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Preservation of Rabbit Hearts With Different Cardioplegic Solutions at Low Temperature

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Summary

An organ-preserving solution, including in its composition also organic molecules, prepared at the University of Wisconsin (UW), has been successfully used for preservation of liver, pancreas and kidney, and has recently been tested for long-term storage of isolated hearts. We have compared the effectiveness of the UW solution with that of a standard crystalloid cardioplegic solution (St. Thomas, ST) in the functional and structural preservation of isolated hearts. The hearts taken from 24 rabbits were mounted on a Langendorff preparation. After assessment of the left ventricular function by an intraventricular balloon, 40 ml of either cardioplegic solution were injected to arrest the hearts (12 UW and 12 ST), which were then immersed in the same solution for 4 h at 4 °C without perfusion. After this period, the hearts were normothermally reperfused with oxygenated Krebs-Henseleit solution for 30 min, and finally left ventricular function was assessed again. An electron microscopic evaluation was performed as well. Significantly higher recovery of left ventricular developed pressure ($p<0.01$) and of negative dP/dt ($p<0.05$), was observed after preservation with UW, while no difference on positive dP/dt was found. After reperfusion, left ventricular end-diastolic pressure significantly rose with ST ($p<0.01$), but did not change with UW; the difference between ST and UW was significant ($p<0.01$). Tissue water content was significantly lower in the hearts preserved with UW ($p<0.05$). Electron microscopic examination revealed generally good preservation with no substantial difference between the two solutions. We conclude that UW cardioplegic solution, rather than ST solution, associated with low temperature, provides better protection of isolated hearts against ischaemia and reperfusion injury after long-term preservation. This is probably due to the combined effects of antioxidants, cell-membrane impermeable substances and oncotic agents.

Key words

Isolated heart – Hypothermic cardioplegia – Ventricular function – Ultrastructural preservation – Rabbits

Introduction

Crystalloid cardioplegic solutions, originally developed for myocardial arrest and improved cardiac preservation during open heart surgery, have also been used for the preservation of explanted hearts for human transplantation (Swanson *et al.* 1980, Darracott-Cankovic *et al.* 1987). Experimental work has been done to optimize the composition of the cardioplegic solutions and to find out the best protocols for arresting the heart, in order to minimize damage during the ischaemic period, and to recover a full function at the end of ischaemia.

Several different compositions of the fluid bathing the heart during the preservation period were tested, including fluids of normal or elevated osmolarity and various modifications of the ionic composition of the Krebs balanced salt solution, which span the range between the composition of the extracellular fluid and that of the intracellular fluid (Maurer *et al.* 1990, Wang *et al.* 1991). Further modifications included addition of a membrane stabilizer, metabolic inhibitors and erythrocytes

(Shlafer *et al.* 1982, Feindel *et al.* 1984, Myers *et al.* 1986, Julia *et al.* 1991).

The current cardiac preservation technique, involving the use of a static hypothermic hyperkalaemic crystalloid solutions, allows fairly good clinical results in cardiac transplantation for up to 4 h storage (Billingham *et al.* 1980, English *et al.* 1984, Molina *et al.* 1985).

Recently, the University of Wisconsin solution has been introduced into clinical practice, both in Europe and in the United States, for the preservation of organs such as the liver, pancreas and kidney (Wahlberg *et al.* 1987, Jamieson *et al.* 1988, Ploeg *et al.* 1988). Its use for heart preservation has also been proposed, in alternative to currently used methods, such as the St. Thomas Hospital cardioplegic solution (Braimbridge *et al.* 1977, Hearse *et al.* 1981). In this study we compared the effectiveness of both solutions during 4 h preservation of isolated rabbit hearts.

Methods

Male New Zeland white rabbits (2.4–2.8 kg) were killed by cervical dislocation, after receiving an injection of heparin (1000 IU i.v.) to prevent microembolism. The heart was rapidly excised through a midline sternotomy, immediately immersed in ice-cold physiological solution and prepared for mounting on a modified non-recirculating Langendorff apparatus. The aorta was cannulated and the heart was retrogradely perfused at 70–80 mmHg constant pressure for 30 min with Krebs-Henseleit solution containing (in mmol/l): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$* $7H_2O$ 1.64, $NaHCO_3$ 25, $CaCl_2$ 2.5, glucose 11.1. The perfusate was bubbled with a mixture of 95% O_2 and 5% CO_2 which maintained pH between 7.38 and 7.42 and oxygen tension between 600 and 760 mmHg. The perfusate was forced through a 2 μm filter, to remove debris, and through a heat exchanger where temperature was adjusted and maintained at the desired level of 37 °C during the preischaemic period and reperfusion. The heart was paced at a constant rate of 180 bpm by electrodes placed on the ventricular epicardium and driven by pulses of 3 V and 3 ms duration (Grass, S-48). Perfusion pressure was continuously monitored through a sidearm cannula connected to a pressure transducer (Gould-Statham P23dB).

Isovolumic systolic and diastolic pressures were measured by introducing a fluid-filled latex balloon, connected to another Statham P23 ID pressure transducer, into the left ventricle *via* the atrium and mitral valve. To study the left ventricular contractile function, the dead volume of the balloon was filled with saline until intraballoon pressure started to rise above 0 mmHg. This zero volume was slightly different depending on individual heart size. Thereafter, the balloon volume was increased in four steps of 0.1 ml. Experiments were recorded on floppy disks after analog-to-digital conversion through a computerized acquisition system (Cevese 1986) that allowed off-line beat-to-beat calculation of peak left ventricular pressure (LVP, mmHg), left ventricular end-diastolic pressure (LVEDP, mmHg), maximal dP/dt (+dP/dt, mmHg/s), minimal dP/dt (−dP/dt, mmHg/s) and perfusion pressure (mmHg). Left ventricular developed pressure (LVDP) was calculated from the difference between LVP and LVEDP.

To avoid variability due to stress relaxation, the same time interval was always used between volume increments, and pressures were measured 30 s after each volume increment in the balloon. Records in each step lasted 20 s.

After completion of a set of control measurements, the intraventricular balloon was deflated and the temperature of the heart was lowered to 27 °C. The perfusion with Krebs-Henseleit solution was discontinued and cardiac arrest was induced by infusing 40 ml of a cardioplegic solution at 4–5 °C over a period of 2 min.

Two groups of 12 rabbits were used. The hearts of the first group received the St. Thomas' cardioplegic solution (ST) (composition in mmol/l: NaCl 110.0, KCl 16.0, $MgCl_2$ 16.0, $CaCl_2$ 1.2, $NaHCO_3$ 10.0, pH 7.8, osmolarity 290 mosm/kg H_2O), the hearts of the second group received the Wisconsin solution (UW) (composition in mmol/l: KH_2PO_4 25.0, $MgSO_4$ 5.0, adenosine 5.0, glutathione 3.0, raffinose 30.0, allopurinol 1.0, K-lactobionate 100.0, pentastarch (%) 5.0, insulin (IU/l) 40, pH 7.4, osmolarity 320 mosm/kg H_2O).

The UW solution was supplied by E.I. du Pont de Nemours & Co., Inc., Medical Products Department, Bannockburn, Ill., and the ST by S.I.F.RA., Verona, Italy.

Each heart received a single cardioplegic infusion and was kept in a state of hypothermic ischaemic arrest, immersed in the same solution, at 4–5 °C, for 4 h. At the end of this period, the chamber containing the heart was progressively rewarmed over a period of approximately 5 min, up to an average myocardial temperature of 27 °C, and the heart was reperfused. Perfusion pressure and temperature were gradually increased up to preischaemic values in the following 5 min.

Left ventricular mechanical function was tested after 30 min of reperfusion with the balloon inflated with the same volumes which had been used before hypothermia.

At the end of the experiment, a slice of left ventricular wall was taken, to assess tissue water content (%) the specimen was weighed, heated to 110 °C for 24 h and weighed again. An additional biopsy was obtained from the upper part of the left ventricle and used for the ultrastructural study.

Table 1
Left ventricular haemodynamic parameters of the hearts preserved with St. Thomas' (ST) and Wisconsin (UW solutions) at different left ventricular balloon volumes

Volume		0.0 ml		0.1 ml		0.2 ml		0.3 ml		0.4 ml	
		Baseline	Reperfusion	Baseline	Reperfusion	Baseline	Reperfusion	Baseline	Reperfusion	Baseline	Reperfusion
LVP (mmHg)	ST	97.0±3.1	81.7±4.1	105.0±3.4	89.9±4.4	113.4±3.9	98.9±4.7	121.5±4.4	107.1±5.0	132.2±4.9	118.6±6.0
	UW	102.9±4.7	81.9±2.6	109.9±5.0	89.6±2.9	118.5±5.2	98.6±2.8	122.9±4.7	107.5±3.2	134.4±4.3	117.1±3.6
LVEDP (mmHg)	ST	0.7±0.3	4.9±2.1	2.4±0.7	9.1±2.2	5.8±2.1	15.0±2.9	10.7±3.6	21.8±3.8	20.9±4.3	32.8±4.8
	UW	0.5±0.3	0.7±0.3	2.5±1.0	2.8±0.9	4.3±1.5	5.3±6.2	7.3±1.5	9.5±1.9	18.9±3.7	20.1±3.7
+dP/dt (mmHg/s)	ST	1445±48	1189±69	1484±43	1295±62	1571±46	1323±62	1580±46	1310±65	1587±44	1302±64
	UW	1532±79	1295±47	1612±64	1384±39	1692±80	1429±43	1671±70	1460±43	1683±64	1475±44
−dP/dt (mmHg/s)	ST	1125±35	989±57	1188±24	997±55	1184±24	1006±50	1206±32	985±44	1186±27	957±48
	UW	1167±29	1096±28	1201±38	1113±27	1235±44	1131±29	1238±36	1140±32	1215±43	1161±29

Values are mean ± S.E.M. (n = 12). LVP, peak systolic pressure, LVEDP, left ventricular end-diastolic pressure, + dP/dt, maximal positive derivative of left ventricular pressure, −dP/dt, minimal derivative of left ventricular pressure.

Myocardial specimens (about 1 mm^3) were immersion-fixed in glutaraldehyde (2 % in 0.1M phosphate buffer) overnight and then post-fixed for 1 h with osmium tetroxide (1 % in distilled water). All fixation procedures were performed at 4 °C. The tissue fragments were then dehydrated through graded concentrations of aceton and embedded in a mixture of Epon/Araldite. Semithin sections (2 μm) stained with toluidine were used to select well-fixed regions for thin sectioning. Thin sections (about 70 nm) were cut on a Ultracut E ultramicrotome (Reichert), placed on copper/rodium grids and stained with lead citrate. Observations were made in a Zeiss EM10 electron microscope operated at 60 kV.

Data are means ± S.E.M. Postischaemic values of LVDP, +dP/dt, −dP/dt are expressed as percentage of values measured before cardioplegic arrest, while LVEDP as absolute differences. Mixed design analysis of variance (ANOVA) and multiple comparisons with Tukey's two-tailed test were used. Differences were considered significant if $P<0.05$.

Results

Absolute values of the haemodynamic parameters before arrest (baseline) and after reperfusion with ST and UW are presented in Table 1. Percentage recoveries of LVDP, +dP/dt and −dP/dt are plotted as a function of left ventricular balloon volume in Fig. 1. In general, recovery of LVDP (Fig. 1a) was about 80 % complete, but with higher balloon volumes recovery was significantly better in the UW group (ANOVA, $p<0.01$). Figs 1b and 1c show that the recovery of +dP/dt and −dP/dt was larger in the UW group at all balloon volumes. However, group statistics indicate significant differences only for −dP/dt ($p<0.05$). The difference in LVEDP absolute values before and after hypothermic ischaemia is plotted as a function of left ventricular balloon volume for ST and UW in Fig. 2: the hearts preserved with ST showed marked significant elevations of LVEDP (ANOVA, $p<0.01$), while with UW the diastolic pressure during reperfusion was not statistically different from the baseline. Also the difference between the two groups was statistically significant. The tissue water content after ischaemia and reperfusion was significantly lower ($p<0.05$) in hearts arrested with UW than in those arrested with ST (82.4 ± 0.6 % and 84.06 ± 0.4, respectively).

The overall morphology of the myocardium was generally well preserved in all specimens both in ST- and UW-preserved hearts. A qualitative ranking of myocardial morphology referred to the myocardial ultrastructure (sarcolemma, contractile material, mitochondria, intracellular membrane systems, nucleus) of the 24 hearts gave: 1 excellent, 7 good, 4 fair preservations for ST, and: 7 good, 5 fair preservations for UW.

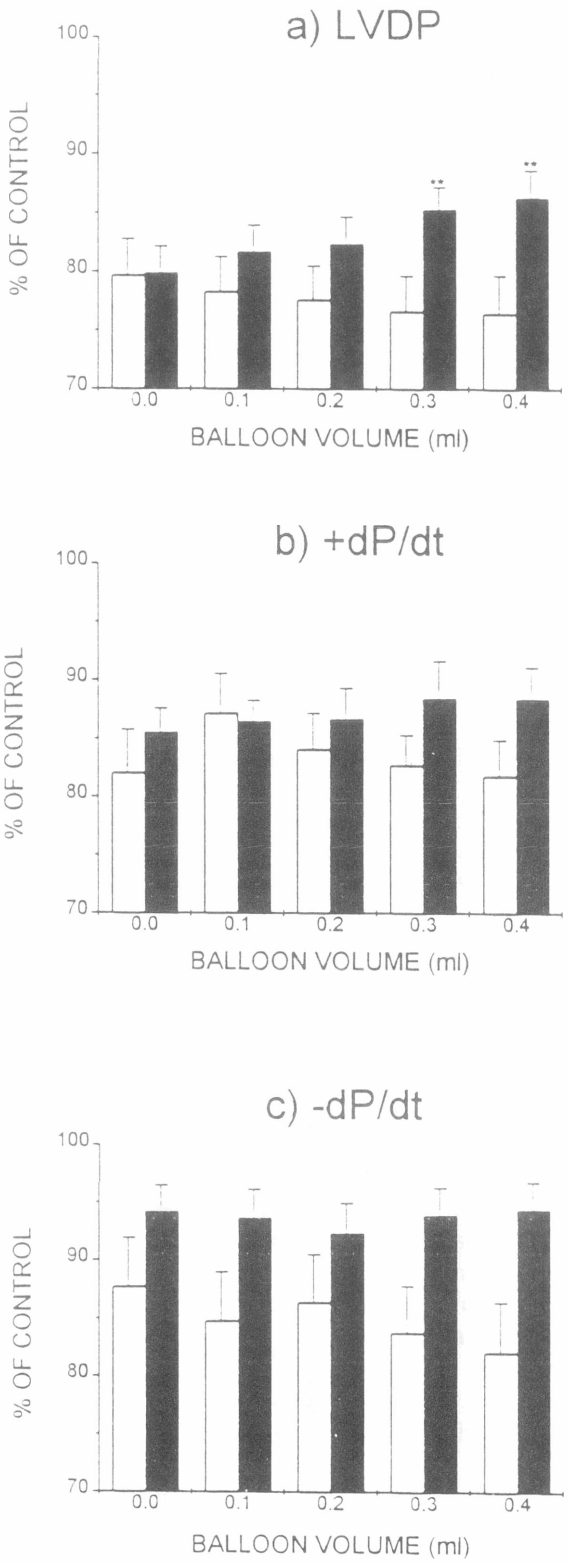

Fig. 1a–c
Recovery of LVDP, +dP/dt and −dP/dt for ST (open columns) and UW solutions (filled columns), in percent of control values, is plotted as a function of increments in left ventricular balloon volume. Each column represents means ± S.E.M. Between columns : ** = $p<0.01$, Fig. 1c : group statistic, $p< 0.05$.

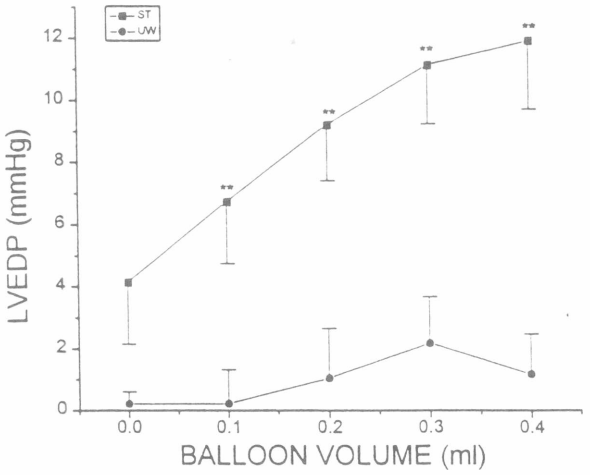

Fig. 2
Difference in LVEDP absolute values before and after hypothermic ischaemia is plotted as a function of increments in left ventricular balloon volume for ST and UW solutions. Data points are means ± S.E.M. Global ANOVA: ** = $p<0.01$.

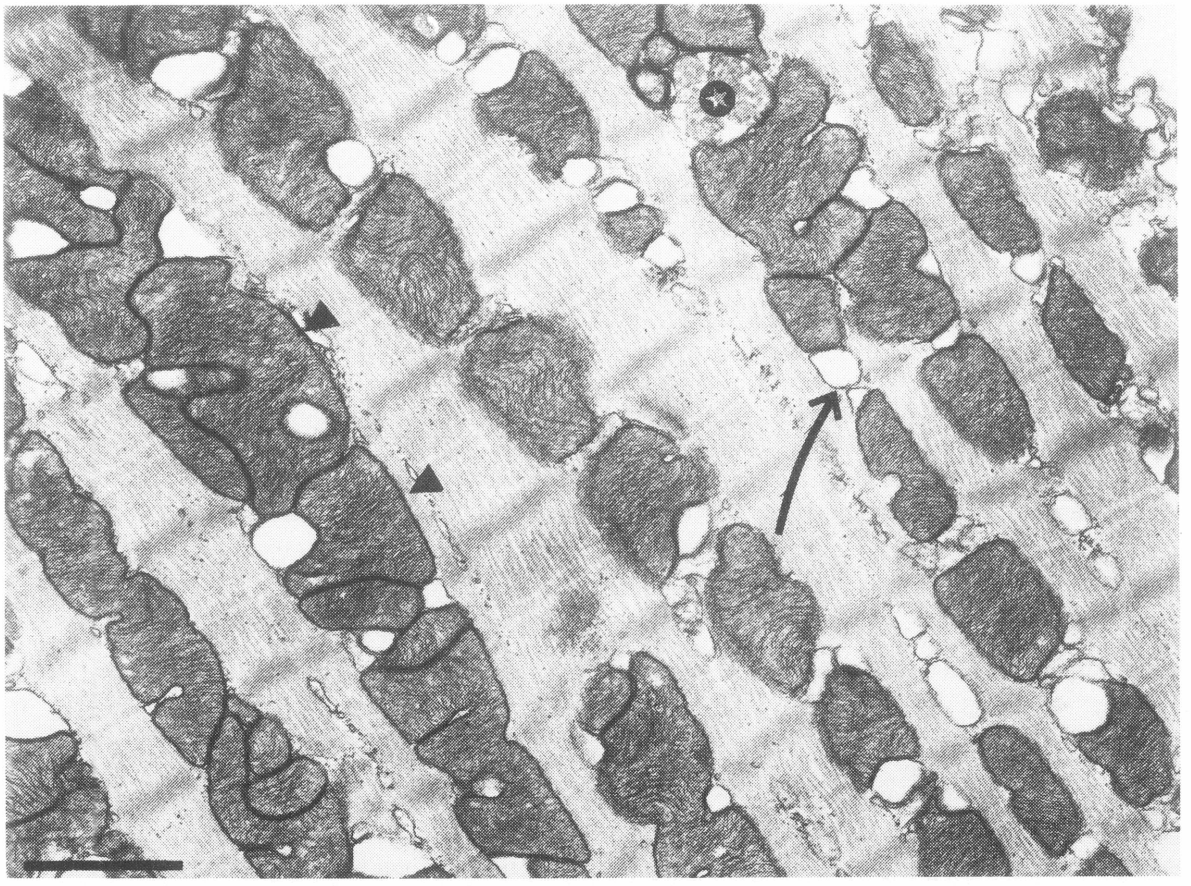

Fig. 3
Sample electron micrograph showing the ultrastructure of a rabbit heart after cold cardioplegia and reperfusion (UW solution). Sarcomeres are slightly contracted, mitochondria (triangles) are orderly packed between adjacent myofibrils, the mitochondrial matrix is normal and cristae are not dilated. Degenerating organelles are seldom observed (star). The tubular system of the myocyte is slightly dilated (curved arrows). In the upper right corner the sarcolemma with a distinct external lamina is visible. Hearts preserved with ST solution had a similar appearance. Bar = 1 μm.

Intercellular edema was present to some extent in all specimens, but it was never prominent. Irreversibly damaged cells were seldom observed. In the large majority of myocytes, the sarcolemma was in contact with the outermost myofibrillar component and mitochondria, and a distinct basal lamina was visible on the extracellular side. In longitudinal section, sarcomeres appeared well organized and partially contracted. The T-tubule system and sarcoplasmic reticulum were always slightly dilated. Glycogen stores appeared almost depleted in the majority of myocytes. Mitochondria were abundant and packed in the cells, nevertheless, the matricial space could slightly vary in different hearts. Cristae were thin, usually encompassing the entire width of the organelle. Electron dense granules were inconstantly found in mitochondria. The structure of intercalated disks was regular. A sample micrograph is shown in Fig. 3.

Discussion

The differences in heart preservation and recovery of cardiac function with the two solutions which have been the object of the present work could not be expected to be striking, because all the hearts were preserved at low temperature. Indeed, structural alterations were scanty and almost equally distributed among the two groups of hearts. To observe more extensive cell damage it might have been convenient to protract the preservation period beyond 4 h. However, we preferred to remain within limits presently accepted in human heart transplantation surgery, in order to obtain results of potentially practical usefulness. Indeed, despite the lack of ultrastructural evidence, we were able to demonstrate that the UW solution improves functional restoration, by allowing reduced edema and normal diastolic compliance. These two elements constitute the most evident difference between the treatments we have tested.

In clinical and experimental practice, hypothermia is considered the main protective agent during prolonged storage (Belzer and Jouthard 1988, Schmid *et al.* 1991). Although many studies on the physiology and biochemistry of the myocardium at low temperature have been done, a complete understanding of the cellular functions at low temperature is not yet available. During extended periods of hypothermic organ preservation with any of the fluids currently used as vehicles for cardioplegia, some degree of myocardial edema has always been reported (Hearse *et al.* 1981, Ko *et al.* 1992). Therefore, hypothermia has been combined with the administration of chemical components which provide additive protective effects on the ischaemic heart (Myers *et al.* 1986, de Jong *et al.* 1990, Ko *et al.* 1992). Some improvements in heart preservation have already been described with the UW solution (Ledingham *et al.* 1990, Yeh *et al.* 1990, Ko *et al.* 1992, Mankad *et al.* 1992), despite its original destination for organs other than the heart. Although the osmotic concentrations of the ST and UW solutions are similar (approximate osmolarities of 290 and 320 mosm/Kg H_2O, respectively), ST does not contain any component relatively impermeable to cell membranes, while in UW solution the correct osmolarity is attained by addition of raffinose and lactobionate, both impermeable to the cells, which limit intracellular edema, and pentastarch, which decreases interstitial edema. The combination of these molecules probably plays an important role in limiting myocardial edema, and in avoiding a large increase in diastolic stiffness. These observations are confirmed by the significantly lower water content of hearts in the UW group.

One of the earliest detectable abnormalities observed after hypothermic ischaemia and reperfusion is the diastolic dysfunction. Changes in diastolic wall thickness and compliance elicit direct mechanical effects which affect slightly postischaemic positive ventricular dP/dt (contractility), but affect especially negative dP/dt. This has been used as an index of left ventricular relaxation (Palacios *et al.* 1978). The better recovery of contractile and relaxation functions obtained with UW solution is most probably related to preservation of the calcium release-uptake cycle. Several authors reported that myofibrillar relaxation may be impaired more than contraction by altered calcium kinetics which occur during moderate or severe ischaemia (Palacios *et al.* 1978, Serizawa *et al.* 1981, Wexler *et al.* 1986).

The cellular mechanisms elevating cytosolic calcium during ischaemic-reperfusion injury have not been well defined, while it is well known that prompt calcium removal from the cytosol is required for normal muscle relaxation. Profound hypothermia (4 °C), is likely to protect effectively the calcium transport activity of the sarcoplasmic reticulum in the ischaemic myocardium up to 3 hours. Thereafter, however, the Ca^{2+}-transport system is markedly depressed (Fukumoto *et al.* 1990). Thus, the sarcoplasmic reticulum inevitably fails to remove calcium from the cytosol after repolarization, causing elevation of free calcium concentration, which allows some activation of the contractile proteins even during the diastole, with incomplete ventricular relaxation. On reperfusion, total cell calcium is more or less increased, depending on the compensation for the impairment of sarcoplasmic reticulum activity by sarcolemmal extrusion. Indeed, it is possible that the calcium influx upon reperfusion is caused by a variation in the permeability of sarcolemmal membranes, as a consequence of the activation of Ca^{2+}-dependent protease and phospholipase (Rao *et al.* 1983, McCord, 1985). Moreover, cell membranes may be damaged by the formation of oxygen-derived free radicals on

reoxygenation (Ferrari *et al.* 1985). The presence of such antioxidants as allopurinol and glutathione in UW solution may have contributed, at least in part, to the improved postischaemic recovery of left ventricular function in our study. Indeed, allopurinol when given in low doses inhibits xantine-oxidase, thus decreasing the formation of oxygen free-radicals by catalysis of hypoxantine (Myers *et al.* 1986, Chambers *et al.* 1987). This mechanism, however, is not likely to play a major role in rabbits, whose endothelial content of xantine-oxidase is scanty (Kopacz *et al.* 1993). On the other hand, since glutathione, one of the most abundant reducing agents normally present in the intracellular compartment (Tsan *et al.* 1985), decreases during myocardial ischaemia, there is a corresponding decrease in the activity of scavenging enzymes. Therefore, the presence of glutathione in the UW solution enhances protection of the cardiac cells against the oxidative stress.

In conclusion, we have shown that cardioplegia and preservation of the heart at low temperature up to 4 h with UW solution, in comparison with the currently used ST solution, limits myocardial edema, decreases cardiac stiffness and improves ventricular performance. It may be expected that under less favourable conditions, such as a longer ischaemic period, or a poorly controlled low temperature environment, the protective superiority of UW solution with respect to ST one would become even more evident.

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