An Investigation Into the Influence of the pH of a Cardioplegic Solution on Markers of Myocardial Damage

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ABSTRACT

Although crystalloid cardioplegic solutions are generally buffered to be alkalotic, some workers have advocated the use of an acidotic solution. In order to evaluate the influence of the pH of a crystalloid cardioplegic solution on post-ischaemic markers of myocardial injury, we studied 30 rat hearts using an isolated perfused heart model, with a one hour ischaemic arrest at 20°C following single dose cardioplegia.

The pH of the cardioplegic solution was varied by adjusting the pCO_2. Six pH levels were studied with five hearts at each level, (group 1 pH = 7.0102 ±0.0106 (±SEM) group 2 = 7.2044 ±0.0077, group 3 = 7.4074 ±0.0068, group 4 = 7.6082 ±0.0071, group 5 = 7.8010 ±0.0082, group 6 = 8.0352 ±0.0129).

Pre-arrest (control) and post-arrest coronary effluent enzyme levels, heart rate, coronary vascular resistance and ECG score were determined. In addition, times to spontaneous defibrillation were noted. There were no statistically significant differences between the control values.

The mean time in seconds from institution of reperfusion to spontaneous defibrillation in groups 1 to 6 respectively were 67.6 ±2.379, 62 ±1.949, 62 ±1.483 52.4 ±3.326, 58.6 ±1.631 and 55 ±2.55 ($p<0.01$ Kruskal-Wallis Test). Post-arrest mean heart rates were 190 (one heart only), 203.33 ±26.034, 217.5 ±17.017, 235 ±26.552, 214 ±16 and 208 ±15.29 (NS). The post-arrest mean ECG scores were 2.4 ±0.5099, 1.2 ±0.5831, 1.2 ±0.7348, 0.2 ±0.2, 0.6 ±0.2449 and 0.6 ±0.2449. (NS). Of note is that ECG scores of two or more were only seen in groups 1, 2 and 3. Post-arrest mean coronary vascular resistances were 134.6 ±18.819, 118.6 ±6.653, 78 ±4.461, 65.8 ±2.01, 68 ±1.342 and 72.2 ±5.774 ($p<0.001$). Post-arrest coronary effluent mean creatine phosphokinase levels were 703.21 ±149.97, 152.34 ±27.959, 141.11 ±12.67 ,80.568 ±16.025, 88.854 ±17.391 and 88.108 ±17.225 ($p<0.005$). Post-arrest coronary effluent mean lactate dehydrogenase levels were 244.02 ±60.516, 192.46 ±68.234, 141.66 ±56.317, 25.55 ±10.974, 41.366 ±4.428 and 35.2 ±8.473 ($p<0.001$).

These data support the conclusion that an alkalotic cardioplegic solution provides superior protection to the arrested heart.

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INTRODUCTION

Optimal conditions for cardiac surgery include a bloodless, relaxed and motionless operative field. This is frequently achieved by cross-clamping the aorta and pharmacologically arresting the heart. Any such period of ischaemia is accompanied by metabolic and structural changes, which determine the functional recovery of the heart in the post-operative period.[1,2] The need for protection of the myocardium during the ischaemic arrest is well recognised, and a number of methods have been employed to achieve this,[3-9] with a hypothermic potassium cardioplegic solution[3,4,9] having gained the widest acceptance.

It has been proposed that the major mechanism of damage associated with ischaemic arrest and reperfusion is an Adenosine Triphosphate (ATP) depletion induced failure of homeostatic mechanisms,[10] and subsequent calcium ion (Ca^{2+}) entry into the cell. It is known that this Ca^{2+} influx is an active process mediated by a sodium ion (Na^+)/Ca^{2+} exchange mechanism.[11,12]

It has been demonstrated, in non-ischaemic models, that hydrogen ions (H^+) are able to antagonise Ca^{2+} uptake.[11,12] The Ca^{2+} binding sub-unit of the Na^+/Ca^{2+} exchanger is able to bind two H^+, therefore H^+ act competitively with Ca^{2+} to bind to the exchanger sub-unit, and thereby reduce Ca^{2+} influx (**Figure 1**).

In apparent conflict with this beneficial effect of a reduced pH (increased [H^+]) is the known myocardial protective effect of increasing pH in line with the proposed ectotherm model during hypothermia.[13,14] Ectotherms (cold blooded animals) undergo normal temperature fluctuations similar to those we impose during cardiac surgery. These animals actively increase blood pH progressively as their body temperature decreases.

The active sites of many important enzymes involved in energy production, are dependent on specific parts of the molecule being ionised. Variations in both temperature and pH affect the level of ionisation of many molecules. It has been

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suggested that varying the pH in line with the ectotherm model maintains the necessary ionisation of enzyme sites under hypothermic conditions.[13]

This pattern of pH change during hypothermia has been studied clinically during hypothermic circulatory arrest procedures, and has been clearly shown to provide improved myocardial protection.

In general, clinical cardioplegic solutions are buffered to be alkalotic at the delivery temperature, though some workers have advocated the use of an acidotic cardioplegic solution,[15,16] both because of its Ca^{2+} antagonistic effects, and for its negative inotropic effects. In an attempt to clarify the situation a series of experiments were performed using an isolated, perfused rat heart model.

MATERIALS AND METHODS

The model used in these experiments is essentially a modified form of that described by Langendorff.[17] This is a non-recirculating, isolated, retro-perfused rat heart, with the perfusate gravity feed system being replaced by a peristaltic pump, which delivers a set flow in the face of a rising coronary vascular resistance. In addition, the whole system is water jacketed in order to maintain a constant temperature.

Male Wistar rats within the weight range 350-450 grams were anaesthetised with chloroform in an oxygen chamber. Once anaesthetised, 300 iu of sodium heparin was injected i.p., and the heart and lungs rapidly excised.

The excised heart and lungs were placed in oxygenated Krebs-Heinslet bicarbonate buffer solution (KH) at room temperature. The aorta was cannulated and Langendorff perfusion with oxygenated, normothermic KH solution, instituted at a flow rate of 10ml/min. Once perfusion was initiated the lungs were removed to allow ejection of the coronary effluent via the pulmonary artery.

Perfusion Sequence

All hearts were subjected to the same perfusion protocol, with the pH and PCO_2 of the cardioplegic solution being the only variables. Hearts were randomly allocated to one of six pH levels (n=5 per group) using random numbers generated by a microcomputer.

Ten minutes were allowed for stabilisation of the heart (**Figure 2**). There then followed a 15 minute control period during which the heart rate (HR) and coronary vascular resistance (CVR) were determined, and the electrocardiograph (ECG) recorded for subsequent ECG scoring and heart rate determination. Coronary effluent was collected for analysis of the enzymes lactate dehydrogenase (LD) and creatine phosphokinase (CK).

At the end of the control period the hearts were made globally ischaemic and cardioplegic solution at 20°C infused into the aortic root at a pressure of 60 cm.H_2O for three minutes. The temperature of the water jacket around the heart was adjusted to 20°C. This temperature was chosen as it represents our clinically acceptable upper limit for ischaemic arrest. At the end of 60 minutes arrest, reperfusion was initiated with normothermic oxygenated KH solution.

A further 15 minute period (RP1) was then allowed for stabilisation of the heart. During this time HR and CVR were determined, the ECG was recorded, and coronary effluent collected for LD and CK leakage determination. The time taken from institution of reperfusion to spontaneous defibrillation was noted.

During a final 15 minute perfusion period (RP2), HR and CVR were determined and the ECG recorded. Coronary effluent was collected for LD and CK leakage determination. At termination of the experiment, the heart was dried at 100°C for 24 hours for determination of heart dry weight.

Perfusates

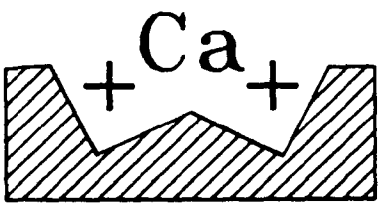
The composition of the perfusate KH by weight in grams per litre was; NaCl - 6.9, KCl - 10.35, $CaCl_2.6H_2O$ - 0.56, $NaHCO_3$ - 2.1, KH_2PO_4 - 0.16, $MgSO_4.7H_2O$ - 0.29, Glucose - 2.0. The pH, PO_2, PCO_2 and potassium concentration for the perfusate in each of the cardioplegic solution groups is detailed in **Table 1.**

The cardioplegic solution was similar to St.Thomas' Type I. The constituents of the cardioplegic solution were; K^+ 20 mMol/l, Na^+ 147.2 mMol/l, Mg^{2+} 16 mMol/l, Cl^- 204.6 mMol/l, Ca^{2+} 2.2 mMol/l, procaine HCl 273 mg/l, 50% glucose 20 ml/l. The PO_2, PCO_2 and pH for each of the cardioplegic solution groups is detailed in Table 2. The pH of the cardioplegic solution was varied by gasing with a $N_2/O_2/CO_2$ mixture via a Boyles' Apparatus and checked using an ABL2ª blood gas analyser.

Statistics

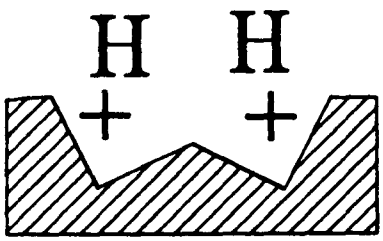
All data analysis was performed using a BBC microcomputer with "in-house" statistical software. The data were initially analysed using analysis of variance (ANOVA) with an F-test for testing for significant effects.

FIGURE 1



Calcium binding sub–unit of Na^{+}/ Ca^{++}exchanger binds divalent Ca^{++}ion

BUT



Can also bind two monovalent hydrogen ions

FIGURE 2

Experimental Time Course

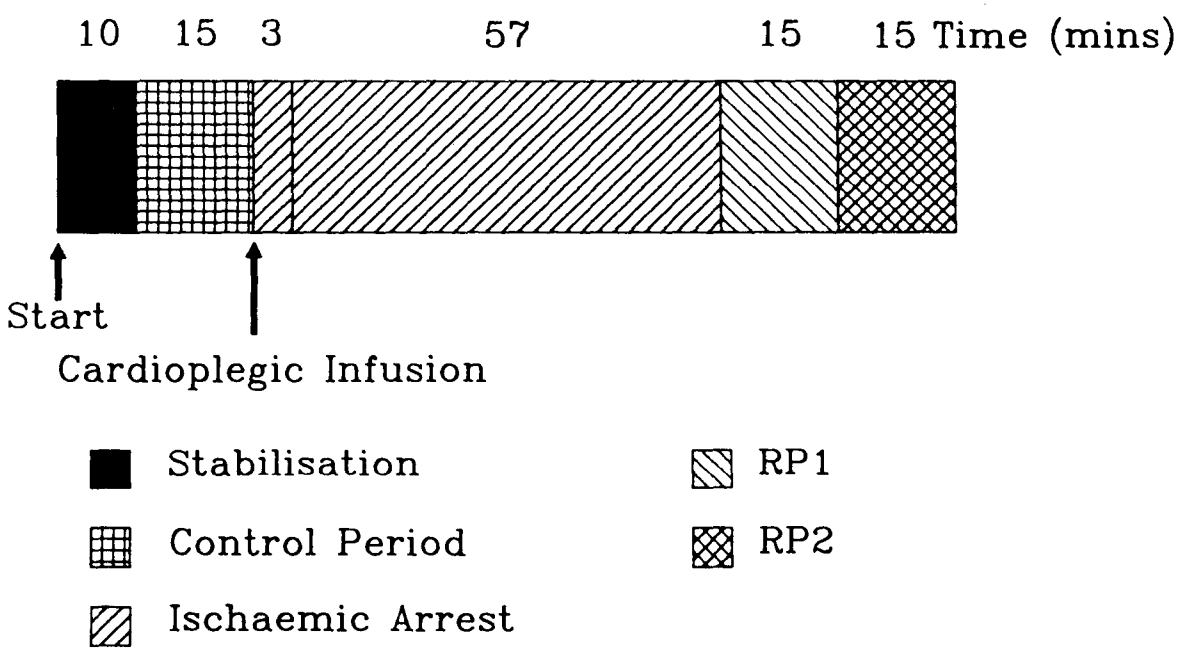


TABLE 1

Group	pH		pO_2 (KPa)		pCO_2 (KPa)		K+ (mMol/l)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1	7.408	0.001	82.74	1.61	5.45	0.17	5.61	0.07
2	7.405	0.004	81.72	0.68	5.44	0.20	5.65	0.06
3	7.397	0.006	81.05	0.35	5.48	0.14	5.59	0.04
4	7.394	0.006	81.43	0.40	5.48	0.07	5.63	0.03
5	7.396	0.005	81.38	0.47	5.67	0.06	5.59	0.05
6	7.397	0.009	83.89	1.43	5.66	0.09	5.70	0.02
K-W test	NS		NS		NS		NS	

TABLE 2

Group	pH		pO_2 (KPa)		pCO_2 (KPa)	
	Mean	SEM	Mean	SEM	Mean	SEM
1	7.01	0.011	11.86	0.36	8.94	0.58
2	7.20	0.007	11.34	0.20	5.91	0.29
3	7.41	0.007	11.70	0.08	3.70	0.09
4	7.61	0.007	10.95	0.13	2.52	0.03
5	7.80	0.008	11.83	0.09	1.61	0.07
6	8.04	0.013	11.29	0.26	1.21	0.05
K-W test	$p < 0.001$		NS		$p < 0.001$	

TABLE 3

ECG Score	ECG Features
0	Normal
1	50% or greater reduction in QRS amplitude when compared to control
2	Abnormally shaped QRS complex
3	Rhythm disturbance/heart block
4	Ventricular asystole/ventricular fibrillation

TABLE 4

	Control		RP1		RP2	
Group	Mean	SEM	Mean	SEM	Mean	SEM
1	228	13.93	156.5	23.5	190*	-
2	256	19.39	230	32.15	203.3	26.03
3	261	15.53	194	29.26	217.5	17.02
4	242	25.43	226	27.67	235	26.55
5	238	18.55	208	10.67	214	16
6	248	23.11	240	14.14	208	15.3
K-W test	NS		NS		NS	

* One heart only

TABLE 5

Group	Control		RP1		RP2	
	Mean	SEM	Mean	SEM	Mean	SEM
1	0	0	2.2	0.58	2.4	0.51
2	0	0	1.2	0.74	1.2	0.58
3	0	0	0.6	0.25	1.2	0.74
4	0	0	0.4	0.25	0.2	0.2
5	0	0	0.8	0.2	0.6	0.25
6	0	0	0.6	0.25	0.6	0.25
K-W test	NS		NS		NS	

TABLE 6

Group	Control		RP1		RP2	
	Mean	SEM	Mean	SEM	Mean	SEM
1	59.4	2.52	102	20.75	134.6	18.82
2	62.8	1.28	91.8	8.10	118.6	6.65
3	59.6	1.94	69.2	4.33	78	4.46
4	61	2.05	70.2	2.52	65.8	2.01
5	59.8	0.66	68.8	3.37	68	1.34
6	56	1.95	65	1.64	72.2	5.77
K-W test	NS		$p < 0.05$		$p < 0.001$	

FIGURE 3

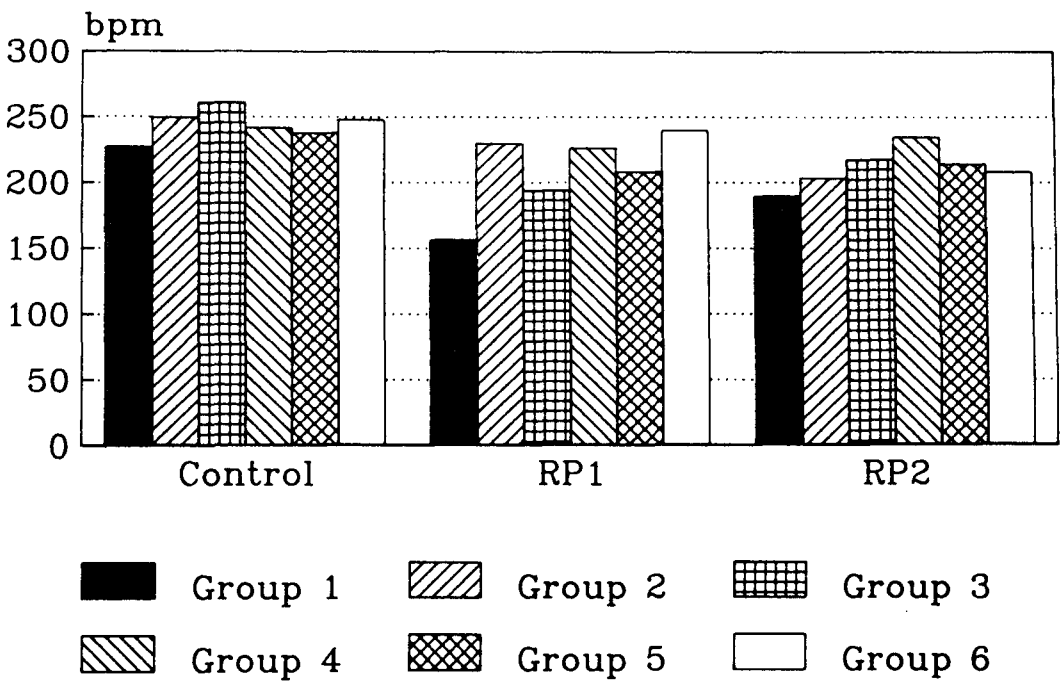


FIGURE 4

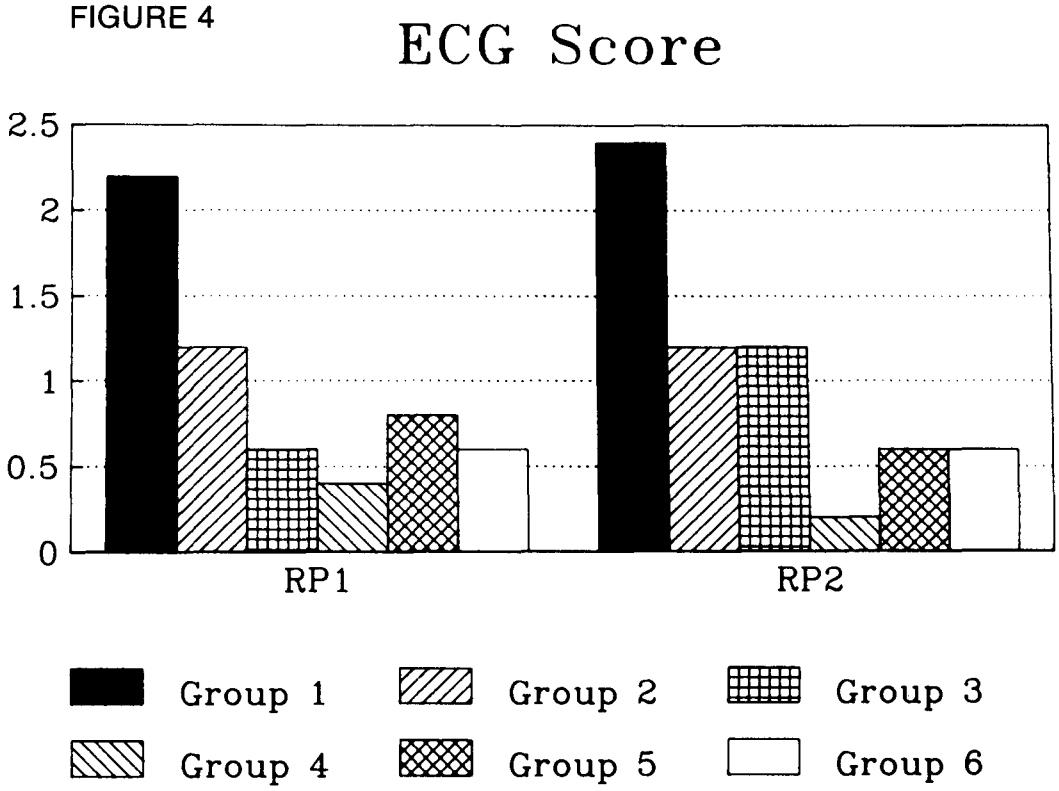


FIGURE 5

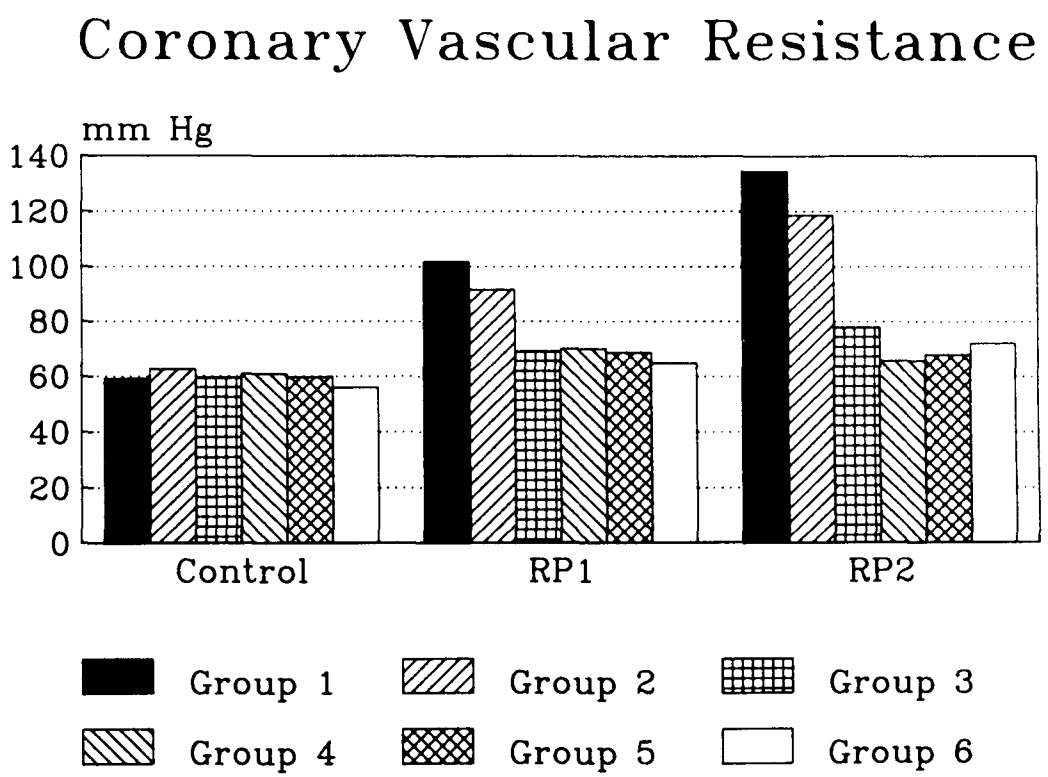


TABLE 7

Group	Control		RP1		RP2	
	Mean	SEM	Mean	SEM	Mean	SEM
1	5.95	3.76	229.08	73.37	244.02	60.52
2	3.99	2.10	221.77	93.45	192.46	68.12
3	4.41	1.81	169.11	68.43	141.66	56.32
4	3.21	1.52	24.59	6.97	25.55	10.97
5	4.26	1.19	49.30	11.36	41.37	4.43
6	4.67	1.73	34.02	3.06	35.2	8.47
K-W test	NS		$p < 0.001$		$p < 0.001$	

FIGURE 6

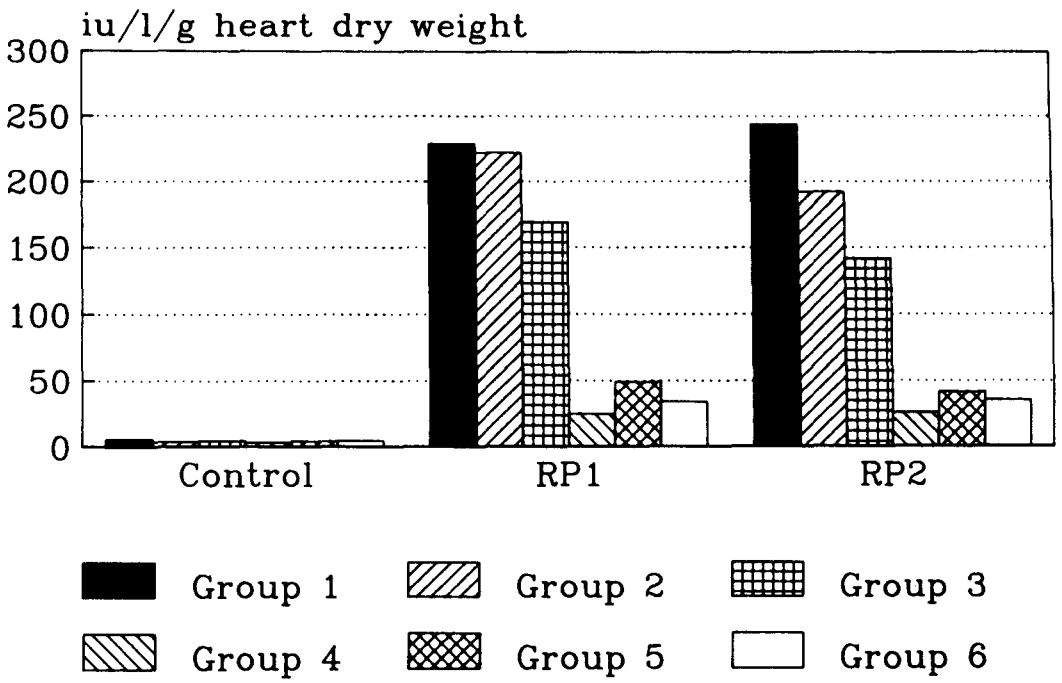


TABLE 8

Group	Control		RP1		RP2	
	Mean	SEM	Mean	SEM	Mean	SEM
1	19.60	6.80	631.61	240.18	703.21	149.97
2	6.94	4.35	262.55	81.14	152.34	27.96
3	16.37	5.59	111.74	26.42	141.11	12.67
4	19.20	10.39	110.19	17.50	80.57	16.03
5	24.27	4.40	97.66	28.97	88.85	17.39
6	28.39	11.99	122.52	36.60	88.11	17.23
K-W test	NS		$p < 0.025$		$p < 0.005$	

FIGURE 7

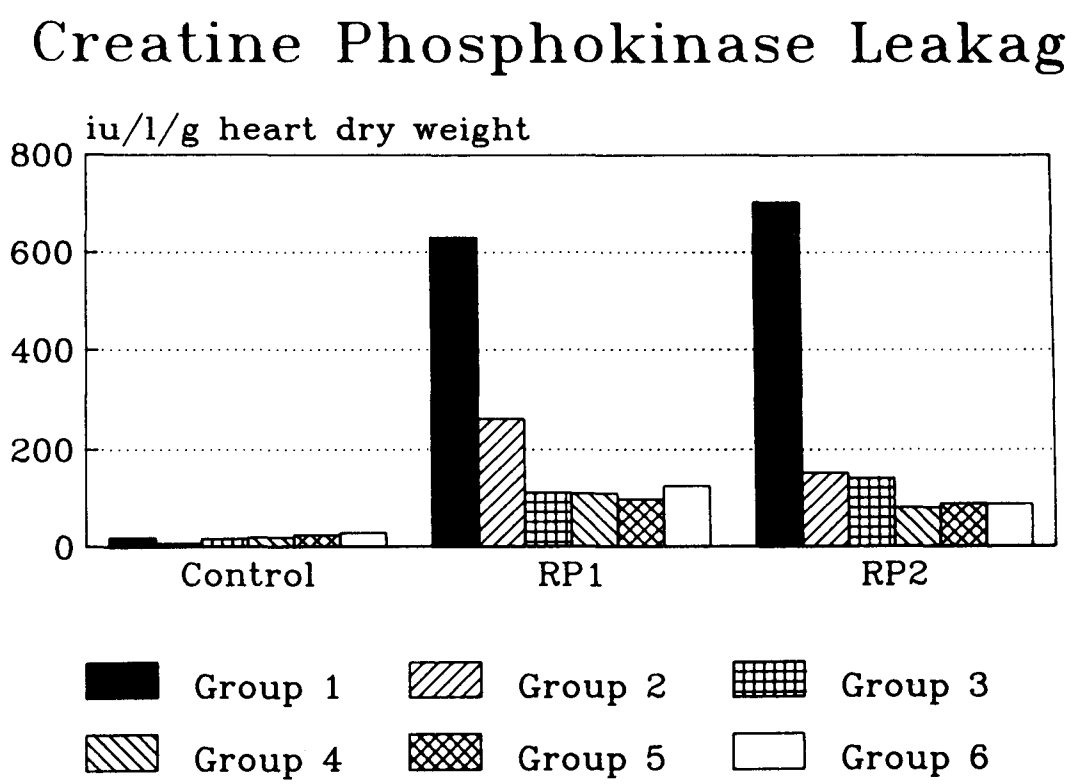


TABLE 9

Group	Mean	SEM
1	67.6	2.38
2	62	1.95
3	62	1.48
4	52.4	3.33
5	58.6	1.63
6	55	2.55
K-W test	$p < 0.01$	

It was found that the nonhomogenicity of the variance of the data made it unsuitable for this method of analysis and so the non-parametric Kruskal-Wallis One Way Analysis Of Variance By Ranks test was used. Statistics for which the level of significance was greater than 0.05 were considered to be not significant (NS).

ECG and Heart Rate

The heart rate was determined from hard copy recordings of the ECG. For each of the recording periods (control, RP1 and RP2) a 60 second recording of the ECG was made mid-way through the period. In addition to determining the heart rate, the ECG was scored by comparison with the control ECG recording for the relevant heart. The scoring protocol is detailed in **Table 3**.

Coronary Vascular Resistance

This isolated perfused rat heart model is of the fixed flow type, and any variation in the coronary vascular resistance will be reflected by a corresponding change in perfusion pressure. We express CVR as the perfusion pressure in mm Hg as measured mid-way through the relevant period.

Lactate Dehydrogenase

Coronary effluent was analysed for the cytosolic enzyme LD according to the method of Richards.[18] LD was measured using a quantitative kinetic assay kit (228-UV)[b] and a spectrophotometer SP30UV.[c] Coronary effluent samples were assayed within two hours of collection and control values checked from Decision[d] clinical chemistry control sera. The assays were performed at 37°C and the results converted to international units per litre, and adjusted for heart dry weight.

Creatine Phosphokinase

Coronary effluent was analysed for the membrane bound enzyme CK according to the methods of Oliver[19] and Rosalki.[20] CK was measured with a quantitative kinetic assay kit (45-UV),[b] using a spectrophotometer.[c] Coronary effluent samples were analysed within two hours of collection and control values checked from Decision[d] clinical chemistry control sera. The assays were performed at 37°C and results converted to international units per litre, then adjusted for heart dry weight.

RESULTS

Table 4 shows the mean heart rate for each of the pH groups, in each of the three sample periods (control, RP1 and RP2). Only hearts with an ECG score of 1 or less (**Table 3**) were used for the calculation of mean heart rate. Although there appears to be an optimum pH at which the heart rate is maintained, (**Figure 3**), this was not of statistical significance, possibly due to the insufficient numbers of hearts in some of the groups (group 1 = 1 heart, group 2 = 3 hearts, group 3 = 4 hearts, groups 4, 5 and 6 = 5 hearts).

The mean ECG score for each of the groups is detailed in **Table 5** and illustrated in Figure 4. Again, a lower ECG score was recorded in the pH 7.6 group, but was not found to be statistically significant. Of note is that ECG scores of two or more were only seen in groups 1, 2 and 3.

An increase in coronary vascular resistance is acknowledged to be a good indicator of myocardial damage.[21] The mean coronary vascular resistance, (expressed as perfusion pressure), for each of the pH groups is detailed in **Table 6**. The difference between the group means is statistically significant in both post-arrest periods ($p<0.05$ in RP1 and $p<0.001$ in RP2) and the graphs (**Figure 5**) show that the alkalotic pH groups demonstrated a more normal coronary vascular resistance.

The difference between the group means for coronary effluent LD leakage, **Table 7** and **Figure 6**, is statistically significant, $p<0.001$ in both post-arrest periods, with less enzyme leakage in the more alkalotic groups.

Table 8 and **Figure 7** show the results for coronary effluent CK leakage. The group means showed statistically significance differences in both post-arrest periods (with $p<0.025$ in RP1 and $p<0.005$ in RP2) with less enzyme leakage in the more alkalotic groups.

Table 9 shows the mean time taken from institution of reperfusion to spontaneous defibrillation. All hearts spontaneously defibrillated within 2 minutes of institution of reperfusion. The difference in these mean times was statistically significant, ($P<0.01$), with the more acidotic groups taking longer to defibrillate.

CONCLUSIONS AND DISCUSSION

The results of these experiments imply that the pH of the cardioplegic solution has an effect on post-arrest markers of myocardial damage. Viewing the results overall, it appears that the optimum pH for the cardioplegic solution at 20°C is in the region of 7.6.

This is supported by the observation that the proposed ectotherm model would predict an optimum pH at 20°C of 7.6 to 7.8.[14]

These results are in apparent conflict with those reported by Bernard et al[15] and Nugent et al,[16] who both advocated the use of an acidotic cardioplegic solution.

Bernard's study demonstrated that an acidotic, glutamate buffered, cardioplegic solution offered superior myocardial protection to an alkalotic, glutamate buffered, cardioplegic solution.

In addition, Bernard's group assessed the effects of a variety of buffers in the cardioplegic solution, (glutamate, bicarbonate, histidine and TRIS), at two pH levels (pH 7.00 and 7.40 at 20°C). Unfortunately their paper drew no conclusions as to the optimum pH for the individual buffers, though they did observe that the variation in the buffer, at a given pH, produced differing degrees of myocardial protection. This may imply that the optimum pH for a bicarbonate buffered cardioplegic solution, (as used in these experiments), differs from that of a glutamate

a. Radiometer A/S Emdrupvej 72 DK-2400 Copenhagen NV Denmark
b. Sigma Chemical Co. Ltd., Poole, Dorset, England
c. Pye Unicam
d. Beckman RRIC, High Wycombe, England

buffered cardioplegic solution.

The study of Nugent et al.[16] reported that a bicarbonate buffered cardioplegic solution of pH 7.7 resulted in depressed post-arrest myocardial performance and metabolism, when compared to pH 7.4 and pH 7.1 cardioplegic solutions. They did not report any differences between the pH 7.4 and pH 7.1 solutions. In addition no mention was made as to the temperature the pH was measured at, and so it is difficult to draw any conclusions from this work. If the measurement temperature was 37°C, then this would equate to more alkaline pHs at the delivery temperature.

The pH of the cardioplegic solutions in our experiments was varied by adjusting the PCO_2. It may be that the progressively increasing PCO_2, in the more acidotic groups, contributed to the observed increase in myocardial damage as pH decreased.

Further work is indicated to address this problem. A series of experiments in which the pH of the cardioplegic solution is adjusted by varying either the amount or type of buffer included, whilst maintaining a constant PCO_2, would be useful in answering this question.

If the improved myocardial protection in the alkalotic cardioplegic solution groups observed in these experiments is due to the pH, then there are several reasons as to why this may be so.

It may be, as suggested by White,[14] that adjusting pH in line with the ectotherm model maintains the correct ionisation of enzymes involved in anaerobic metabolism thus optimising conditions for maintained energy production during ischaemic arrest.

Finally, a further explanation of the superiority of the alkalotic cardioplegic solution may be that the end product of anaerobic metabolism, lactic acid, would take longer to reduce intracellular pH (pH_i) to levels where anaerobic metabolism is inhibited.

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