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Development and regression of pulmonary arterial lesions after experimental air embolism

A light and electronmicroscopic study

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Summary. Repeated systemic venous air embolism produces pulmonary vascular lesions, the nature of which is still a subject of controversy. We investigated the pulmonary arterial lesions produced by repeated air embolism in rabbits, both at light and electron microscopic level. We found that they form a remarkable histopathological entity, consisting of initial pronounced vasoconstriction, combined with severe intimal inflammatory changes. Within 4 days after the last injection of air, peculiar sheet-like structures consisting of oedematous tissue and lined by endothelium, projected into the lumen. These structures probably resulted from the shearing stress of the blood, streaming over the severely oedematous intima. They subsequently became thinner and disappeared after two weeks. Various types of blood-borne and mesenchymal cells were present in the thickened intima and within the sheets. The origin of the latter cells remained undecided. They may originate from medial smooth muscle cells penetrating the internal elastic lamina as well as by transition from blood-borne cells into mesenchymal cells, or both.

Key words: Pulmonary artery – Air embolism – Intima – Oedema – Electron microscopy

Introduction

Intimal fibrosis of muscular pulmonary arteries may occur in a variety of pathological conditions, particularly in various forms of pulmonary hypertension. Depending upon the underlying condition and aetiology, it varies not only in its morphological aspects, but also with regard to its clinical and prognostic significance for the patient (Wagenvoort and Wagenvoort 1977; Wagenvoort et al. 1984).

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Although several investigators have described the ultrastructure of intimal fibrosis, there remains controversy on the nature and the origin of the intimal cells in these lesions (Esterly et al. 1968; Balk et al. 1979; Kondo et al. 1979; Meyrick and Reid 1980; Smith and Heath 1980; Schaub et al. 1981). Whereas there is now general agreement that fibroblasts are not the predominant constituents of intimal thickening, as previously assumed, smooth muscle cells, myofibroblasts and undifferentiated cells have all been described. The question whether all these intimal cells originate from medial smooth muscle cells or also from blood-borne cells, has remained unsettled.

In the present study repeated air embolism in the rabbit was used as an experimental model since it has been shown (Barnard 1957; Boerema 1965) that in this way intimal proliferation may be produced in a relatively short time. In the course of this study we came across certain peculiar lesions that, to our knowledge, have not been described before. This report deals with the development and regression of these changes as well as with the nature and origin of the cells therein.

Material and methods

Twelve New Zealand white rabbits, weighing approximately 2,000 g, were used. In ten animals, during 5 consecutive days, 0.5 ml of air per kg body weight was injected into an ear vein. One of the animals died 15 min after the second injection. The remaining animals were killed by an intramuscular injection of Hypnorm® at 1, 2, 3, 4, 7, 14, 21, 28 and 35 days after the last dose of air. Two control animals, without prior air embolism, were killed in the same way. The lungs were fixed by intratracheal instillation of undiluted Karnovsky's fixative (Karnovsky 1965), while the thorax was still closed. By this procedure, the lungs are fixed in an expanded state and vascular collapse is fully prevented (Dingemans 1973). Specimens for light and electron microscopy were taken from each lobe of the lungs. For light microscopy, the tissue samples were postfixed in 6% buffered formalin. This was done under vacuum, in order to extract remaining air. From the specimens to be used for electron microscopy, 50 μm thick sections were cut on a Vibratome. The blood vessels therein were dissected and, after postfixation with osmium tetroxide and uranyl acetate, embedded in Epon. Subsequently 1 μm thick sections, stained with toluidine blue were used for selecting vessels with vascular lesions, if present, but at least 5 vessels per animal. Ultrathin sections were then stained with lead citrate and uranyl magnesium acetate, and some also with tannic acid to visualize elastin (Kajikawa et al. 1975).

Results

Most pulmonary arteries were of the muscular type, although it must be noted that in the rabbit, there are often some elastic fibres within the media in muscular arteries. Light and electron microscopic study revealed that their internal elastic lamina was continuous except for a limited number of small fenestrations. Intrathoracic fixation had essentially preserved its smooth outline, with only minimal crenation in a few vessels. Generally, the endothelium rested directly on the internal elastic lamina but sometimes a few cells, usually with smooth muscle characteristics, were found in the subendothelial intimal layer. In such areas, the internal elastic lamina might show a slight degree of reduplication. No erythrocytes or inflammatory cells were found within the vessel walls in the control animals. Medial smooth

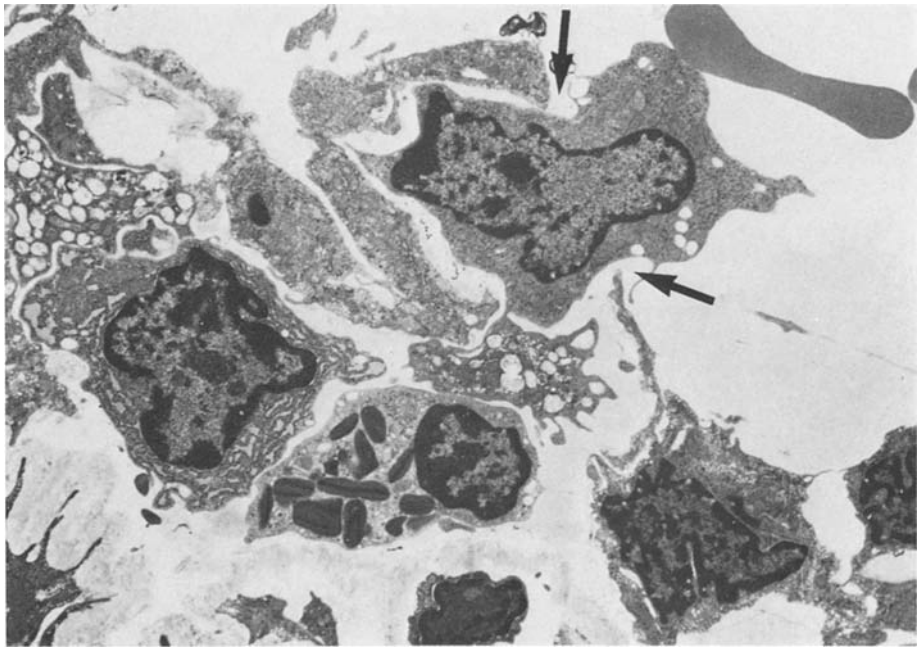

Fig. 1. Part of wall of muscular pulmonary artery during acute contraction. Crenation of internal elastic lamina in left lower corner. Within intima leucocytes of several types, one of them in process of invasion through endothelium (*arrows*). Electron micrograph, ×9,700

muscle cells were regularly shaped with hardly any cytoplasmic protrusions. No regional differences were found between the vessels in various lobes of the lungs.

In the rabbit that died after the second administration of air, light and electron microscopic investigation showed severe constriction of muscular pulmonary arteries. The lumen in these arteries generally was narrow, the media considerably thickened, the internal elastic lamina prominently crenated, while the endothelial cells protruded far into the lumen. Often, the endothelial cells lost the direct contact with the internal elastic lamina over part of their surface. Sometimes the endothelium was lifted up by large, balloon-shaped, empty-appearing subendothelial spaces. Granulocytes, monocytes and lymphocytes had marginated to the vascular wall and diapedesis of these cells into the subendothelial spaces and other parts of the vascular wall often occurred (Fig. 1). Fibrin or platelet adhesion was not observed at this or at any of the following stages. The fenestrations within the internal elastic lamina were increased in number and in width. Through these fenestrations small cytoplasmic evaginations of medial smooth muscle cells protruded. On a few occasions, these evaginations were large and electron-lucent, resembling those that have been described in severe vasoconstriction due to various causes, e.g., hypoxia (Dingemans and Wagenvoort 1978) or Crotalaria administration (Wagenvoort et al. 1974; Dingemans and Wagenvoort 1976).

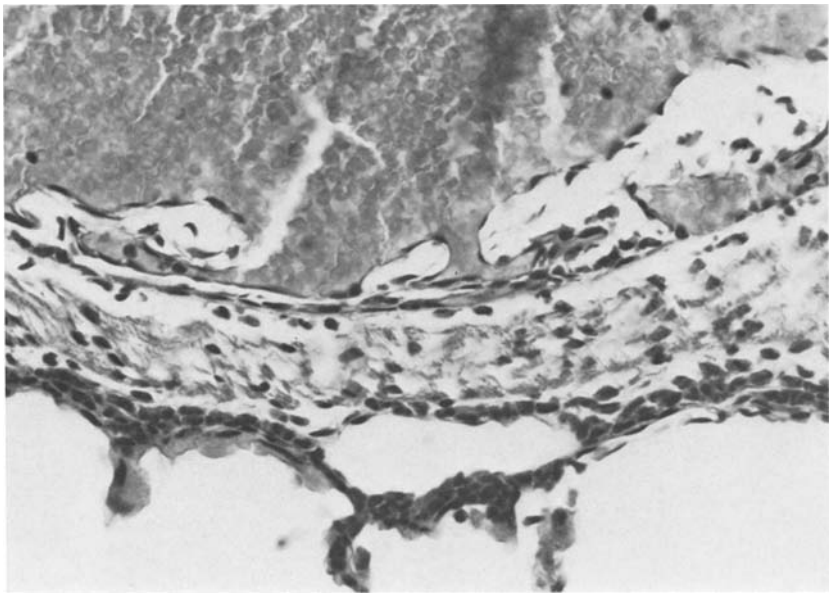

Fig. 2. Muscular pulmonary artery with oedematous protrusions of intima and early formation of sheet-like structures. H. and E., ×380

In the lungs from rabbits killed 1 to 4 days after the last injection of air, morphological signs of vasoconstriction were less conspicuous. However, there were other prominent structural alterations, especially near branching points of large and medium-sized muscular arteries. In these areas, the internal elastic lamina was usually fragmented, with large discontinuities. The media was thickened and oedematous and many large protrusions of the cytoplasm of smooth muscle cells, penetrated the internal elastic lamina, so that, in fact, parts of these cells were lying within the intima. There was also prominent intimal thickening from oedema, resulting in wide separation of the endothelium form the internal elastic lamina, a feature not observed or only to a minimal extent in the control animals. In many arteries, the subendothelial spaces were locally enlarged to such an extent that conspicuous endothelium-covered protrusions had developed. Often these became undermined so that large sheet-like structures were formed (Fig. 2). These structures tended to be oriented parallel to the vascular wall with luminal channels in between. Subendothelial spaces, including those of the protrusions, were electron-lucent (Fig. 3) and contained varying number of granulocytes and monocytes with occasional lymphocytes and erythrocytes. Some leucocytes penetrated over a short distance into the media. Some intimal cells with the ultrastructural characteristics of fibroblasts were observed in the intima as well as occasional myofibroblasts and smooth muscle cells, the latter cells lying more closely to the internal elastic lamina. The fenestrations in this lamina, however, were still wide and numerous with many smooth muscle cell evaginations.

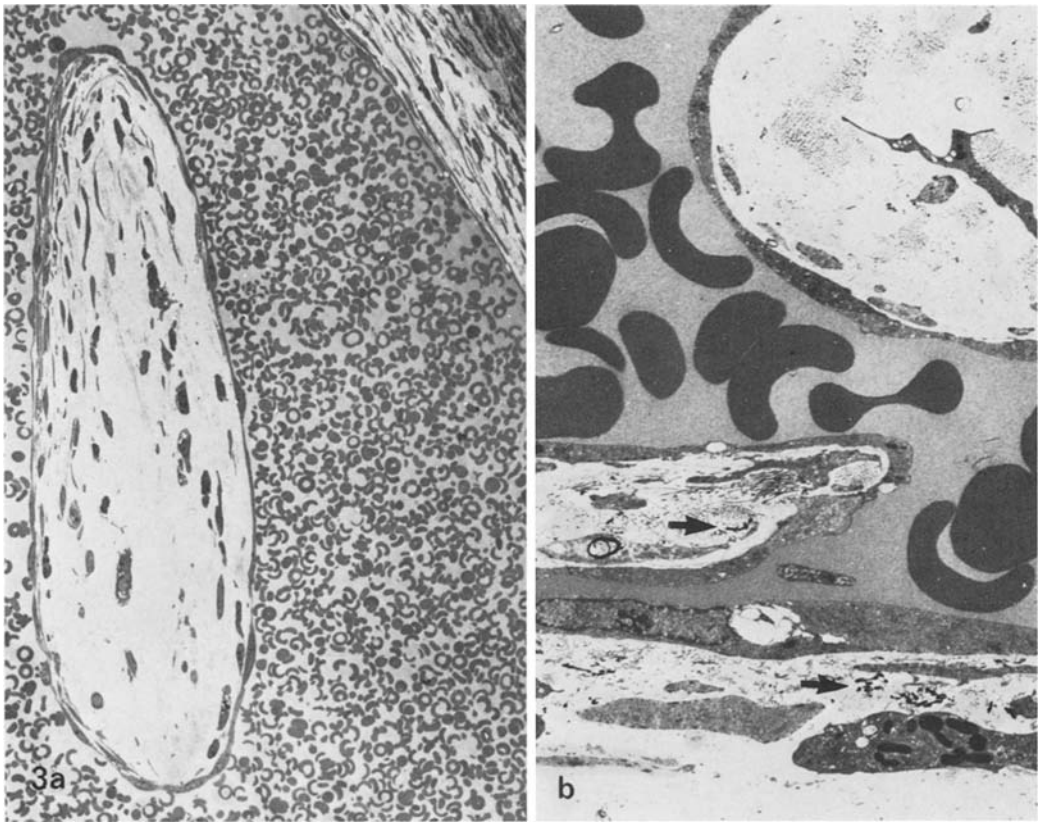

Fig. 3A, B. Electron micrographs of similar areas as in Fig. 2. Large intimal protrusions and sheet-like structures project far into vascular lumen. **A,** conventional staining with uranyl acetate and lead citrate. **B,** tannic acid staining demonstrating small patches of elastin (*arrows*). **A,** ×400; **B,** ×3,500

In the lungs of rabbits killed 1 to 2 weeks after the last injection of air, there was gradual regression of the vascular lesions. Initially, the intimal layer and the sheets became thinner as the oedema somewhat diminished, leading to a condensation of collagen and elastic fibres. While the number of subendothelial blood cells decreased, there was some increase of elongated fibroblast-like cells. These cells had a well developed rough endoplasmic reticulum but inconspicuous intracytoplasmic filaments (Fig. 4). Again smooth muscle fibers penetrating the internal elastic lamina were found regularly (Fig. 5). The sheet-like structures gradually became discontinuous, eventually leading to the formation of delicate interconnecting septa traversing the lumen and consisting of a double endothelial layer with hardly any stroma in between (Fig. 6).

Three to five weeks following the last injection no traces of the sheet-like structures could be found any longer and blood borne cells became rare. Within the intimal layer smooth muscle cells, although still fairly scarce,

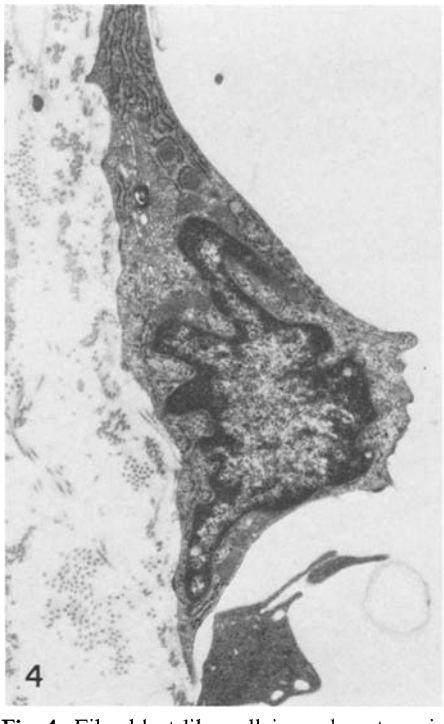

Fig. 4. Fibroblast-like cell in oedematous intima. Collagen fibers are seen adjacent to cell. Electron micrograph, × 12,000

Fig. 5. Smooth muscle cell penetrating internal elastic lamina. Notice collagen fibers in thickened intima. Electron micrograph, × 16,000

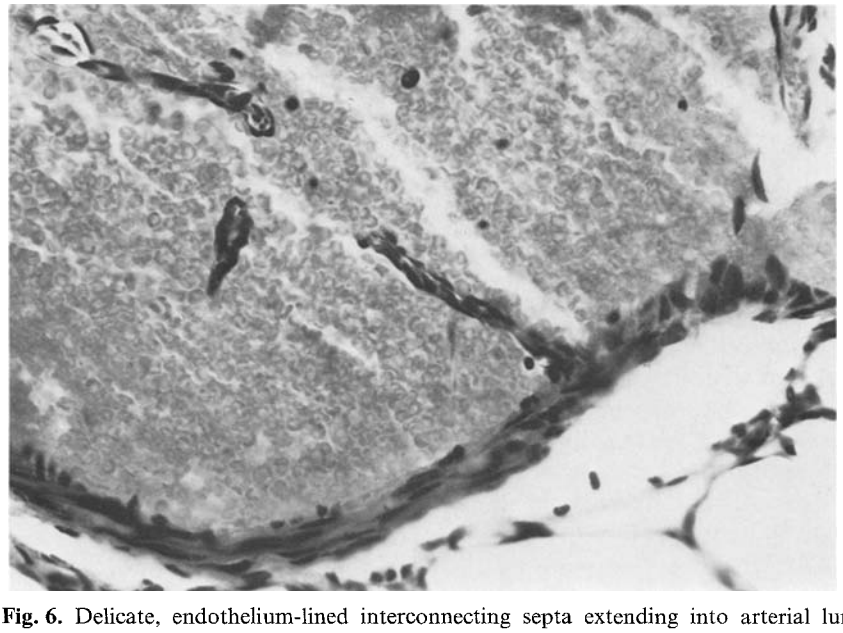

Fig. 6. Delicate, endothelium-lined interconnecting septa extending into arterial lumen. H. and E., × 380

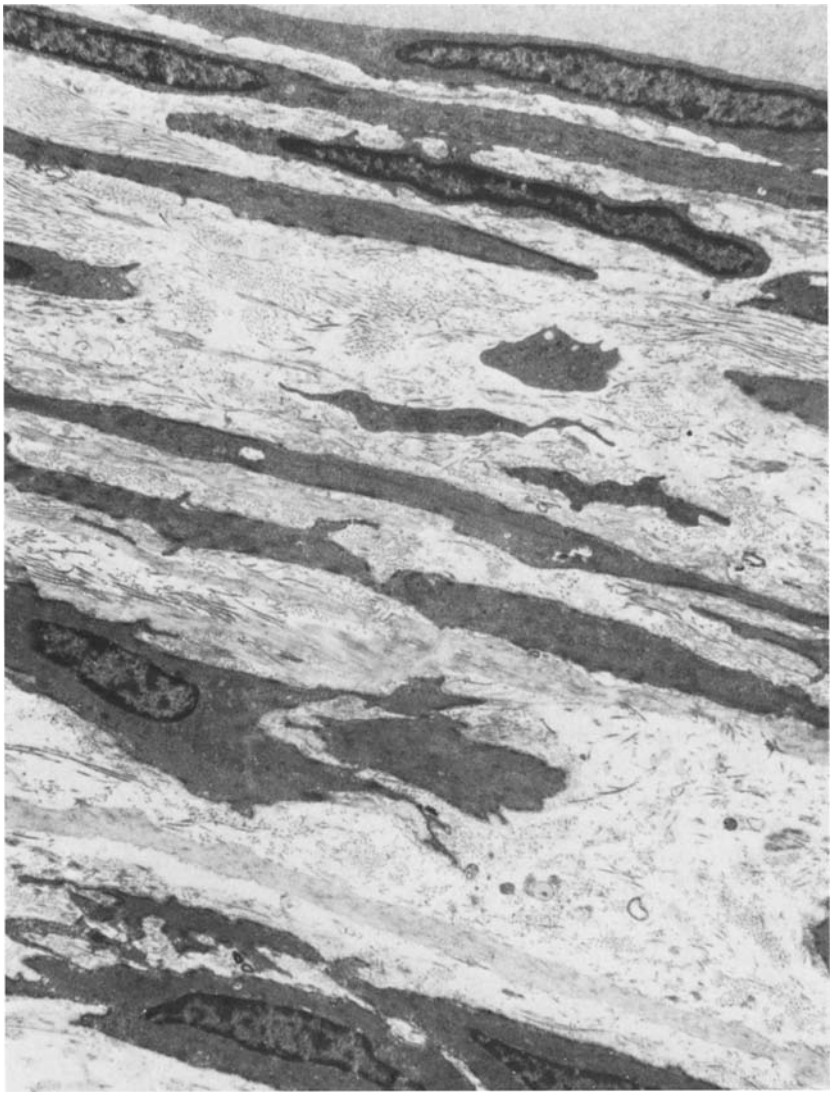

Fig. 7. Part of wall of muscular pulmonary artery in stage of regression. Flattened intimal smooth muscle cells, separated by slightly oedematous extracellular matrix containing much collagen. Electron micrograph, × 6,000

now predominated over the fibroblasts and myofibroblasts (Fig. 7). Eventually only traces of intimal thickening remained. The internal elastic lamina became smooth again and its fenestrations decreased in width and number, while the smooth muscle cell evaginations gradually disappeared. The medial thickness regressed to normal.

Discussion

The experimental model of repeated air or gas embolism to produce pulmonary vascular lesions has been used by various authors since the original paper by Barnard in 1957. Whereas most authors agree that significant damage to the pulmonary arteries is produced by this method, the exact nature of this damage has remained subject of controversy. Indeed, it has not become clear whether the lesions are thrombotic in origin, as has been suggested (Barnard 1957; Boerema 1965; Hartveit et al. 1968; Moosavi et al. 1981) or not. It has even been maintained that some of the lesions represented plexiform lesions (Wright 1962; Gilbert et al. 1968).

In order to ascertain the nature of the lesions produced by this method, we essentially repeated the original experiments, now including electron microscopy. It appeared that the arterial lesions showed a complex pattern, consisting of severe vasoconstriction in the acute phase, followed by inflammatory changes, including severe intimal oedema and diapedesis of blood cells. We assume that the inflammatory reaction was elicited by direct damage to the endothelium, either mechanical or hypoxic, as a result of the air bubbles. The damage to the vessel wall was probably enhanced by the intense vasospasm seen in the acute phase. The intimal inflammatory reaction consisting of oedema and cellular infiltration, was especially prominent near bifurcations of the arteries, where the air emboli most likely got stuck. In our opinion, the subsequent development of sheet-like structures projecting into the vascular lumen, may have resulted from the shearing stress of the blood streaming over the severely oedematous intima. These structures were unlike the intraarterial band- or weblike fibrous septa characteristic of recanalized thrombi or thromboemboli but, more important, there were no signs of early thrombosis at any stage. There were no expressions of plexogenic arteriopathy. It important to point out that the air embolism-induced lesions depicted and interpreted as plexiform lesions by others (Wright 1962; Gilbert et al. 1968), were far from convincing, in our view. Severe intimal inflammatory eodema is not a feature of thrombotic or plexogenic pulmonary arteriopathy.

The nature and origin of the cells found in the thickened intima are of some importance in the understanding of the lesions and possibly of intimal fibrosis in general. Especially in the early phases, blood-borne cells were relatively numerous in the intima. In later stages, other types of cells were added to these, e.g. fibroblasts and myofibroblasts. Since, in the acute phase, smooth muscle cells were regularly found lying partly in the media and partly in the intima, using the wide fenestrations in the internal elastic lamina, it is likely that the intimal mesenchymal cells were derived from medial smooth muscle cells. In other models, a migration of such cells to the intima has been well documented (Benditt 1976). The possibility of a transformation of blood-borne cells into intimal mesenchymal cells has been proposed by others (Esterly et al. 1968; Lee et al. 1970). However, because blood-borne cells were initially fairly numerous in the intima, the

second possibility, though difficult to prove morphologically, cannot be completely excluded.

The present study shows that the intimal lesions produced by repeated air embolism in pulmonary arteries are of an inflammatory nature, probably secondary to direct damage by the air emboli, rather than thrombotic in origin. In part, and particularly with regard to the sheet-like structures, they constitute a special type of pulmonary vascular lesion, distinct from other vascular lesions of the lung.

References

Balk AG, Dingemans KP, Wagenvoort CA (1979) The ultrastructure of the various forms of pulmonary arterial intimal fibrosis. Virchows Arch [Pathol Anat] 382:139–150

Barnard PJ (1957) Pulmonary arteriosclerosis due to oxygen, nitrogen, and argon embolism, An experimental study. Arch Pathol 63:322–332

Benditt EP (1976) Intimal thickening after ligature of arteries. An electronmicroscopic study. Circ Res 9:418–426

Boerema B (1965) Appearance and regression of pulmonary arterial lesions after repeated intravenous injection of gas. J Pathol Bacteriol 89:741–744

Dingemans KP (1973) Behavior of intravenously injected malignant lymphoma cells: a morphologic study. J Natl Cancer Inst 51:1883–1897

Dingemans KP, Wagenvoort CA (1976) Ultrastructural study of contraction of pulmonary vascular smooth muscle cells. Lab Invest 35:205–212

Dingemans KP, Wagenvoort CA (1978) Pulmonary arteries and veins in experimental hypoxia. An ultrastructural study. Am J Pahol 93:353–368

Esterly JA, Glagov S, Ferguson DJ (1968) Morphogenesis of intimal obliterative hyperplasia of small arteries in experimental pulmonary hypertension. An ultrastructural study of the role of smooth muscle cells. Am J Pathol 52:325–347

Gilbert JW, Berglund E, Dahlgren S, Overfors CO (1968) Experimental pulmonary hypertension in the dog. A preparation involving repeated air embolism. J Thorac Cardiovasc Surg 55:565–571

Hartveit F, Lystad H, Minken A (1968) The pathology of venous air embolism. Br J Exp Pathol 49:81–88

Kajikawa K, Mamaguchi T, Katsuda S, Miwa A (1975) An improved electron stain for elastic fibers using tannic acid. J Electr Micr 24:287–289

Karnovsky MJ (1965) A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. J Cell Biol 27:137A

Kondo Y, Niwa Y, Takizawa J, Akikusa B, Sano M, Shigematsu H (1979) Accelerated serum sickness in the rabbit. IV. Characteristic endarteritis in the pulmonary artery. Lab Invest 41:119–127

Lee KT, Lee KJ, Lee SK, Imai H, O'Neal RM (1970) Poorly differentiated subendothelial cells in swine aortas. Exptl Mol Pathol 13:118–129

Meyrick B, Reid L (1980) Ultrastructural findings in lung biopsy material from children with congenital heart defects. Am J Pathol 101:527–542

Moosavi H, Utell MJ, Hyde RW, Fahey PJ, Peterson BT, Donnelly BS, Jensen KD (1981) Lung ultrastructure in non-cardiogenic pulmonary edema induced by air embolization in dogs. Lab Invest 45:456–464

Schaub RG, Rawlings CA, Keith JC (1981) Platelet adhesion and myointimal proliferation in canine pulmonary arteries. Am J Pathol 104:13–22

Smith P, Heath D (1980) The ultrastructure of age-associated intimal fibrosis in pulmonary blood vessels. J Pathol 130:247–253

Wagenvoort CA, Dingemans KP, Lotgering GG (1974) Electronmicroscopy of pulmonary vasculature after application of fulvine. Thorax 29:511–521

Wagenvoort CA, Wagenvoort N (1977) Pathology of Pulmonary Hypertension. J Wiley & Sons, New York

Wagenvoort CA, Wagenvoort N, Draulans-Noë Y (1984) Reversibility of plexogenic pulmonary arteriopathy following banding of the pulmonary artery. J Thorac Cardiovasc Surg 87:876–886

Wright RR (1962) Experimental pulmonary hypertension produced by recurrent air emboli. Med Thorac 19:423–427

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