Prolonging myocardial preservation with a modified University of Wisconsin solution containing 2,3-butanedione monoxime and calcium

The University of Wisconsin solution is an effective preservative for cold storage of the cardiac allograft. In an earlier study we showed that addition of calcium and 2,3-butanedione monoxime, a reversible inhibitor of myocardial contracture, further improved preservation of the rabbit heart. In this study we investigated the following: (1) the effects of different concentrations of 2,3-butanedione monoxime and calcium on function of the preserved rabbit heart, (2) how heart preservation is affected when 2,3-butanedione monoxime and calcium are added to the St. Thomas' Hospital and Stanford solutions, and (3) how 2,3-butanedione monoxime and calcium, at optimal concentrations in University of Wisconsin solution, affect hearts preserved up to 48 hours. Rabbit hearts were flushed with preservative and stored at 4° C for 24, 30, 40, or 48 hours. Myocardial function was assessed during 60 minutes of isolated reperfusion, and myocardial adenine nucleotide content was measured after completion of reperfusion. Three concentrations of 2,3-butanedione monoxime (15, 30, and 60 mmol/L) in the University of Wisconsin solution were studied in hearts preserved for 30 hours. Storage with 2,3-butanedione monoxime at 30 mmol/L resulted in significantly better left ventricular developed pressure ($p < 0.01$), left ventricular end-diastolic volume ($p < 0.01$), rate of left ventricular pressure rise ($p < 0.01$), coronary flow ($p < 0.05$), rate-pressure product ($p < 0.001$), and adenine nucleotide regeneration ($p < 0.05$) than with 60 mmol/L, although function was not significantly different when the osmolality of the solutions was equalized. There was significant reduction in end-diastolic volume ($p < 0.05$) and adenine nucleotide recovery ($p < 0.01$) when 2,3-butanedione monoxime was lowered to 15 mmol/L. Decreasing the calcium concentration from 1.0 to 0.1 mmol/L also had a deleterious effect on myocardial function ($p < 0.05$). The addition of 30 mmol/L 2,3-butanedione monoxime and 1.0 mmol/L calcium to the St. Thomas' or Stanford solutions improved preservation of the heart when compared with the unmodified solutions, but to a lesser degree than with the modified University of Wisconsin solution. After 24 to 48 hours of storage in University of Wisconsin solution containing 30 mmol/L 2,3-butanedione monoxime and 1.0 mmol/L calcium, there was substantial improvement in developed pressure ($p < 0.001$), end-diastolic volume ($p < 0.05$), and rate pressure product ($p < 0.001$), although there was little effect on heart rate and coronary flow, when compared with the unmodified University of Wisconsin solution. We conclude that modification of the University of Wisconsin solution with 30 mmol/L 2,3-butanedione monoxime and 1.0 mmol/L calcium significantly improves long-term preservation of the rabbit heart. Further extension in preservation might be accomplished by optimizing the osmolality of this modified solution. (*J THORAC CARDIOVASC SURG* 1994;107:764-75)

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Development of the University of Wisconsin solution has had a marked impact on the transplantation of intraabdominal organs. The kidney, liver, and pancreas can be successfully stored for extended periods,¹ allowing virtual coast-to-coast searches for suitable replacement organs and providing the time necessary to improve immunologic matching and decrease logistic and manpower needs. Most of these transplantations can now be performed on a semiselective basis. University of Wisconsin solution has virtually become the standard for intraabdominal organ preservation.

University of Wisconsin solution has also successfully preserved the human cardiac allograft,² but significant extension of the currently accepted limits for ischemic storage has not yet been realized in the clinical setting. Experimental studies have suggested, however, that heart storage with University of Wisconsin solution may safely increase the current limits of 4 to 6 hours up to 8 to 12 hours.^{3,4} Although this method is clearly an improvement over previous methods of myocardial cold storage, there still remains a rather stark contrast between this interval and the experimental storage intervals of 24 to 72 hours obtained for the kidney, liver, and pancreas.⁵⁻⁷

A major difference between prolonged ischemic storage of the heart and these other organs is the gradual development of myocardial contracture as a consequence of adenosine triphosphate depletion.⁸ We recently reported that modification of University of Wisconsin solution with calcium chloride and a reversible contractile inhibitor, 2,3-butanedione monoxime (BDM), resulted in elimination of ischemic contracture and resumption of near-normal myocardial function after 24-hour cold storage of the rabbit heart.⁹ It is still unclear, however, what the optimal concentration of BDM and calcium should be and whether equivalent protection is afforded by adding BDM and calcium to other commonly used preservation solutions. This study addresses these issues and quantitates the amount of myocardial protection afforded by BDM and calcium when preservation is extended beyond 24 hours.

Materials and methods

Experimental animals were treated humanely in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH publication No. 86-23, revised 1985).

Our experimental protocol has been previously described in detail⁹ and was used in this study with only minor modification. In brief, hearts were harvested from adult New Zealand White rabbits (1.6 to 2.7 kg) via a median sternotomy after receiving

intravenous ketamine anesthesia (25 mg/kg) and intravenous heparin (1000 U), and undergoing tracheostomy, chemical paralysis, and mechanical ventilation of the lungs. Hearts were excised after achieving mechanical arrest with an intravenous injection of KCl (4 mEq) and were immediately placed in 4° C storage solution. The coronary circulation was then flushed with 50 ml of storage solution at 4° C at a perfusion pressure of 50 cm H₂O.

After flushing, hearts were weighed, immersed in storage solution, surrounded with crushed ice, and stored in a refrigerated room at 4° C. The hearts of each experimental group were stored for 30 hours because previous storage for 24 hours resulted in near-control function,⁹ and differences between experimental groups were not readily apparent with the minor damage associated with 24 hours of cold ischemia. Control hearts were flushed but underwent no storage. All hearts were then mounted on a modified Langendorff column for reperfusion and functional assessment. Reperfusion was performed for 60 minutes at 37° C with a diastolic perfusion pressure of approximately 70 mm Hg. The perfusate consisted of a modified Krebs-Henseleit buffer containing NaHCO₃, 25 mmol/L; KH₂PO₄, 1.2 mmol/L; NaCl, 118 mmol/L; KCl, 4.7 mmol/L; MgSO₄, 1.2 mmol/L; CaCl₂, 2 mmol/L; dextrose, 10 mmol/L, and was bubbled to equilibration with 95% oxygen and 5% carbon dioxide (oxygen tension was 450 to 600).

Ventricular function was assessed with a fluid-filled left ventricular latex balloon, which approximated the normal rabbit ventricular volume when inflated to 1.0 ml. Hemodynamic data were continuously monitored on a multichannel recorder, and the maximum values attained during reperfusion were compared. Measurement of heart rate and systolic pressure was recorded at 15-minute intervals by inflating the balloon to an end-diastolic pressure of 20 mm Hg. The balloon was deflated to 0.1 ml between data collection points to avoid subendocardial ischemia from continuous pressure of the inflated balloon on the irregular inner ventricular contour. Left ventricular end-diastolic volume (LVEDV), an indicator of postischemic diastolic relaxation, was measured from the balloon volume that was necessary to create an end-diastolic pressure of 20 mm Hg. Coronary flow was determined at the same intervals by a 30-second collection of effluent from the heart, and the maximum rate of left ventricular pressure rise (dp/dt) was recorded by a direct differentiation of the pressure waveform.

Comparisons of systolic function were performed at constant end-diastolic pressure instead of constant LVEDV because of the variability in the size of hearts studied and the severe postischemic contracture that developed in some hearts. Pilot studies showed that, had measurements at constant LVEDV been attempted, supranormal pressures of 50 to 60 mm Hg would have been reached in hearts that had undergone contracture. These measurements would have resulted in mechanical stretching of the myocardium, thus making comparisons of either systolic or diastolic function under these circumstances unreliable and irreproducible on later measurements. Evaluation of systolic function at constant end-diastolic pressure allowed comparison under more physiologic conditions without mechanical damage to the myocardium but may have introduced a small degree of bias into interpretation of the data because of the variability in diastolic volumes.

Left ventricular developed pressure (LVDP) was calculated by subtracting end-diastolic pressure from systolic pressure. Coronary flow and LVEDV were corrected for myocardial mass

Table I. Composition of preservation solutions

University of Wisconsin solution	Concentration	St. Thomas' Hospital solution	Concentration	Stanford solution	Concentration
K-lactobionate	100 mmol/L	NaCl	110 mmol/L	Dextrose	50 gm/L
Raffinose	30 mmol/L	KCl	16 mmol/L	KCl	15 mmol/L
Na*	25 mmol/L	NaHCO ₃	10 mmol/L	NaHCO ₃	25 mmol/L
KH ₂ PO ₄	25 mmol/L	MgCl ₂	16 mmol/L	Mannitol	12.5 gm/L
Adenosine	5 mmol/L	CaCl ₂	1.2 mmol/L		
MgSO ₄	5 mmol/L				
Glutathione	3 mmol/L				
Allopurinol	1 mmol/L				
Hydroxyethyl starch	50 gm/L				
Insulin	100 U/L				
Dexamethasone	16 mg/L				
TMP-SMZ	160 mg-32 mg/L				
pH	7.4		7.8		8.1-8.4
Osmolarity	320 ± 5 mOsm/L		324 mOsm/L		440 mOsm/L

TMP-SMZ, Trimethoprim sulfamethoxazole.

*pH of University of Wisconsin solution adjusted with NaOH (25 mmol/L) and KOH as needed.

by dividing each value by the prepreservation heart weight. The rate-pressure product was calculated by multiplying the heart rate and LVDP. After 60 minutes of reperfusion, hearts were rapidly removed from the column and frozen in liquid nitrogen. The frozen myocardium was then crushed and stored at -70° C. Adenine nucleotide content was later determined in duplicate from these samples by high-performance liquid chromatography.⁹

Storage solutions (Table I) were prepared fresh in our laboratory and used within 24 hours. BDM was added to University of Wisconsin solution in concentrations of 15 mmol/L (Group 1), 30 mmol/L (Group 2), and 60 mmol/L (Group 3). Addition of 60 mmol/L BDM resulted in a calculated osmolarity of approximately 380 mOsm/L, which was based on the known osmolarity of standard University of Wisconsin solution of 320 mOsm/L. K⁺ and lactobionate concentrations were both reduced by 15 mmol/L (Group 4) to compensate for this discrepancy to create an osmolarity equivalent to that of Group 2—approximately 350 mOsm/L. Calcium concentration was compared at 1.0 mmol/L (Group 2) and 0.1 mmol/L (Group 5), with BDM concentration at 30 mmol/L in both groups. Control hearts were flushed with University of Wisconsin solution containing BDM 30 mmol/L and CaCl₂ 1.0 mmol/L and immediately reperfused. Groups 1 to 5, with their respective solution modifications, are listed in Table II. University of Wisconsin solution, with and without BDM 30 mmol/L and CaCl₂ 1.0 mmol/L, was compared with both the Stanford solution and the St. Thomas' Hospital cardioplegic solution, either alone or modified with BDM 30 mmol/L and CaCl₂ 1.0 mmol/L. The composition of Stanford solution¹⁰ and St. Thomas' Hospital solution¹¹ was described previously (Table I). Calcium was not added to the modified St. Thomas' Hospital solution because it normally contains CaCl₂ 1.2 mmol/L. Finally, standard University of Wisconsin solution was compared with modified University of Wisconsin solution containing BDM 30 mmol/L and CaCl₂ 1.0 mmol/L after 24, 30, 40, and 48 hours of preservation to assess the length of protection afforded by these modifications.

Results for each group are expressed as the mean ± standard error. Multiple group comparisons were performed by analysis

of variance with adjustment by the Bonferroni method. The means of two corresponding groups were compared by a two-tailed Student's *t* test. Statistical analysis was performed with Instat statistical software (Graphpad, San Diego, Calif.), and differences were considered significant at *p* < 0.05.

Results

After 30 hours of storage, all hearts defibrillated spontaneously and resumed a sinus rhythm. All groups exhibited some degree of myocardial dysfunction (Table II) as a result of the prolonged ischemic storage interval.

BDM concentration. The addition of 30 mmol/L BDM to University of Wisconsin solution resulted in superior postischemic function compared with other BDM concentrations tested (Fig. 1). LVDP returned to 75% of control compared with 66% of control with 15 mmol/L, and 41% with 60 mmol/L (*p* < 0.01). LVEDV returned to 74% of control with BDM 30 mmol/L, 38% with 15 mmol/L (*p* < 0.05), and 35% by adding BDM 60 mmol/L in the preservative (*p* < 0.01). Most hearts experienced some degree of sinus tachycardia that did not correlate with the concentration of BDM in solution. Heart rates ranged from 117% of control value with BDM 30 mmol/L, to 109% with 15 mmol/L, and 100% of baseline with 60 mmol/L. Thus, the use of BDM was associated with a pronounced effect on the automaticity and integrity of the conduction system, as bradycardia is often the result of ischemic myocardial damage and was not seen in any group.

Coronary flow was reduced in all hearts studied, returning to 69% of control in hearts stored with BDM 30 mmol/L, 60% with 15 mmol/L, and 38% for 60 mmol/L (*p* < 0.05 versus 30 mmol/L). dP/dt was also best with 30 mmol/L, resuming 78% of control value versus 64%

Fig. 1. Function of hearts cold stored 30 hours in University of Wisconsin solution modified with varying concentrations of BDM. Values shown represent the mean $\pm$ standard error for each group, as a percentage of control. Absolute values are shown in Table II. Statistical comparisons performed by analysis of variance, comparing each group that containing BDM 30 mmol/L. HR, Heart rate; CF, coronary flow; dP/dt, rate of left ventricular pressure rise; RPP, rate pressure product. * $p < 0.05$, † $p < 0.01$, ‡ $p < 0.001$.

Table II. Function of hearts cold stored 30 hours in University of Wisconsin solution modified with BDM and calcium

	Control (n = 7)	Group 1 (n = 6)	Group 2 (n = 7)	Group 3 (n = 7)	Group 4 (n = 6)	Group 5 (n = 6)
BDM (mmol/L)	30	15	30	60	60*	30
Ca (mmol/L)	1.0	1.0	1.0	1.0	1.0	0.1
LVDP (mm Hg)	121.0 $\pm$ 3.8	79.5 $\pm$ 8.8	91.1 $\pm$ 5.4	49.0 $\pm$ 8.7	80.0 $\pm$ 7.9	60.0 $\pm$ 9.3
LVEDV (μ l/gm)	227.4 $\pm$ 6.7	84.8 $\pm$ 18.4	167.1 $\pm$ 20.9	78.9 $\pm$ 15.6	140.0 $\pm$ 15.7	93.2 $\pm$ 37.1
HR (min ⁻¹)	191 $\pm$ 7	208 $\pm$ 18	224 $\pm$ 5	191 $\pm$ 8	231 $\pm$ 18	192 $\pm$ 24
CF (ml/gm/min)	12.6 $\pm$ 0.9	7.6 $\pm$ 0.8	8.7 $\pm$ 0.8	4.8 $\pm$ 0.7	7.7 $\pm$ 0.6	7.8 $\pm$ 0.3
dP/dt (mm Hg/sec)	2097 $\pm$ 75	1348 $\pm$ 160	1646 $\pm$ 142	761 $\pm$ 167	1190 $\pm$ 129	933 $\pm$ 143
RPP (mm Hg/min)	23100 $\pm$ 1376	16138 $\pm$ 1684	20474 $\pm$ 1376	9596 $\pm$ 1984	18114 $\pm$ 1806	10863 $\pm$ 1704

Control hearts were immediately reperfused without storage. Values represent the mean $\pm$ standard error for each group. HR, Heart rate; CF, coronary flow; RPP, rate pressure product.

*Reduced osmolality by 30 mmol/L.

with 15 mmol/L and 36% with 60 mmol/L ($p < 0.01$). Rate-pressure product followed the same trend, with 89% of control with 30 mmol/L, compared with 70% with 15 mmol/L, and only 42% with 60 mmol/L ($p < 0.001$).

With respect to regeneration of adenine nucleotide stores on reperfusion, total adenine nucleotides, adenosine triphosphate, adenosine diphosphate, and adenosine monophosphate content all returned to control values when hearts were stored 30 hours in the presence of BDM 30 mmol/L (Fig. 2). In contrast, storage with BDM 15 mmol/L resulted in return of only 43% of adenosine triphosphate content ($p < 0.01$) and 65% of total adenine nucleotides content ($p < 0.01$). Storage with BDM 60

mmol/L produced recovery of 68% of adenosine triphosphate and 74% of total adenine nucleotides content ($p < 0.05$).

Osmolality. When the osmolality of the solution containing BDM 60 mmol/L was reduced from 380 to 350 mOsm/L, to equal that containing BDM 30 mmol/L, LVDP returned to 66%, LVEDV to 62%, heart rate to 121%, coronary flow to 61%, dP/dt to 57%, and rate-pressure product to 78% of control values (Fig. 3). This was a marked improvement over hearts stored with BDM 60 mmol/L, but at a higher osmolality, and reached statistical significance in LVDP, coronary flow, and rate-pressure product ($p < 0.05$). In addition, these values

Fig. 2. Adenine nucleotide regeneration after 30 hours of cold storage in University of Wisconsin solution with varying concentrations of BDM and 60-minute reperfusion. Values shown represent the mean $\pm$ standard error for each group. Statistical analysis performed by analysis of variance, comparing each group to that containing BDM 30 mmol/L. Control values are shown for reference only; $n = 7$ for controls, 6 for 15 mmol/L, 10 for 30 mmol/L, and 7 for 60 mmol/L BDM. *ATP*, Adenosine triphosphate; *ADP*, adenosine diphosphate; *AMP*, adenosine monophosphate; *TAN*, total adenine nucleotides. * $p < 0.05$, $^{\dagger}p < 0.01$.

Fig. 3. Function of hearts after 30 hours of cold storage in University of Wisconsin solution modified with BDM 60 mmol/L and CaCl_2 1.0 mmol/L at varying osmolarity. Values shown represent the mean $\pm$ standard error for each group, as a percentage of control. Absolute values shown in Table II; * $p < 0.05$. See Fig. 1 for abbreviations.

were only slightly less, but not significantly different, than those obtained by storage with BDM 30 mmol/L. The nucleotide regeneration capacity of this group was also improved, regaining 112% of control adenosine triphosphate, and 96% of control total adenine nucleotides (Fig. 4). Adenosine diphosphate and adenosine monophosphate, however, remained at similar concentrations.

Calcium concentration. Micromolar amounts of calcium were insufficient to protect the myocardium during prolonged storage. Hearts stored with CaCl_2 0.1 mmol/L

in solution recovered only 50% of control LVDP, 41% of LVEDV, 62% of coronary flow, 45% of dP/dt, and 47% of rate-pressure product but maintained 100% of baseline heart rate (Fig. 5). Storage with CaCl_2 1.0 mmol/L resulted in superior function, with 75% of control LVDP ($p < 0.01$), 74% of LVEDV ($p < 0.05$), 117% of heart rate ($p = \text{not significant}$), 69% of coronary flow ($p = \text{not significant}$), 78% of dP/dt ($p < 0.01$), and 89% of RPP ($p < 0.001$).

Regeneration of adenine nucleotides was also reduced

Fig. 4. Myocardial adenine nucleotide content after 30 hours of cold storage in University of Wisconsin solution containing BDM 60 mmol/L and CaCl_2 1.0 mmol/L at varying osmolarity and 60-minute reperfusion. Values shown represent the mean $\pm$ standard error for each group. Experimental groups were compared by analysis of variance. Control values shown for reference only; $n = 7$ for controls, 7 for 380 mOsm/L, and 6 for 350 mOsm/L, * $p < 0.05$. See Fig. 2 for abbreviations.

Fig. 5. Function of hearts after 30 hours of cold storage in University of Wisconsin solution modified with BDM 30 mmol/L and CaCl_2 , either 1.0 mmol/L or 0.1 mmol/L. Values shown represent the mean $\pm$ standard error for each group, calculated as a percentage of control. Absolute values are shown in Table II; * $p < 0.05$, † $p < 0.01$, ‡ $p < 0.001$. See Fig. 1 for abbreviations.

with CaCl_2 0.1 mmol/L (Fig. 6), reaching 47% of control adenosine triphosphate, and 65% of total adenine nucleotides. In contrast, hearts exposed to CaCl_2 1.0 mmol/L during storage regained 106% of baseline adenosine triphosphate ($p < 0.05$) and 117% of total adenine nucleotide content ($p < 0.01$).

Preservation solutions. Preservation solutions modified with BDM 30 mmol/L and CaCl_2 1.0 mmol/L were

found to be consistently superior in preservation capability when compared with their respective unmodified counterparts (Table III). After 30 hours of storage, hearts preserved in University of Wisconsin solution containing BDM and calcium recovered superior LVDP ($p < 0.0001$), LVEDV ($p < 0.0001$), heart rate ($p < 0.001$), dP/dt ($p < 0.0001$), and rate-pressure product ($p < 0.0001$) than did those with standard University of

Fig. 6. Myocardial adenine nucleotide regeneration after 30 hours of cold storage in University of Wisconsin solution containing BDM 30 mmol/L and CaCl₂, either 1.0 mmol/L or 0.1 mmol/L. Values shown represent the mean $\pm$ standard error for each group. Experimental groups were compared by analysis of variance. Control values shown for reference only; $n = 7$ for controls, 10 for CaCl₂ 1.0 mmol/L, and 6 for CaCl₂ 0.1 mmol/L; * $p < 0.05$, † $p < 0.01$. See Fig. 2 for abbreviations.

Table III. Function of hearts cold stored 30 hours in commonly used preservation solutions with and without BDM and calcium

	University of Wisconsin solution ($n = 8$)	Modified University of Wisconsin solution ($n = 7$)	St. Thomas' Hospital solution ($n = 6$)	Modified St. Thomas' Hospital solution ($n = 7$)	Stanford solution ($n = 6$)	Modified Stanford solution ($n = 6$)
LVDP (mm Hg)	37.0 $\pm$ 8.1	91.1 $\pm$ 5.4*	34.2 $\pm$ 10.8	79.1 $\pm$ 8.2†	20.8 $\pm$ 4.8	49.3 $\pm$ 12.6
LVEDV (μ l/gm)	31.9 $\pm$ 4.8	167.1 $\pm$ 20.9*	47.2 $\pm$ 15.3	150.1 $\pm$ 11.8‡	28.0 $\pm$ 4.5	74.8 $\pm$ 25.4
HR (min^{-1})	181 $\pm$ 7	224 $\pm$ 5‡	180 $\pm$ 13	197 $\pm$ 6	172 $\pm$ 16	171 $\pm$ 16
CF (ml/gm/min)	6.8 $\pm$ 0.6	8.7 $\pm$ 0.8	6.3 $\pm$ 0.4†	5.0 $\pm$ 0.1	7.2 $\pm$ 0.8	7.1 $\pm$ 0.6
dP/dt (mm Hg/sec)	474 $\pm$ 115	1646 $\pm$ 142*	489 $\pm$ 148	1316 $\pm$ 141†	293 $\pm$ 76	773 $\pm$ 224
RPP (mm Hg/min)	6500 $\pm$ 1569	20474 $\pm$ 1376*	5625 $\pm$ 1574	15799 $\pm$ 1839†	3692 $\pm$ 1099	8883 $\pm$ 2403

See Methods section. Values represent the mean $\pm$ standard error for each group. Statistical comparisons were performed between each solution and its respective modified counterpart with Student's t test. HR, heart rate; CF, coronary flow; dP/dt, rate of left ventricular pressure rise; RPP, rate pressure product.

* $p < 0.0001$.

† $p < 0.01$.

‡ $p < 0.001$.

Wisconsin solution. Coronary flow in this group was slightly but not significantly higher than that with the unmodified solution.

In hearts preserved with St. Thomas' Hospital solution, addition of BDM resulted in better LVDP ($p < 0.01$), LVEDV ($p < 0.001$), dP/dt ($p < 0.01$), and rate-pressure product ($p < 0.01$). Heart rate was unchanged, and coronary flow remained slightly more depressed ($p < 0.01$) than with unmodified St. Thomas' Hospital solution. When Stanford solution was the preservative, LVDP, LVEDV, dP/dt, and rate-pressure product were all improved with addition of BDM and calcium, but

these differences did not reach statistical significance. Heart rate and coronary flow were unchanged.

Myocardial adenine nucleotide content was improved only after storage in modified University of Wisconsin solution (Fig. 7). Adenosine triphosphate recovery improved from 8.8 to 19.0 nmol/mg protein ($p < 0.001$), adenosine diphosphate from 5.5 to 12.8 nmol/mg protein ($p < 0.001$), adenosine monophosphate from 1.1 to 5.2 nmol/mg protein ($p < 0.001$), and total adenine nucleotides from 15.3 to 37.1 nmol/mg protein ($p < 0.0001$) with storage in University of Wisconsin solution and modified University of Wisconsin solution, respectively.

Fig. 7. Adenine nucleotides regenerated after 30 hours of cold storage in solutions with and without BDM 30 mmol/L and CaCl_2 1.0 mmol/L and subsequent 60 minutes of reperfusion. Values shown represent the mean $\pm$ standard error for each group. Each solution was compared with its respective modified counterpart by Student's *t* test; *n* = 6 for University of Wisconsin solution (UWS), 10 for modified University of Wisconsin solution (mUWS), 6 for St. Thomas' Hospital solution (STS), 7 for modified St. Thomas' Hospital solution (mSTS), 6 for Stanford solution (STNF), and 6 for modified Stanford solution (mSTNF). **p* < 0.001, †*p* < 0.0001. See Fig. 2 for other abbreviations.

Although minor differences existed with the other solutions, none of these reached statistical significance.

When comparisons were made among the three modified solutions, modified University of Wisconsin solution was consistently superior to modified St. Thomas' Hospital solution, and modified St. Thomas' Hospital solution was superior to modified Stanford solution. Differences between modified University of Wisconsin solution and modified St. Thomas' Hospital solution, however, reached statistical significance only when comparing coronary flow (*p* < 0.01) and adenine nucleotide content (adenosine triphosphate: 19.0 ± 1.3 versus 10.9 ± 1.6 ; *p* < 0.01; total adenine nucleotides 37.1 ± 2.6 versus 20.8 ± 1.7 ; *p* < 0.001). Hearts stored in modified St. Thomas' Hospital solution performed better than those stored in modified Stanford solution in all parameters except coronary flow, but reached statistical significance only in adenosine triphosphate concentration (20.8 ± 1.7 versus 4.2 ± 0.6 ; *p* < 0.05). Storage in modified University of Wisconsin solution resulted in better performance than storage in modified Stanford solution in LVDP (*p* < 0.05), LVEDV (*p* < 0.05), heart rate (*p* < 0.01), dP/dt (*p* < 0.05), rate-pressure product (*p* < 0.01), and adenine nucleotide recovery (adenosine triphosphate: 19.0 ± 1.3 versus 4.2 ± 0.6 ; *p* < 0.001; total adenine nucleotides: 37.1 ± 2.6 versus 12.1 ± 2.0 ; *p* < 0.001).

Duration of protection. Hearts stored in standard University of Wisconsin solution demonstrated loss of

function after 24 hour storage, whereas storage in modified University of Wisconsin solution resulted in substantially improved function which gradually decreased as storage was prolonged to 48 hours (Fig. 8). LVDP dropped to 32%, 32%, 17%, and 9% of control after 24, 30, 40, and 48 hours of storage in University of Wisconsin solution but fell to only 81% (*p* < 0.001), 75% (*p* < 0.0001), 61% (*p* < 0.001), and 56% (*p* < 0.001) of control after 24, 30, 40, and 48 hours of storage in modified University of Wisconsin solution, respectively. Similar differences in function were observed in LVEDV for modified University of Wisconsin solution compared with University of Wisconsin solution at 24 hours (94% versus 36%, *p* < 0.001), 30 hours (74% versus 15%, *p* < 0.0001), 40 hours (51% versus 10%, *p* < 0.01), and 48 hours of storage (34% versus 7%, *p* < 0.01). This pattern was also evident in rate-pressure product for modified University of Wisconsin solution compared with University of Wisconsin solution at 24 hours (100% versus 33%, *p* < 0.001), 30 hours (89% versus 27%, *p* < 0.0001), 40 hours (65% versus 17%, *p* < 0.001), and 48 hours of storage (58% versus 7%, *p* < 0.0001).

Differences in other parameters, however, were not as dramatic. Heart rate after storage in modified University of Wisconsin solution was higher than that of control at all intervals of storage but was significantly higher than hearts stored in University of Wisconsin solution only at 24 and 30 hours of storage (*p* < 0.001). In addition, cor-

Fig. 8. Function of hearts stored for 0, 24, 30, 40, and 48 hours with University of Wisconsin solution with and without BDM 30 mmol/L and CaCl_2 1.0 mmol/L. Groups were compared at each time point by Student's *t* test; *n* for University of Wisconsin solution = 6 at 0 hours, 6 at 24 hours, 8 at 30 hours, 6 at 40 hours, and 6 at 48 hours; *n* for modified University of Wisconsin solution = 7 at 0 hours, 6 at 24 hours, 7 at 30 hours, 8 at 40 hours, and 6 at 48 hours; **p* < 0.05, †*p* < 0.001, ‡*p* < 0.0001. See Figs. 1 and 7 for abbreviations.

onary flow decreased in both groups at nearly an equivalent rate, remaining only slightly higher in hearts stored in modified University of Wisconsin solution at 24 hours (83% versus 53%) and 30 hours (65% versus 46%), differences which did not reach statistical significance.

Discussion

Current myocardial preservation strategies of cardioplegic arrest, hypothermic storage, and short ischemic intervals are aimed primarily at preserving levels of high-energy phosphate compounds and avoiding the devastating effects of myocardial energy depletion. The liver, kidney, and pancreas have been stored for extended periods with the University of Wisconsin solution¹ without regard to energy levels. Successful preservation of the heart, however, is much more sensitive to falling levels of high-energy compounds, mainly because of the development of myocardial contracture as energy levels are depleted.¹² Our approach in this study was not aimed at preserving adenosine triphosphate, but rather at preventing the deleterious myocardial events that result from energy depletion during extended storage.

BDM was selected as an adjunct to the University of Wisconsin solution because it reversibly inhibits myofilament contraction in cardiac muscle.¹³ BDM has also been shown to inhibit activity of the myofibrillar adenosinetriphosphatase,¹⁴ and to preserve high-energy phos-

phates during myocardial ischemia.⁹ This probably is a result of BDM's inherent phosphatase activity,¹⁵ which appears to spare adenine nucleotides but may affect regulatory proteins which require phosphorylation to remain active in intracellular metabolism. In addition, Gwathmey, Hajjar, and Solaro¹⁶ have shown BDM to have inhibitory effects on intracellular calcium transients, and Wiggins and colleagues¹⁷ have reported that BDM inhibits sarcolemmal calcium channels. These effects might result in amelioration of the intracellular calcium overload associated with myocardial stunning and ischemic damage.

We have shown in this study that myocardial preservation is optimized when the University of Wisconsin solution is modified with BDM 30 mmol/L. Without BDM in the preservative, myocardial function was depressed by approximately 50%. Addition of only 15 mmol/L produced insufficient protection, resulting in poor contractile function and severe contracture. When BDM concentration was raised to 60 mmol/L, myocardial function was also diminished. Lowering the osmolarity of the 60 mmol/L group, however, resulted in markedly improved myocardial function, tending to be slightly (but not significantly) less than with 30 mmol/L. Other than injury from hyperosmolarity, another possible reason for this effect is the reduction in either lactobionate or potassium concentration, which was used to decrease the

osmolality of the solution. It is improbable, however, that this was the cause of the improved function because the change was small (15 mmol/L), and the concentrations of these ions remained high (lactobionate at 85 mmol/L, K^+ at 115 mmol/L).

These data indicate that a BDM concentration of at least 30 mmol/L is necessary for extended myocardial preservation, and higher concentrations appear to be no more effective at protecting the heart from ischemic damage. The higher osmolality resulting from addition of other compounds to the University of Wisconsin solution, however, is detrimental to the ischemic heart. The sensitivity of these hearts to changes in osmolality is in agreement with the findings of Batty and colleagues,¹⁸ who reported that hyperosmolality of a storage solution is detrimental to myocardial preservation. Armstrong and Ganote¹⁹ have also suggested that BDM may render the myocyte more susceptible to osmotic stress. The correct osmolality of a solution containing BDM therefore appears to be extremely important in preservation quality. A more detailed study of the osmolality of this modified solution may lead to further improvement in myocardial preservation.

The optimal concentration of calcium for myocardial preservation has remained controversial. Proponents of adding calcium to cardioplegic solutions have suggested that the calcium paradox may occur with storage in acalcemic solutions, and the addition of calcium results in superior postischemic myocardial function.²⁰ More recent reports have argued that only micromolar amounts of calcium (50 to 100 μ mol/L) are necessary for prevention of this phenomenon, and higher concentrations can cause increased energy expenditure and structural myocardial contracture.²¹⁻²⁴ In fact, improvement in 7-hour heart preservation with the University of Wisconsin solution containing calcium 0.25 mmol/L has been reported.²⁵ The calcium paradox, however, has not been observed as a clinically important problem in cardiac surgery, which is probably due to other factors that mitigate the absence of calcium, such as moderate hypothermia,²⁶ trace calcium contamination of cardioplegic solutions, or noncoronary collateral blood flow, which may provide adequate calcium to the sarcolemma. Rich and Langer²¹ have reported, however, that as the calcium-free exposure time is prolonged, hypothermia becomes increasingly less effective at preventing the calcium paradox. With extended intervals of ischemic storage of approximately 24 to 48 hours and in an isolated environment, the calcium paradox may become more of a problem for the cardiac allograft than has been recognized in the past.

We have previously reported evidence strongly sugges-

tive of a calcium paradox occurring after 24 hours of cold storage.⁹ Systolic dysfunction, severe myocardial contracture, and failure of diastolic relaxation were observed on reperfusion when rabbit hearts were stored in University of Wisconsin solution modified with BDM in the absence of calcium. These phenomena were completely reversed by the addition of calcium to the preservative. We also found, however, that the presence of calcium alone (without BDM) caused accelerated loss of high-energy phosphates and worsened myocardial contracture during the storage period. These sequelae were completely inhibited by the addition of BDM to the solution.

It appears, therefore, that calcium is a necessary component of a preservative for extended myocardial storage but will be effective only if inhibition of calcium-sensitive adenosine triphosphatases can be provided by a substance such as BDM. Although we did not perform an in-depth dose-response study of calcium concentrations, our data indicate that micromolar amounts of calcium are insufficient for extended myocardial preservation with this solution, which is likely due to the calcium-chelating effect of lactobionate, an integral component of the University of Wisconsin solution. Lactobionate may act as a "calcium buffer," allowing only minute amounts of free ionized calcium to bathe and diffuse through the sarcolemma. In fact, the free ionized calcium content of the University of Wisconsin solution containing $CaCl_2$ 1.0 mmol/L has been measured in our laboratory to be approximately 0.1 mmol/L (unpublished data). Similar results of the calcium-chelating properties of the University of Wisconsin solution have been recently published.²⁷

If modification with BDM and calcium is able to potentiate the preservation capability of the University of Wisconsin solution, then a similar effect should be apparent with other preservation solutions. We have shown, in fact, that preservation is significantly enhanced in both the St. Thomas' Hospital solution and the Stanford solution with addition of BDM and calcium. Hearts stored with the modified University of Wisconsin solution, however, exhibited significantly better preservation of function and regeneration of adenine nucleotides than did those stored in either of the other modified solutions. These findings are not surprising in light of previous reports that have shown the University of Wisconsin solution to be superior to either the Stanford solution^{28, 29} or the St. Thomas' Hospital solution^{30, 31} for hypothermic myocardial preservation of shorter duration. This may be due in part to the components in the University of Wisconsin solution, which protect the cell from ischemic damage, such as cellular impermeants which reduce cell swelling (lactobionate, raffinose), a vascular impermeant which protects from interstitial edema (hydroxyethyl

starch), precursors of high-energy phosphates for improved regeneration (adenosine, phosphate), antioxidants to ameliorate reperfusion injury caused by generation of oxygen free radicals (glutathione, allopurinol), and an "intracellular" ionic composition to reduce energy expenditure and a physiologic intracellular accumulation of ions as a consequence of diffusion. BDM has only potentiated the preservation capabilities of these components by adding its own protective effect specific for muscular tissue.

We also showed in this study that hearts stored in this modified University of Wisconsin solution were able to withstand prolonged periods of ischemic cold storage. Function was maintained at 80% to 100% of control at 24 hours, 70% to 89% at 30 hours, and 50% to 65% after 40 hours of cold storage. After 48 hours of storage, hearts stored in modified University of Wisconsin solution had function superior to hearts stored in standard University of Wisconsin solution after only 24 hours of storage. These findings do not indicate, however, that a blood-reperfused transplanted heart would function adequately after such prolonged ischemia. Further studies of an orthotopically transplanted cardiac allograft, which is able to support the recipient circulation for extended periods, will be necessary to demonstrate the importance of our findings.

Several other limitations of this study should be addressed. Although we have been able to substantially improve myocardial contractility, diastolic relaxation, and mitochondrial function, we have done little to eliminate the progressive deterioration of coronary flow observed during reperfusion. Hearts stored in either modified or standard University of Wisconsin solution demonstrated nearly identical reduction in coronary flow. Current study of endothelial and microvascular ischemic dysfunction may soon reveal methods of overcoming this problem. In addition, maintenance of postischemic heart rates above control levels is not necessarily beneficial. This finding may suggest that hearts stored in the presence of BDM may be prone to develop supraventricular tachycardia and catecholamine hypersensitivity, effects which may be attributable to alteration in membrane channels or intracellular regulatory proteins. Additional studies on these effects of BDM may be necessary before optimization of extended preservation with BDM can be attained.

In conclusion, the University of Wisconsin solution modified with BDM 30 mmol/L and CaCl_2 1.0 mmol/L is effective for long-term preservation of the heart in this experimental model. Preservation might be further improved by optimization of the solution osmolality. Further study of these and other solution characteristics

is warranted to tailor the preservative specifically to the needs of the ischemic heart. With modifications such as these, truly successful long-term myocardial preservation may eventually become a reality.

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