NMR Studies of Long-Term Hypothermic Preservation of Porcine Hearts: Effect of Slow Perfusion on High Energy Phosphates and Developed Force

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INTRODUCTION

Hypothermic preservation of cardiac tissue destined for transplant is currently limited to approximately 4-5 hours [1-3]. In order to increase the allowable time between harvest and transplant of the donor organ a number of strategies including optimization of preservation temperature, composition of cardioplegic preservation solutions as well as preservation modality are currently under investigation [4].

We have been using ^{31}P and ^{1}H nuclear magnetic resonance (NMR) spectroscopy to gain a better understanding of energetics in preserved human [5,6] and porcine [7] myocardium. In this study, we used ^{31}P NMR to monitor the high energy phosphates of hypothermic pig heart preserved by slow perfusion at 5° or 12°C. We have correlated the results of these studies with biochemical assays and performance upon reperfusion with Krebs-Henseleit solution.

The results of these studies have shown that although preservation of high energy phosphates is improved with a slowly flowing hypothermic cardioplegic solution [8,9], the rate-pressure products (RPP = heart rate x developed pressure) are significantly depressed compared to hearts freshly perfused with Krebs-Henseleit solution. We have not observed any difference in RPP of hearts preserved at 5°C or 12°C for up to 12 hours. The functional depression in the preserved hearts could in part be due to edema formation during the preservation period which limits oxygen availability to the heart.

METHODS AND MATERIALS

Surgical Procedures

Pigs (10-20 kg) were obtained from Agriculture Canada. The animals were pre-sedated with Diazepam (i.m. 0.5 mL of a solution containing 5 mg/mL) and anesthetized with Ketamine (i.m. 2-5 mL of a solution containing 100 mg/mL). Fluorothane, 3% initially, was given by mask and maintained at 1.25% in a 50% mix of O_2/N_2 with a flow of 2 L/min. The pig was intubated. The heart was exposed via a medial sternotomy and the brachiocephalic trunk was dissected approximately 1 inch from the aorta. Two thousand units of heparin were injected into the inferior vena cava. To arrest the heart Plegisol with 10 mM additional KCl (Table I) was injected via the brachiocephalic artery at a pressure of 260 mm Hg for one minute. The total volume was 200-300 mL. The chest cavity was filled with cold saline which was removed by suction and the heart excised. The aorta was joined to a cannula attached to the removable top portion of the NMR probe for Langendorff perfusion. A balloon catheter was inserted into the left

Table I

Solutions for Pig Heart Preservation and Reperfusion

	Plegisol	Slow flow preservation	Krebs-Henseleit	
NaCl	110.0	136.4	118.0	mM
$NaHCO_3$	10.0	-	20.0	"
KCl	26.0	-	4.7	"
KH_2PO_4	-	1.8	1.2	"
K_2HPO_4	-	6.4	-	
$MgSO_4$	-	14.1	0.6	"
$MgCl_2$	16.0	-	-	
$CaCl_2$	1.2	1.1	1.8	"
glucose	-	11.1	11.0	"
sucrose	-	7.3	-	
glycerol	-	136.8	-	
taurine	-	4.0	-	
procaine HCl	-	1.0	-	g/L
chloropromazine	-	3.1	-	mg/L
phenoxybenzamine	-	2.5	-	mM
Dextran		-	-	2%
pH	7.8	7.0	7.4	

ventricle and secured with a purse string suture placed around the mitral valve. The pulmonary artery was vented. A catheter and a thermocouple were placed in the right atrium to monitor coronary perfusate and temperature. The left ventricular balloon was inflated to 10 mm Hg. The heart was placed in a plastic bag and transported on ice to the NMR facility. Usually 1-1.5 hours elapsed between severing the aorta and acquisition of the first NMR spectrum. During this time the heart was kept in Plegisol and on ice. Unless otherwise stated, preservation time begins the moment the aorta is severed.

Heart Preservation

Slow flow conditions were established in a separate series of experiments in which the flow rate of the preservation solution (Table I) to the heart was varied while ATP and phosphocreatine (PCr) were monitored. It was found that at least 12 mL per minute were required to stabilize the PCr/β-ATP at a normal value (between 2 and 3). Heart temperature was recorded with a thermocouple adjacent to the septum. The heart was kept at the desired temperature by passing the preservation solution through a heat exchanger prior to entering the magnet and by passing cool N_2 through a jacket surrounding the heart chamber. Heart temperature, coolant temperature, line pressure and balloon pressure were continuously recorded at 10 minute intervals. pH of the perfusate was adjusted to 7.0 prior to the experiment and was not readjusted during the storage procedure. The slow flow solution was continuously bubbled in the storage reservoir with 100% O_2.

NMR Spectroscopy

All experiments were performed with a Bruker BMT 47/30 BIOSPEC spectrometer. The heart was suspended in an 8 cm diameter single turn solenoid coil with 4 loops in parallel double-tuned to phosphorus and protons (Morris Instruments Inc.). The proton free-induction decay was observed to shim the magnet. Phosphorus free-induction decays were acquired with a cyclops phase

sequence, the excitation pulse adjusted for maximum signal (typically 200 μsec) and a five second recycle time. Signals were averaged for five or ten minutes. A spherical sample of a phosphonate standard affixed to the heart was used for calibration. Since the entire heart was within the coil, the signal intensity was a measure of the total amount of a compound in the heart. The amount of the various phosphate compounds was measured from the heights of the peaks in the spectra transformed with a 20 to 30 Hz line-broadening.

The T_1 values (±S.D.) determined in pig hearts perfused with Krebs-Henseleit solution (Table I) at 37°C were as follows: P_i 3.4±0.8 s. (n=4), phosphocreatine (PCr) 4.2±1.1 s (n=4), and ß-ATP 1.3±0.4 s (n=4). In hypothermically preserved hearts, the T_1 values for P_i, PCr and ß-ATP were 3.7±0.4 s. (n=5), 4.1 s (n=1) and 1.7±0.3 s. (n=5), respectively.

Chemical shifts were measured with respect to that of PCr at -2.52 ppm. pH values were determined from the chemical shift of P_i as described previously [6].

Functional Assessment

Hearts were reperfused with a modified Krebs-Henseleit solution. Hearts were slowly rewarmed from the storage temperature to 36°C using the Krebs-Henseleit solution over a 45-60 minute period. Viable hearts generally showed ventricular activity at 26°C. Initial perfusion pressure was 50 mm Hg and was increased to 90-100 mm Hg once rhythmic ventricular activity was achieved. Fibrillating hearts were removed from the magnet for cardioversion. The Krebs-Henseleit solution was bubbled continuously with 95% O_2, 5% CO_2 to maintain a buffer pO_2 of 500-600 mm Hg and a pH of 7.3-7.4 at 37°C. Coronary sinus samples were obtained from a catheter placed in the right ventricle.

Biochemical Assays

The freeze-clamped heart samples (left atrium (LA), right atrium (RA), left ventricle (LV) and right ventricle (RV)) were stored at -70°C prior to weight determination, extraction, and analysis. The samples were weighed and then freeze-dried on a Termovac lyophilisation unit for 20 hours. The samples were ground with silica gel in a 1:1 (w/w) ratio. The powdered samples (25-40 mg) were extracted in an ice bath with 250 μL of 7% perchloric acid for 5 minutes. The samples were centrifuged, 200 μL of supernatant were removed and neutralized with 200 μL of 1.0 M KOH and 50 μL of 1.0 M K_3PO_4 containing 10 mM EDTA. Creatine phosphate and ATP were determined biochemically by the method described by Bergmeyer [10] using the following modifications: The substrates were added as a combined substrate composed of 25 mg of NADP, 36 mg of glucose and 0.2 ml of ADP stock solution (10 mg of ADP in 2 mL) made up to 2 ml of water. A volume of 100 μL was added to each cuvet. The buffer was composed of 50 mM triethanolamine hydrochloride, 20 mM magnesium sulfate, 10 mM potassium chloride and 5 mM EGTA. The pH was adjusted to 7.4. The assays were performed at a wavelength of 340 instead of 366 nm [6].

RESULTS

NMR Studies of Preservation and Reperfusion

Figure 1 shows a typical 12 hour pig heart preservation experiment. ATP and PCr levels are well maintained during the preservation period. The increase in the signal intensity of P_i correlates with the total heart weight measured continuously during slow perfusion. Edema formation results in the accumulation of phosphate-containing perfusate within the heart.

Figure 2 shows the results of an experiment in which a pig heart was preserved by slow perfusion [8] for 3 hours at 12°C prior to reperfusion at 37°C. The heart was then removed from the NMR probe and samples were quick-frozen with a Wollenberger clamp for biochemical assay. Figure 2a shows the first spectrum obtained from the heart upon reperfusion with Krebs-Henseleit solution. Figure 2b shows that there was little change in ATP over the course of the experiment. PCr increased 20% over the 1.5 hour slow perfusion period as a consequence of increased oxygen supply

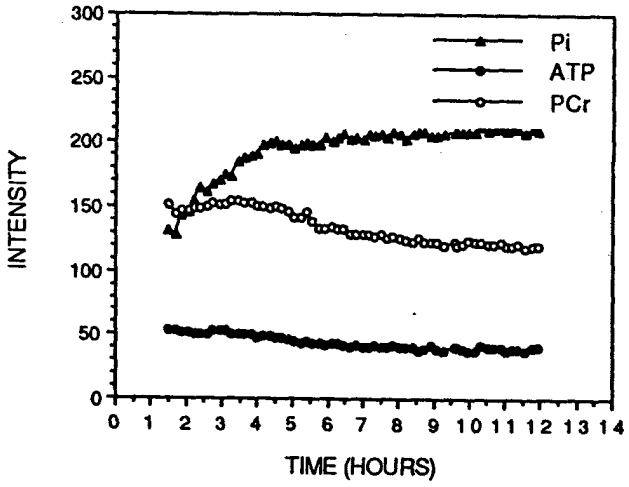

Figure 1 Relative peak heights of ATP, PCr, and P_i during slow perfusion of a pig heart with preservation solution. The heart was isolated at time zero.

from the slowly flowing preservation solution compared to that available during static storage in Plegisol. P_i increased 50% due to gradual accumulation of the perfusion medium (which contains 8 mM phosphate) in the heart. Upon reperfusion the P_i decreased dramatically because the Krebs-Henseleit medium contains only 1.2 mM phosphate. PCr levels decrease in the beating heart compared to levels observed in the preserved resting heart. At the time of freeze-clamping the rate pressure product of the heart was 10800. Biochemical assays showed the final average (LA, LV, RA, RV) ATP and PCr levels were 19±3 and 29±5 µmol/g dry weight, respectively. It may be more relevant to use only data for the left ventricle since it represents the major tissue mass in the heart. The ATP and PCr values for the left ventricle were 16 and 23 µmol/g dry weight respectively. At the end of the experiment the dry/wet weight ratio for the heart was 11±1% (n=4). The heart had swollen considerably over the course of the experiment. However, because the NMR coil encompassed the whole heart, the NMR data for ATP and PCr was not influenced by swelling. This is not

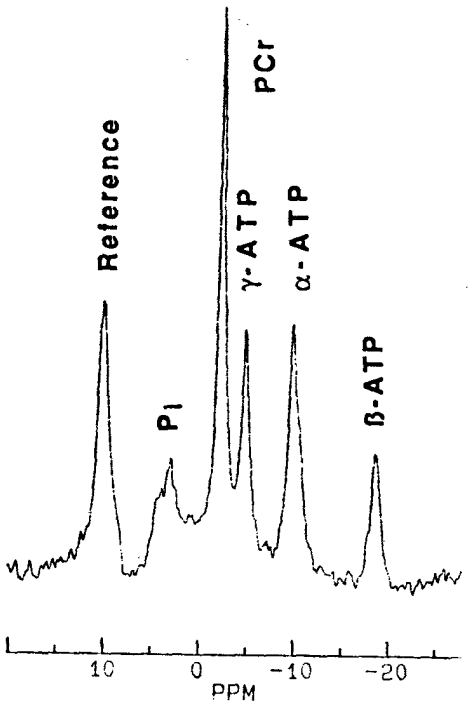

Figure 2a ^{31}P NMR spectrum of a preserved pig heart reperfused with Krebs-Henseleit solution (pH 7.4 at 37°C).

necessarily the case in studies which use surface coils.

Figure 2c illustrates the pH derived from the chemical shift of P_i. During the preservation period, the pH which is measured is that of the preservation solution containing 8 mM phosphate. During reperfusion however, the P_i peak will increasingly monitor intracellular pH. The best estimate of intracellular pH (pH_i) during this period is the first measurement during reperfusion, i.e. 7.4. This has been confirmed using a preservation solution in which phosphate was replaced by PIPES buffer at the same pH in order to observe intracellular phosphate during hypothermic preservation. As the PCr level decreases in the beating heart compared to the resting, preserved state, the P_i level increases intracellularly. The drop in pH during reperfusion correlates with decreasing levels of PCr in the heart.

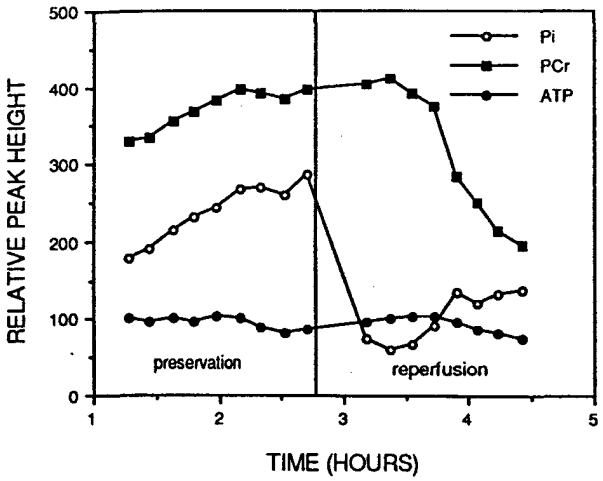

Figure 2b Relative peak heights of ATP, PCr, and P_i from a pig heart subjected to slow perfusion preservation at 12°C followed by reperfusion at 37°C.

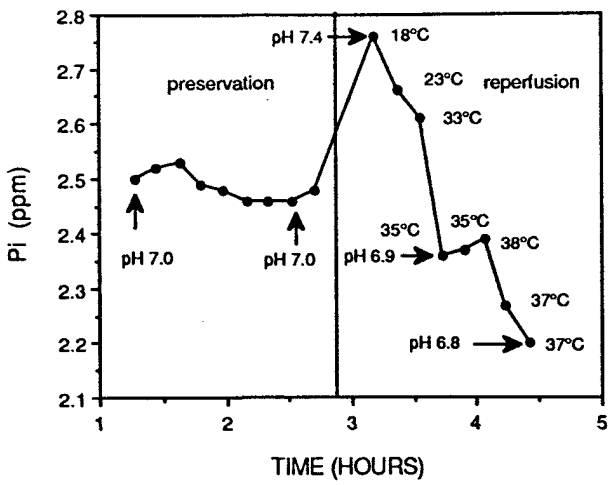

Figure 2c pH in the pig heart during preservation and reperfusion.

Measurements of coronary effluents show that the pH of the medium perfusing the coronaries decreases from 7.3 to 7.1 whereas the pH measured by NMR decreases from 7.4 to 6.8 during the 1.5 hour reperfusion period. Under our experimental conditions, it appears that the buffer capacity of the Krebs-Henseleit reperfusion medium is insufficient to prevent a decrease in pH of the coronary perfusate.

Similar results have been obtained for all hearts preserved with slow perfusion for up to 24 hours or flushed with Plegisol prior to reperfusion. Table II shows the pH changes measured by NMR for hearts reperfused with Krebs-Henseleit solution following slow perfusion preservation.

NMR and Biochemical Analysis of Preserved Hearts

Table III shows a correlation between NMR data and biochemical assays of hearts preserved for 6 hours at 12°C with slowly flowing preservation solution (35 mL/min). The ATP levels are expressed as % of the initial ATP value. The PCr values are given as % of the maximal level measured during slow perfusion preservation. The results indicate that maximal levels of ATP and PCr in non-working hypothemic preserved and oxygenated hearts, are 24±2 and 53±8 µmol/g dry weight, respectively.

Langendorff Perfused Hearts

Table IV shows the relation between NMR and biochemical data obtained from Langendorff-perfused hearts following 3 hours slow perfusion preservation at 12°C. The data show that the perfused pig heart preparation is capable of developing RPP values similar to those observed in vivo. The results show that after reperfusion, average ATP and PCr levels determined biochemically are 18 and 29 µmol/g dry weight, respectively. The average intracellular pH is 6.8. The NMR data on ATP and PCr parallel those obtained biochemically.

Long-Term Preservation and Reperfusion

Table V shows the RPP and levels of high energy phosphates measured biochemically in reperfused hearts. The most striking feature of the table is the consistent decrease in RPP following 12 hours preservation at either 5°C or 12°C, despite good maintenance of ATP and PCr levels prior to reperfusion.

The diastolic pressure on the ventricular balloon at the end of reperfusion was <7 mm Hg for hearts

Table II
pH of Pig Hearts Reperfused with Krebs-Henseleit Solution

Preservation Conditions			Final RPP	pH on Reperfusion		Final pH	
Time (hrs)	Temp °C	(n)	x 10^3	NMR+	Perfusate	NMR	Perfusate
3	12	(4)	10±2	7.6±0.2	7.3±0.0	6.8±0.2	7.1±0.1
12	5	(4)	2±1	7.6±0.1	7.4±0.1	6.6±0.1	7.0±0.1
12	12	(7)	2±0	7.4±0.2	7.4±0.1	6.7±0.2	7.1±0.1

+NMR spectra were obtained within the first 10 minutes of reperfusion: the temperature of the warming hearts was between 12° and 20°C.

Table III
NMR and Biochemical Data for Pig Hearts
Preserved for 6 hr at 12°C with Flowing Preservation Solution

ATP		PCr	
NMR (% of init)	Biochem (µmol/g d.w.)	NMR (% of max)	Biochem (µmol/g d.w.)
100	20	100	41
89	23	90	52
87	24	94	57
Avg(n=3) 92±7	22±2	95±5	50±8

preserved for 3 hours at 12°C (n=4). These hearts developed an average RPP of $10\pm2\times10^3$ mm Hg min^{-1}. Hearts preserved for 12 hours at 5°C had diastolic pressures of 48± 10 mm Hg min^{-1} (n=4) and corresponding RPP were $2\pm0\times10^3$ mm Hg min^{-1}. Similarly, hearts preserved for 12 hours at 12°C showed diastolic pressures of 29±20 mm Hg (n=5) with RPP values of $2\pm0\times10^3$ mm Hg min^{-1} after reperfusion at 37°C.

DISCUSSION

High Energy Phosphates

A number of studies have indicated that recovery of ventricular function in hearts subjected to global ischemia and reperfusion correlates with tissue content of ATP at the end of ischemia [11-13]. Furthermore, there appears to be a threshold ATP level below which irreversible functional damage occurs [7,13,14]. In this perspective, it would seem beneficial to ensure optimal preservation of high-energy phosphates in cardiac tissue destined for transplant. We have shown in pig hearts [7] that under hypothermic ischemic conditions, ATP levels decay linearly with time following loss of PCr which usually occurs after 5 hours of hypothermic preservation

In order to improve long-term preservation of hearts destined for transplant, we used ^{31}P NMR to investigate the effect of temperature and slow perfusion of an oxygenated solution on the levels of high-energy phosphates. A cardioplegic solution devised by Wicomb and co-workers [8] was chosen for study

Table IV

NMR and Biochemical Analysis of High Energy Phosphates in Pig Hearts after 1.5 Hours Reperfusion following 3 Hours Slow Perfusion at 12°C.

RPP x10^3	ATP NMR+ (% of init.)	ATP Biochem# (µmol/gdw)	PCr NMR+ (% of max)	PCr Biochem# (µmol/gdw)	pHi (final)
10	80	15±2	50	24±6	6.7
8	75	13±8	46	26±12	6.7
11	70	19±3	50	29±5	6.8
12	80	23±2	63	35±6	7.0
avg.	76±5	18±4	52±7	29±5	6.8±0.1

+ The ATP levels are given as the % of the initial value measured in the first spectrum. The PCr levels are given as % of the maximal level reached.

Each biochemical determination was performed on 4 tissue samples.

because it had been demonstrated effective for long-term preservation (24-48 hours) of pig and baboon hearts as evidenced by recovery of contractile function of hearts reperfused with blood and by survival of pig and baboons following transplantation of preserved hearts.

NMR and biochemical data have shown that ATP and PCr values in the hypothermic, arrested pig heart are 24 and 53 µmol/g dry weight. Following reperfusion control pig hearts which had developed RPP>10000 mm Hg min^{-1} at the time of freeze-clamping had ATP and PCr levels of 18±6 and 35±15 µmol/g dry weight, respectively.

Intracellular pH

The present NMR results have enabled us to follow intracellular pH upon reperfusion in the Langendorff mode. Using Krebs-Henseleit solution, fresh hearts developed RPP values similar to those observed in the pig heart in situ and had an intracellular pH of 7.0 [7]. After 3 hours of hypothermic preservation followed by reperfusion, the intracellular pH decreased to 6.8. The pH of the coronary perfusate was 7.1. These data demonstrate significant acidification both intracellularly and in the coronary perfusion medium compared to the pH of the perfusion medium in the reservoir (pH 7.3). The apparent alkaline shift observed upon reperfusion results from the fact we are measuring intracellular pH at low temperature (12-20°C) at the beginning of reperfusion.

Previous studies have shown that hearts preserved in the ischemic state with Plegisol for 5 hours at either 5°C or 12°C developed RPP values similar to control hearts after perfusion with Krebs-Henseleit solution [7]. In all cases, the intracellular pH was acidic following preservation 6.4-6.5 (n=9). Upon reperfusion, the pH increased to reach a value of approximately 7.0 measured by NMR. The pH remained at this value during reperfusion phase. Lactate levels in the left-ventricle at the end of the perfusion period were <8 µmol/g dry weight.

Rate-Pressure Products

Twelve hours of hypothermic preservation with flowing cardioplegic solution results in RPP values following reperfusion which are considerably less than fresh controls. Biochemical analyses show the left ventricular values of ATP and PCr decrease selectively compared to either left or right atrial values measured

Table V

RPP and High Energy Phosphates* Observed in Hearts Reperfused with Krebs-Henseleit Solution Following Slow Perfusion Preservation

RPP x10^3	ATP (µmol/gdw) RA	LA	RV	LV	Avg	PCr (µmol/gdw) RA	LA	RV	LV	Avg	Dry Weight%
	Control+										
14	23	21	13	18	19±4	32	33	20	31	29±6	12±1
11	4	21	23	17	16±9	9	49	61	57	44±24	15±2
10	25	22	15	17	20±5	42	26	29	29	32±7	13±4
12±2	17±12	21±1	17±5	17±1	18±6	28±17	36±12	37±22	39±16	35±15	13±3
	Preserved 3 hours at 12°C										
10	15	15	13	18	15±2	33	21	20	21	24±6	9±1
11	18	23	20	16	19±3	32	33	29	23	29±5	11±1
12	25	24	22	22	23±2	43	33	34	30	35±6	13±2
11±1	19±5	21±5	18±5	19±3	19±4	36±6	29±7	28±7	25±5	29±7	11±2
	Preserved 12 hours at 12°C										
2	10	21	8	9	12±6	13	24	9	10	14±7	10±1
2	23	17	16	16	18±3	39	29	19	13	25±11	10±2
1	18	18	10	10	14±5	28	34	15	19	24±9	11±2
2±1	17±7	19±2	11±4	12±4	15±5	27±13	29±5	14±5	14±5	21±10	10±1
	Preserved 12 hours at 5°C										
2	15	16	15	7	13±4	29	26	12	4	18±12	11±1
2	15	19	10	6	13±6	23	31	26	13	23±7	10±1
2	18	18	11	13	15±4	39	34	14	8	24±15	10±1
2±0	16±2	18±2	12±3	9±4	14±4	30±8	30±4	17±8	8±5	22±11	10±1

*Determined on tissue samples quick-frozen with Wollenberger clamps following reperfusion in the Langendorff mode for 1.5 hours.

+Control hearts are reperfused immediately following arrest with Plegisol and mounting in the perfusion apparatus; this usually requires 0.5 hours.

after reperfusion. One of the reasons for the lower RPP upon reperfusion of the 12 hour preserved hearts could be edema causing lack of adequate O_2 supply to the tissue. Edema may be a contributing factor but it is certainly not the only one because, as seen in Table V, perfused hearts which were not preserved also show considerable edema following reperfusion, yet have RPP values at the time of freeze-clamping similar to the pig heart in situ.

Edema

Ex vivo functional evaluation of pig hearts following 24 hours preservation with hypothermic perfusion of Wicomb solution has shown that the myocardial function of the blood perfused working hearts is similar to that of freshly excised hearts [9]. The preserved hearts were

markedly edematous with 82% increase in weight at the end of the perfusion period. This level of edema is similar to our findings following 24 hours of preservation [7]. Histological examination of hearts under similar conditions revealed interstitial edema [9].

Effect of Perfusate Composition

The discrepancy between our results after 12 hours of preservation followed by reperfusion and those of Wicomb may in part be due to our use of Krebs-Henseleit solution for functional testing within the NMR magnet. It has been shown in rat hearts that blood perfusion is superior to oxygenated Krebs-Henseleit buffer solution for studies of cardioplegia-induced myocardial deterioration [15]. Rat hearts perfused with Krebs-Henseleit buffer have relatively poor hemodynamic function and high values of coronary flow with limited oxygenation and edema formation leading to lactate formation to meet their energy demands. This is not the case in normal hearts.

In freshly perfused pig hearts, the presence of red blood cells in the perfusate does not improve the maintenance of systolic pressures above 100 mm Hg (data not shown). Krebs-Henseleit solution containing 0.5% bovine serum albumin (BSA), 3% Dextran and 11 mM sucrose can be used to perfuse freshly excised hearts which yield RPP>$10x10^3$ mm Hg min^{-1} with little edema formation over a 2 hour experimental period. Addition of 12% red cells (v/v) with 6% plasma to the above Krebs-Henseleit solution did not improve heart performance. Similar results were also obtained with the addition of 6% plasma to the Krebs-Henseleit solution.

Effect of Acidosis on Heart Function.

Based on our NMR data, it is possible that improved buffering capacity of Krebs-Henseleit solution could help reduce the intracellular acidosis observed in perfused hearts. Production of intracellular acidosis can itself lead to changes in the inotropic state of the heart which are not directly related to changes in high-energy phosphate metabolism but to alterations in excitation-contraction coupling. It has been suggested that even mild intracellular acidosis (0.2 pH units) can lead to impairment of diastolic compliance and/or relaxation [16]. Severe intracellular acidosis (pH 6.6-6.7) can lead to a marked increase in vascular resistance further diminishing oxygen delivery to the heart which then leads to a large decrease in energy levels [17]. Not all studies however are in agreement regarding the effect of acidosis during preservation on the function of the myocardium upon reperfusion. At least one study on hearts of puppies has shown that during long-term hypothermic (0°C) preservation (14-24 hours), hearts flushed with an acidic intracellular-type solution and immersed in a cardioplegic solution bubbled with O_2 showed better maintenance of ATP stores [18].

ATP and Compliance

The present studies are not unique in reporting a discrepancy between ATP levels at the end of hypothermic cardioplegic arrest and function upon reperfusion [18]. After five hours of hypothermic arrest with oxygenated cardioplegic solution rat hearts reperfused with Krebs-Henseleit solution only achieved work levels of ca. 50% of control levels in spite of complete preservation of high-energy phosphates. The mitochondrial oxidative function after 5 hours of preservation was indicative of ischemic damage: oxygen uptake (state 3) of isolated mitochondria was depressed. Starling curves for these preserved hearts showed both a decrease in compliance and in contractility after as little as 3 hours of hypothermic preservation. In these studies where preservation was accomplished with non-flowing cardioplegic solution the hearts would not be edematous prior to reperfusion (as was the case in our studies). Thus factors other than edema prior to reperfusion are important in determining cardiac function upon reperfusion.

Reperfusion Protocol.

Our chosen experimental protocol for reperfusion could be less than optimal for some of the hearts which were studied. The present studies were performed without using inotropic and/or chronotropic agents upon reperfusion. In many cases of long-term preservation, this type of intervention is required to achieve maximal cardiac function [18]. In the present studies, the hearts were reperfused for a period of 1.5 hours. It has been reported [19] that dog hearts preserved hypothermically (4°C) with modified Collins' solution for up to 24 hours required longer periods to recover maximal function than hearts preserved for shorter times.

CONCLUSION

We have shown the maintenance of high energy phosphates in hearts perfused with a slowly flowing oxygenated preservation solution for up to 12 hours. In spite of this, heart function assessed by the rate pressure product is less than 20% of that in the hearts reperfused with no delay or only short (3 hr) preservation period. Clearly the maintenance of high energy phosphates is not by itself adequate to preserve function. Some other factor perhaps related to increased diastolic pressure must be responsible for the decreased ability to develop force. The discrepancy between our results and those of Wicomb and co-workers [9] who reperfused 24-hour preserved pig hearts with blood, may be a consequence of increased susceptibility of preserved hearts to ischemia/anoxia caused by low O_2 availability in Krebs-Henseleit solution compared to blood at 37°C.

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