Erythrocyte-Containing Versus Crystalloid Cardioplegia in the Rat: Effects on Myocardial Capillaries

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Background. **The purpose of this study was to investigate the effects of crystalloid and erythrocyte-containing cardioplegia on capillary morphology of the isolated erythrocyte-perfused rat heart.**

Methods. **Hearts from adult Sprague-Dawley rats were perfused throughout with resuspended sheep erythrocytes and subjected to the following protocols (n = 6, all groups): (1) 15 minutes nonworking and 30 minutes working heart mode (control; group 1); (2) as for group 1, with 30 minutes erythrocyte-containing (BL) or crystalloid (CR) cardioplegic arrest without reperfusion (groups 2BL and 2CR); (3) as for group 2, with 30 minutes nonworking reperfusion (groups 3BL and 3CR); and (4) as for group 3, with 30 minutes working heart mode (groups 4BL and 4CR). After each protocol troponin I from coronary effluent was measured. Corrosion casts were then made of the coronary microvasculature. Cast density was calculated as cast volume per left ventricular dry weight. Casts also underwent scanning electron microscopy. Analysis was by analysis of variance. Values are mean ± standard deviation.**

Results. **Prearrest working heart coronary flow averaged 15.1 ± 4.7 mL/min without any differences among groups. Coronary flow in group 4 working hearts was the same before and after either cardioplegia. Cardiac outputs were similarly consistent in all groups. Cast density in group 1 (control) was $9.60 \pm 1.17 \times 10^{-2}$ mm^3/mg. It was unaltered by erythrocyte-containing cardioplegia, but after crystalloid cardioplegia (group 2CR), it was $6.52 \pm 0.93 \times 10^{-2}$ mm^3/mg ($p = 0.0001$ versus group 1 and $p = 0.0007$ versus group 2BL). With 30 minutes of nonworking reperfusion (group 3CR, there was slight improvement in cast density at $7.60 \pm 0.90 \times 10^{-2}$ mm^3/mg ($p = 0.0072$ versus group 1; $p = 0.0242$ versus group 3BL). No further improvement was seen in group 4CR. Electron micrographs showed circumferential angularities or narrowings in crystalloid-perfused, arrested hearts, consistent with ischemic damage. Troponin I rose significantly after reperfusion in all groups, but it was higher in crystalloid-perfused, arrested hearts: 0.054 ± 0.013 μg/L versus 0.024 ± 0.017 μg/L ($p = 0.0273$).**

Conclusions. **Erythrocyte-containing cardioplegia maintained capillary density and morphology. Crystalloid cardioplegia produced capillary loss, visible abnormalities, and higher troponin I release. These hearts may be more vulnerable to myocardial damage during reperfusion than hearts perfused with erythrocyte-containing cardioplegic solution.**

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Hyperkalemic cardioplegic arrest (either with blood or crystalloid solutions) is the commonest method used to protect the heart during cardiac surgical procedures. Since its first description by Melrose and associates in 1955 [1], there have been numerous changes to composition and delivery, although the principle common to all cardioplegia protocols is the rapid induction of diastolic arrest to allow operations to be carried out in a still and relatively bloodless field. Extensive experimental and clinical data have shown that cardioplegic arrest protects the cardiac myocyte against loss of contractile function in the face of ischemia [2]. Effects on the myocardial microvasculature are less clear [3].

We have previously shown evidence of damage to capillaries by crystalloid cardioplegia that does not resolve immediately after reperfusion [4]. In an animal model we showed a 30% loss of the capillary bed and the appearance of bypass channels after 90 minutes of St. Thomas' No. 1 crystalloid cardioplegic arrest. After 30 minutes of reperfusion there was some recovery of capillary numbers but not of caliber. It was notable that no resistance changes were seen [4].

The presence of blood in cardioplegic solutions appears to preserve the endothelium-dependent [5–7] but not the endothelium-independent vasodilator responses [8]. These responses, although important, are a reflection of changes in the conductance arteries and resistance arterioles and do not provide a true picture of what occurs in the capillary beds [9]. Little morphologic data exist on the effect of cardioplegic solutions on coronary capillary anatomy. Damage to the capillary beds may

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impair the ability of the heart to recover from episodes of ischemia and reperfusion [3].

The addition of red blood cells to a perfusate is known to improve myocardial perfusion [10–12] and provide a more physiologic environment with preservation of coronary flow reserve [13, 14]. Perfusion of the heart with cardioplegic formulas that include red blood cells results in increased mechanical and metabolic stability [15], stable conditions for mechanical and energetic studies [16], more stable electrophysiology [17], and improved diastolic function [18] when compared with crystalloid cardioplegic solution–perfused hearts. Even a 2% hematocrit produces demonstrably better coronary perfusion than an asanguinous perfusate [12]. Addition of washed erythrocytes to cardioplegic solutions has been reported to provide superior myocardial protection in an isolated, whole blood–perfused dog heart preparation [19]; also, deformable red blood cells have been shown to be essential for optimal capillary bed perfusion [20]. Thus, we hypothesized that the presence of red blood cells in cardioplegic solution should result in preservation of the myocardial capillary bed and thus improve myocardial protection, as suggested by Daggett and colleagues [21] and Bing and coworkers [19].

To elucidate the effects of erythrocytes in cardioplegia we used a red blood cell–perfused isolated working heart model to study the effect of blood and crystalloid cardioplegia as well as reperfusion on the myocardial capillary bed. Microvascular corrosion casting was used to assess the volume of perfused myocardial capillary beds [4, 22–26] as well as their topography [27]. Hemodynamic effects and troponin I release were also studied. Scanning electron microscopy was used to provide qualitative assessment of luminal changes in the capillaries of the microvascular casts.

Material and Methods

Isolated Washed Erythrocyte–Perfused Working Heart Model

We used a sheep erythrocyte–perfused isolated working rat heart model based on the description of Neely and associates [28], and, except during periods of cardioplegic arrest, hearts were continually perfused with resuspended red cells.

Red Cell Perfusate

A washed erythrocyte perfusate, based on the description of Duvelleroy and colleagues [11], was used. Sheep blood was obtained from a local abattoir and collected in phosphate-buffered saline at pH 7.4 containing 20,000 IU sodium heparin and 100,000 U penicillin per liter. The blood was then filtered and centrifuged at 3,000 rpm for 15 minutes. The packed red blood cells obtained were washed an additional three times in phosphate-buffered saline. The erythrocyte concentrate thus obtained was stored in citrate-phosphate-dextrose-adenine preservative (CPDA-1) at 4°C for up to 4 days. On the morning of the experiments the erythrocyte concentrate was washed three times in phosphate-buffered saline before resuspension in a modified dextran-albumin–based Krebs-Henseleit buffer solution. The final composition of the resuspended washed erythrocyte perfusate was (in mmol/L unless otherwise indicated) NaCl, 118; KCl, 3.8; KH_2PO_4, 1.2; $NaHCO_3$, 25; $MgSO_4$, 1; $CaCl_2$, 1.8; glucose, 10; dextran (molecular weight, 70,000), 4%; albumin, 4 g/L; and heparin, 500 IU/L. The final hematocrit was 20%.

The perfusate was exposed to a 95% O_2 and 5% CO_2 humidified gas mixture by means of a polytetrafluoroethylene disc oxygenator (gift of Dr MB Segal, Sherrington Department of Physiology, Kings College, University of London, UK). The perfusate was passed through a heating coil (37°C) by means of a Watson-Marlow (Falmouth, Cornwall, UK) roller pump and debubbled and filtered with a 40-μm arterial line filter (PallMedical LPE1440, Pall Biomedical, Portsmouth, UK), before entering either the Langendorff or working heart reservoir. The perfusate was also filtered downstream from the reservoirs. The hearts were only installed on the apparatus once optimum biochemistry and temperature of the perfusate had been achieved (pH 7.4).

Isolated Rat Heart

All procedures were carried out in strict accordance with the guidelines of the Animals (Scientific Procedures) Act UK 1986. Adult male Sprague-Dawley rats weighing 350 to 500 g were anesthetized with 2% halothane administered in 1:1 oxygen and nitric oxide. Five hundred international units of sodium heparin was injected into a femoral vein. The heart-lung block was excised through a thoracotomy, and the heart was arrested by immediate immersion in Krebs-Henseleit buffer solution at 4°C.

The cold-arrested heart was then transferred to the perfusion apparatus, and the aorta was cannulated. Perfusion was commenced at 37°C at a constant pressure of 100 cm H_2O. Perfusion for the first 2 minutes was with Krebs-Henseleit buffer solution (pH 7.4) to wash out any residual rat blood in the heart before commencing perfusion with resuspended sheep erythrocytes. The pulmonary artery was incised to vent the right heart, and the lungs were removed. The left atrium was cannulated and connected to a reservoir with a filling pressure of 18 cm H_2O. After stabilization in the Langendorff nonworking mode for 15 minutes the preparation was converted to the working mode as described by Neely and colleagues [28]. The left ventricle was allowed to eject spontaneously through a compliance chamber against a 100-cm column of perfusate. Subsequent perfusion details were dependent on the protocol used.

Experimental Protocol

The perfusion was converted to the working mode for 30 minutes as described previously, and the performance of the heart was assessed. If satisfactory, the relevant protocol was proceeded with (n = 6, all groups). Control (group 1) experiments were terminated at this point (Fig

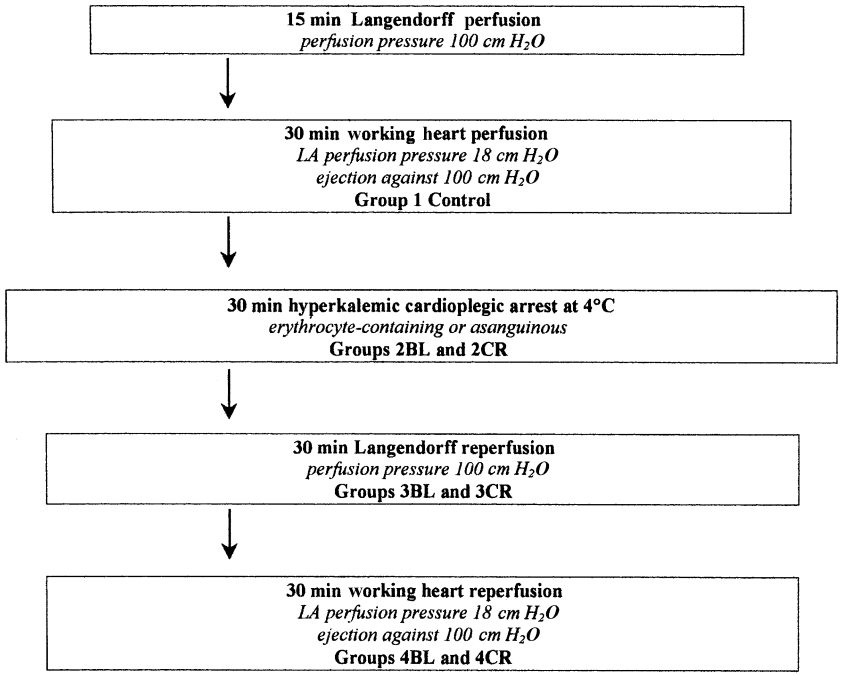

Fig 1. Experimental protocol: measurements were taken at the end of each perfusion period just before casting. Perfusion protocol: retrograde Langendorff perfusion at 100 cm H_2O. For antegrade (working heart) perfusion, left atrial (LA) filling pressure was 18 cm H_2O, ejecting against a 100-cm column of perfusate.

1). Hearts belonging to the other groups (2, 3, and 4) were arrested with either erythrocyte-containing (BL) or crystalloid (CR) hyperkalemic cardioplegic solution (as detailed subsequently) at 4°C for 30 minutes. Experiments terminated at the end of the arrest period (without reperfusion) formed groups 2BL and 2CR. To examine recovery, some hearts were reperfused in the Langendorff mode for 30 minutes at 37°C, which approximates the time taken for proximal anastomoses on the beating nonworking heart in a cardiac operation. Experiments that were stopped here form groups 3BL and 3CR. The experiment was then extended in some hearts with further reperfusion for 30 minutes in the working mode to simulate the effect of removing cardiopulmonary bypass support, thus giving a total reperfusion time of 60 minutes for groups 4BL and 4CR.

Cardioplegic Solutions

The cardioplegic solution was given as a single 30-mL bolus at 4°C. St. Thomas' Hospital no.1 solution (Ivex Pharmaceuticals, Larne, Northern Ireland, UK) cardioplegia (KCl, 20 mmol/L; NaCl, 144 mmol/L; $MgCl_2$, 16 mmol/L; $CaCl_2$, 2.2 mmol/L; procaine hydrochloride, 0.05 mg) was used in the crystalloid (CR) arm of the study and diluted in the modified dextran-albumin–based Krebs-Henseleit buffer solution. Erythrocyte-containing cardioplegia (BL) was made by mixing the washed erythrocyte perfusate with undiluted St. Thomas' no. 1 cardioplegia solution in a 4:1 ratio to give the same electrolyte composition as the crystalloid cardioplegic solution with a hematocrit of 16%. All crystalloid solutions used were filtered (0.4 μm) before use.

Hemodynamic Measurements

Coronary effluent flow and aortic flow were measured at all stages of perfusion by collection into a graduated cylinder for 1 minute. Cardiac output was the sum of coronary effluent flow and aortic flow.

Myocardial Capillary Density

Microvascular corrosion casts were created and used to determine myocardial capillary density. At the designated end points, the coronary circulation was flushed with isotonic Krebs-Henseleit buffer solution. If the heart remained beating, it was fibrillated electrically. Batsons no. 17 polymer (Polysciences Inc, Warrington, PA) with added methylmethacrylate (Merck Ltd, Poole, Dorset, UK) was injected into the aortic root at a pressure of 60 to 70 mm Hg until it could be seen escaping from the right atrium. The cast material was then allowed to polymerize at room temperature for 2 hours. A cylinder of left ventricle (still containing intraluminal cast material) was dissected free, and the epicardial vessels and any cast material inside the ventricle were removed. Because coronary blood volume represents only approximately 12% of left ventricular mass, 90% of which is in the capillaries [29], we were concerned about avoiding potential inaccuracies induced by rapid drying or by surface tension effects keeping excess surface water on the preparation, which would make a large difference to such small wet specimens. We also found that the weight of wet specimens declined even within 1 hour. In addition, after injection of cast material, the vascular component of ventricular mass became a dry part, and we therefore elected to work with dry weights only. These were approximately 31% of wet weights. The resultant prepa-

ration was dried at 28°C for 48 hours and weighed (dry weight of left ventricular myocardium and cast cylinder). Next the myocardium was macerated using alternate baths of 15% KOH and distilled water, until all organic material was removed. The resultant cast cylinder, which was a skeleton of the microvasculature of the left ventricle, was washed, dried at 28°C for 48 hours, and weighed (dry weight of cast cylinder). Using the polymer density, which was found to be 1.051 g/mL, the volume of microvascular cast per milligram of left ventricular muscle was calculated as follows:

Dry wt of LV myocardium (mg)

$$= \text{dry wt of LV myocardium and cast cylinder} - \text{dry wt of cast cylinder} \quad (1)$$

Dry wt of cast material per mg LV myocardium

$$= \text{dry wt of cast cylinder} / \text{dry wt of LV myocardium} \quad (2)$$

Volume of cast material per mg LV myocardium

$$= \text{dry wt of cast material per mg LV myocardium} / \text{polymer density} \quad (3)$$

The volume of cast material per milligram of left ventricle (LV) is therefore equivalent to the perfused capillary density (cubic millimeters per milligram) of the left ventricle.

Scanning Electron Microscopy of Capillary Casts

Corrosion casts of the left ventricle were divided vertically into three parts, mounted on a conducting plate, and coated with gold using a sputter coater (VG Microtech, East Grinstead, West Sussex, UK). Using an ESEM (Environmental Scanning Electron Microscope; Philips, Eindhoven, The Netherlands), representative ×2,000 magnification electron micrographs were made for qualitative estimation of capillary anatomy and topography.

Troponin I Release

In group 4CR and 4BL hearts, which were undergoing the full reperfusion protocol, coronary effluent was collected before cardioplegic arrest and at 60 minutes of reperfusion. It was centrifuged at 3,000 rpm, and the resultant supernatant was stored at −70°C for later batch analysis. This was performed at the routine biochemistry laboratory of the Victoria Infirmary at Glasgow (Alan Reid, MD) using the Beckman Coulter (Brea, CA) Access sandwich enzyme-linked immunosorbent assay.

Statistical Analysis

Data are expressed as the mean ± standard deviation unless otherwise stated. For cast data the groups were treated as k independent samples. For hemodynamic and troponin data, because measures were repeated until that particular experiment was terminated by casting, these variables were treated as a repeated measures problem. Direct, paired comparisons were made by paired Student's t tests. For between group comparisons, analysis of variance with appropriate transformations and Bonferroni correction for significant p values was used; if data sets were incompatible for treatment in this way, Kruskal-Wallis (one level) distribution-free analysis of variance was performed using appropriate corrections for multiple comparisons or ties, by use of Arcus Quickstat Biomedical software (Addison Wesley Longman, trading as Research Solutions, Cambridge, UK).

Results

Hemodynamics

CORONARY BLOOD FLOW. Coronary blood flow increased when hearts were working compared with hearts in the nonworking mode. Flow in Langendorff (nonworking) mode was approximately 35% of that in working mode. The values for coronary blood flow in working heart mode before ischemia were consistent in groups 2BL (15.4 ± 3.8 mL/min), 3BL (15.8 ± 5.2 mL/min), 2CR (15.7 ± 5.5 mL/min), and 3CR (16.9 ± 4.2 mL/min). Before arrest coronary flow in group 4BL (15.1 ± 4.6 mL/min) and group 4CR (12.3 ± 5.0 mL/min) was not significantly different from that during reperfusion (13.7 ± 3.4 mL/min and 12.7 ± 4.0 mL/min, respectively), nor were nonworking flows different as shown in Figure 2. There were no significant between-group differences in coronary blood flow.

CARDIAC OUTPUT. Control (group 1) cardiac output averaged 86 ± 7 mL/min. This was no different from any prearrest values in groups 2BL, 2CR, 3BL, 3CR, 4BL (79.6 ± 13.2 mL/min), or 4CR (81.1 ± 9.0 mL/min). There were no postischemic between-group differences on reperfusion in groups 4BL (74.8 ± 6.7 mL/min) and 4CR (78.5 ± 10.1 mL/min; Fig 3).

Perfused Capillary Density

Perfused capillary density in control hearts and after hypothermic cardioplegic arrest and reperfusion is shown in Figure 4.

CONTROL GROUP. In control hearts (group 1) the perfused capillary density was $9.60 \pm 1.17 \times 10^{-2}$ mm^3/mg.

ERYTHROCYTE-CONTAINING CARDIOPLEGIA. After 30 minutes of erythrocyte-containing cardioplegic arrest (group 2BL), perfused capillary density was similar to that of control hearts at $9.53 \pm 2.91 \times 10^{-2}$ mm^3/mg. After 30 minutes of reperfusion in the Langendorff mode (group 3BL), capillary density in erythrocyte-containing cardioplegic solution–arrested hearts was $9.18 \pm 1.66 \times 10^{-2}$ mm^3/mg. After a further 30 minutes of reperfusion, now in the working heart mode (ie, a total of 60 minutes of reperfusion), cast density for the erythrocyte-containing cardioplegia group (group 4BL) was $9.78 \pm 1.99 \times 10^{-2}$ mm^3/mg.

CRYSTALLOID CARDIOPLEGIA. In the hearts that received crystalloid cardioplegic solution, 30 minutes of hypothermic arrest (group 2CR) resulted in a significant loss of per-

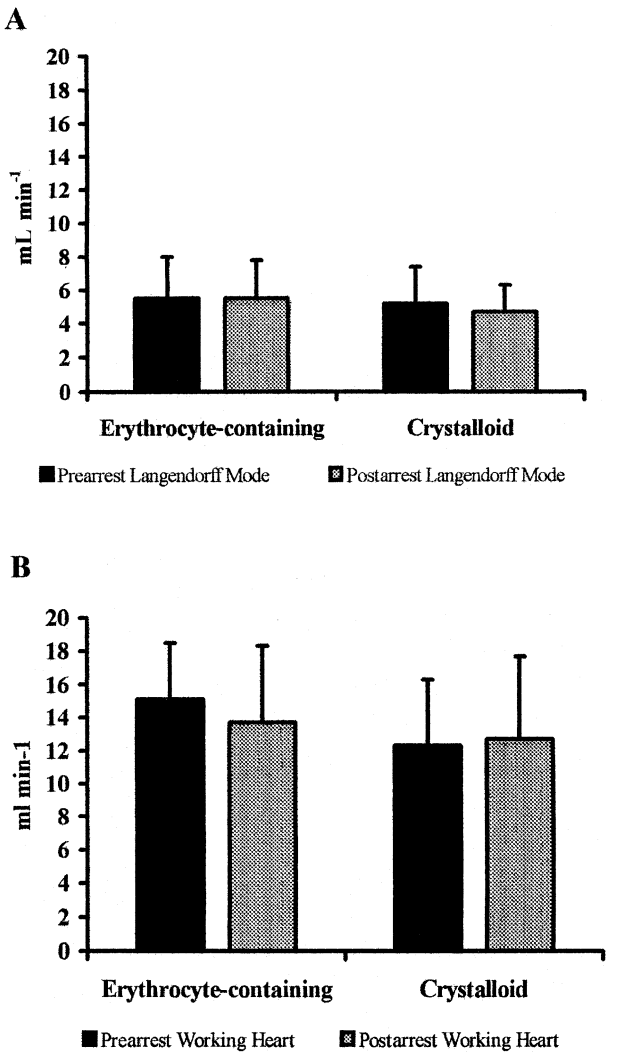

Fig 2. (A) Mean and standard deviations of grouped coronary blood flow for hearts in nonworking mode before and after cardioplegic arrest by both methods. There were no differences seen. (B) Coronary blood flow in working mode before and after arrest by both methods. There were no significant differences.

fused capillary density measured at $6.52 \pm 0.93 \times 10^{-2}$ mm^3/mg ($p < 0.0001$ versus control). After 30 minutes of reperfusion in nonworking mode (group 3CR), the perfused capillary density was $7.60 \pm 0.90 \times 10^{-2}$ mm^3/mg (not significant versus group 2CR; $p = 0.0072$ versus group 1). With a further 30 minutes of reperfusion in working mode (group 4CR), the mean cast density was $7.77 \pm 1.06 \times 10^{-2}$ mm^3/mg at 60 minutes ($p = 0.1340$ versus control), possibly indicating some recovery.

At all points of the investigation perfused capillary density was significantly less in the crystalloid cardioplegia group than that seen in the blood cardioplegia group ($p = 0.0007$, $p = 0.0242$, and $p = 0.0168$, respectively, at 0, 30, and 60 minutes of reperfusion).

Scanning Electron Microscopy of Capillary Casts

Representative scanning electron micrographs of microvascular casts are shown in Figure 5 and illustrate the alterations in topography of capillaries after crystalloid cardioplegic arrest (group 2CR) compared with control (group 1) or erythrocyte-containing cardioplegic solution–arrested hearts (group 2BL). Capillaries from erythrocyte-containing cardioplegic solution–arrested hearts closely resembled control capillaries, whereas crystalloid cardioplegic solution–arrested capillaries had several abnormal features similar to those seen in ischemic damage [30].

Troponin I Release

In group 4BL and 4CR hearts, just before cardioplegic arrest, troponin I concentrations in coronary effluent were 0.008 ± 0.02 $\mu g/mL$ and 0.013 ± 0.003 $\mu g/mL$, respectively ($p = 0.1866$). After 60 minutes of reperfusion, troponin I was significantly lower in the erythrocyte-containing cardioplegic solution–arrested hearts of group 4BL at 0.024 ± 0.02 $\mu g/mL$ than the levels seen in the crystalloid-arrested hearts of group 4CR at 0.054 ± 0.016 $\mu g/mL$ ($p = 0.0273$; Fig 6). These values were, however, significantly higher than their respective prearrest levels ($p = 0.0281$ and $p = 0.0141$).

Comment

The main findings of this study were that crystalloid cardioplegia, as opposed to erythrocyte-containing cardioplegia, was associated with loss of capillary density. This was slow to recover and was accompanied by significantly higher troponin I release (indicating increased myocyte damage) on reperfusion than erythrocyte-containing cardioplegia, which caused no loss of capillary density. Despite these findings coronary flow and cardiac output were unaffected.

We have previously demonstrated, in a large animal morphologic study, that crystalloid cardioplegic arrest resulted in the temporary disappearance of 30% of the myocardial capillaries [4]. Some recovery was seen after 30 minutes of reperfusion in terms of capillary number but not of caliber. It should be noted that coronary resistance to controlled reperfusion was unaffected, and cardiac performance was good. These findings were, however, disquieting because, at the time, we did not establish whether recovery was complete. Furthermore, in the situation of decreased capillary reflow after crystalloid cardioplegic arrest, despite overall normal coronary blood flow, it is possible to envisage that myocytes are being rewarmed without being properly supplied with oxygen and other nutrients. They may therefore be more vulnerable to further damage [8] with serious immediate as well as long-term effects. This suggestion is supported by the association between troponin I release and loss of capillary density that we observed.

Troponin I release has been shown to be a reliable marker of ischemic injury in the rat heart [31], and the raised levels during reperfusion in crystalloid-arrested

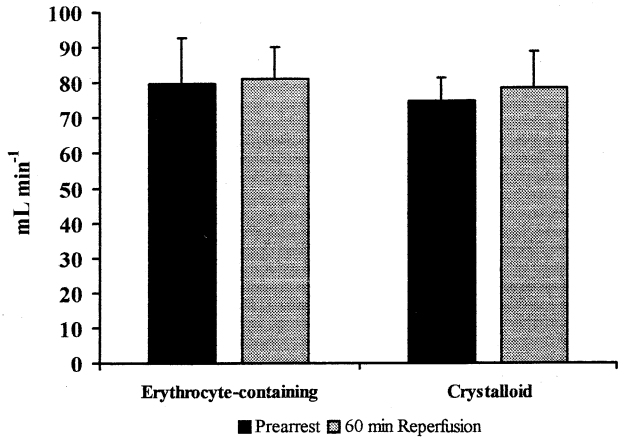

Fig 3. Cardiac output values for hearts before and after arrest by both methods. No differences were seen.

hearts suggest the presence of increased ischemic myocardial damage. The loss of capillary density seen in the present study may be an anatomic correlate of previous studies that show that heterogeneity of microvascular perfusion is determined at the capillary level [32], more especially in compromised hearts [30]. This heterogeneity of tissue oxygenation has been demonstrated in humans after cardioplegic arrest with evidence of microvascular no-reflow even though there were no correlations with cardiac performance or inotropic agent requirement [33]. Ince and coworkers [32] have concluded that there are circulatory units within the vasculature of the rat heart at the capillary level that result in the temporary persistence of hypoxic areas during recovery from hypoxia. These areas probably survive owing to diffusion of nutrients until they recover.

Microvascular preservation may therefore be an important aspect of myocardial protection as the diversion

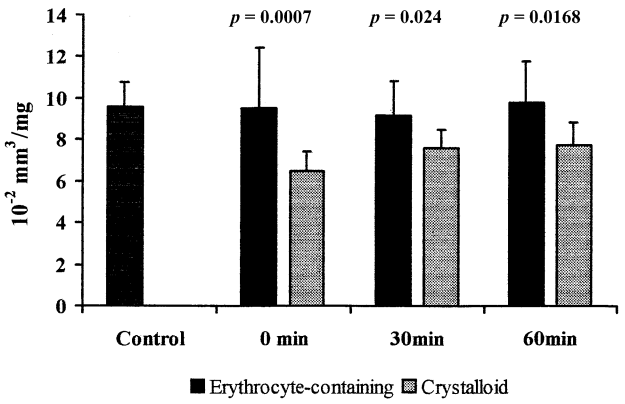

Fig 4. Cast density values for all experimental protocols. Erythrocyte-containing cardioplegic solution–arrested hearts showed no loss of capillary density compared with hearts from the control group or at other stages. In comparison, crystalloid-arrested hearts had significant loss of capillary density at all stages as well as significant loss compared with hearts from the control group at 0 and 30 minutes of reperfusion.

of oxygenated blood from ischemic myocytes [3] is potentially deleterious. The mechanism of reversible capillary loss is unclear: it has been shown that ischemia results in decreased myocardial total (wall plus lumen) capillary diameter, although the luminal diameter decreases to a greater extent. This is not a result of endothelial cell swelling, but is suggestive of true contractile elements within capillary endotheliocytes [34]. Further investigation by the same group has established that 45 minutes of normothermic ischemia resulted in abrupt constrictions of capillaries, which could be abolished by pretreatment with the F-actin stabilizer, phalloidin, which stops the actomyosin system in endothelial cells [27]. Our finding that, after 30 minutes of crystalloid as opposed to erythrocyte-containing cardioplegia, there was persistent capillary narrowing as well as discrete contracted areas and topographic abnormalities in the corrosion casts was remarkably similar. This suggests that the protection of myocytes by crystalloid cardioplegia may be imperfect. It is noteworthy that the capillaries of erythrocyte-containing cardioplegic solution–arrested hearts were indistinguishable from capillaries in control hearts (Fig 4).

The use of cold sanguinous St. Thomas' cardioplegic solution ($[K^+]$ 20 mmol/L) has been shown to provide better preservation of the vasodilator reserve than asanguinous St. Thomas' solution [26], indicating a potential advantage for blood cardioplegia. Cold by itself has been shown to have very little influence on recovery of vasomotor function [6].

The isolated rat heart preparation, used in our experiments, was perfused by red blood cells only, without white blood cells. This allowed strict comparison of the methods of arrest and reperfusion although it does not precisely mimic the clinical situation. Our previous study in this field, using a large animal model designed to mimic the clinical use of crystalloid cardioplegia [10], specifically examined whether neutrophil deposition was associated with capillary loss. We were unable to confirm this, but it is possible that neutrophil effects may be distant and mediated by released compounds. However the capillary loss seen in the crystalloid cardioplegia-arrested isolated rat hearts very closely resembled that seen in the whole blood–perfused pig heart. Nonetheless it is reported that in the isolated rat heart a small but significant improvement in coronary blood flow after reversible ischemic damage can be attributed to leukopenic reperfusion [35]. We do not doubt the contribution that neutrophils make to reperfusion injury, but this investigation was intended to examine the microvascular effects of a relatively short period of cardioplegic arrest after which one would be unlikely to observe no-reflow [36].

The presence of red blood cells in a perfusate is reported to improve both skeletal and heart muscle perfusion [10–12]; additional protection appears to be conferred by the presence of washed red blood cells in cardioplegia [19]. Improved myocardial electrical stability has also been shown [17]. Work in which a 2% hematocrit produced demonstrably better perfusion than

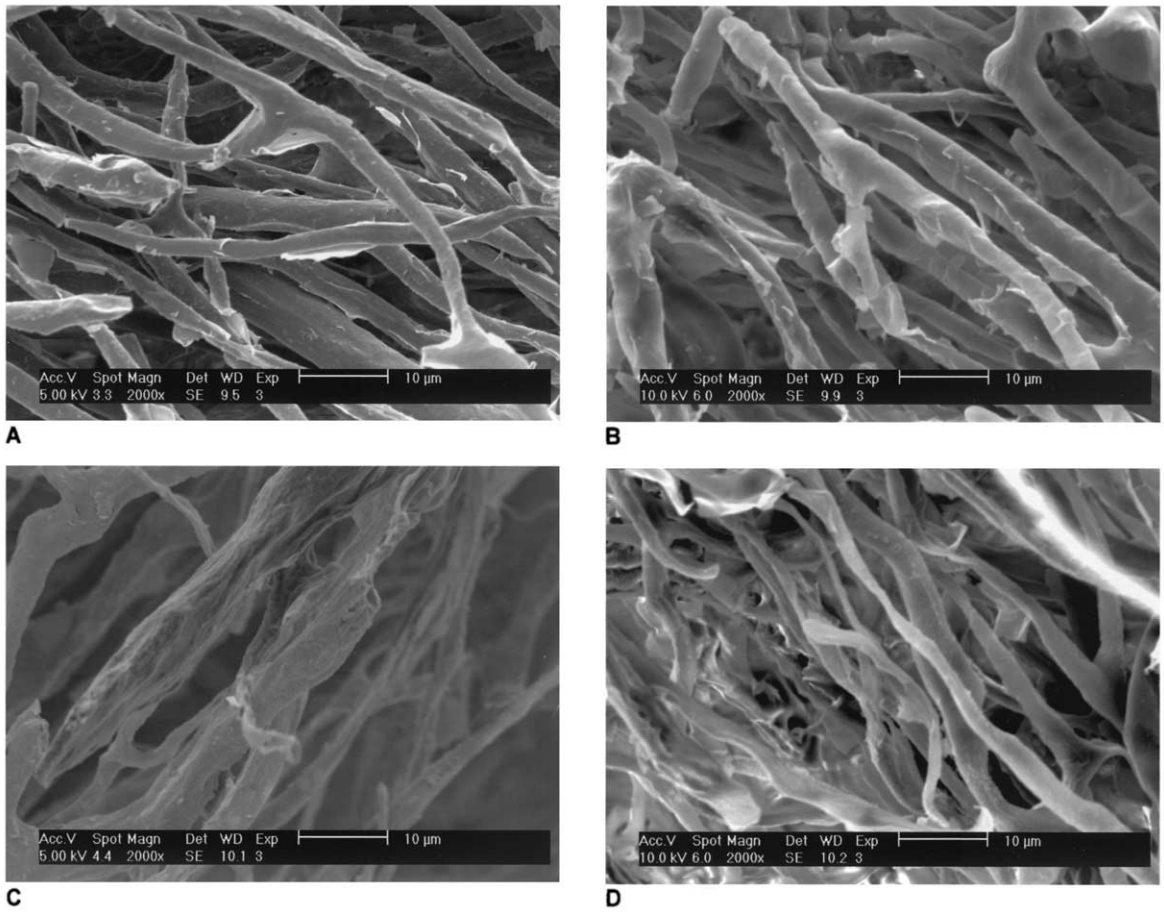

Fig 5. Scanning electron micrographs of individual casts of left ventricular microvasculature at 2,000 magnification: (A) Control (group 1, rat 156) heart subjected to 15 minutes of Langendorff perfusion and 30 minutes in working heart mode. Note the circular circumference and smooth lumen. (B) Immediately after 30 minutes of erythrocyte-containing cardioplegic arrest without reperfusion (group 2BL, rat 123). Note also a circular and smooth lumen. (C) Immediately after crystalloid cardioplegic arrest without reperfusion (group 2CR, rat 179). These vessels are more angular and irregular. This resembles the findings of Glyn and Ward [27], who saw similar deformities in myocardial capillaries in a model of pure (45 minutes) ischemia. (D) After crystalloid cardioplegic arrest with 30 minutes of nonworking reperfusion (group 3CR, rat 161). Note specific narrowings of capillaries with generalized small caliber. This again is similar to the findings of Glyn and Ward [27] after 45 minutes ischemia. AccV = *accelerating voltage;* Det = *detector;* Exp = *internal reference number for photomicrograph;* Spot Magn = *spot size or beam width;* WD = *working distance.*

an asanguinous perfusate [12] has been followed by clinical demonstration of the superiority of cold, oxygenated, dilute blood cardioplegia [21]. In contrast, perfusion with formalin-fixed and therefore undeformable red blood cells resulted in decreased coronary blood flow [20]. It could be that sanguinous cardioplegic solutions ensure better and speedier cooling of the myocyte and delivery of the altered ionic gradient necessary to protect the myocyte and possibly the endothelial cell. In clinical practice, cardioplegic solutions are usually injected into the aortic root, which contains blood. Mixing with this may improve delivery of crystalloid cardioplegia in a way not seen in the carefully controlled conditions in which the isolated rat heart preparation was used in these experiments.

The behavior of blood-perfused hearts is markedly different from that of crystalloid-perfused hearts, and coronary blood flow is higher with crystalloid rather than blood perfusate [14]. Hemodynamics observed in our experiments were consistent with published data [11, 37]. It has also been shown that perfusate lacking red blood cells produces deficient oxygenation of myocardial cells [37]. Blood-perfused hearts also show better recovery from normothermic ischemia as well as a better endothelial function and reactive hyperemic response [38], which may imply a larger available capillary bed. This improved reactive hyperemic response has also been shown by Murphy and colleagues [5]. The maximum reactive hyperemic response is a suitable indicator of the coronary blood flow capacity as 90% of the myocardial blood volume resides in the capillaries [29]. A recent study by Jayaweera and associates [9] indicates that during maximum vasodilation of the coronary vascular bed, the total vascular resistance is equal to the capillary resistance.

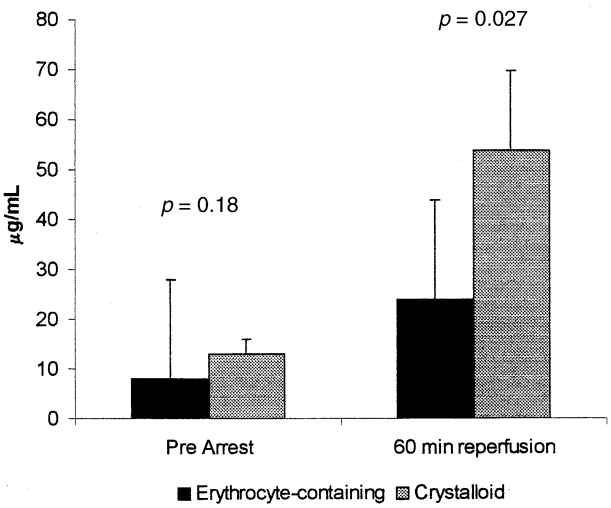

Fig 6. Troponin I release before and after cardioplegic arrest. There was significant troponin I release after reperfusion in both groups but this was significantly higher in the crystalloid arrested hearts.

Thus, the limiting factor of such vasodilation is capillary resistance, which will not necessarily affect resting blood flow.

The use of a red blood cell–perfused preparation was mandated by the experimental hypothesis that erythrocyte-containing cardioplegia would exhibit preferential flow compared with crystalloid. It was considered that prolonged exposure to crystalloid perfusion before arrest might influence results. We are aware that this preparation can show signs of deterioration after prolonged perfusion [16, 39] but there was no sign of this in the hemodynamics (Figs 1 and 2), and both groups were otherwise treated equally.

The use of the isolated heart means that all these hearts have undergone a short period of ischemia and thus should be considered to have been preconditioned in the first place. Our hearts had therefore undergone one cycle of preconditioning but had not undergone the additional preconditioning cycles described by Juggi and colleagues [40], who, after ischemic preconditioning in the isolated heart, demonstrated no differences between crystalloid or blood perfusion in degree of necrosis or recovery of function. This is similar to the findings of Kolocassides and coworkers [41], who compared cardioplegic arrest with ischemic preconditioning and suggested different protective mechanisms.

We conclude that, although cardiac output and coronary blood flow are equivalent after crystalloid or erythrocyte-containing cardioplegic arrest, crystalloid arrest is associated with areas of reduced capillary perfusion, demonstrable capillary abnormalities, and evidence of myocyte damage.

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