Morphogenesis of Intimal Obliterative Hyperplasia of Small Arteries in Experimental Pulmonary Hypertension

An Ultrastructural Study of the Role of Smooth-Muscle Cells

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CHARACTERISTIC LESIONS of small pulmonary artery branches are associated with pulmonary hypertension in some forms of congenital heart disease [1] and in experimentally established systemic-to-pulmonary-artery shunts.[2-4] These include medial hypertrophy and sclerosis, and focal intimal hyperplasia with eventual obliteration of the vessel lumen; thrombi and intramural inflammatory cells are sometimes noted. The pathogenesis of the fibrocellular obliterative reaction is poorly understood; [2-4] attempts to inhibit thrombosis and proliferation of fibrous tissue by the administration of combinations of heparin, corticosteroids, and fibrinolysin to dogs with surgical systemic to pulmonary artery shunts did not modify formation of the lesions.[5]

In the present study, serial lung biopsies of dogs with systemic-to-pulmonary-artery shunts were examined by electron microscopy in order to ascertain the extent to which various cellular and fibrous components participate in the development of luminal obliteration of small pulmonary arteries. Three phases were observed. In the first phase, diffuse endothelial cellular changes predominated. The second stage was characterized by focal but extensive projection of portions of medial smooth-muscle cells into the subendothelial space through gaps in the internal elastin lamella. In the third or obliterative phase, the lumen was occluded by poorly differentiated cells apparently derived from smooth-muscle cells. There was no evidence that endothelial proliferation, accumulation of circulating blood cells, or thrombus formation played any role in the luminal occlusion.

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Materials and Methods

Pulmonary hypertension was induced in 12 young adult dogs of both sexes by anastomosing the left subclavian artery to the distal end of the divided left main pulmonary artery as described elsewhere.[2] Two weeks later, the artery to the lower lobe of the left lung was ligated, directing all of the shunted systemic blood into the upper lobe; blood flow and pressure were measured immediately after the establishment of the anastomosis, at the time of lower lobe arterial ligation, and at the time of sacrifice, 10–40 weeks after anastomosis. A calibrated magnetic flow meter [6] was used to measure flow; pressure was recorded simultaneously from a cannula 0.5 cm. distal to the anastomosis by means of a Statham (P23G) strain gauge and a Grass recorder. The criterion for an adequate shunt was a pulmonary artery pressure equal to or greater than 30 mm. Hg at the time of ligation of the artery to the left lower lobe.

The upper lobe of the left lung of dogs with systemic-pulmonary shunts was biopsied immediately prior to anastomosis, at the time of lower lobe pulmonary artery ligation, at 2-week intervals thereafter, and at sacrifice. A cube of tissue, approximately 1 cm. on each edge, was removed at the time of each biopsy. For light microscopy, a slice 0.2 cm. thick was cut from each of these samples, fixed in 1.5% glutaraldehyde in Sorensen's phosphate buffer solution at pH 7.4 for 6 hr., washed, dehydrated, and embedded in paraffin. Sections 7 μ thick were stained with hematoxylin and eosin, and the Weigert-van Gieson procedure for connective tissue. For electron microscopy, the remainder of each biopsy specimen was cut into smaller cubes, approximately 0.2 cm. on each edge. Half of these were fixed in 1% osmium tetroxide in sucrose buffer solution [7] at 4°C.; the others were fixed in 1.5% glutaraldehyde in phosphate buffer solution at 4°C. for 6 hr., washed in buffer solution, and fixed again in 1% osmium tetroxide in sucrose buffer solution for 1½ hr. Tissue blocks were dehydrated and embedded in Epon; sections were cut on a Porter-Blum ultramicrotome. "Thick" sections (0.5–1.5 μ) were stained with a 1% aqueous solution of toluidine blue and screened by light microscopy in order to locate small pulmonary artery branches 50–200 μ in diameter which had been cut in transverse section. (The toluidine blue staining solution consisted of a mixture of 10 parts of a 1% solution of toluidine blue in 1% sodium borate added to 3 parts of 1% pyronine B. This stains elastin membranes extremely well and permits easy identification of pulmonary arteries.)

This report deals with the early progressive changes in the small pulmonary artery branches preceding and including the cellular obliteration of the lumen. The observations recorded and discussed below are based on the examination of serial biopsies from 6 dogs, all of which developed pulmonary pressure of at least 30 mm. Hg at the time of ligation of the artery to the left lower lobe.

Results

In the dogs which form the basis of this report, pressure in pulmonary arteries distal to systemic-pulmonary artery anastomoses ranged from 30 to 90 mm. Hg. The evolution of the intimal changes was clearly and uniformly related to the duration of the hypertension and to the rate of the progression to the level of hypertension. Increased pulmonary blood flow without increased blood pressure did not result in obliterative arterial lesions.

Of the dogs which subsequently developed small-artery lesions, none had had any lesions at the time of the pre-anastomosis biopsy. The earliest ultrastructural changes were detected in endothelial cells in the biopsies

made 2 weeks after anastomosis. The first changes detectable by light microscopic examination were not obvious until 6–8 weeks after anastomosis and consisted of slight-to-moderate medial and adventitial thickening.

Light Microscopy

The uniform progression of the changes leading to obliteration was evident on sections studied by light microscopy. In addition, so-called "plexiform" lesions resembling vascular glomera [1,3,8] were seen occasionally but only after luminal cellular occlusive lesions were abundant. The morphogenesis of the cellular obliterative lesions will be discussed in this communication. A typical small-artery branch from a biopsy taken 2 weeks after systemic-to-pulmonary-artery anastomosis is shown in Fig. 1A. The vessel is indistinguishable from those seen in biopsy specimens taken before anastomosis. The media is sharply bounded on both intimal and adventitial aspects by an elastin lamella. The medial smooth-muscle fibers are arranged in 1–3 circumferential layers. Endothelial cells form a single layer, apparently closely applied to the internal elastin lamella. Adventitial cells and fibers, mainly collagen, form a sheath around the vessel outside of the external elastin layer. Two to 3 months after anastomosis, intimal thickening consisting of focal accumulations of cells becomes evident (Fig. 1B); concomitantly, the mural thickening, already discernible during the second month after anastomosis, appears to include medial smooth-muscle hypertrophy and increased numbers of medial and adventitial collagen fibers. By the end of the fourth month, arteries in which intimal cellularity had progressed to complete obliteration of the lumen were easily found (Fig. 1C). The internal elastin lamella is thinned and interrupted; the media is thickened. The luminal plug is uniform and usually composed of a single cell type with occasional slight accumulation of intercellular material. At no time in the development of the lesions to this stage was there any evidence of thrombosis.

Electron Microscopy

The electron microscopic appearance of the normal small pulmonary arteries (50–200 μ in diameter) of the dog as they appeared in biopsy specimens taken before anastomosis will be presented in some detail since other descriptions of these vessels are not available.

Before Anastomosis

Small Arteries. Depending on the state of vascular distention as judged by straightening of the internal elastin lamella, endothelial cells were flat, ovoid, or spherical. They formed a single layer in close apposi-

tion to the focally fenestrated internal elastin layer. The intervening subendothelial space contained amorphous material, fine fibrillary strands, and occasional collagen and elastin fragments. Rarely, smooth-muscle cells were found in this space, sometimes adjacent to gaps in the elastin membrane. The surface of the internal elastin lamella directed toward the intima was much more irregular than the surface directed toward the media; the subendothelial and medial interstitial spaces were continuous at the numerous fenestrations. In contrast, the thinner external elastin layer had fewer fenestrations than the internal elastin layer. Smooth-muscle cells of the media were disposed in 2 or 3 circumferential layers; occasionally, interstitial collagen fibers and elastin fragments were noted. The adventitia was composed of dense collagen bundles in which scattered fibroblasts were arranged circumferentially.

Endothelium. Before anastomosis, endothelial cells contained relatively few microsomes. A typical normal endothelial cell is shown in Fig. 2. The occasional profiles of smooth endoplasmic reticulum, the scattered ribosomes, and the small numbers of elongated mitochondria are characteristic; pinocytotic vesicles are only moderately abundant. Golgi apparatuses were sparse and small. No intracellular cytoplasmic fibrils were seen. Basement membranes were not demonstrable on luminal surfaces. Cytoplasmic vacuoles were occasionally seen, but these were small and, rarely, electron dense. Junctions between adjacent endothelial cells usually appeared as overlapping cytoplasmic extensions with attachments consisting of focal thickenings and blurring of apposed membranes, similar to those described by others.[9]

Medial Smooth Muscle. Cells located between the internal and external elastin layers had distinct basement membranes and abundant pinocytotic vesicles. Dense bodies, most often located peripherally, were associated with myofilaments. Smooth endoplasmic reticulum profiles, free ribosomes, elongated mitochondria, and small Golgi complexes were noted, but these were infrequent and nearly always confined to perinuclear zones. The appearance of medial smooth-muscle cells was uniform; no other types of medial cells could be identified in any of the sections.

Adventitia. Fibroblasts were found only in the adventitia among abundant collagen fibers. Profiles of rough-surfaced endoplasmic reticulum were abundant in these cells; mitochondria were also plentiful. Golgi apparatuses were somewhat more numerous than in endothelial cells. Basement membranes were not prominent.

After Anastomosis but Before Intimal Proliferation

Electron microscopic study of the interval biopsy specimens revealed changes prior to the onset of intimal proliferation which were not evident

by light microscopy. By 2 weeks after establishment of hypertension by systemic-to-pulmonary-artery shunt, changes could be observed in each small-artery branch studied.

Endothelium. Pinocytotic vacuoles in endothelial cells were generally more numerous than normal. Villiform cytoplasmic processes projected from the luminal surfaces of many cells. An endothelial cell showing typical early reactive changes is shown in Fig. 3. Free ribosomes and profiles of rough-surfaced endoplasmic reticulum are greatly increased in number; Golgi apparatuses are more frequent and larger; small, osmiophilic vacuoles bounded by single membranes occur frequently. The subendothelial space was usually widened. During the first month after shunting, occasional vesicles were noted in the subendothelial space. No definite continuity between these structures and endothelial cells could be observed, and they were absent from the biopsies taken after the first month.

Medial Smooth Muscle. Some evidence of hyperactivity consisting of increase in the size and number of mitochondria, and the augmentation of free ribosomes and endoplasmic reticulum was noted. Unlike the endothelial cells, these changes were minimal at first and increased gradually, as seen in subsequent biopsy specimens. Medial collagen fibers were somewhat increased in number by this time, but there were no modifications of either the internal or external elastin lamellae.

Adventitia. There was increased adventitial collagen, and profiles of rough-surfaced endoplasmic reticulum seemed to be increased in adventitial fibroblasts. Circumferential orientation of these cells was prominent during this phase.

Intimal Proliferation Onset

In contrast to the diffuse nature of the initial endothelial and medial cellular changes described above, intimal thickenings were distinctly focal and were not seen in biopsy specimens taken earlier than 2 months after anastomosis. In some animals, no intimal hyperplastic lesions were found until 3 months after the shunting procedure.

Endothelium. With the appearance of intimal thickenings and often in association with them, many endothelial cells showed changes indicative of degeneration or involution. Although some cells contained increased numbers of organelles, others contained dense irregular osmiophilic cytoplasmic clumps and few recognizable organelles with scattered dilated cisternae of the endoplasmic reticulum. A typical "dark" or "shriveled" endothelial cell is shown in Fig. 4. Still other endothelial cells had diffusely vacuolated, poorly stained cytoplasm almost entirely devoid of organelles. No transitional forms between endothelial cells and the cell types forming the subendothelial intimal thickenings could be found.

Subendothelial Space. The focal intimal thickenings consisted of cells and portions of cells between the endothelium and the internal elastin lamella. Three types were present: (1) cells with distinct morphologic features of smooth muscle, (2) poorly differentiated cells with structures resembling those found in smooth-muscle fibers, and (3) blood cells. The intimal cellular elements with morphologic features of smooth muscle were most numerous near the internal elastin lamella and contained peripheral cytoplasmic myofilaments and dense bodies. Basement membranes and surface microvesicles or vacuoles were evident. Mitochondria, free ribosomes, and rough-surfaced endoplasmic reticulum profiles were much more numerous than in medial smooth muscle of vessels of lungs sampled before anastomosis; however, the cells were entirely comparable to many medial smooth-muscle cells of vessels from lungs with established pulmonary hypertension. A considerable number of these intimal smooth-muscle cells were partially medial and partially subendothelial, with connecting cytoplasmic bridges occupying fenestrations in the internal elastin lamellar membrane. Where the plane of section did not reveal connecting bridges, the cells appeared to be aligned with their long axes in the direction of the vessel axis. In Fig. 5, modified smooth-muscle cells form an intimal thickening. The plane of the section shows a cell which is partially in the intima and partially in the media. Between the focally degenerating endothelium and the intimal smooth-muscle cells adjacent to the internal elastin layer, modified smooth-muscle cells, and even less-differentiated cells intermingled to form 2–4 layers. Basement membranes or occasional focal extracellular condensations resembling basement membranes were seen about all of the cells at this stage. Cytoplasmic fibrils resembling myofibrils occurred in small groups or as single strands, but not as dense bundles and, rarely, in association with dense bodies. Peripheral vacuoles were rare but free ribosomes, rough endoplasmic reticulum, and mitochondria were abundant.

The subendothelial blood cells were polymorphonuclear granulocytes and monocytes. These were few, scattered, and superficial, occurring only in the presence of marked endothelial degenerative changes.

Medial Smooth Muscle and Adventitia. Ribosomes and profiles of rough endoplasmic reticulum were increased in medial smooth-muscle cells at this stage. Basement membranes were focally interrupted or absent, especially from the intimal projections of medial cells underlying intimal thickenings. Adventitial collagen appeared to be increased around all arterial segments examined at this time interval, and increased numbers of adventitial fibroblasts were aligned circumferentially.

Luminal Obliteration

Approximately 6 weeks after focal intimal thickening was first noted in the arteries of a particular animal, totally obliterated vessels could be found. At this stage, sections with no arterial intimal thickening could still be found, but focal intimal proliferative lesions were abundant.

Endothelium. No identifiable endothelial cells could be found in completely obliterated vessels. However, in occasional sections which showed a minute central orifice in an artery, portions of involuted endothelial dark cells similar to those described above were somtimes seen.

Obliterating Intimal Cells. The cells filling the lumen were almost exclusively of the poorly differentiated type, similar in most respects to those which occupied the innermost layer of the earlier focal subendothelial thickenings. In addition, however, the cells forming the center of the luminal cellular occlusion contained osmiophilic vacuoles; occasional darker osmiophilic particulate inclusions were noted in these vacuoles. Extracellular finely granular or amorphous material of various degrees of density occurred throughout the occluding cellular mass; occasional fine fibrillar strands were seen. Deeper cells, nearer to the internal elastin lamella, were identical to the more differentiated cells containing recognizable smooth-muscle cytoplasmic components seen in the focal thickenings prior to complete occlusion. Modified smooth-muscle cells, often with cytoplasmic projections crossing through gaps in the atrophic internal elastin lamella, formed the deepest intimal cell layer. The ultrastructural features of the components of a typical luminal occlusion are shown in Fig. 6.

Medial Smooth Muscle and Adventitia. Smooth-muscle cells confined to the media of totally occluded vessel segments were qualitatively similar to those described in vessels with focal intimal thickenings. However, medial cytoplasmic myofibrils and dense bodies were greatly reduced, and ribosomes and rough endoplasmic reticulum profiles much more numerous. Focal laminated membranous cytoplasmic configurations in close association with mitochondria were numerous in some medial cells and resembled areas of focal cytoplasmic degradation described in degenerating hepatocytes by Hruban *et al.*[10] Most of the medial cells were still recognizable smooth-muscle cells 20 weeks after the onset of hypertension. Interstitial accumulations of granular osmiophilic material and finely osmiophilic globules were seen adjacent to almost every smooth-muscle cell in the media of vessels with obliterated lumens. The internal elastin lamina was markedly narrowed and fragmented, and the fenestrations increased in number and width. These ultrastructural medial changes are illustrated in Fig. 7 and 8.

The external elastin lamella was also attentuated but far less than

the internal layer, and interruptions and fenestrations were not numerous. Adventitial collagen and fibroblasts surrounded the media and were aligned circumferentially as those found in sections from vessels with focal intimal thickenings.

Discussion

Morphogenesis of Small-Artery Cellular Obliteration

Focal obliteration of small pulmonary arteries occurred 2 months after the establishment of experimental pulmonary hypertension by systemic-to-pulmonary-artery shunting. Serial studies of lung biopsy specimens taken at 2-week intervals following anastomosis revealed 3 distinct phases in the development of the occlusions. During the *first phase*, endothelial cells showed increased cytoplasmic activity, and the subendothelial space widened. In the *second phase*, beginning 1 month after the onset of hypertension, focal intimal thickenings were seen. These consisted mainly of cells accumulated in the subendothelial space. Although the cells resembled smooth-muscle fibers, those closest to the lumen had relatively few myofibrils or dense bodies and only fragmentary basement membranes; those closest to the internal elastin lamella had more myofibrils and nearly complete basement membranes. Portions of medial smooth-muscle cells extended into the subendothelial space through gaps in the internal elastin lamella. Overlying the focal intimal thickenings, endothelial cells often showed degenerative changes, and the subjacent internal elastin lamella was usually attenuated and atrophic. There was an acccompanying general reduction in myofibrils and dense bodies and an increase in ribosomes in the medial smooth-muscle cells of involved arteries. In the *third phase*, that of focal total occlusion, the vessel lumen was obliterated by closely packed, poorly differentiated cells. Although cells at the center of the plug had very few cytoplasmic fibrils and no basement membranes, ribosomes were abundant; more peripheral cells retained remnants of myofibrillar bundles and fragments of basement membrane. Bridging modified smooth-muscle cells could still be identified about a markedly atrophic internal elastin lamella; immediately subjacent smooth-muscle cells showed cytoplasmic involutional changes. No normal or abnormal endothelial cells could be identified among the luminal-occluding elements.

Nature of Obliterating Cell

Serial observations of the development of the lesions suggest that the luminal obliteration resulted mainly from focal invasion of the subendo-

thelial space by medial smooth-muscle cells. As the vessel segments became obstructed, intimal subendothelial cells showed fewer of the characteristic morphologic features of smooth muscle, i.e., bundles of peripheral myofibrils with dense bodies, pinocytotic vesicles, and basement membranes decreased; structures such as profiles of rough endoplasmic reticulum and free ribosomes were increasingly abundant. Many cells, interpreted as transitional forms, were found. This process is suggestive of "dedifferentiation," i.e., the reversion of cells with distinct specialized structures to simpler embryonal forms, which are presumably multipotent.

Occasionally during the phase of intimal thickening, portions of some of the subendothelial cells resembled endothelial cells, but associated overlying luminal endothelial cells were always easily distinguishable from these elements. In most instances of intimal thickening, severely altered and often degenerated endothelium covered well-preserved poorly differentiated subendothelial cells. No extensions of endothelial cells into the subendothelial space were seen, though such extensions from medial cells were numerous. Bensch, Gordon, and Miller [11] have described filamentous structures within endothelial cells of normal pulmonary arteries of guinea pigs, and human bronchial arterioles as small as 20 μ in diameter. They could not find attachments of the fibrils to either cell membranes or desmosomes. No such filaments were seen in any of the endothelial cells of the small pulmonary arteries studied by us in either normal or hypertensive dogs.

The possibility that circulating blood cells contributed to the intimal thickening should also be considered, for such cells may be involved in the pathogenesis of atherosclerotic lesions in large systemic arteries.[12] In the present study, occasional recognizable leukocytes were seen only when intimal thickening was advanced and beneath markedly altered endothelium; none were seen in early lesions. Most were mature segmented granulocytes rather than the mononuclear forms which might be expected to be precursors of the poorly differentiated obliterating subendothelial elements.

Subendothelial cells did not resemble mature fibroblasts and no fibroblasts were ever seen within the intima or media, either before or after the onset of hypertension.

The possibility that the hyperplastic intimal reaction under consideration is a consequence of earlier thrombosis at some unobserved stage is unlikely but it cannot be ruled out. None of the occluding cells resembled thrombocytes, nor was there any evidence of fibrin accretion in any of the sections. In general, intimal thickening which follows thrombus formation

is eccentric;[13] the lesions in the present study were characteristically concentric.

Reaction of Arterial Wall to Elevated Pressure

The sequential changes noted in the vessels probably reflected the reaction of the vessel to increased intraluminal pressure and/or flow.[14] The initial and early increases in endothelial pinocytotic vesicles, rough endoplasmic reticulum, ribosomes, mitochondria, and Golgi formations reflect increased cellular metabolism and protein synthesis. The associated accumulation of vacuoles and the apparent widening of the subendothelial space suggest increased passage of materials from the vessel lumen. That the capacity of the endothelial transport system may be exceeded by the increased filtration, and that the endothelial cells are eventually injured irreversibly, are suggested by the subsequent appearance of degenerated cells containing condensed and shriveled organelles, accumulated inclusions, and disrupted vacuoles. Thus, the extended duration of an initial stimulus to cellular hyperreactivity may prove injurious.

Medial smooth-muscle cells also showed evidence of early stimulation to increased activity with relative increase of ribosomes, rough endoplasmic reticulum, Golgi formations, and mitochondria, and a relative decrease of myofibrils. However, in contrast to the endothelial cells, there was no increase in cytoplasmic vacuoles; however, the interstitial spaces were enlarged and contained poorly defined granular material. Medial smooth-muscle cells may have been stimulated to increased protein synthesis and energy production corresponding to increased medial tension, but the presumed increase in filtration through the intima seemed to have been diverted into the intercellular ground substance. In material from rats observed by light microscopy, Töndury and Weibel[15] found that medial smooth-muscle cells may bridge the internal elastic lamella of mesenteric arteries which have been stretched by attaching them to the diaphragm. In the present study, smooth-muscle cells which bridged the elastin lamella and projected into the intima showed more morphologic evidence of increased cytoplasmic anabolic activity and decreased contractile function than did cells which remained entirely medial. Those subendothelial cells which were associated with focal intimal thickening or formed the uniform luminal cellular plugs bore somewhat equivocal resemblances to smooth-muscle fibers.

Thus, the medial smooth-muscle cell may be considered to have reacted initially to compensate for the increased mural tension and eventually to cause a reduction in mural tension by occluding the lumen. Finally, with mural tension absent from the obliterated vessel, the luminal cell

population becomes undifferentiated. At this stage, smooth-muscle cells confined to the media did show evidences of cytoplasmic degradation in the form of myelin figures associated with mitochondria;[10] this, together with the apparent resorption of the internal elastin lamella, could correspond to the reduced mural tension and/or interference with mural nutrition. Occasional vacuoles resembling lipid droplets in these cells probably represent phagocytized material or accumulated metabolites. Some of the focal medial interstitial granular or globular material could be debris derived from damaged medial cells.

Since fibroblasts were not found in the media, the increase in medial collagen may also be attributed to compensatory function of modified smooth-muscle cells. These cells resemble partially differentiated medial cells of embryonal and fetal vessels. Such elements are associated with early collagen and elastin formation and only gradually increased their myofibrillar content during the later phases of medial morphogenesis.[16] Presumably, medial cells could revert to relatively increased fibrillogenesis under suitable conditions such as the increased mural tensions encountered in hypertension.

Summary

Serial lung biopsies were performed at biweekly intervals on dogs with systemic-to-pulmonary-artery anastomoses. The morphogenesis of the focal hyperplastic obliterative lesions in small pulmonary artery branches (50–200 μ) was studied by electron microscopy. The earliest changes, 4 weeks after the shunting procedure, occurred in endothelial and medial smooth-muscle cells; increased numbers of ribosomes, rough endoplasmic reticulum profiles, Golgi formations, and mitochondria indicated cellular hyperactivity; the subendothelial space was widened. In the second phase, after 2–4 months of pulmonary hypertension, there were accumulations of cells in the intima; portions of medial smooth-muscle cells extended into the subendothelial space through gaps in the internal elastin membrane; other cells in the subendothelial space and closer to the lumen had some features of smooth-muscle cells but contained increased numbers of ribosomes; endothelial cells overlying focal intimal thickenings showed evidence of degeneration. There was no evidence of thrombosis or endothelial proliferation. Totally occlusive lesions occurred after 4 months. The cells obliterating the lumens were uniformly poorly differentiated but had some cytoplasmic features of smooth-muscle cells and contained occasional osmiophilic vacuoles. At this stage, medial smooth-muscle cells also appeared less differentiated and contained cytoplasmic degenerative structures; interstitial amorphous or granular osmiophilic

material was also present, and elastin lamellae were attenuated and atrophic. The findings suggest that focal cellular occlusions of small arteries in pulmonary hypertension probably result from the invasion of the intima by altered medial smooth-muscle cells which subsequently lose their characteristic morphologic features. These changes appear to follow an initial hyperplastic reaction of the endothelium and media to increased blood pressure.

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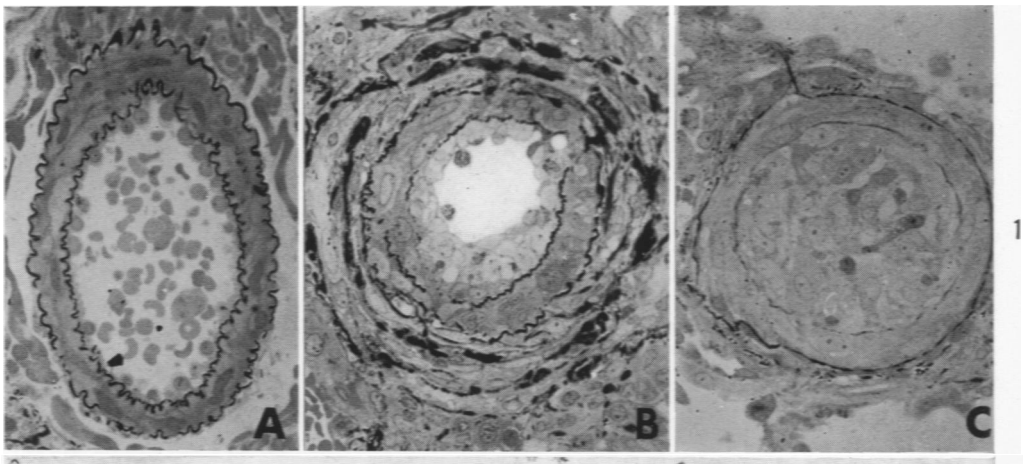
[*Illustrations follow*]

Legends for Figures

All photographs were made from sections cut from material fixed in osmium tetroxide and embedded in Epon. Thick sections for photomicrography were stained with toluidine blue-pyronin B. Sections for electron microscopy were stained with uranyl acetate.

Fig. 1. Small pulmonary artery branches in lung biopsy specimens of dogs with systemic-to-pulmonary-artery anastomoses. **A.** Artery from biopsy specimen taken 2 weeks after shunting procedure. Endothelial cells appear intact; there is no intimal thickening; media is sharply delineated by internal and external elastin lamellae. **B.** Artery after 2 months of pulmonary hypertension. Intima is thickened by accumulated cells. Internal elastin membrane is focally atrophic and interrupted, medial cells are enlarged, and adventitia is thickened. **C.** Occluded artery 4 months after systemic-to-pulmonary-artery anastomosis. Luminal cells are of uniform type. Internal elastin layer is marked atrophic. Fig. 1A, B, C, × 200.

Fig. 2. Typical endothelial cell (E) of small pulmonary artery before systemic-to-pulmonary-artery anastomosis. Very few profiles of endoplasmic reticulum, small numbers of mitochondria and scattered ribosomes; surface pinocytic vesicles (arrows) are moderately abundant. Internal elastin lamella (ie) is of fairly uniform thickness. Medial smooth-muscle cells (M) contain abundant myofibrils and associated dense bodies, few endoplasmic reticulum profiles, and few mitochondria. Subendothelial space (ss) occasionally appears widened. × 14,000.



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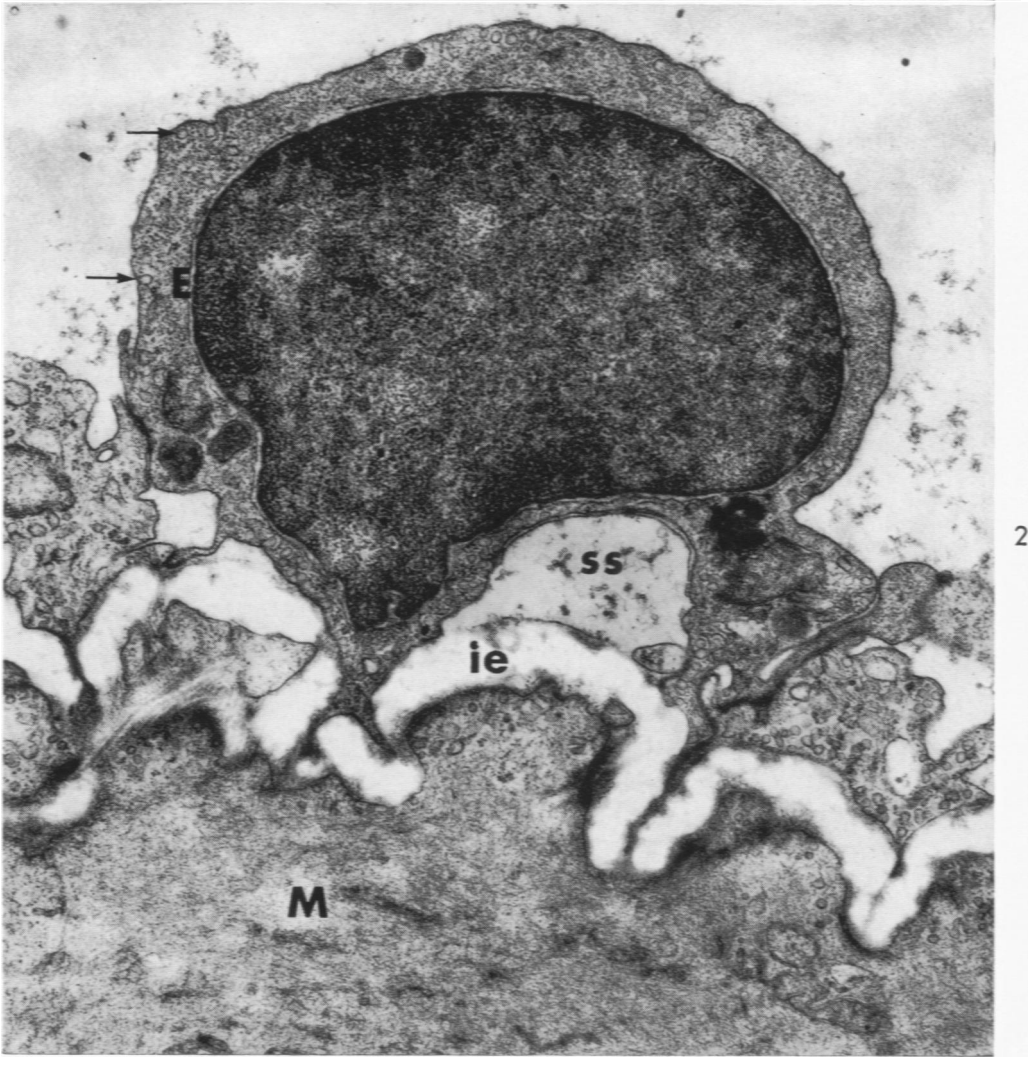

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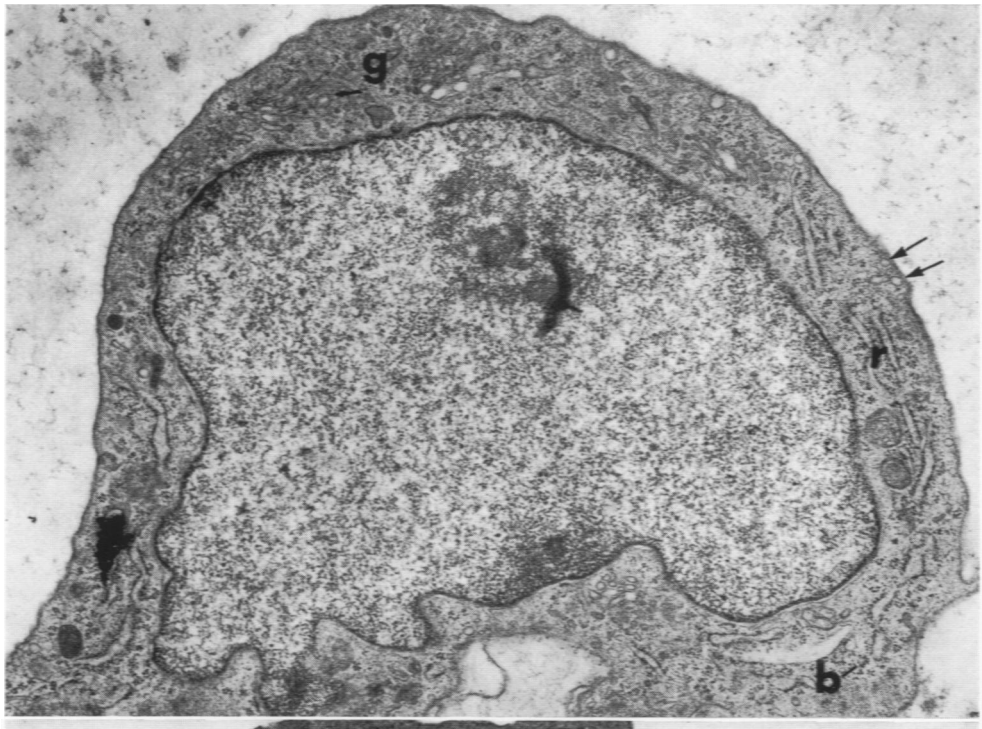
Fig. 3. Endothelial cell of artery after 2 months of pulmonary hypertension. Pinocytotic vesicles (arrows), profiles of rough endoplasmic reticulum (r), free ribosomes (b), and Golgi formations (g) are much more numerous than in endothelial cells of pulmonary arteries of normotensive dogs. These changes are already apparent 2 months after shunting. × 14,000.

Fig. 4. Endothelial cells of artery after 3 months of pulmonary hypertension. Dark, irregular osmiophilic cytoplasmic densities and few recognizable organelles are scattered among dilated cisternae (arrows) of endoplasmic reticulum. Similar cells could be found as early as 2 months after onset of pulmonary hypertension and were often overlying intimal thickenings. × 14,000.



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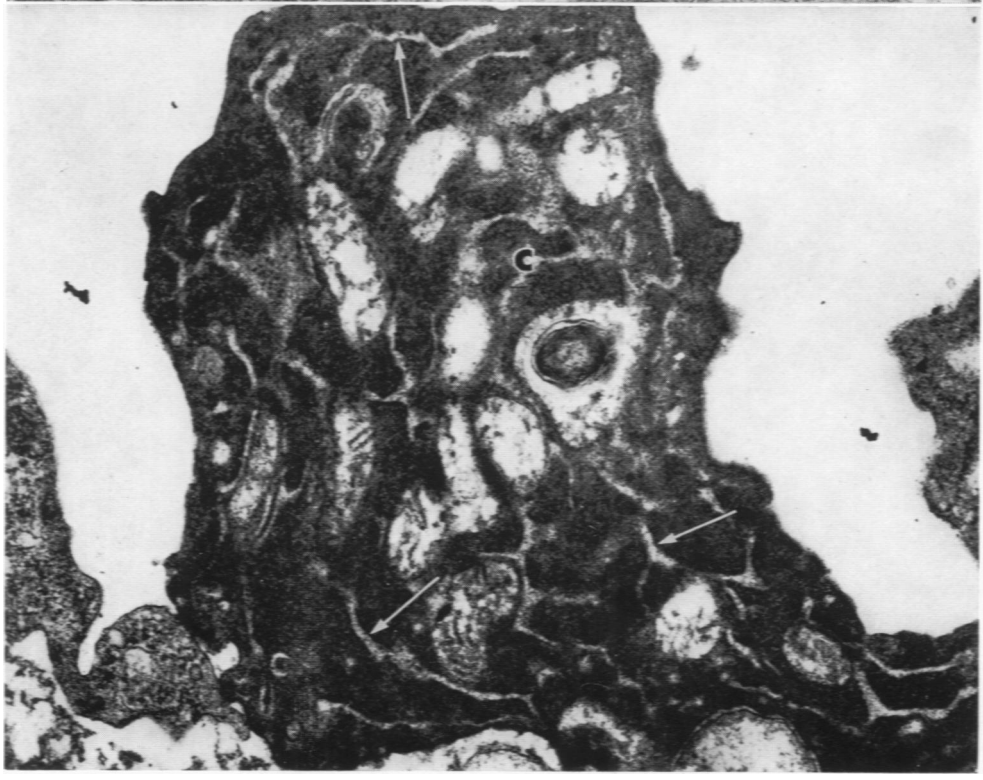

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Fig. 5. Focus of intimal thickening in small artery after 2 months of pulmonary hypertension. Portions of modified smooth-muscle cells occupy subendothelial space between endothelium (E) and internal elastin lamella (ie). Many of the cells show cytoplasmic myofibrils (f), associated dense bodies (d), and fragments of basement membrane. Some cells are partially intimal and partially medial with a joining cytoplasmic bridge lying in a gap in the internal elastin lamella (opposing arrows). × 11,000.


E
E
f
d
ie
ie

Fig. 6. Intimal cells adjacent to internal elastin lamella in a completely occluded small pulmonary artery 4 months after shunting procedure. Markedly atrophic internal elastin lamella (ie) forms an incomplete boundary between the intima (right) and the media (left). The intimal cells are fairly uniform and contain prominent profiles of rough endoplasmic reticulum (r), many ribosomes, and occasional osmiophilic vacuoles (v). There are occasional cytoplasmic fibrils, focal condensations resembling basement membranes, and scattered extracellular amorphous osmiophilic deposits (p). One cell (M) bridges a gap in the internal elastin lamella; pinocytotic vesicles are much more numerous on the medial side (arrows) than on the intimal side. × 8000.

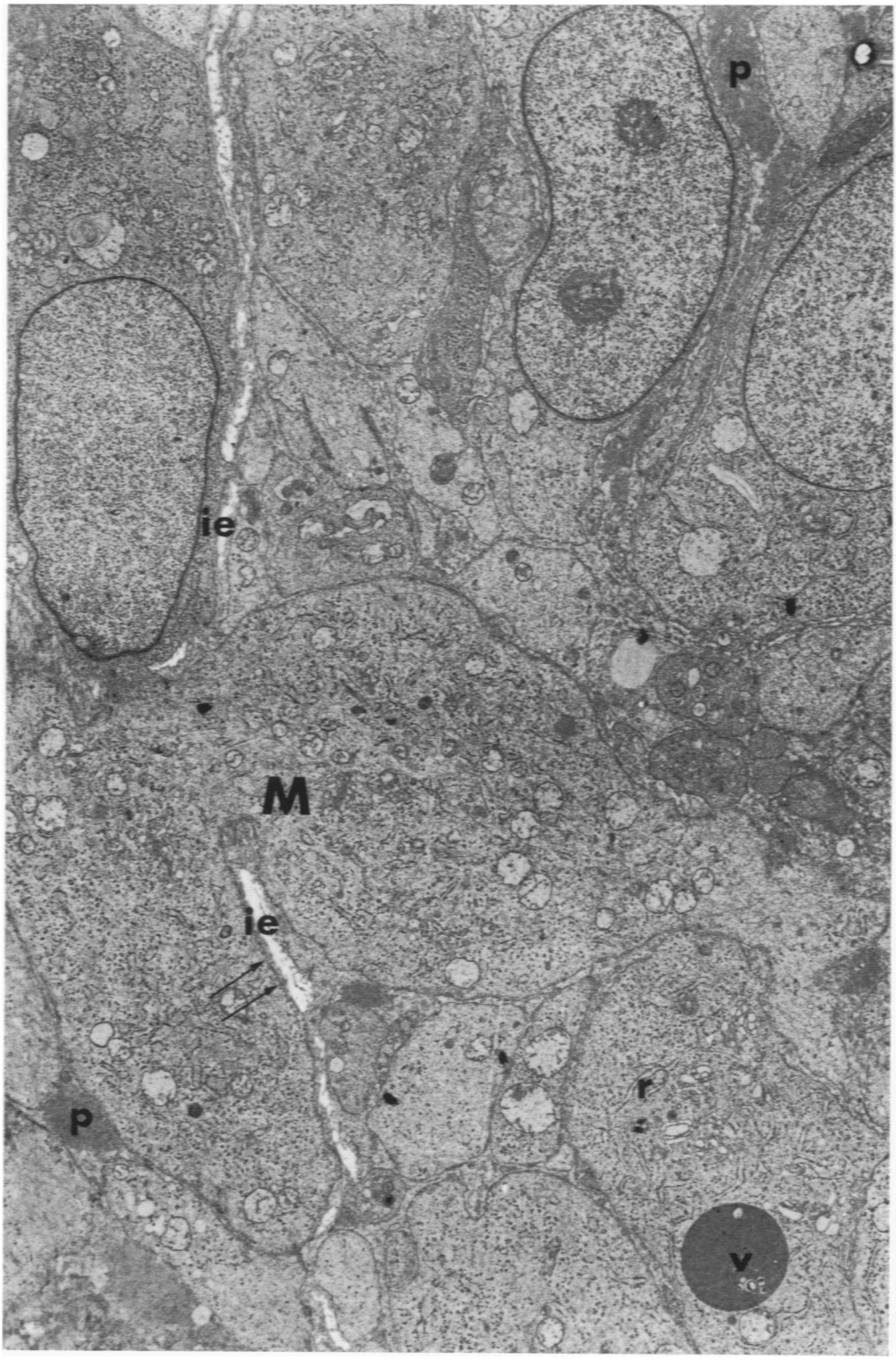
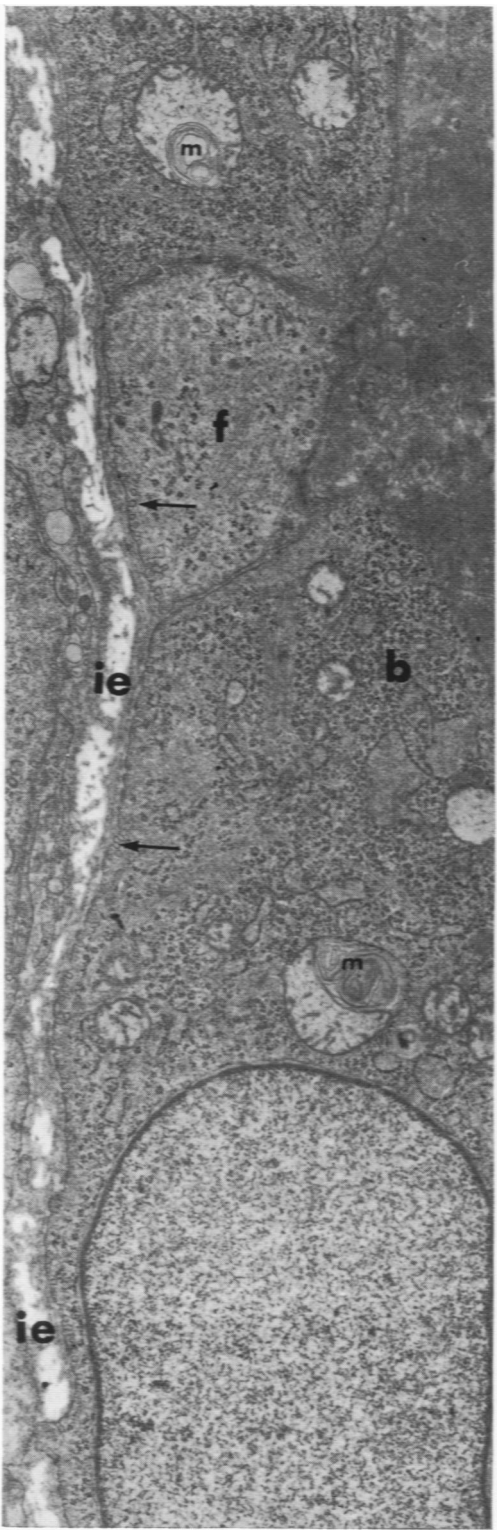

p
ie
M
ie
p
r
v

Fig. 7. Higher magnification of portion of Fig. 6. Medial smooth-muscle cells to right of internal elastin lamella (ie) of totally occluded pulmonary artery after 4 months of pulmonary hypertension. Ribosomes (b) and profiles of endoplasmic reticulum are greatly increased and some mitochondria show closely associated myelin figures (m). Myofibrils (f) and dense bodies are greatly reduced in number, but basement membranes and pinocytotic vesicles (arrows) are still prominent. × 17,000.

Fig. 8. Media of occluded pulmonary artery after 4 months of pulmonary hypertension between atrophic internal (ie) and external (ee) elastin membranes. Interstitial accumulations of osmiophilic material (g) are numerous. Medial smooth-muscle cells contain scattered fibrils (f) and dense bodies (d), abundant ribosomes (b) and pinocytotic vesicles (arrows), and numerous mitochondria. × 18,000.



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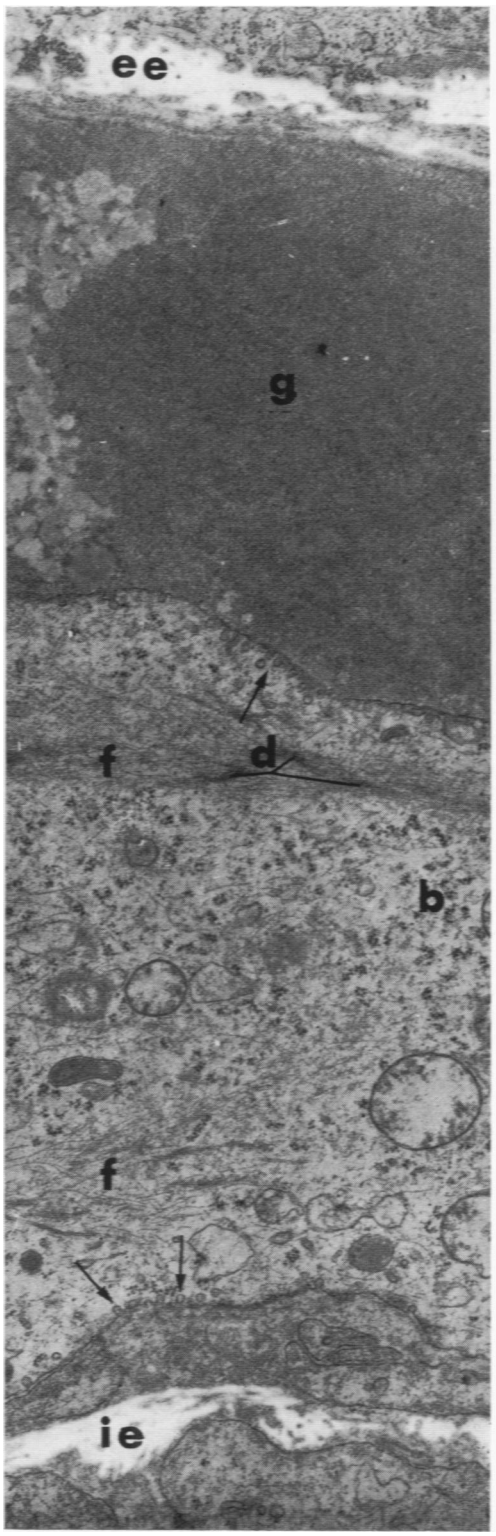

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