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Cape Town solution in prolonged myocardial preservation: structural and ultrastructural study

E. García-Poblete[1], L. Alvarez[2], H. Fernández[1], C. Escudero[2] and A. Torralba[3]
[1]Department of Cellular Biology, Complutensis University of Madrid,
[2]Services of Experimental Surgery and [3]Hospital Pharmacy, Clínica Puerta de Hierro, Madrid, Spain

Summary. This study deals with myocardial preservation after 24 hours of continuous, hypothermic and oxygenated perfusion with Cape Town (CT) solution, focusing on the morphological changes produced by preservation and reperfusion, and their possible relationship to the composition of the solution and the immediate hemodynamic findings after orthotopic heart transplantation in dogs. After preservation, aside from mild or moderate mitochondrial changes, the most relevant lesions included edema and vasoconstriction. Reperfusion was followed by the development of areas of necrosis forming contraction bands and an increment in the mitochondrial damage; the intercalated disks conserved their normal structure; edema became more prominent and was invariably accompanied by hemorrhage; vasoconstriction was very pronounced and was accompanied on occasion by evidence of capillary rupture; and inflammatory cells were observed in the interstitium. These results indicate that colloid must be added to Cape Town solution and that reperfusion probably requires selective approaches to deal with vasoconstriction and inflammation.

Key words: Prolonged myocardial preservation, Cape Town solution, Continuous perfusion, Reperfusion injury

Introduction

The standard method of myocardial preservation consists of the rapid induction of cardiac arrest using a cold cardioplegic solution and hypothermic storage until implantation of the organ into the recipient. With this approach, the margin of safety in terms of graft viability is estimated to be approximately 4 to 6 hours (Mankad et al., 1992; Masuda et al., 1992; Oz et al., 1993; Rubin et al., 1995). An increase in the safe ischemia time would allow: a) the acceptance of organs from long-distance and high-risk donors, thus increasing the availability; b) cross-matching of the donor and recipient tissues, reducing the risk of rejection; and c) improved planning of the procedure for the purpose of reducing costs (Choong et al., 1992; Mankad et al., 1992; Masuda et al., 1992; Menasché et al., 1993; Oz et al., 1993).

Experimental studies involving continuous perfusion of the graft have shown that this measure can prolong myocardial viability to 72 hours, depending on the composition of the preservation solution employed (Nutt et al., 1991; Choong et al., 1992; Masuda et al., 1992).

This report assesses canine myocardial preservation after 24 hours of continuous gravity-driven (low-pressure), hypothermic, oxygenated perfusion, using Cape Town (CT) solution (Wicomb et al., 1984). The study focuses on the structural and ultrastructural myocardial changes (cellular and/or interstitial edema, loss of mitochondrial structure, presence of contraction bands in the myocardial fibers, status of the intercellular junctions and the blood vessels, and presence of inflammatory cells) produced in the postpreservation and post-reperfusion periods of orthotopic heart transplantation.

Materials and methods

Animals

The trials were performed in 11 healthy adult dogs of unknown age and breed, weighing between 20 and 25 kg. Care of the animals complied with the rules stipulated by the Principles of Laboratory Animal Care and the Guide for the Care and Use of Laboratory Animals issued by the National Society for Medical Research and the National Academy of Sciences, respectively.

Surgical technique

Following systemic heparinization, cardiac arrest was induced in the donor by injection of a crystalloid cardioplegic solution (K^+=30 mEq/L). The procurement of the donor heart included the aorta up to the commencement of the descending segment. Once the heart was harvested, the pulmonary artery was transected

Offprint requests to: Dr. L. Alvarez Ayuso, Rafael Bergamín, 9, 10-B, 28043 Madrid, Spain

at the level of its bifurcation, and both atria were opened wide in preparation for implantation. The heart, suspended by the aorta, was placed in a gravity-driven continuous perfusion chamber, where it remained for 24 hours at 4 °C. After this period of time, the organ was implanted orthotopically into the recipient using a technique similar to that employed in humans.

Preservation technique

Preservation involved the use of the gravity-driven continuous perfusion technique (maximum pressure 15 cm H_2O) with hypothermia (4 °C) and oxygenation (95% O_2, 5% CO_2) described by Wicomb et al. (1984). The heart was perfused with CT solution: NaCl (7.98g/L), $MgSO_4.7H_2O$ (3.48g/L), $CaCl_2.2H_2O$ (0.16g/L), KH_2PO_4 (0.235g/L), K_2HPO_4 (1.105g/L), procaine hydrochloride (0.27g/L), chlorpro-mazine (0.005g/L), phenoxybenzamine (0.01g/L), glucose (2.0g/L), sucrose (2.5g/L), glycerol (12.6g/L) and taurine(0.5 g/L); pH 7.3; osmolarity 410 mOsM.

Determinations

In the preservation solution, pH, lactate, creatine kinase (CK) and lactic dehydrogenase (LDH) were determined 0, 6, 20 and 24 hours after continuous perfusion. In the donor heart, temperature and myocardium weight were measured pre- and post-preservation. Heart rate and arterial blood pressure were also assessed in basal situation and post-transplantation.

Histological study

Samples were taken of both ventricles for light microscopy and transmission electron microscopy studies after 24 hours of continuous perfusion and 60 minutes after revascularization of the graft.

For viewing under light microscopy, the samples were fixed in 10% formaldehyde for 4 days, embedded in paraffin and cut into 7-micron-thick slices in a Minot microtome. Once freed from the paraffin, they were stained with hematoxylin-eosin and studied under a Leitz photomicroscope (Dialux model).

The samples to be used in the ultrastructural study were immersed for 2 hours in 2% glutaraldehyde in phosphate buffer and cut into 1-mm^3 blocks; then they were washed in phosphate buffer and postfixed with 2% osmium tetroxide for 1 hour. After dehydration in a graded acetone series, they were embedded in Epon 812, cut into semithin slices (0.5 to 1 micron thick) using a Reichert Jung ultramicrotome, and stained with Richardson's methylene blue for light microscopy study. Likewise, ultrathin slices (70 nm thick) were stained with a water-based solution of 2% uranyl acetate and lead citrate for study under a Zeiss 902 electron microscope.

Results

Functional findings

The pH decreased and the lactate levels rose during the first 6 hours of perfusion; thereafter, they remained stable. The enzyme levels increased progressively over the 24-hour preservation period (Table 1). The weight of the myocardium increased by 35% (from 168 to 226 g) during this period.

With respect to recovery of the heart rhythm following revascularization, sinus rhythm was reestablished in only 3 dogs; the remainder presented sinus arrest or atrioventricular dissociation. The arterial pressure fell 35% with respect to the preoperative values; only 4 of the 9 animals achieved a systolic arterial pressure that surpassed 100 mmHg. Left ventricle presented an edematous aspect with reduced contractility in every case, and one animal presented signs of no-reflow and stone heart.

Morphological findings

After the 24-hour preservation period with continuous perfusion, the myocyte structure and the intercellular junctions remained intact. Interstitial edema was observed. Intracellular edema was also present in some cases, mainly beneath the sarcolemma, and was more marked in right ventricle than in left. The mitochondria tended to be arranged in clusters and presented cristae changes that were usually mild or moderate. There was occasional evidence of capillary and arteriolar vasoconstriction during this phase (Fig. 1).

After transplantation and subsequent reperfusion, areas of necrosis forming contraction bands were produced (Fig. 2). The intercalated disks maintained their normal structure (Fig. 3). Both cellular and interstitial edema increased notably, and were invariably accompanied by interstitial hemorrhage (Fig. 2). The mitochondrial lesions became more widespread and

Table 1. Determination in the CT solution.

	0 hours		6 hours		20 hours		24 hours
pH*	7.3±0.02	<0.01	6.5±0.03	ns	6.5±0.03	ns	6.5±0.05
Lactate nmol/L	0.38±0.27	ns	0.45±0.14	ns	0.42±0.13	ns	0.34±0.18
LDH U/L	4±7	ns	22±11	<0.01	61±26	ns	74±25
CK U/L	0	ns	7±12	ns	15±7	ns	25±15

*: at 5 °C; CT: Cape Town solution; LDH: lactic dehydrogenase; CK: creatine kinase; ns: not significant.

severe (Fig. 4). Together with hemorrhage, the most prominent and persistent change was vasoconstriction (Fig. 5); among the vascular changes, there were even signs of capillary rupture. Inflammatory cells - leukocytes, macrophages and mast cells - were also observed in the interstitium.

Discussion

Given their ionic composition, nearly all the solutions used in organ preservation are intracellular; that is, they contain more potassium than sodium and more magnesium than calcium. The purpose is to avoid transmembrane ionic gradients, thus helping to maintain the intracellular ionic composition during the ischemic period (Choong et al., 1992; Menasché et al., 1993; Rubin et al., 1995). Osmotically active molecules are also added to the preservation liquid in an attempt to prevent the development of intracellular edema, and the addition of colloids is indicated when continuous perfusion systems are employed since the latter are associated with a considerable production of interstitial edema (Menasché et al., 1993). The composition of CT solution is deficient in this respect, as was demonstrated in the postperfusion histological study by the presence of cellular and interstitial edema, in accordance with the marked increment in the weight of the heart at the end of this phase, a circumstance that has a negative impact on postreperfusion functional recovery (Hsu et al., 1993).

When an organ is subjected to continuous perfusion, there is an initial decrease in the vascular resistances, which subsequently undergo a progressive increase. The various components of CT solution include chlorpromazine and phenoxybenzamine, an antagonist and a blocker, respectively, of α-adrenergic receptors and, therefore, substances that are capable of acting as vasodilators (Gabauer et al., 1994; Tsuchida et al., 1994). Both should have contributed to maintaining a satisfactory tissue perfusion during ischemia; however, vasoconstriction was evident at the end of the ischemic period, revealing the limited efficacy of these substances; the slight rise in the lactic acid and intracellular enzyme concentrations, especially during the last 4 hours of continuous perfusion, would agree

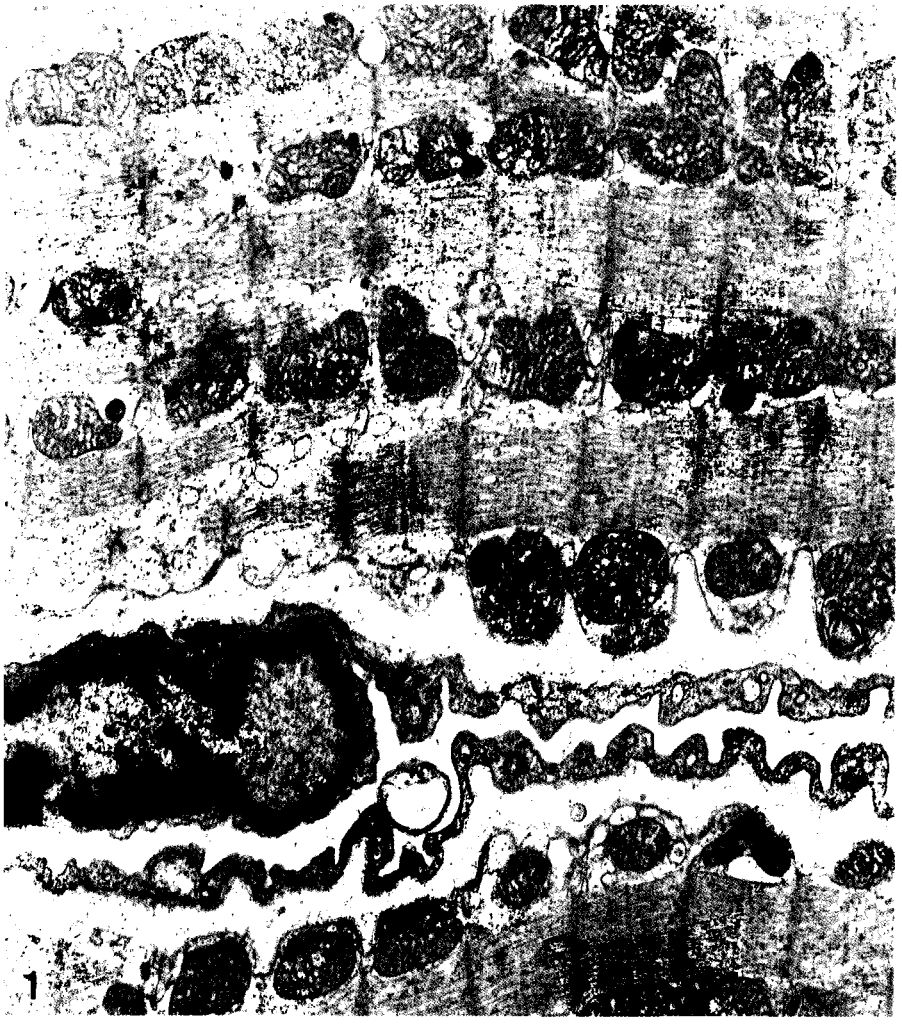


Fig. 1. Left ventricle after 24 hours of preservation with continuous perfusion of Cape Town solution. Vasoconstriction can be observed in a capillary cut open lengthwise. Mitochondria are arranged in clusters and show slight to moderate changes in their cristae. There is evidence of the onset of subsarcolemmal edema (arrow). x 7,000

with the above findings, indicating the inability of the microcirculation to eliminate cellular debris.

On the other hand, it is known that both the α_1-adrenergic receptors and the adenosine-A1 receptors are activated during prolonged ischemia to trigger myocyte-protecting mechanisms (preconditioning) mediated by protein kinase C, and that phenoxybenzamine effectively blocks this protection (Tsuchida et al., 1994); thus, the presence of phenoxybenzamine in the preservation solution may have provoked additional myocytic damage.

Reperfusion augmented the damage produced during continuous perfusion. Only the intercellular junctions, which are calcium-dependent, remained undamaged, indicating that the concentration of this cation in the preservation solution was probably adequate.

An important factor implicated in reperfusion injury is the production of free radicals (Barrabés et al., 1995; Mezzetti et al., 1995; Pesonen et al., 1995). Although these products were not determined in the present study, their existence correlates with the presence of inflammatory cells within the tissue (Mazer, 1993; Barrabés et al., 1995; Mezzetti et al., 1995; Pesonen et al., 1995). Among the latter are mast cells, which release platelet-activating factor which, in turn, stimulates leukocyte adherence to the endothelium and the production of microvascular damage; and proteases which, in addition to the endothelial lesion, trigger or promote extracellular matrix degradation (Kurose and Granger, 1994; Kovanen et al., 1995).

The comparison between the University of Wisconsin (UW) solution, studied previously by our group under identical experimental conditions (García Poblete et al., 1997), and CT solution does not clearly indicate one or the other as more suitable. The functional results revealed no significant differences between the two groups and while the morphologicalal findings demonstrate that the UW solution produced no edema

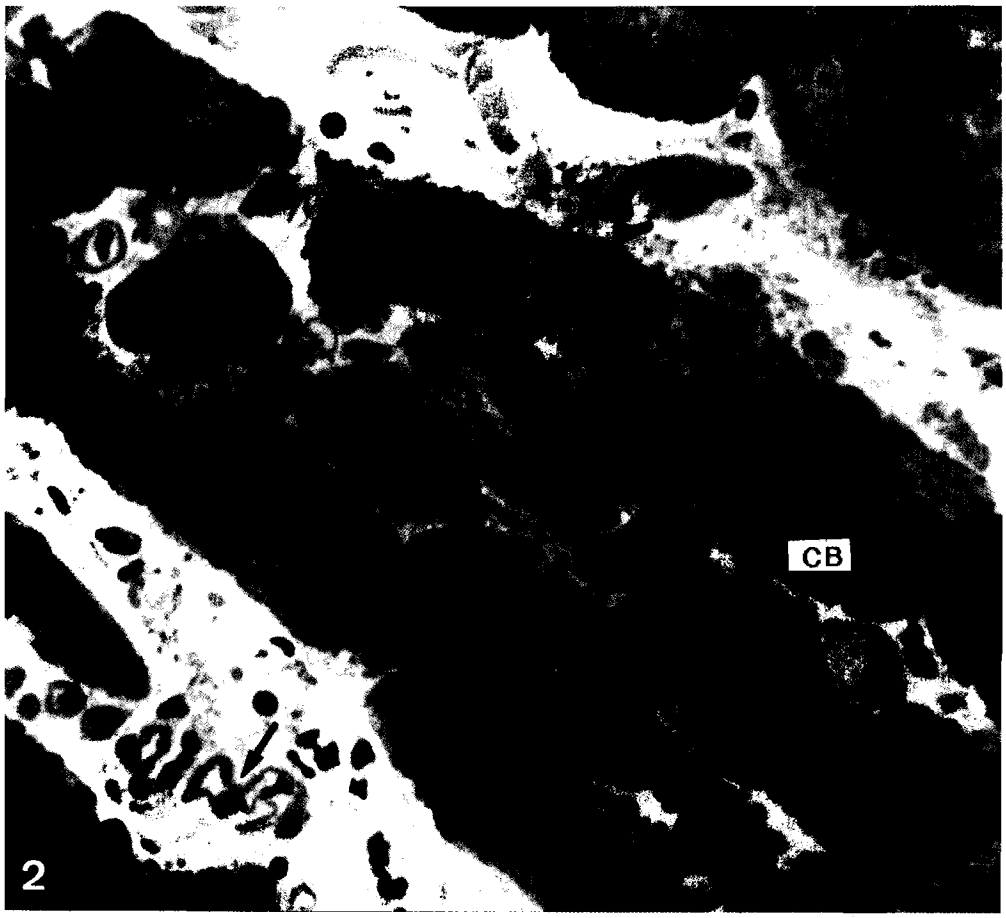
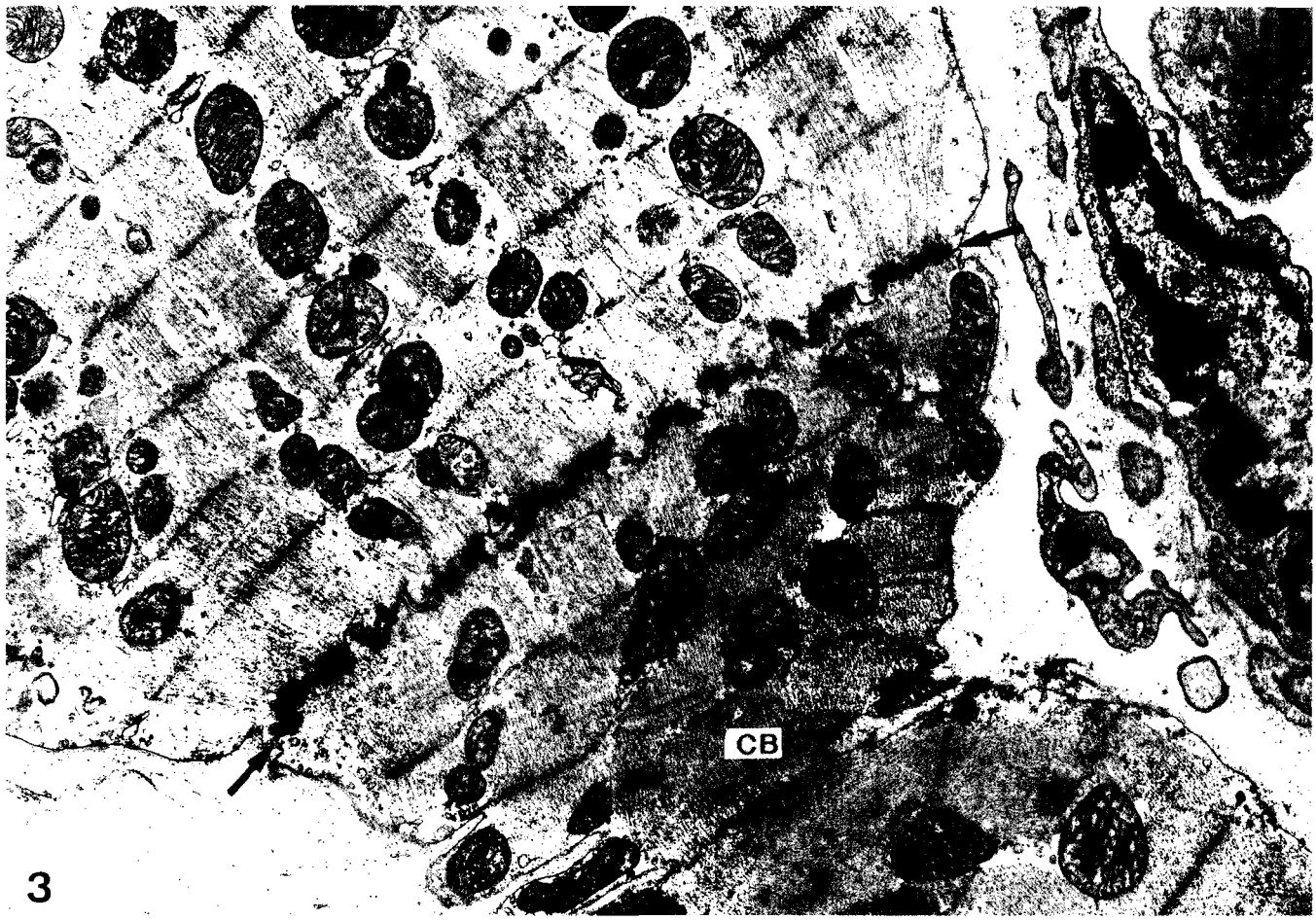


Fig. 2. Left ventricle after reperfusion. Necrosis forming contraction bands (CB), cellular and interstitial edema, hemorrhage and capillary vasoconstriction (arrow) can be observed . x 400

Fig. 3. Left ventricle after reperfusion showing the normal structure of an intercalated disk (arrows), the presence of contraction bands (CB), cellular and interstitial edema and moderate mitochondrial changes. x 7,000

Fig. 4. Left ventricle after reperfusion. The mitochondria present very severe changes: marked swelling is evident in some, accompanied by decreased matrix density and loss of structure in cristae x 12,000



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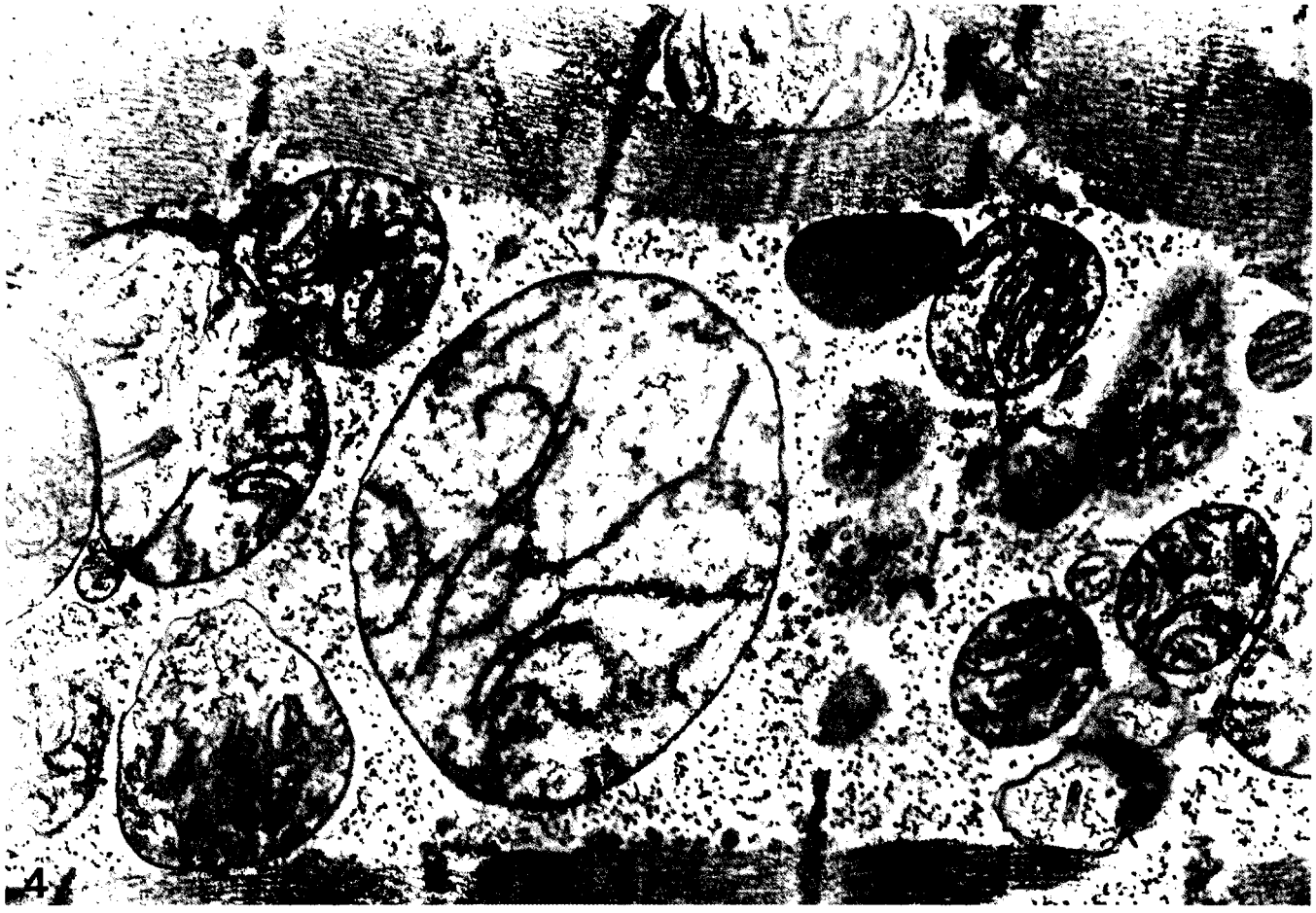

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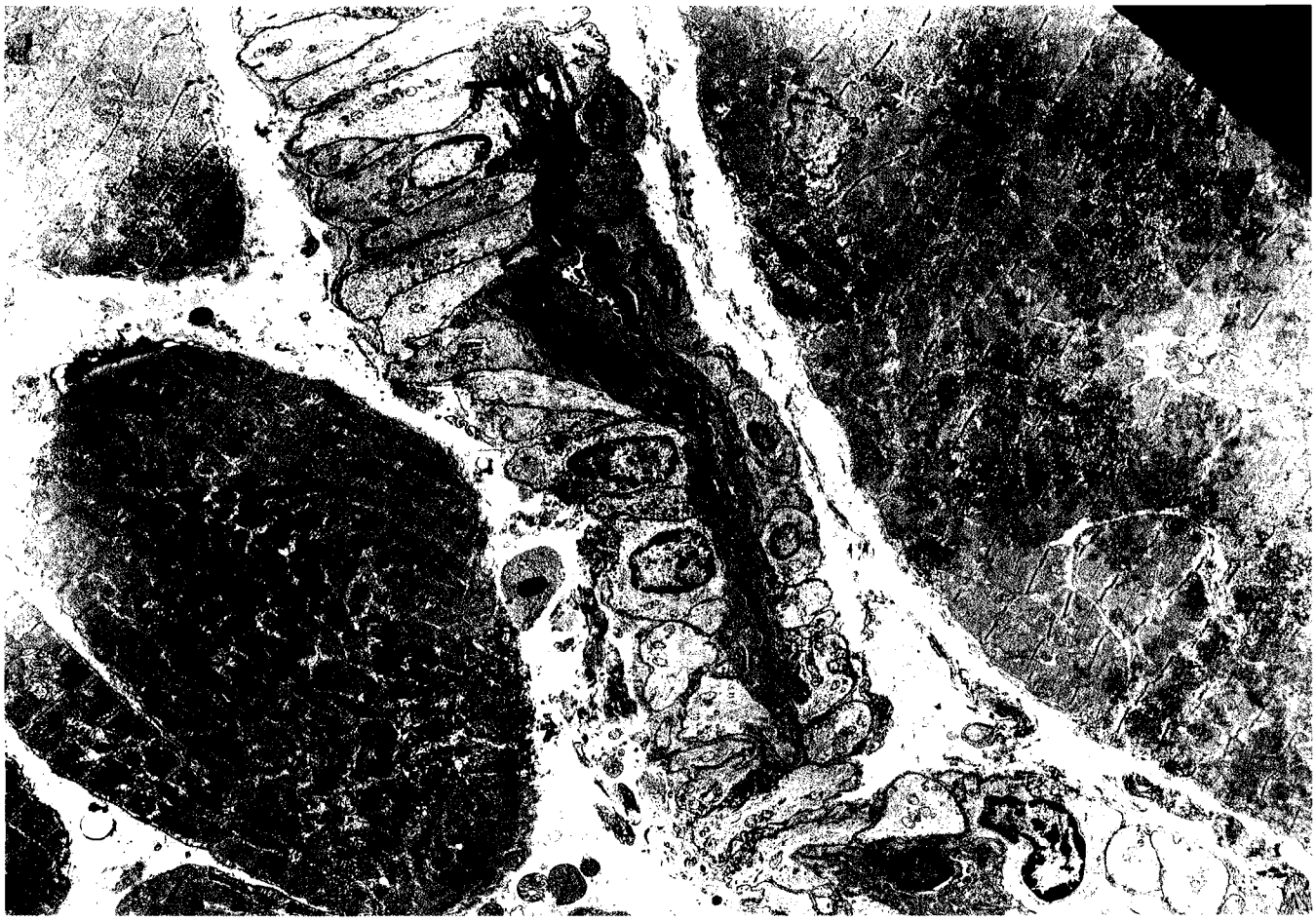

Fig. 5. Left ventricle after reperfusion, showing the lengthwise section of a severely contracted arteriole with a nearly virtual lumen (arrow). x 1,100

during the preservation phase, both solutions left marked, although differing, lesions during the reperfusion phase.

From the results obtained in the present study, the need for two basic strategies can be deduced: 1) a substantial modification of the composition of CT solution, removing components of dubious efficacy and adding colloids; and 2) the application of differentiated and selective approaches to managing the effects of reperfusion, a strategy that has already been recommended by Menasché et al. (1993).

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