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Fine structure of the muscular wall of rat pulmonary veins

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INTRODUCTION

A century ago Peaslee (1857) had already pointed out that the main branches of pulmonary veins are furnished with an external layer of striated muscular fibres 'like those of the heart itself'. Stieda (1877) showed that in small animals such as mice and bats pulmonary veins have a wall made of striated muscle down to the smallest branches, whereas in man, dog and guinea-pig this muscle extends only to the hilum. The physiological meaning of this fact is not well understood. Arnstein (1877) identified the characteristic morphological structures of cardiac muscle in pulmonary vessels of the mouse and rat. Granel (1921) studied venous intrapulmonary branches in the rat and pointed out that cardiac muscle disappears in the terminal branches of veins with a diameter of approximately 110 μm.

Karrer (1959, 1960) described in detail the fine structure of cardiac muscle in mouse pulmonary veins, but saw no smooth muscle cells even by electron microscopy. However, in the rat the situation is different and smooth muscle fibres have even been seen by light microscopy (Granel, 1921).

In the rat no detailed investigation of the ultrastructure of intrapulmonary veins is yet available. The presence of striated muscle has been reported but intercalated discs have not been seen (Policard, Collet & Prégermain, 1959; Klavins, 1963). The organization of the striated muscle fibres and the size of the smallest pulmonary venule with a cardiac muscle layer in rat lung is one of the aims of this study.

MATERIALS AND METHODS

Adult Buffalo rats were killed by decapitation. Dalton's chrome-osmium fixative was immediately injected into the trachea. Pieces of tissue approximately 1 mm^3 were removed from both lungs, immersed in fixative and intermittently agitated for 1$\frac{1}{2}$ h. After ethanol dehydration, blocks were embedded in Dow epoxy resin, according to Lockwood (1964). Sections approximately 1 μm thick were cut on a Porter–Blum MT-1 microtome using glass knives. The veins were identified by phase microscopy. The block was subsequently trimmed and serially sectioned for electron microscopy. Following ten thin sections, a thick section was removed and examined in the phase microscope and the diameter of the vascular lumen was measured by means of a Leitz micrometer. In fortunate blocks a larger vein could be pursued to its smallest ramifications, usually 25 μm in diameter or less. Thin sections were stained with lead citrate or lead acetate and examined in an RCA EMU-3F electron microscope.

OBSERVATIONS

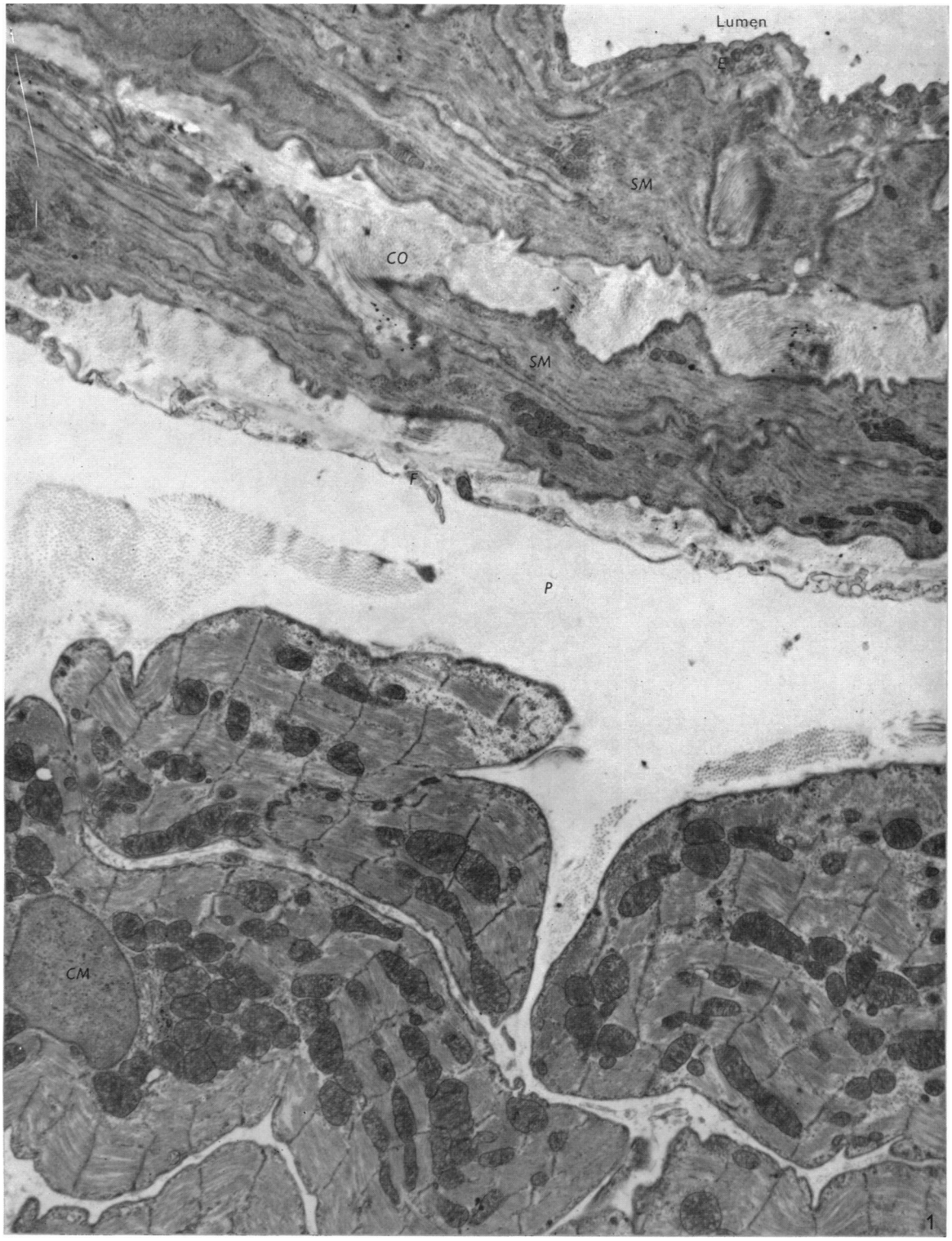
General architecture of the muscular wall

In the rat lung, veins of all sizes, including the smallest ramifications, have an internal subendothelial layer composed of smooth muscle and an external layer composed of striated cardiac muscle (Fig. 1). The smooth muscle layer is one or two cells thick in the smallest vessels, its width usually increasing proportionally to the lumen, so that in veins about 400 μm in diameter there are several superimposed smooth muscle cells (Fig. 1). Variations in thickness of the muscular layer were encountered as would be expected from the variability in thickness of venous walls. Collagen fibrils and elastic lamellae embedded in a matrix substance surround smooth muscle cells (Figs. 1, 2), and mark the meagre boundary between endothelial cells of intima and the smooth-muscle layer (Figs. 1, 2). Attenuated fibroblasts delineate the outer margin of the smooth muscle wall (Fig. 1). A space about 7–15 μm wide containing collagen separates the smooth-muscle layer from the outer layer of cardiac muscle. This large space is probably an artefact due to the elasticity of the lung as well as to contraction of the cardiac muscle. The space is sizeable in small veins but sometimes is narrow or discontinuous in large veins. Figure 1 shows a space between the two muscle layers in a vein 350 μm in diameter. However, in further sections of this same vessel there is close contiguity between smooth and cardiac muscle (Fig. 2). Near the hilum the cardiac muscle layer is six to ten cells thick in pulmonary veins and more prominent than the smooth muscle stratum. The width of the cardiac muscle layer decreases proportionally to the size of vascular lumen and is usually only two cells thick about small venules. Here again, as with the smooth muscle layer, the relation to size of lumen is not absolute and variations are encountered. In vessels of 20–30 μm diameter the cardiac muscle is only one cell thick before finally becoming discontinuous. Before disappearing, short strands of 2–3 cells are recognizable only with the electron microscope.

Detailed architecture of the cardiac muscle wall

Cardiac muscle cells in the rat lung closely resemble heart muscle in the rat (Porter & Palade, 1957; Moore & Ruska, 1957; Muir, 1965). No structural differences could be seen in cardiac muscle cells along the length of the pulmonary venous channel although in those about the small vessels (30 μm) some patterns disappear. The free surface of muscle cells often exhibit uniform indentations of the sarcolemma with the deepest part extending toward the *Z* band (Fig. 7). Numerous capillaries are encountered in the intercellular space between branching muscle fibres. Mitochondria are numerous and have abundant closely arranged cristae (Figs. 3, 4). The *A* bands

Fig. 1. Electron micrograph of the wall of a pulmonary vein 350 μm in diameter. Scattered elastic lamellae and collagen fibrils (*CO*) are interposed between the thin endothelium (*E*) and smooth muscle (*SM*) as well as between the smooth muscle cells. The smooth muscle wall is cut longitudinally. An extracellular space (*P*) containing collagen separates smooth muscle from cardiac muscle (*CM*). Collagen and extremely attenuated fibroblastic cells (*F*) outline the outer margin of the smooth muscle layer. × 8000.



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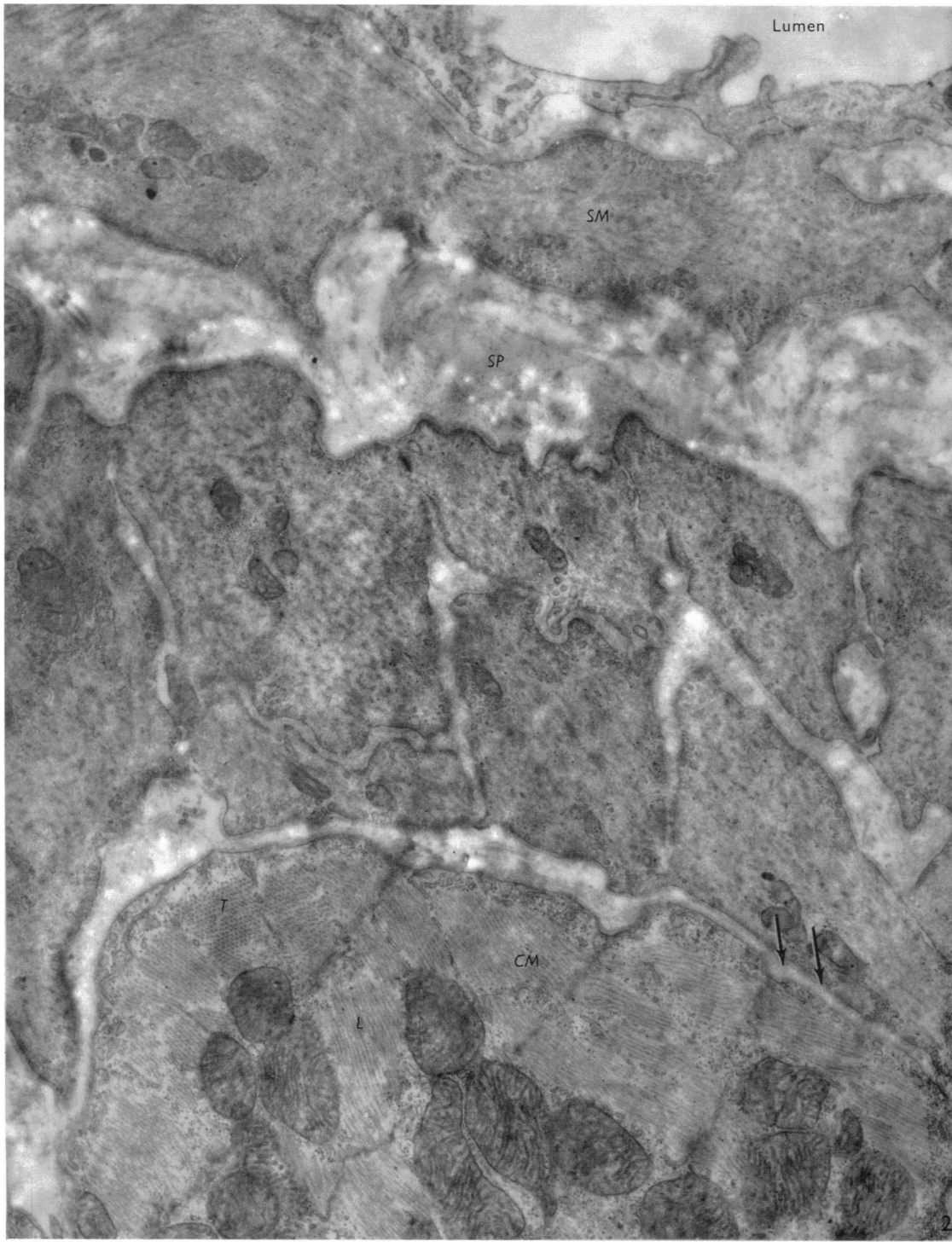
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often show *M* lines at their mid-portion (Fig. 6). The *I* band is frequently identified only by a prominent *Z* line (Figs. 3, 4).

The nucleus is round or ovoid and centrally located. Sarcoplasm at one pole of the nucleus often contains glycogen granules, smooth vesicles and dense bodies containing lipid. Glycogen granules also occur beneath the plasma membrane and around mitochondria (Fig. 3).

Intercalated discs marking contiguous cell margins in cardiac muscle of pulmonary veins are variable in structure. Their detailed morphology is much as shown in rat heart muscle by Muir (1965), and in mouse pulmonary veins by Karrer (1960). Intercalated discs often have a wavy pattern where they traverse *Z* bands of several myofibrils (Fig. 3). The transverse portion of the disc is associated with a greater amount of outer dense material appearing either as myofibrillar insertion plaques, as termed by Muir (1965), where myofilaments attach on the dense material forming the plaque (Figs. 4, 5), or as desmosomes. In the complete form of the disc, the longitudinal part of the interconnecting surface is parallel with the myofilaments and occurs as a continuous unit with the transversely oriented disc (Fig. 5). The longitudinal part of the intercalated disc is provided with an outer condensed material more like that of a desmosome (Fig. 5). Continuity between the intercalated disc and the surface plasma membranes of adjacent cells is especially evident at the end of the cells where they are separated by a larger space (Fig. 5). Intercalated discs are present in veins and venules as small as 30 μm across. Only lateral cell interconnexions occur in terminal ramifications where the cardiac muscle layer is one cell thick. These lateral interconnexions possess desmosomes of various length (Fig. 8) and correspond to the specialized longitudinal connecting surfaces of the intercalated disc referred to as the S regions by Sjöstrand & Andersson-Cedergren (1960).

The sarcoplasmic reticulum in many respects reproduces the random orientation of tubules described in rat cardiac muscle by Porter & Palade (1957). In longitudinal sections elongated tubules appear parallel to the myofilaments of the *A* band, especially at the mid-portion of the sarcomere, precisely in front of the *M* line (arrow, Fig. 6). The tubule opposite the *M* line is present in each sarcomere exemplifying the similarity to the tubular units in rat myocardium at the *H*-band level described by Porter & Palade (1957) as a structure with a 'repeating pattern'. Other narrow tubules and oval vesicles are oriented transversely to the myofilaments at the level of the *Z* band (*t*, Figs. 6, 7, 9). They correspond to the transverse intermediary vesicles of the *Z* line described in *Amblystoma* (Porter & Palade, 1957). These transverse tubules sometimes show a longitudinal orientation after leaving the *Z* band (Fig. 9). At this point a few ribosomes are attached to the longitudinally oriented tubule (arrow, Fig. 9). A similar appearance of transverse tubules at the *Z* band level

Fig. 2. Higher magnification of the same vein shown in Fig. 1. A single layer of smooth muscle (SM) is present immediately outside the endothelium. These smooth muscle cells have abundant pinocytotic vesicles and are surrounded by basement membrane. This inner layer of smooth muscle is separated from the thicker outer layer by a space (*SP*) containing both elastic lamellae and collagen. The outer longitudinally arranged muscle layers here are cut in cross-section and are closely related (arrows) to the outermost muscle layer composed of cardiac muscle (*CM*). Transversely sectioned myofilaments (*T*). Longitudinally sectioned myofilaments (*L*). ×22000.

Fig. 3. An intercalated disc in the cardiac muscle of a vein 400 μm in diameter is present between two muscle cells. The intercalated disc has a wavy pattern and is transversely oriented to the myofilaments. The somewhat longitudinal portion of the disc is parallel to the myofilaments (*L*). Glycogen granules are dispersed around mitochondria. $\times$ 30000.

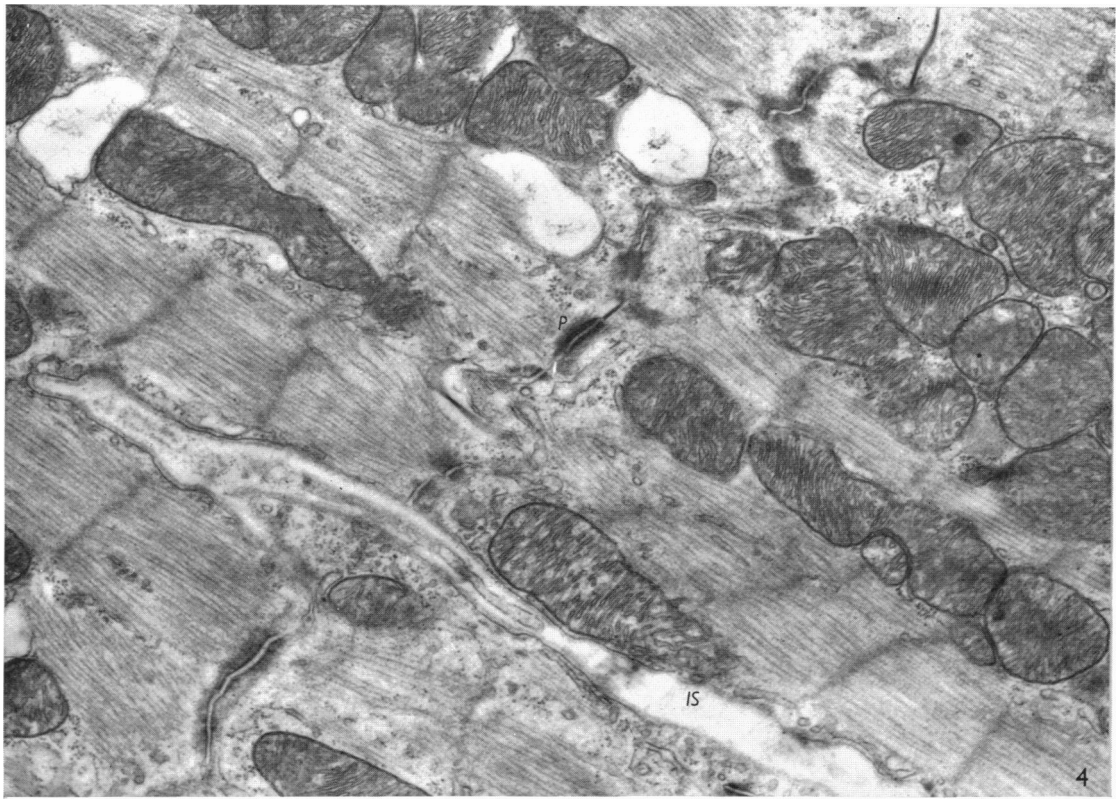
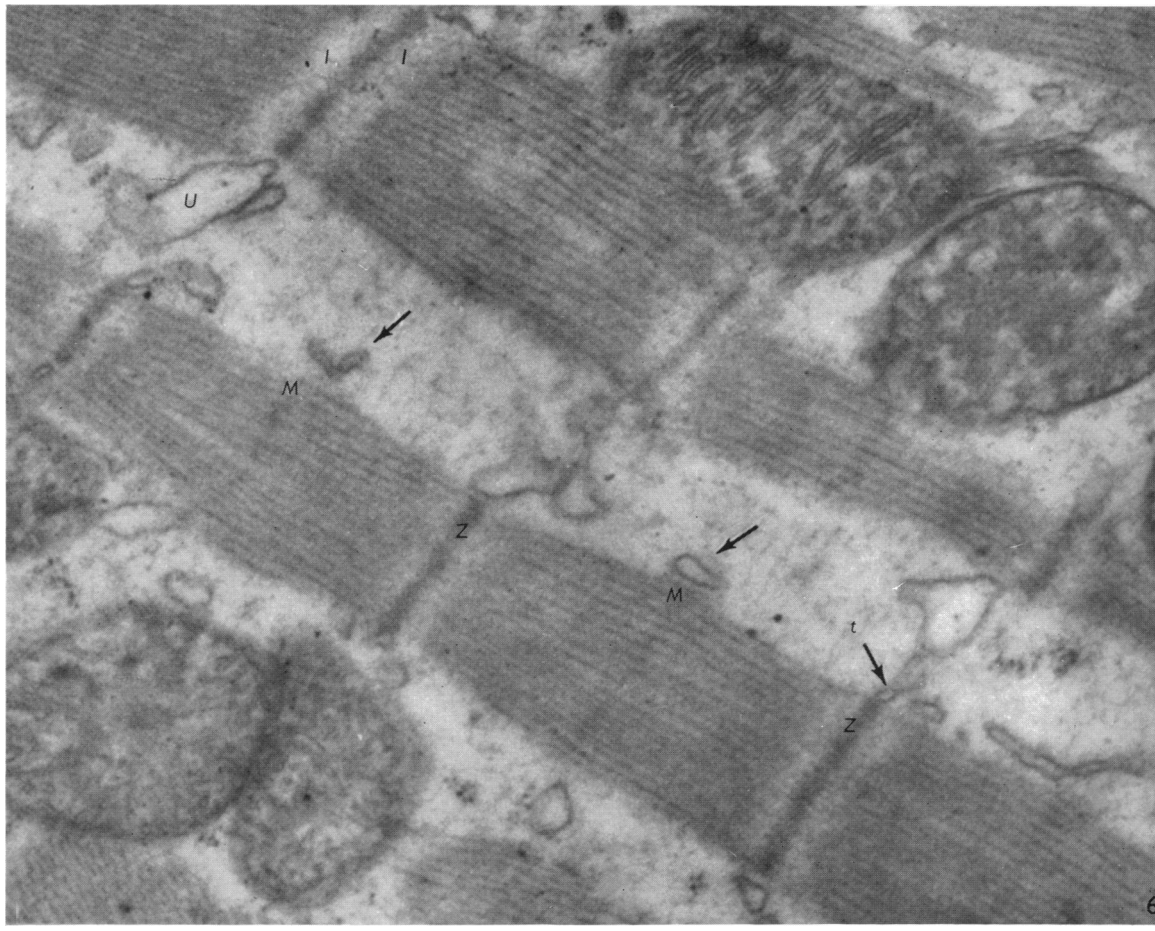


Fig. 4. This micrograph is from a vein 320 μm in diameter. An intercalated disc connects marginal cells of a branching muscle fibre to two other muscle cells. Mitochondria are rich in cristae. Dense material is applied on the transversally oriented disc to form a myofibrillar insertion plaque at *P*. *IS*, Intercellular space. $\times$ 22000.

Fig. 5. In the same vein shown in Fig. 4 an intercalated disc is present between three adjacent cells (M_1, M_2, M_3). The longitudinal connecting surfaces of the disc are parallel to myofilaments and have desmosomes (*D*). Myofibrillar insertion plaques (*P*) are present at the transverse portion of the disc. Surface plasma membrane is continuous with the disc (arrow). Marginal sarcoplasm (*S*) contains vesicles and glycogen granules. $\times$ 30000.



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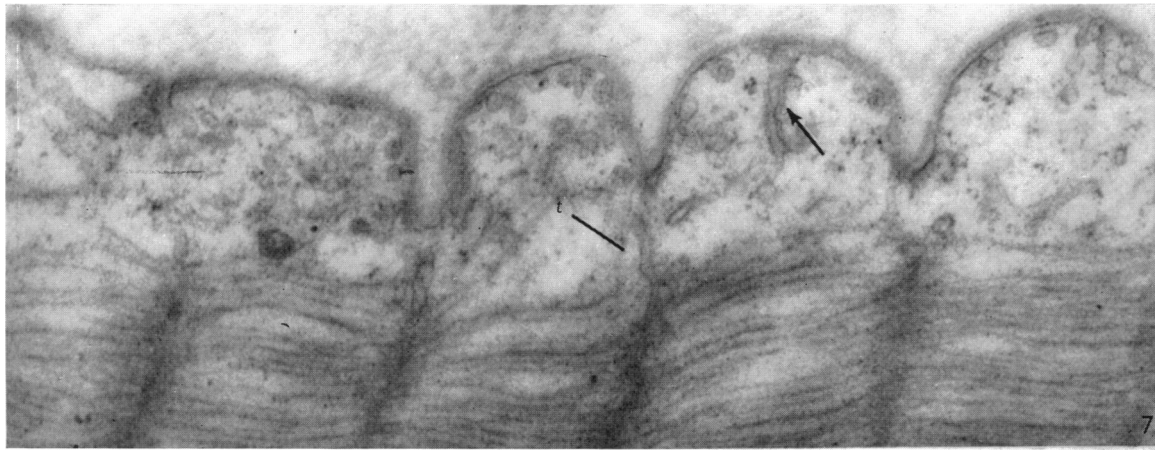

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has been seen in mouse veins (Karrer, 1959). Sometimes the slender transverse tubules at the *Z* line level are seen close to the inner face of indented sarcolemma (*t*, Fig. 7), but neither continuity nor communication between indented sarcolemma and transverse tubules is evident in the present work. In addition, there were dilated transverse tubules which sometimes appear U-shaped, with one extremity pointing to the *Z* line and the opening of the U facing one-half of the *I* band and part of the *I–M* interspace (Fig. 6), much as described in rat myocardium by Porter & Palade (1957). These dilated transverse tubules have an appearance somewhat similar to the large transverse tubules at the *Z*-band level described as T-tubules in sheep (Simpson & Oertelis, 1962), and in other species (Nelson & Benson, 1963; Simpson, 1965). However, the T-tubules described by these authors show obvious communication with the invaginated sarcolemma at the *Z*-line region, whereas in our material such a communication or continuity was not demonstrated.

In veins near the lung hilum structures containing granulated vesicles with the appearance of nerve endings occur in close relation to the plasma membrane of cardiac muscle cells.

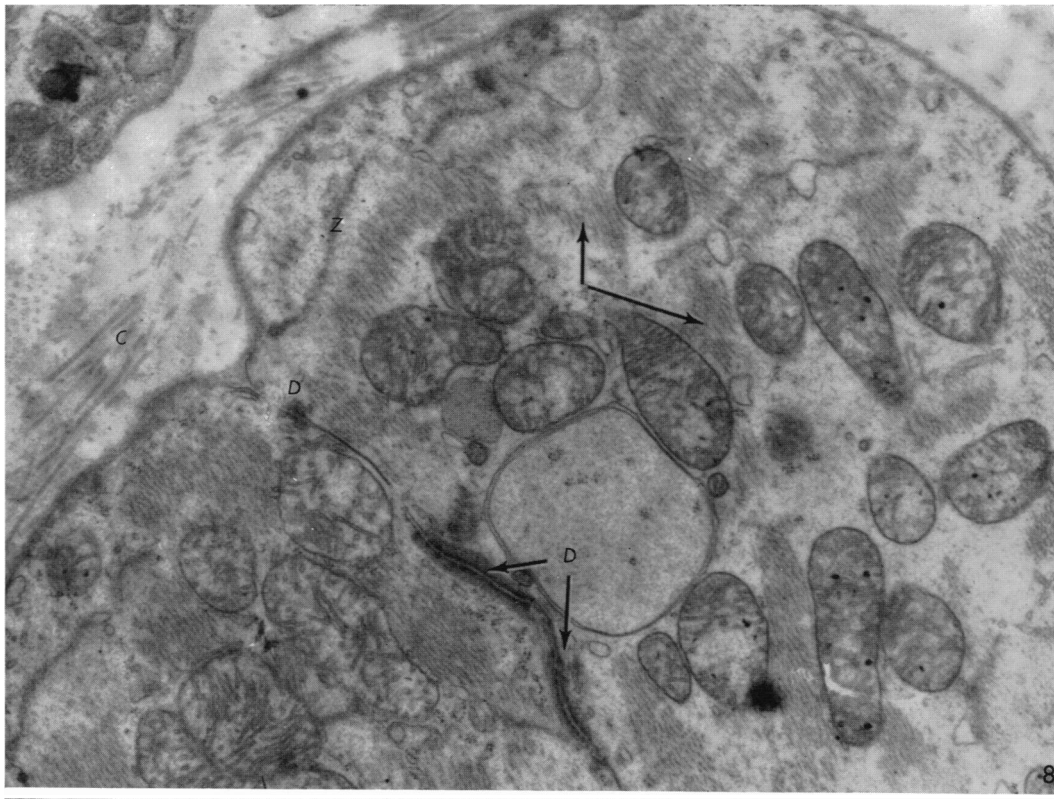
DISCUSSION

The cardiac muscle of rat pulmonary veins extends as a continuous wall to the smallest venules. This striated layer becomes discontinuous and begins to disappear in ramifications of vessels 20–30 μm in diameter. As the ultrastructural cytology of rat myocardium has already been elaborated in detail, in the present work emphasis is given only to features that point out the resemblance between myocardial muscle and that forming the wall of pulmonary veins in the rat. Characteristic structures of myocardial muscle (mitochondria, sarcoplasmic reticulum and intercalated discs) are easily identified in striated muscle of pulmonary veins.

The role played by the sarcoplasmic reticulum in transmission of impulses to the interior of muscle cells has been postulated by many (Porter & Palade, 1957; Huxley & Taylor, 1958; Huxley & Straub, 1958). Barrnett & Palade (1959) found enzymic activity at the *M*-band level of rat cardiac muscle. Karnovsky & Hug (1963) pointed out that the enzyme at the *M*-band level is a non-specific esterase and has a role in acyl transfer reactions, although its role in cardiac physiology is not yet established. Recently Rostgaard & Behnke (1965) demonstrated in rat myocardium enzymes which split ATP in the terminal sacs of the sarcoplasmic reticulum and in subsarcolemmal cisterns. They postulated that the close relationship of these structures to the plasma membrane, to the intercalated disc and to the T system may signify a

Fig. 6. Electron micrograph from cardiac muscle of a vein 100 μm in diameter. The *I* bands (*I*) have prominent *Z* lines. *M* lines are present at the middle of the *A* bands. Longitudinal sarcoplasmic tubules occur at the mid-portion of *A* bands, precisely in front of the *M* line of each sarcomere (arrow). Transverse U-shaped dilated tubules (*U*) have one extremity directed toward the *Z* line with the opening of the U facing one-half of the *I* band and part of the *I–M* interspace. Transverse intermediary vesicles occur at the *Z*-line level (*t*). × 32000.

Fig. 7. Indented sarcoplasm and sarcolemma of a cardiac muscle cell in a venule 50 μm in diameter. The sarcoplasm is rich in vesicles. Narrow transversely oriented tubules (*t*) near the inner face of indented sarcolemma extend toward the *Z* line. Other tubules (arrow) appear to join the plasma membrane at positions distant from the *Z* line. × 30000.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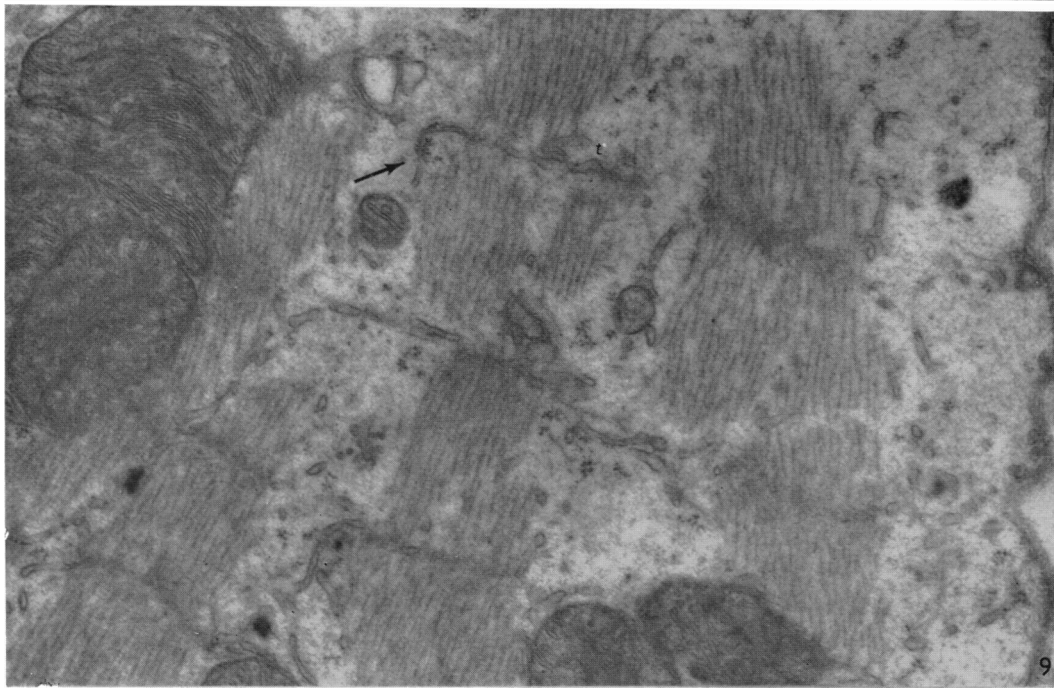
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role in excitation–contraction coupling in cardiac muscle. On the other hand in some species (Simpson & Oertelis, 1962; Nelson & Benson, 1963) there is accumulating evidence of a possible pathway for spread of impulses from the sarcolemma to the myofilaments via the T-tubules of the *Z* line. Simpson (1965) pointed out that although in ox and guinea-pig myocardium communication between T-tubules and sarcolemma is apparent, in the rat such evidence is less readily encountered. The lack of continuity between the large transverse tubules and the sarcolemma described in the present work, might be due to the fact that T-tubules are less frequent in cardiac muscle of rat pulmonary veins than they are in rat myocardium.

In any case, in the cardiac muscle cells studied here there are abundant transverse tubules at the *Z*-line level. Therefore the intermediary vesicles and transverse tubules seen in the present work and described in rat cardiac muscle by Porter & Palade (1957) may well correspond to the transverse tubular system (T-tubules) described in other species, as suggested by Simpson & Oertelis (1962) and Nelson & Benson (1963).

Although previous investigators (Policard *et al.* 1959; Klavins, 1963) failed to demonstrate intercalated discs in the muscle of rat pulmonary veins, there is no doubt that these structures exist in various forms (Figs. 3, 4, 5). Intercalated discs in both lateral and transverse portions have zones of dense material applied on the outer membrane. Most of these structures are identified as desmosomes or S regions when located along the longitudinal connecting surfaces, as described in guinea-pig myocardium by Sjöstrand *et al.* (1958).

The problem of conduction of impulses across membranes at cell junctions has been the core of many studies (Sjöstrand & Andersson-Cedergren, 1960; Bourne, 1953). Transmission of impulses across intercalated discs of Purkinje fibres has been discussed in the sheep's heart by Muir (1957). Rhodin, Del Missier & Reid (1961) postulated that in the bovine conducting system the more rapid movement of impulses is probably explained by the increased number of desmosomes between fibres.

In rat pulmonary veins the lateral connecting surfaces of cells within the intercalated disc have desmosomes throughout the striated muscle layer even to the smallest venules (Fig. 8). This observation suggests that conduction between cells may occur in cardiac muscle of pulmonary veins.

Fig. 8. Electron micrograph showing part of two muscle cells from a venule 20 μm in diameter shortly before the muscle disappeared from the vessel. The myofilaments (arrows) have been cut obliquely and therefore no banding is apparent except for thin *Z* lines (*Z*). Mitochondria and sarcoplasmic vesicles are evident. The longitudinal connecting surfaces of adjoining cells have desmosomes (*D*) of various length representing an intercalated disc. The large pale vesicle near the cell junction is encircled by two membranes and may represent a deep infolding of plasma cell membranes. Collagen (*C*) is present in the interstitial space surrounding the muscle fibre. × 15000.

Fig. 9. Electron micrograph from a venule 30 μm in diameter showing narrow transverse tubules (*t*) at the *Z*-band level. These tubules are continuous with longitudinally oriented ones (arrow). × 22000.

SUMMARY

1. The detailed architecture of the muscular wall of rat pulmonary veins was studied by serial sections. Veins of varied size, including the smallest ramifications usually 25 μm in diameter, were examined by phase and electron microscopy.

2. All veins examined had an internal layer composed of smooth muscle and an external layer of cardiac muscle. The cardiac muscle layer was more prominent than the inner layer.

3. The striated muscle in the wall of veins down to the smallest ramifications had the characteristic structures of cardiac muscle.

4. The sarcoplasmic reticulum was prominent.

5. Intercalated discs were common but cardiac muscle cells near their termination had only lateral cell interconnexions.

6. It is suggested that conduction of impulses between cells is transmitted along the cardiac muscle of pulmonary veins in a manner analogous to that in muscle of the heart.

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