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Changes in vascular morphology associated with the no-reflow phenomenon in ischaemic myocardium

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Summary. To investigate the pathogenesis of the reperfusion defect which develops in ischaemic myocardium, intravascular casts were prepared by injection of methyl methacrylate into the coronary arteries of isolated heparinised rat hearts. Using a scanning electron microscope, the vascular morphology following 60 min of global ischaemia at 37° C was compared to that of non-ischaemic control hearts injected immediately after stopping perfusion with oxygenated Krebs-Henseleit buffer. Complete casts were obtained from control hearts and from all parts of ischaemic hearts except the subendocardial half of the left ventricular wall of ischaemic hearts where the blood vessels were not filled. At the border between the perfused subepicardial and unperfused left subendocardial regions, the resin which filled the radial penetrating arteries and their branches projected from the filled capillary plexus to an extent proportional to their diameter. Intravascular events such as erythrocyte plugging and thrombosis were excluded as causative factors by the use of a cell-free perfusate. Also, there was no morphological evidence that endothelial cell swelling or constriction of any particular population of vessels was involved. The observed pattern of vascular occlusion suggests that, during global ischaemia, blood vessels in the endocardial half of the left ventricular myocardium lose their ability to be reperfused because of extravascular compression.

Key words: Myocardial ischaemia – Intravascular casts – Reperfusion

Kloner, Ganote and Jennings (1974) observed that blood failed to enter ischaemically injured canine myocardium when a temporary ligature of a branch of the coronary arteries was removed after 90 min of occlusion. They termed this reperfusion defect the no-reflow phenomenon. Various possible explanations for it, including endothelial cell swelling, myocardial cell swelling, tissue oedema, collapse or compression of capillaries, alter-

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ations in blood viscosity and thrombosis, have been proposed and discussed (Kloner et al. 1974; Gavin et al. 1978a). These changes occur during ischaemia and probably all contribute to an increased resistance to coronary blood flow on reperfusion. However, none of them has yet been proved conclusively to cause complete no-reflow, usually defined by the inability of injected tracer molecules to pass into the affected vascular bed.

The chronological development of no-reflow in the ischaemic dog heart has been defined (Gavin et al. 1978a) and its onset shown to be closely associated with a decrease in compliance of the affected myocardium (Gavin et al. 1978b). More recent investigations of the contribution of ischaemic contracture of cardiac muscle to the development of no-reflow in isolated rat hearts (Alanen et al. 1979; Humphrey et al. 1980) suggest that both contraction of the myocardium and some secondary change, probably rigor mortis, are necessary to prevent vascular reperfusion. But the observed patterns of no-reflow also could be explained by plugging of myocardial capillaries with erythrocytes (Gavin et al. 1978b) or by spasm of contractile vessels. The methods so far used have not determined in which population of myocardial blood vessels the occlusion occurs.

We therefore used scanning electron microscopy and compared intravascular casts of normal and ischaemic hearts to elucidate the pathogenesis of the no-reflow phenomenon in myocardium.

Methods

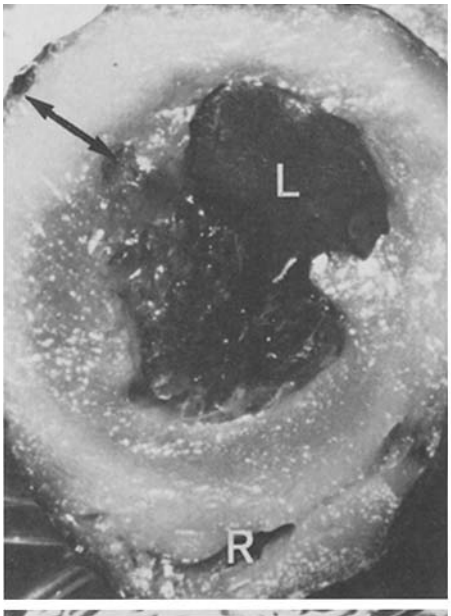
Sixteen male albino Wistar rats (250–350 g) were each lightly anaesthetised with diethyl ether before opening the right femoral vein and injecting 200 I.U. of heparin. One min later the heart was excised, rinsed with ice-cold perfusion medium (see below), mounted by ligation of the aorta on a stainless steel cannula and subjected to non-recirculating perfusion (Langendorff 1895). The right atrium was incised to facilitate drainage of the coronary perfusate and each heart was surrounded by a water-jacketed chamber and maintained at 37° C. All hearts were perfused at a pressure of 100 cm of water with bicarbonate buffer (Krebs and Henseleit 1932) pH 7.4 at 37° C which had been gassed with oxygen and carbon dioxide (95:5). Perfusion continued for 10 min.

Following this equilibration period, the flow of buffer to eight control hearts was stopped and methyl methacrylate, prepared according to Murakami (1971) to give a viscosity of 70–80 centipoises, was injected into the aortic cannula until it began to discharge from the right atrium. Another eight hearts were made globally ischaemic at 37° C by stopping the flow of buffer for 60 min before injecting methyl methacrylate.

The preparations were allowed to polymerise at room temperature for 60 min before dividing each heart transversely through the ventricles (Figs. 1–5) at the level of the origin of the first diagonal branch from the left anterior descending branch of the left coronary artery. After examination with a dissecting microscope, the half hearts were immersed for 2 h in concentrated sodium hydroxide solution at 60° C to dissolve the tissue. The casts were then washed in distilled water (Fig. 6) and dried in air. After re-examination by light microscopy, they were mounted on stubs, coated with carbon and gold in a vacuum evaporator and studied in a scanning electron microscope (Stereoscan 2A, Cambridge Instrument Co. Cambridge, Great Britain) using an accelerating voltage of 20 KV.

Results

One millilitre of methyl methacrylate was sufficient volume to perfuse the hearts, and with a hand lens resin could be observed passing down the epicardial arteries and back up the epicardial veins (Fig. 2).



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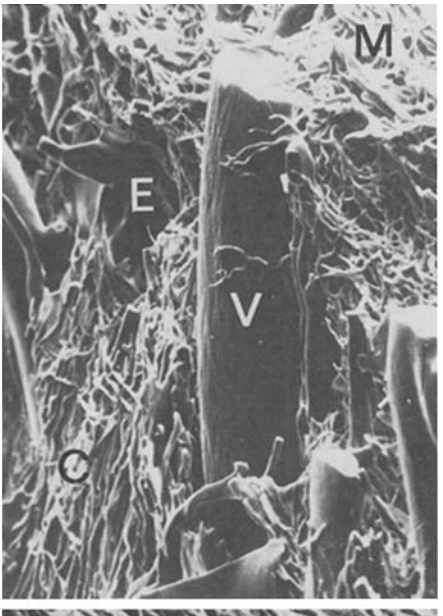

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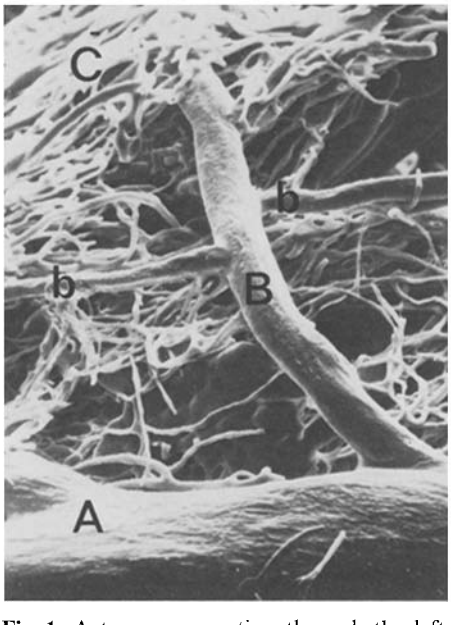

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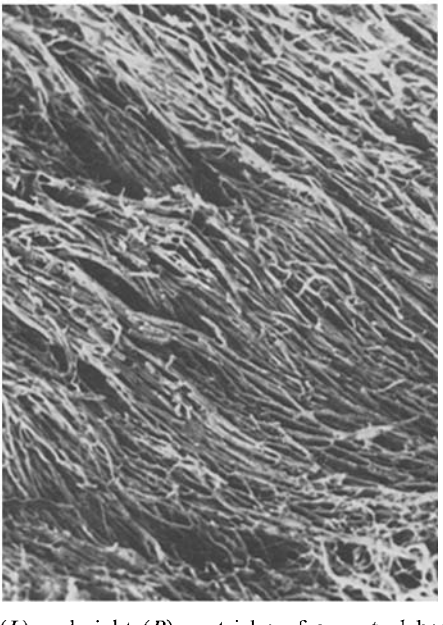

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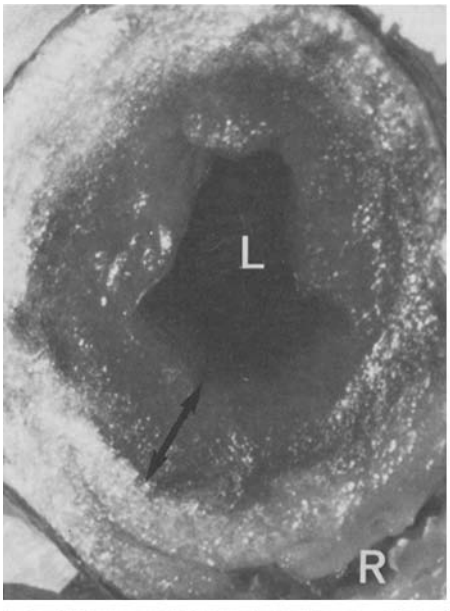
Fig. 1. A transverse section through the left (*L*) and right (*R*) ventricles of a control heart injected intravascularly with methyl methacrylate which has perfused the full thickness of the left ventricular wall (*arrows*). × 10

Fig. 2. The epicardial surface (*E*) and part of the cut surface through the myocardium (*M*) of a vascular cast from a control heart. It shows large vessels (*V*) lying on or partly within a dense meshwork of capillaries (*C*). SEM × 110

Fig. 3. The cut surface of a cast of vessels in the mid-myocardium of a control heart showing a radial penetrating artery (*A*) 90 m in diameter giving rise to a 30 m branch (*B*) and it in turn to 10 m branches (*b*). These larger vessels lie in a capillary meshwork (*C*). SEM × 240

Fig. 4. The endocardial surface of a vascular cast of a control heart showing a dense capillary plexus with a few larger vessels. SEM × 120



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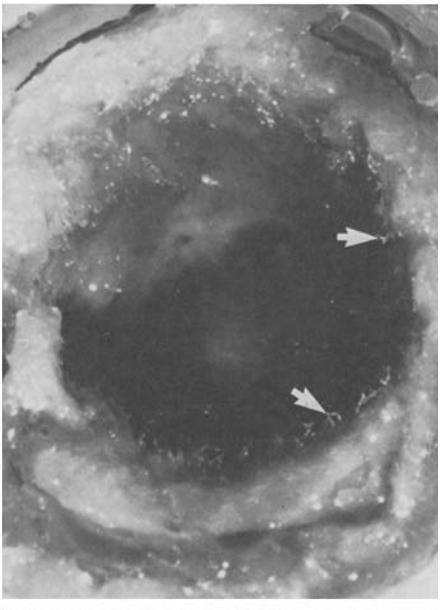

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Fig. 5. Transverse section through the left (*L*) and right (*R*) ventricles of a heart ischaemic for 60 min showing that injected methyl methacrylate has not entered vessels in the subendocardial half (*arrows*) of the left ventricular myocardium. × 10

Fig. 6. A transverse section through a preparation similar to that shown in Fig. 5 following 2 h digestion in caustic soda. The resin cast shows projections representing relatively large vessels (*arrows*) extending from the perfused zone. × 10

Fig. 7, 8. The interface between the left subendocardial zone in which vascular lumina were not filled (*N*) and the perfused subepicardial zone. The vessels which extend from the capillary plexus (*C*) are the same size as the penetrating arteries (*P*) and their branches. Their ends are smoothly rounded (large arrows), sometimes tapered (double arrow-heads). Their branches are filled for only a short distance (*small arrows*). Some vessels show focal narrowing (*double arrows*) near points of branching. SEM × 240

The extent of perfusion of the myocardium by the resin was evident from the light microscopic examination of the cut surface of the ventricles both before and after tissue digestion. In the control group the resin extended through the entire thickness of the walls of both ventricles (Fig. 1), but the ischaemic group showed a subendocardial zone, comprising 50–60% of the cross-sectional area of the left ventricular wall, which was not infiltrated (Fig. 5). This zone corresponds very closely to the pattern and extent of no-reflow indicated by fluorescent tracers (Alanen et al. 1979; Humphrey et al. 1980).

Control hearts

The epicardial surface of the vascular casts showed larger vessels lying on or near the surface of a dense meshwork of capillaries (Fig. 2). These main vessels (up to 220 μm in diameter) gave rise to branches of smaller size which either continued on or near the surface, or penetrated the myocardium to run radially towards the ventricular lumen.

Vessels within the myocardium consisted of a capillary plexus in which larger vessels lay (Fig. 3). The larger penetrating vessels (80–100 μm in diameter) ran towards the endocardium and gave rise more or less perpendicularly to relatively straight branches (40–50 μm). These in turn formed smaller, relatively straight perpendicular branches (20–30 μm) which were connected by tortuous vessels (10–15 μm) with the network of capillaries (3–7 μm).

The endocardial surface of control casts (Fig. 4) showed a dense plexus of capillaries (3–7 μm) in the form of elongated loops, but relatively few larger vessels were evident.

Ischaemic hearts

In the right ventricular wall and the subepicardial half of the left ventricular myocardium the same numbers and types of vessels were demonstrated as in control hearts. However, in the middle of the left ventricular myocardium there was a marginal zone which showed a distinctly different pattern, and the vessels in the endocardial half of the left ventricular walls were not demonstrated (Figs. 6–8).

The marginal zone was up to 400 μm wide. It showed no capillary vessels, only the truncated ends of the larger penetrating vessels which projected radially towards the lumen of the left ventricle, together with short lengths of their primary and secondary branches (Figs. 7, 8). In general, the larger the diameter of these projecting vessels, the further resin within them had extended beyond the filled capillary network. Similarly, the larger the branches of these projecting vessels, the further resin within them had flowed beyond the point of branching (Figs. 7, 8). The ends of the projecting vessels either tapered to a gently rounded point or were not tapered and had hemispherical ends (Figs. 7, 8). Although some vessels showed focal narrowing near points of branching, the majority of the projecting vessels were circular

in cross-section and of fairly uniform diameter for most of their length. Endothelial cell outlines were indistinct on the surface of the casts and there was no evidence of endothelial cell protrusions or swelling.

Discussion

This study has demonstrated distinct differences between ischaemic and non-ischaemic hearts in the capacity of the myocardial microvasculature to conduct fluid, and indicates that intravascular obstruction is unlikely to be the essential cause of the no-reflow phenomenon which develops in ischaemic myocardium.

Although the methyl methacrylate had a viscosity less than that of glycerin when injected (Murakami 1971), it did polymerize quite quickly on contact with the heart, and injection become impossible after only 1 min. However, despite the relatively high injection pressures this may have caused, there was no evidence of resin leakage or vessel rupture, nor was there any obvious difference between the viscosity of excess resin emerging from normal and ischaemic hearts. Using the conditions of Murakami (1971), polymerisation of the monomer (methyl methacrylate) is initiated by a free radical process and accelerated greatly by the addition of substantial amounts (2–3% by volume) of a tertiary base (dimethylaniline). This process is unlikely to be differentially affected by altered metabolite levels diffusing into the capillaries from adjacent normal or ischaemic tissue. This base-promoted polymerisation would, if anything, be retarded in capillaries within ischaemic tissue due to ingress of protons which immobilise the activity of the free tertiary amine. The use of this technique in studies of the coronary vascular bed has been discussed in detail by others (Phillips et al. 1979; Ohtani 1981; Thomson et al. 1982).

Despite the lack of histological information about the structure of vessel walls, the diameters of the various types of vessel we observed fell into distinct categories. The smallest group lay within the size range of capillaries (2–7 μm. Sobin 1972). Larger vessels were similar to mammalian precapillary sphincters (average 10 μm), terminal arterioles (< 50 μm) or arterioles (50–100 μm. Rhodin 1967) which are the contractile vessels which normally control blood flow to capillaries. None of these groups was observed to be selectively occluded.

The results of this study indicate that several factors previously suggested as an explanation are not essential causes of the no-reflow phenomenon. Intercellular and intracellular oedema (Krug et al. 1966; Willerson et al. 1972; Camilleri et al. 1979) seem unlikely to be sufficiently developed within 60 min of ischaemia, or to alter sufficiently abruptly in the middle of the uniformly ischemic ventricular wall, to produce the relatively sharp border of the no-reflow zone. Furthermore, the use of heparinised rats and a cell-free perfusate demonstrated that both intravascular thrombosis (Ruegsegger et al. 1960; Webb and Howard 1975) and erythrocyte plugging of capillaries (Gavin et al. 1978b) were not responsible. Although the intravascular cast method has been used to study endothelial cell morphology (Levesque et al.

1979), the absence, after 60 min of ischaemia, of any evidence of the swelling and protrusion of endothelial cells which develops later (Hausamen and Poche 1965; Poche et al. 1969; Poche 1970) indicates that this mechanism does not occlude vessels at this stage of ischaemia.

The flow of methyl methacrylate further toward the endocardium along the larger penetrating vessels and their branches than along the smaller ones, is in accordance with Poiseuille's equation (White 1979) which indicates that the decline in pressure along tubes is related to the inverse of the fourth power of their diameter.

Explanation of the limitation of the perfusion defect to the subendocardial half of the left ventricular wall is more difficult. However, within 60 min, globally ischaemic rat hearts develop contracture (Alanen et al. 1979; Humphrey et al. 1980) which is likely to cause an increase in intramyocardial pressure. With normal systolic contraction there is a tissue pressure gradient from highest near the endocardium to lowest near the epicardium (Stein et al. 1980). Vessels closed by such a transmural tissue pressure gradient would show a variation in their resistance to reopening consistent with the observed distribution of flow across the myocardium and with the appearance of vessels at the margin of the reperfusion defect.

The findings of this study therefore support explanations of the reperfusion defect which postulate that extravascular factors such as ischaemic contracture (Hearse et al. 1970; Hauschild et al. 1970), perhaps followed by rigor mortis (Nevalainen et al. 1978), lead to the closure of the affected vessels and resist their reopening when blood flow is restored to the ischaemic vascular bed. It is acknowledged that the experimental system employed in this study involves global ischaemia in vitro, and that additional factors, such as continued function of the adjacent myocardium, may have an influence on the development of no reflow in regional infarcts in vivo.

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