulmonary hypertension.

A more diffuse form of intimal fibrosis, usually eccentric although sometimes circumferential, is found over long sections of pulmonary arteries in areas of pulmonary fibrosis and in patients with obstructed pulmonary venous outflow. In the former instance it is usually not associated with pulmonary hypertension, in the latter this association is common although there is not generally a distinct correlation between the degree of intimal fibrosis and the elevation of the pressure.

Finally, a characteristic intimal alteration is found in plexogenic pulmonary arteriopathy, a form of *hypertensive pulmonary vascular disease* found in patients with congenital cardiac defects with a left to right shunt and in patients with primary pulmonary hypertension. Intimal fibrosis in these instances is concentric-laminar, with an onion-skin configuration. It tends to be progressive and occlusive and it is closely correlated with the pressure in the pulmonary arteries. Intimal fibrosis of this type shows little tendency to regression when the cause is removed, in contrast to that observed in pulmonary venous hypertension.

Since pulmonary arterial intimal fibrosis not only presents in various morphological forms but also has varying haemodynamic and clinical implications, we decided to study it at the ultrastructural level in order to see if morphological differences in composition of the intimal layer and in the type of constituting cells could be demonstrated in different groups. We have carried out an electron microscopic study of pulmonary arterial intimal fibrosis in normal, aged individuals, in patients with pulmonary fibrosis and in patients

Table 1

No.	Sex	Age (years)	Condition
1	m	53	age changes
2	m	72	age changes
3	m	56	age changes
4	m	34	age changes
5	f	57	chronic thromboembolism
6	m	63	chronic thromboembolism
7	f	69	*chronic thromboembolism
8	m	42	*chronic thromboembolism
9	m	51	pulmonary fibrosis
10	m	41	pulmonary fibrosis
11	f	61	mitral stenosis
12	f	50	mitral stenosis
13	f	52	mitral stenosis
14	f	35	mitral stenosis
15	f	37	mitral stenosis
16	f	35	mitral stenosis
17	f	45	mitral stenosis
18	m	18	*primary pulmonary hypertension
19	m	62	*primary pulmonary hypertension
20	m	45	*atial septal defect I
21	f	12	patent ductus arteriosus

* Necropsy material

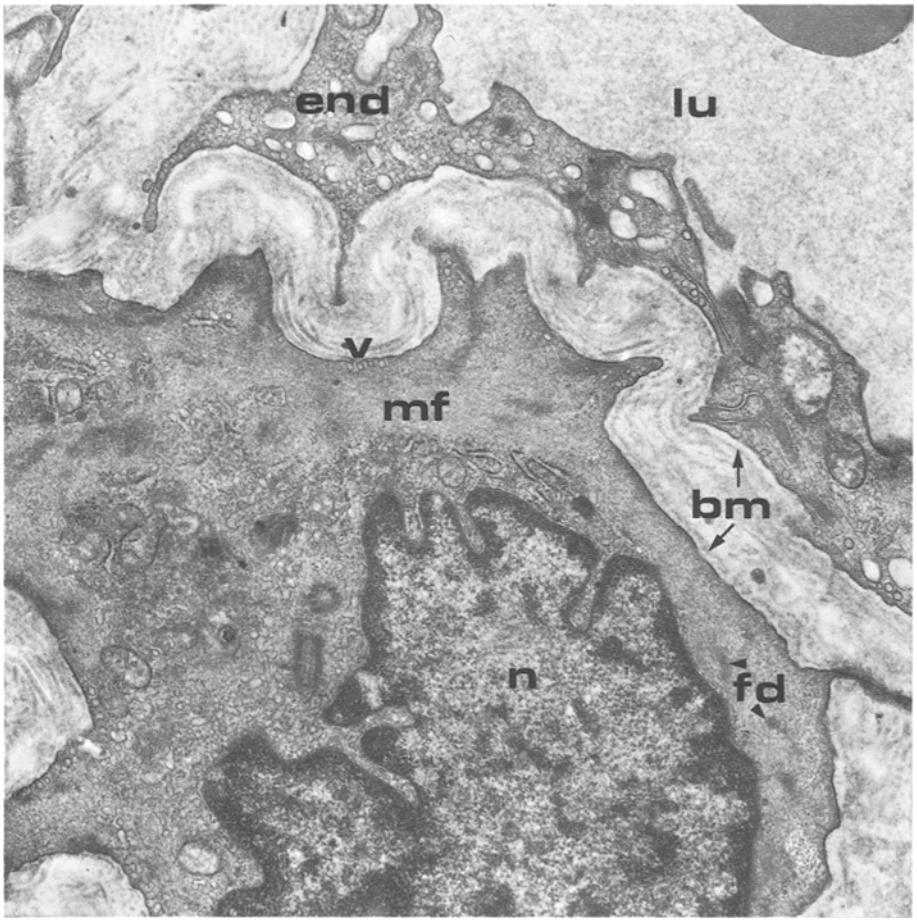

Fig. 1. Detail of subendothelial intimal smooth muscle cell. Cell periphery is mainly occupied by myofilaments (*mf*) with fusiform densities (*fd*), whereas organelles are mainly localized around the nucleus (*n*). *v*, pinocytotic vesicles; *bm*, basement membrane; *end*, endothelium; *lu*, vascular lumen. Woman, 12 years, Plexogenic pulmonary arteriopathy, ×16,300

with various forms of pulmonary venous hypertension, primary pulmonary hypertension and pulmonary hypertension due to congenital cardiac disease.

Material and Methods

Tissue was collected from the lingula of the left lung of 21 patients. In 16 of these, lung biopsies were taken, during thoracotomy directly after opening of the pleural cavity. As it was difficult to obtain biopsy material in sufficient numbers for some conditions, in five instances lung tissue was obtained from necropsy material.

In a group in which normal age changes were studied, lung biopsies were taken from a normal lingula in four patients who were operated upon for a lung tumour. In two patients with interstitial fibrosis of the lung, biopsy was made for diagnostic purposes. Seven patients had isolated mitral

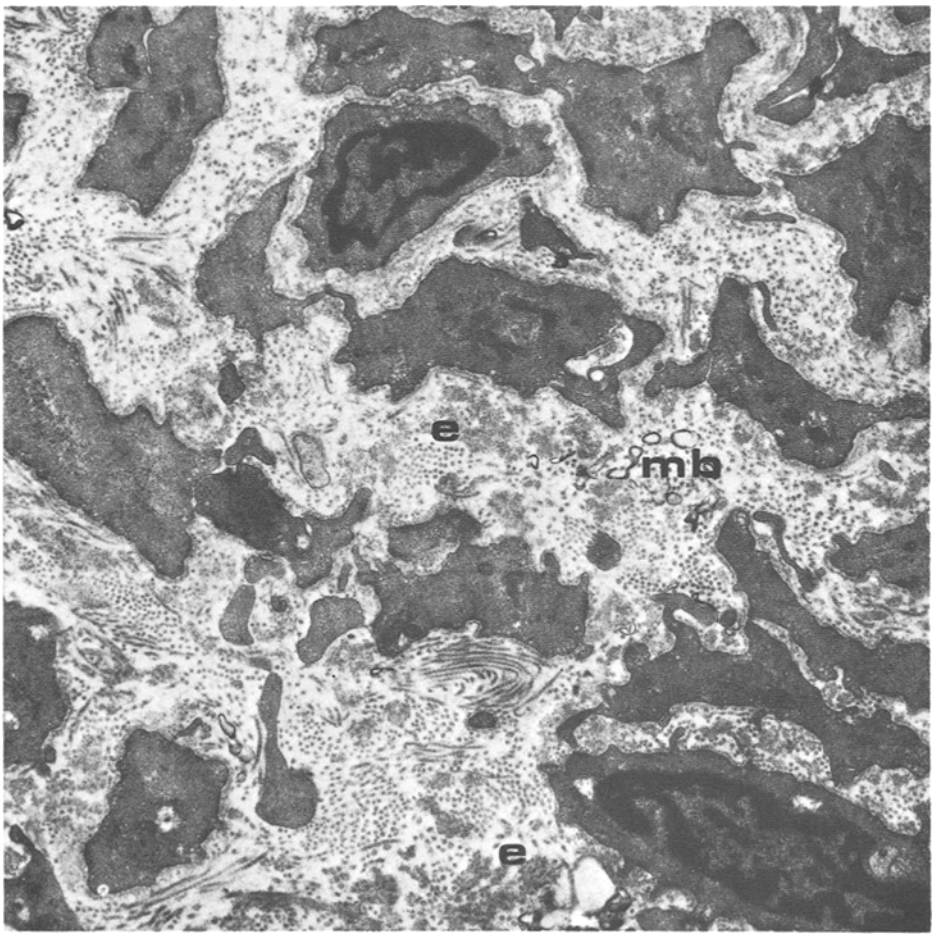

Fig. 2. Intimal smooth muscle cells surrounded by ground substance containing numerous collagen fibres, patches of elastin (*e*) and some vesicular or myelin-like bodies (*mb*). Man, 58 years, Chronic thromboembolism, × 11,600

valve stenosis with pulmonary hypertension. Four patients suffered from chronic thromboembolism. Four patients had plexogenic pulmonary arteriopathy including two with primary pulmonary hypertension, one with an atrial septal defect and one with persistent ductus arteriosus. All patients, except for the normal aged individuals, were shown to have pulmonary hypertension by cardiac catheterization or the diagnosis was suspected on the basis of clinical and/or pathological data.

The patients are summarized in Table 1. In each case one block, approximately perpendicular to the course of the bronchi and pulmonary arteries, was used for light microscopy. It was fixed in a 10% buffered formalin solution. After embedding in paraffin, sections were cut at 5 μ and stained with haematoxylin and eosin, van Gieson's elastic stain and Perl's iron stain. A second block of lung tissue measuring approximately 10 × 10 mm with a thickness of 2 mm was fixed in Karnovsky's fixative for electron microscopy. From the blocks used for electron microscopy slices were cut at a thickness of 50 μ by means of an Oxford Vibratome. From these slices, small pieces of lung tissue measuring 2 × 2 mm and containing pulmonary arteries or arterioles were selected under a binocular microscope. The selected pieces were postfixed in 1% osmium tetroxyde, dehydrated in a graded series of alcohol and embedded in Epon. Light microscopic survey sections of 1 μ thickness were stained with toluidin blue. Small segments were selected from muscular

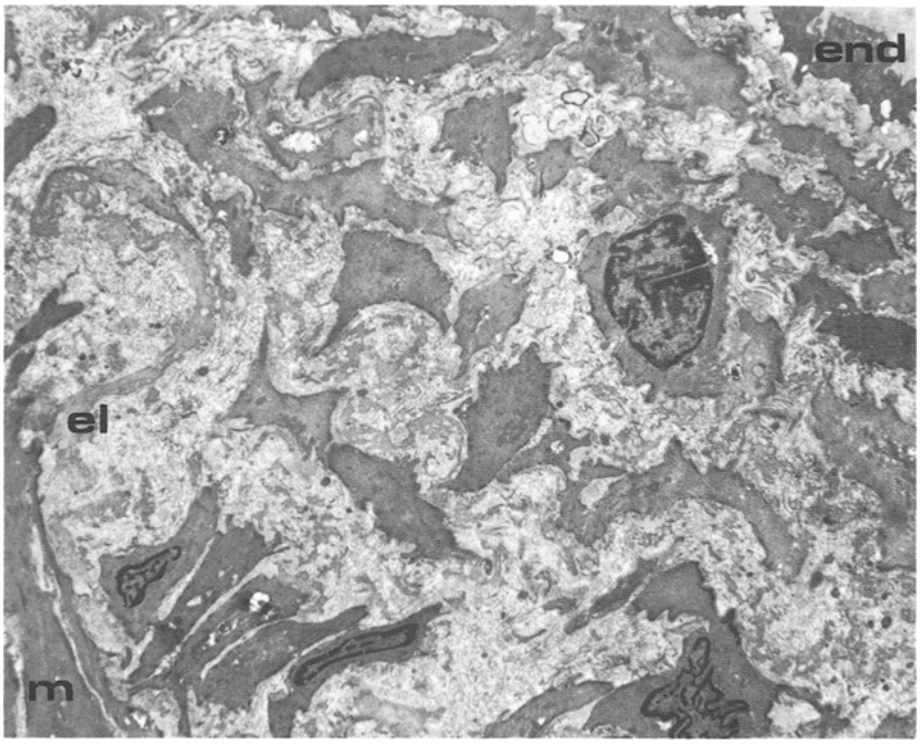

Fig. 3. Thickened intima of 37 years old woman with mitral stenosis in which smooth muscle cells are haphazardly arranged. Immediately adjacent to the internal elastic membrane (*el*) there is a perpendicular arrangement to this laminar (*lower left*). *end,* endothelium; *m,* media. ×4,100

pulmonary arteries and trimmed out with a modified LKB Pyramitome. Ultrathin sections were cut on a LKB ultramicrotome, stained with uranyl magnesium acetate and lead citrate and examined with a Philips EM 300 electron microscope. From each of the patients 2 to 5 muscular pulmonary arteries were studied.

Results

In all conditions the endothelium was seen to be of normal thickness and consisted of a single layer of cells interconnected by junctions, when examined ultrastructurally. The ultrastructure of the endothelium in the different groups was similar.

On the luminal side the surface often had a cobblestone appearance and was provided with few microvilli. The cytoplasm contained pinocytotic vesicles, small mitochondria and a moderate amount of smooth endoplasmic reticulum. In some patients the rough endoplasmic reticulum was well developed. There were many free ribosomes. A general finding was the occurrence of groups of fine microfilaments in the cytoplasm. The endothelial nuclei had a prominent electron-dense chromatin deposition at their periphery and many indentations of the nuclear membrane.

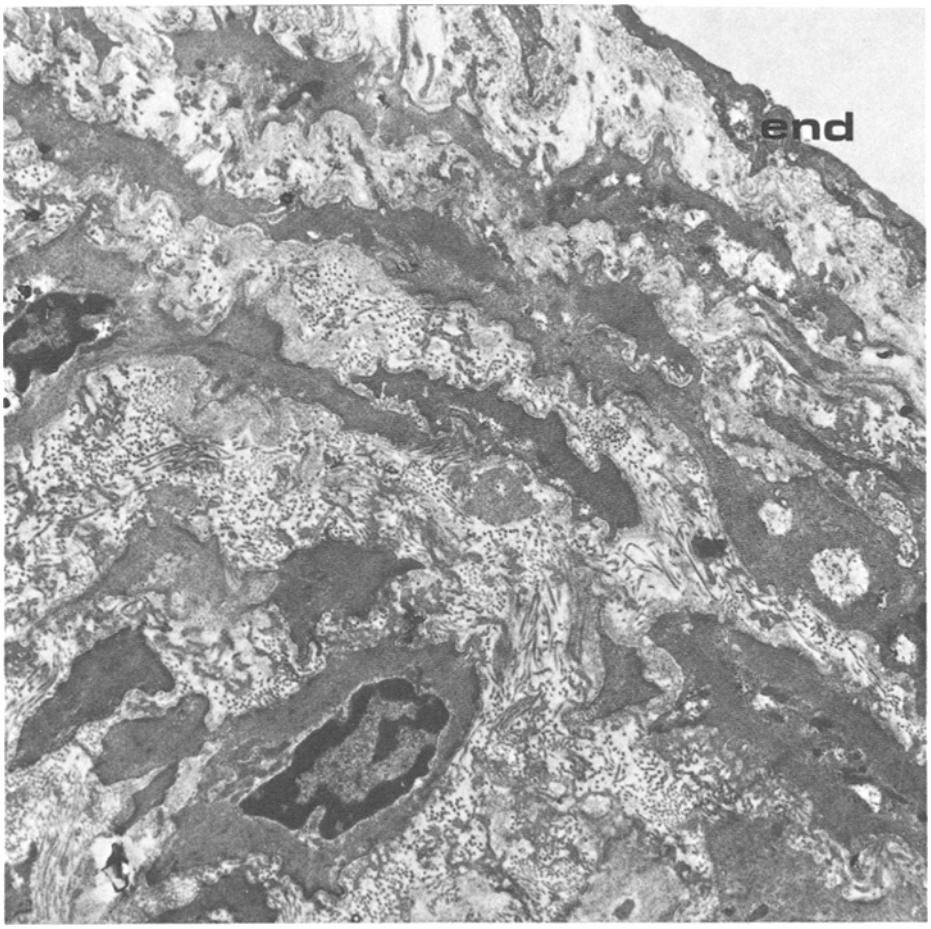

Fig. 4. Innermost layer of thickened intima of 37 years old woman with mitral stenosis. Note circumferential arrangement of several layers of smooth muscle cells beneath the endothelium (*end*). ×4,800

The *endothelial cell layer* was clearly distinguished from the underlying layer of proliferated intimal cells. The sub-endothelial basement membrane was not thickened, indeed in some instances such a membrane was not clearly detectable. The thickness of the subendothelial intimal layer varied from 4 to 105 μ, that is from a single cell to approximately 20 cell layers thick.

In every condition studied, the cells within the subendothelial intima had the characteristics of *smooth muscle cells* (Fig. 1). Their form was oval or elongated, often with irregular cytoplasmic protrusions. The cells were surrounded by a distinct basement membrane. Usually, but not invariably, there were many micropinocytotic vesicles associated with the plasma membrane. The cytoplasm was largely occupied by microfilaments with fusiform electron-dense bodies that tended to be arranged in the longitudinal axis of the cells. Along the plasma membranes there were many attachment sites. Mitochondria, rough

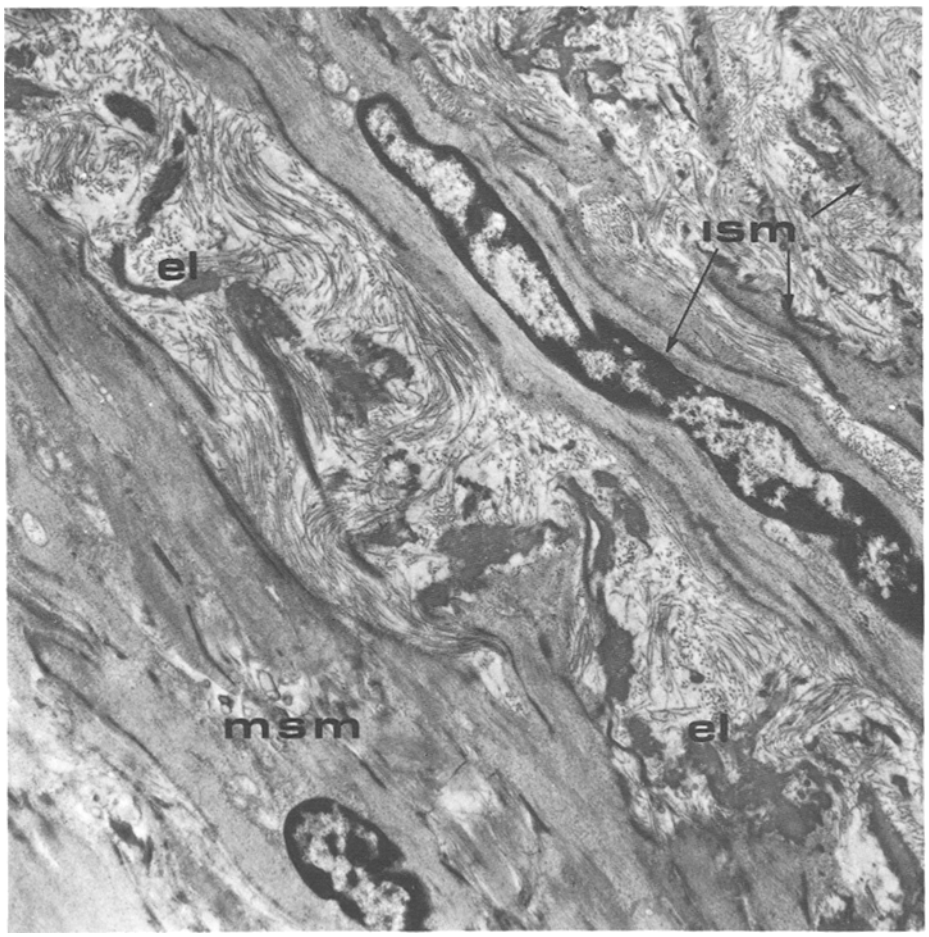

Fig. 5. Intimal smooth muscle cell (*ism*) oriented circumferentially indicated by arrangement parallel to medial smooth muscle cell (*msm*). *el*, internal elastic lamina. Man, 18 years. Primary plexogenic pulmonary arteriopathy, ×4,250

endoplasmic reticulum and free ribosomes were usually localized around the nuclei. Between the microfilaments glycogen granules were observed in varying quantities. The nuclei had many, often deep, indentations and dense chromatin was present, especially at their periphery. All of these features were variable in extent and there was no constant relationship of any one of them and any of the conditions included in this study.

Among the thousands of subendothelial intimal cells identified as smooth muscle cells there were only four with a different morphology, namely two mast cells, one macrophage and one cell differing from smooth muscle cells by the absence of distinct microfilaments and dense bodies.

Between the cells of the subendothelial intima there was a varying amount of electron-lucent ground substance with many collagen fibres, irregular patches

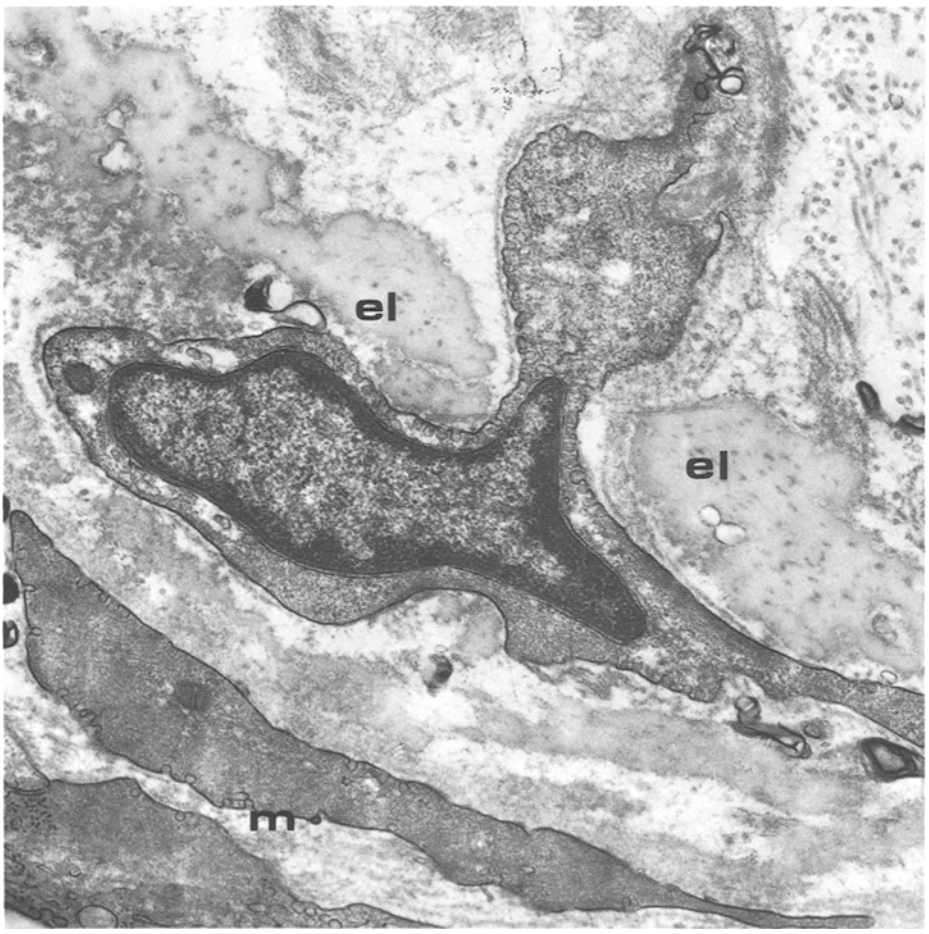

Fig. 6. Smooth muscle cell penetrating through internal elastic lamina (*el*). *m*, media. Man, 58 years. Age change, ×25,900

of elastin and small electron-dense laminar or myelin-like bodies (Fig. 2). The amount and arrangement of intercellular substance and fibres was not characteristic for any of the various forms of intimal thickening.

The arrangement of the cells differed according to their localisation in the intima. In all the conditions investigated, the intimal smooth muscle cells immediately adjacent to the internal elastic lamina were often perpendicular to it. At some distance from the elastic lamina, the cells had an irregular and haphazard arrangement in intimal thickening in those cases where age changes were seen and in patients with pulmonary fibrosis, mitral stenosis and chronic thromboembolism (Fig. 3).

In some patients with mitral stenosis and thromboembolism the innermost layer of intimal cells immediately underlying the endothelium was circumferentially arranged (Fig. 4).

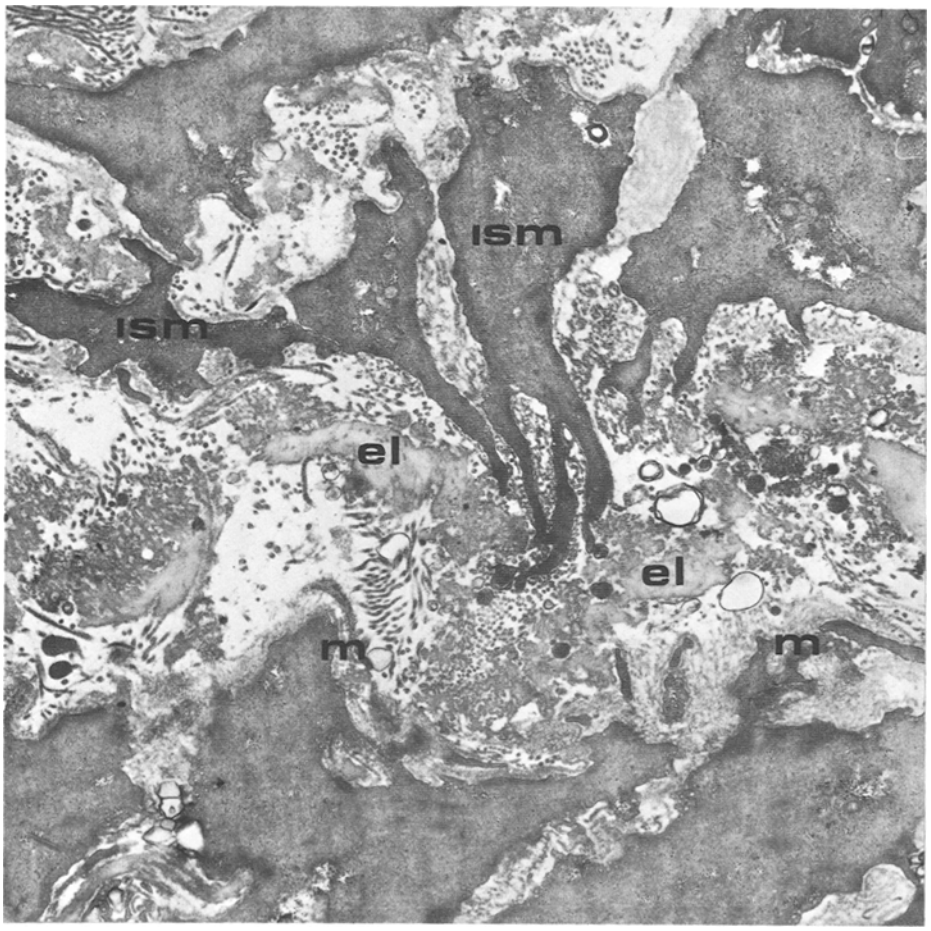

Fig. 7. Electron-dense intimal smooth muscle cells (*ism*) perpendicular to and in part penetrating the internal elastic lamina (*el*). *m*, media. Woman, 45 years. Mitral stenosis, ×11,700

In plexogenic pulmonary arteriopathy whether associated with primary pulmonary hypertension or with congenital heart disease with a shunt there was a definite circumferential arrangement of cells throughout the subendothelial intima, at some distance from the internal elastic lamina (Fig. 5). This was the only constant difference in intimal composition between the various types of intimal thickening.

The internal elastic lamina generally showed crenation. It was well-formed with regular fenestrations in some areas, but with large discontinuities or fragmentation in others. The smooth muscle cells of the media were generally larger and more regularly shaped than those in the intima. In the pulmonary arteries of all conditions investigated, including the normal, aged individuals, we observed some smooth muscle cells penetrating the internal elastic lamina, so that part of the cell was situated within the media and part within the

intima (Fig. 6). These cells, apparently migrating through the internal elastic membrane, and intimal cells located near this membrane, were often very electron-dense (Fig. 7).

Fragmentation and disruption of the internal elastic lamina was particularly prominent in areas where smooth muscle cells were penetrating or arranged perpendicular to the lamina.

Discussion

Intimal fibrosis in pulmonary arteries is a very common lesion and may be an expression of a variety of conditions (Wagenvoort and Wagenvoort, 1977). It is almost universally present in individuals over 40 years of age in a mild form and over short lengths of vessel. It is much more prominent in patients with fibrosis of the lungs, mainly in or near fibrotic areas and irrespective of the cause of the fibrosis. Particularly severe intimal fibrosis is found in patients with elevated pulmonary arterial pressure but is variable in extent and structure, depending on the type of pulmonary hypertension.

In plexogenic pulmonary arteriopathy, as encountered in patients with congenital heart disease with a left to right shunt or with primary pulmonary hypertension, intimal fibrosis is usually very pronounced, often occlusive and present over long distances within the arteries and also has a peculiar onion-skin configuration. This type of intimal fibrosis tends to be progressive and shows little tendency to regression after removal of the cause. However, the diffuse intimal changes of chronic pulmonary congestion, as seen in mitral stenosis, have no laminar arrangement, are often eccentric and probably regress easily after elimination of the obstruction to the pulmonary venous outflow. In chronic thromboembolism the intimal fibrosis is usually eccentric but extends over short distances within the artery, while its reversibility is limited.

In spite of this variability in extent and structure of the intimal lesions, the ultrastructural features of the intimal layers show little difference in the various conditions studied. In all forms of intimal fibrosis investigated, the cells within the thickened subendothelial intimal layer were not distinguishable from ordinary smooth muscle cells, even though they were smaller and more irregularly shaped than those in the media. In this respect we confirm previous reports dealing with the ultrastructure of lung vessels (Hatt and Rouiller, 1958; Hatt et al., 1959; Widgren, 1977) describing smooth muscle cells in the intimal fibrosis of patients with mitral stenosis, with congenital cardiac shunts and with primary pulmonary hypertension. Our study also shows that in patients with fibrosis of the lung and chronic pulmonary thromboembolism as well as in elderly "normal" individuals the intimal fibrosis contains exclusively or almost exclusively smooth muscle cells.

In systemic arteries it has been known, for some time that in intimal thickening the subendothelial intimal cells have some of the features of smooth muscle cells. Buch (1961) introduced the term "myointimal cells". These cells have been demonstrated in atherosclerotic and reparative processes in experimental animals (Parker, 1960; Buck, 1961; Lee et al., 1970; Laden and Sinclair, 1971;

Webster et al., 1974) as well as in man (Geer et al., 1961; Geer, 1965; Balis et al., 1964; Stehbens, 1975). They have also been described in muscular arteries (Sinclair et al., 1976). Most authors have indicated that, although intimal cells have some characteristics of smooth muscle cells, they differ in other respects. Notably it has been emphasized that there are less differentiated forms containing less filaments. Benditt (1976) however, observed only fully differentiated intimal smooth muscle cells in his studies.

In muscular pulmonary arteries of dogs in which a shunt was made berween the subclavian and the pulmonary artery, Esterly et al. (1968) found three types of intimal cells: smooth muscle cells or poorly differentiated smooth muscle cells, poorly differentiated cells and blood cells. In human pulmonary arteries, Widgren (1977) found smooth muscle cells as well as myointimal cells in the intima. The myointimal cells contained somewhat more rough endoplasmic reticulum than the ordinary smooth muscle cells. In our material we observed only a single cell that possibly represented a myointimal or dedifferentiated smooth muscle cell. Cells derived from the blood were only found in three instances and were one macrophage and two mast cells.

With regard to the origin of the intimal smooth muscle cells, our observations do not support the suggestion that they are derived from endothelial cells (Hatt et al., 1958, 1959) from undifferentiated or reserve cells (Stein, 1969) from fibroblasts (Geer, 1965) or from blood-borne cells (Balis et al., 1964; Lee et al., 1970). In particular the observation that the intimal cells adjacent to the internal elastic lamina are often arranged perpendicularly to this lamina suggests that they are derived from medial smooth muscle cells. This is further suggested by the position of many cells lying partly in the media and partly in the intima, apparently penetrating through fenestrations or discontinuities of the internal elastic lamina. This would confirm the hypothesis of several others (Buck, 1961; Spirio et al., 1965; Backwinckel et al., 1973; Webster et al., 1974; Benditt, 1976) that intimal smooth muscle cells are derived from the media.

In all conditions investigated the intimal cells at a distance from the internal elastic lamina were haphazardly arranged, although the cells immediately beneath the endothelium tended to be circumferential. Only in plexogenic pulmonary arteriopathy, whether in cases of congenital heart disease or of primary pulmonary hypertension all the intimal cells have a strikingly regular and concentric arrangement.

We do not know whether the intimal smooth muscle cells have contractile properties, but even if they are not contractile, it is likely that the regular concentric arrangement produces an intima less subject to deformation and thus less pliable than an intima composed of haphazardly arranged cells. This may explain why the effects of intimal fibrosis in plexogenic pulmonary arteriopathy are more serious than in other forms of pulmonary hypertension, and why the effect persists after corrective surgery.

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