Effects of Imidazole Buffered Cardioplegia on Isolated Guinea Pig Hearts

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Abstract

The imidazole moiety of histidine is responsible for the alpha-stat theory of intracellular pH regulation, which conserves the function of essential enzymes at different temperatures. This study tests the hypothesis that the addition of imidazole to cardioplegia enhances myocardial protection.

Eighteen isolated guinea pig hearts were randomly divided into 3 groups and perfused with Krebs-Henseleit solution at 35°C using the Langendorff technique. Perfusion was discontinued after 25 minutes, and each group was arrested by the infusion of cold (4-6°C) oxygenated cardioplegic solution at 2.8 mL/min/g. Group 1 acted as a control, while Groups 2 and 3 were infused with a cardioplegic solution containing 15 mmol/L and 30 mmol/L imidazole respectively. After 30 minutes of hypothermic (< 16°C) ischemia, perfusion was resumed for 15 minutes. Hemodynamic measurements and analysis of perfusate and effluent were made prior to arrest and after 15 minutes of reperfusion. Post-ischemic lactate levels were linearly related to cardioplegia pH ($r = 0.7148$) and non-bicarbonate buffer capacity ($r = 0.8006$). Cardiac edema was lower in Group 3 ($P < 0.05$). Persistent arrhythmic activity was exhibited by 83% of Group 1 throughout reperfusion, compared to 33% and 14% of Groups 2 and 3 respectively. These results suggest that the addition of imidazole enhanced anaerobic metabolism during ischemia, facilitating the preservation of membrane integrity and function.

Introduction

Myocardial preservation during cardiopulmonary bypass procedures is currently achieved by slowing cardiac metabolism. The typical technique combines multiple doses of chemical cardioplegia with profound hypothermia to arrest electromechanical activity, which accounts for 80 to 85% of the oxygen consumption of a normal myocyte (1). The resulting basal metabolic demands of the myocardium are then theoretically reduced to a level where cellular energy stores and anaerobic glycolysis can provide sufficient adenosine triphosphate (ATP) to maintain ion gradients across plasma and organelle membranes as well as preserve essential cellular function and structure (2). Studies have shown that metabolite accumulation during global myocardial ischemia is associated with increased intraoperative morbidity (3,4). In particular, hydrogen ions produced by the glycolytic end-product lactate, cytoplasmic ATP hydrolysis, CO_2 accumulation, glycogenolysis, and lipid metabolism result in progressive intracellular acidosis (5). Additionally, the acidification of the extracellular space inhibits the ability of the ATP-calmodulin-dependent Na^+/H^+ antiport, which regulates intracellular pH, to extrude hydrogen ions from the myocytes (6). Glycolytic failure induced by a falling intracellular pH follows the inhibition of the control enzymes, phosphofructokinase (PFK) and glyceraldehyde-3-phosphate dehydrogenase (G3PD) (7). Intracellular acidosis also inhibits lactate dehydrogenase (LDH), which converts pyruvate to lactate and simultaneously oxidizes the protonated form of nicotinamide adenine dinucleotide (NADH) to its unprotonated form (NAD^+). Unprotonated nicotinamide adenine dinucleotide is essential for the oxidation of glyceraldehyde-3-phosphate (G3P) in the glycolytic pathway (8). The subsequent decline in ATP levels cannot sustain myocardial metabolism during cardioplegic arrest and can lead to the loss of membrane integrity and irreversible injury (1). Additionally, intracellular acidosis causes many essential bio-synthetic intermediates to become un-ionized (9). These molecules, now being lipophilic, diffuse, unimpeded by the plasma membrane, down a concentration gradient and into the extracellular space (10). The loss of these molecules, which include high energy phosphate precursors, hamper the ability of myocytes to regenerate sufficient levels of ATP to fuel adequate electromechanical activity upon reperfusion (11,12). Several studies have shown that the addition of buffers, such as histidine, tricine, HEPES, THAM, and imidazole to cardioplegia solutions maintains and occasionally increases the rate of glycolytic ATP synthesis because they do not require perfusion dependent bicarbonate-CO_2 flux to function effectively (3,4,13,14,15,16).

Vander Woude (16) demonstrated that isolated rabbit hearts arrested by imidazole buffered cardioplegic solution exhibited better post-ischemic hemodynamic performance when compared to those arrested by bicarbonate buffered cardioplegia. Our study tests the hypothesis that imidazole buffered cardioplegic solution enhances myocardial preservation by facilitating anaerobic glycolysis during hypothermic global ischemia.

Materials and Methods

Experimental design

The Langendorff isolated perfused guinea pig heart preparation was used to study the effect of increasing the non-bicarbonate buffer capacity of modified Saint Thomas' Hospital cardioplegic solution by adding specific amounts of imidazole (Table 1). Eighteen guinea pigs (range 400-600 g) were randomly selected and divided into 3 groups of 6 animals. The hearts of Group 1 acted as a control and were arrested with Cardioplegic Solution #1, which contained no imidazole. The hearts of Group 2 were arrested with Cardioplegic Solution #2 containing 15 mmol of imidazole. This amount of imidazole increased the non-bicarbonate buffer capacity to a level corresponding to that of 50% hemodiluted whole blood. The hearts of Group 3 were arrested with Cardioplegic Solution #3, which contained 30 mmol of imidazole and had a non-bicarbonate buffer capacity equivalent to whole blood (17). The studied variables between the groups were the pH and buffer capacity of the cardioplegic solutions.

Experimental procedure

All animals utilized in this study received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals," published by the National Institutes of Health (NIH Publication No. 85-23, revised 1985). Each guinea pig was anesthetized with 50 mcg/g of pentobarbital administered by intraperitoneal injection. Heparin (1000 Units) was administered similarly to prevent coagulation. The heart of each animal was subsequently excised through a parasternal thoracotomy and immediately immersed in iced saline to induce hypothermic arrest. The hearts were rapidly weighed on a digital balance[a] and then suspended from the Langendorff perfusion apparatus[b] by inserting a grooved metal cannula into the aortic root. The cannula insertion site was secured with a silk ligature. Cardiac activity was reestablished by the retrograde perfusion of oxygenated (95% O_2 and 5% CO_2) Krebs-Henseleit solution (KHS) (Table 1) through the ascending aorta and coronary vasculature at 35°C and a constant hydrostatic pressure of 50 mmHg.

All hearts were allowed a 20 minute period of recovery and equilibration following cannulation and initiation of Langendorff perfusion prior to beginning the actual experiment. During this period, a balloon catheter was introduced into the left ventricle via the left atrium and attached to a pressure monitor system[c] and variable speed strip chart recorder[d] to measure left ventricular peak pressure (LVPP), left ventricular end diastolic pressure (LVEDP), dP/dt, and heart rate (HR). The volume of fluid in the balloon was adjusted to approximate a LVEDP of 15 mmHg. Viability of the hearts was also assessed during the stabilization period. Those having persistent arrhythmias, coronary flow indices (CFI) less than 2.5 mL/min/g, or heart rates less than 120 beats/min, were excluded from the study. The 18 isolated hearts that were studied met all viability criteria.

After 20 minutes of Langendorff perfusion, pre-arrest hemodynamic data, comprising HR, LVPP, LVEDP, and dP/dt, were measured and recorded. Coronary flow index was simultaneously measured by collecting the coronary sinus effluent (CSE) in a graduated cylinder. Gas partial pressures (PO_2 and CO_2) and ion concentrations (pH, K^+, and Ca^{++}) of the CSE and KHS were analyzed with a blood gas and electrolyte autoanalyzer.[e] The CSE was also analyzed for lactate, LDH, and creatine phosphokinase (CPK) by a certified chemistry laboratory.

Langendorff perfusion was discontinued after 25 minutes. The hearts were then arrested by the infusion of cold (4-6°C), oxygenated cardioplegic solution delivered via an infusion pump[f] at a rate of 2.8 ml/min/g heart weight for three minutes through a side arm of the aortic root cannula. Cardiac and solution temperatures were measured with thermistors.[g] The times to the first escape from sinus rhythm and to complete arrest were recorded. Global hypothermic (< 16°C) ischemia was maintained for 30 minutes, whereupon Langendorff perfusion was resumed. Samples of immediate post-arrest CSE were analyzed for gases, ions, enzymes, and lactate. The KHS was analyzed for gases and ions. The time to the first mechanical event was recorded. Throughout the period of reperfusion, the contractile activity of the hearts was observed, and any arrhythmic activity was noted and recorded. After 15 minutes of reperfusion, measurement of hemodynamic parameters and analyses of KHS and CSE gases and ions were repeated. The hearts were then removed from the Langendorff perfusion apparatus and weighed.

Data analysis

All measured data as well as calculated data, including net heart weight gain (post-ischemic heart weight - pre-ischemic heart weight) and left ventricular developed pressure (LVDP mmHg = LVPP mmHg - LVEDP mmHg) were expressed either as indices of pre-ischemic heart weight, percent differences between pre-ischemic and post-ischemic values, or both. Myocardial oxygen consumption was calculated as follows:

$(MVO_2 = CFI \times [0.0227/760 \times 100 \times (KHS\ PO_2 - CSE\ PO_2)])$

where MVO_2 = myocardial oxygen consumption, mL O_2/min/100 g heart weight

CFI = coronary flow index, mL/min/g heart weight

0.0227/760 = O_2 solubility constant per atmosphere

KHS PO_2 = Krebs-Henseleit solution oxygen partial pressure, mmHg

CSE PO_2 = coronary sinus effluent oxygen partial pressure, mmHg

a Model PM480, Mettler, Hightstown, NJ 08520
b #K-884600, Kontes, Vineland, NJ 08360
c Series 8800, Hewlett Packard, Walthan, MA 02254
d Model 7758A, Hewlett Packard, Walthan, MA 02254
e GEM 6, Diamond Sensor Systems, Ann Arbor, MI 48104
f Model 904, Harvard Apparatus, Millis, MA 02054
g Model DP23, Shiley Inc., Irvine, CA 92714

All data are presented as the mean plus or minus (+/-) the standard error of the mean (SEM). Tests of significance between Groups 1 and 2, Groups 1 and 3, and Groups 2 and 3, were determined using Student's pooled two sample t test, where a P value equal to 0.05 level was considered the limit of significance. Linear regression was used to assess whether the immediate post-ischemic lactate levels of the CSE and the percent difference of the pre-ischemic and post- ischemic heart weight of each group were dependent upon the pH and the non-bicarbonate buffer capacity of the infused cardioplegic solution.

Results

Hemodynamic data

The mean percent differences between pre-ischemic and post-ischemic hemodynamic performance parameters are presented in Table 2. The mean percent differences of MVO_2 for all three groups were found to be lower after 15 minutes of reperfusion. The analysis also showed that the mean percent difference of HR, CFI, LVDP, and dP/dt of Group 1 increased, while those of Groups 2 and 3 decreased. Additionally, the mean percent difference of MVO_2, CFI, LVDP, and dP/dt varied inversely with the pH and non-bicarbonate buffer capacity of the infused cardioplegic solutions. However, Student's pooled two-sample t tests comparing the mean percent differences of the hemodynamic performance parameters of each revealed no statistical significance because of a large degree of variance. Table 2 also shows that the percentage of subjects within each group that exhibited persistent arrhythmic activity throughout the reperfusion period varied inversely with the pH and non- bicarbonate buffer capacity of the infused cardioplegic solutions. The most common arrhythmia observed was bigeminy.

Biochemical data

The biochemical data are presented in Table 3. The mean percent differences of the pre- ischemic and post-ischemic heart weight, which indicate the level of cardiac edema following global ischemia and reperfusion, increased in all three groups. Application of Student's pooled two sample t test revealed that the cardiac edema of Group 3, which contained the non-bicarbonate buffer capacity of whole blood, was significantly less than that of Group 1, which contained no non-bicarbonate buffer capacity.

The levels of the cardiac enzymes, CPK and LDH, in the CSE increased in all three groups, indicating that some myocardial tissue injury had occurred during global ischemia. Although the pre-ischemic CPK levels of Group 3 were statistically less than those of Groups 1 and 2, differences between the post-ischemic CPK levels were not found to be statistically significant.

The differences between the pre-ischemic levels of lactate in the CSE of all three groups were not statistically significant. Importantly, the differences between the post-ischemic levels of lactate were statistically significant and varied directly with the pH and non-bicarbonate buffer capacity of the infused cardioplegic solutions. Linear regression demonstrated that post-ischemic lactate levels were highly correlated with the pH ($r = 0.7148$) and non-bicarbonate buffer capacity ($r = 0.8006$) of the infused cardioplegic solutions. The linear relationship of these parameters is graphically depicted in Figure 1.

Discussion

The prevention of myocardial acidosis, particularly while the aorta is crossclamped, enhances myocardial preservation during cardiopulmonary bypass procedures. Several studies have demonstrated that the addition of non-bicarbonate buffers enhance post-ischemic hemodynamic performance (15,16). These and other studies (7,8) suggest that the mechanism of this effect was the buffering of accumulating hydrogen ions that would otherwise inhibit the glycolytic enzymes, PFK and G3PD. Tait (14) suggests that the buffer capacity of a cardioplegic solution is more important than pH adjustment in preventing myocardial acidosis. Our study demonstrates that the increased buffer capacity afforded by the addition of physiological amounts of imidazole to bicarbonate buffered cardioplegic solution resulted in enhanced anaerobic glycolysis during hypothermic cardioplegic arrest, less evidence of post-ischemic myocardial hypercontractility, less post-ischemic arrhythmic activity, and reduced myocardial edema upon reperfusion.

Anaerobic glycolysis is stimulated by the lower intracellular levels of ATP resulting from the inhibition of oxidative phosphorylation in the mitochondria by hypoxia (1). Under anaerobic conditions, the end product of glycolysis is lactate instead of pyruvate. The reduction of pyruvate, which produces lactate, allows the regeneration of NAD+, which is essential for the synthesis of 1,3-bisphosphoglycerate from glyceraldehyde-3-phosphate and inorganic phosphate in the glycolytic pathway. Lactate leaks out of the cell into the extracellular space in order to avoid concentrations that would rapidly inhibit glycolysis. Accordingly, the lactate level in the CSE reflects the rate of anaerobic glycolysis during global myocardial ischemia. The fact that immediate post-ischemic CSE lactate levels were found to be highly correlated ($r = 0.8006$) and directly proportional to non-bicarbonate buffer capacity indicates that ATP production by anaerobic glycolysis was enhanced. The mechanism by which imidazole increased ATP production was almost certainly the buffering of accumulated hydrogen ions in the cytosol that would otherwise inhibit the glycolytic control enzymes, PFK and G3PD.

The increased availability of ATP during global myocardial ischemia allows better maintenance of normal transmembrane ion gradients. Calcium sequestration in the extracellular space and in the sarcoplasmic reticulum and the mitochondria is of particular importance in order to avoid reperfusion injury (1,18). The increased post-ischemic values for the mean percent difference of LVDP

and dP/dt of Group 1, which had no non-bicarbonate buffer capacity, are indicative of post-cardioplegic myocardial hypercontractility associated with elevated levels of cytosolic calcium (1,11).

ATP availability also fuels essential DNA expression and protein synthesis as well as the maintenance of the structure and function of cell organelles and plasma membranes. The persistent arrhythmias exhibited in inverse proportion to non-bicarbonate buffer capacity in our study are indicative of post-cardioplegic conduction disturbances in the A-V node and His-Purkinje system. Bigeminy, the predominant arrhythmia noted, and other arrhythmias generated by desynchronized activation and slow conduction, such as premature ventricular contractions, are characteristically produced by acidosis and/or ATP deprivation (19).

The occurrence of profound post-ischemic edema upon reperfusion is associated with irreversible damage to the integrity of the plasma membrane (11). The statistically significant reduction of cardiac edema in Group 3 when compared to Group 1 ($P = 0.014$) suggests that the former suffered less damage to plasma membrane integrity than did the imidazole free group. This type of injury is secondary to hypoxia, intracellular acidosis, and ATP deprivation. The actual mechanism of destruction has been traced to the generation of cytotoxic oxidants from molecular oxygen when the tissue is reperfused with blood or oxygenated perfusate (20).

Imidazole possesses several characteristics that theoretically make it a more effective buffer system for a cardioplegic solution. First, the imidazole moiety of histidine accounts for as much as 90% of the non-bicarbonate buffering capacity in the body (10). Second, the pK of imidazole and that of water are approximate and vary inversely with changes in temperature (21). Consequently, during hypothermia, the rise in both extracellular and intracellular pH is largely due to a concurrent and linear alkaline shift of the pK of imidazole with that of water (10). This shift in pK results in an effective buffer range that circumscribes the changes in physiological pH (Table 4) and assures the net charge on proteins, such as the essential glycolytic enzymes PFK and LDH, remains unchanged, thereby, maintaining tertiary structure and cellular function (22). Such pH regulation is referred to as alpha-stat control (21). In addition, because most cardioplegia solutions are alkalotic, over 50% of the imidazole would not be protonated and thus capable of diffusing across the lipoprotein bilayer. Theoretically, this would allow imidazole to buffer both the extracellular and intracellular milieu.

Imidazole is theoretically superior to many commonly used buffers. Imidazole has been shown to be a more effective buffer of tissue acidosis under conditions of hypothermic cardioplegic arrest than bicarbonate (16). Unlike imidazole, histidine, an amino acid with imidazole as an R-group, requires active transport to enter the cell, thereby consuming ATP. THAM, another commonly used buffer, is a less effective hydrogen ion acceptor at lower pH values than imidazole or histidine (1,23). Imidazole does have other biochemical actions besides buffering. Under the laboratory conditions of a pH equal to 9.0 at 20oC, imidazole stimulated the release of calcium from the sarcoplasmic reticulum, inducing contracture (24). These conditions, however, are not present during hypothermic cardioplegic arrest. Imidazole also inhibits thromboxane synthetase (16). The actions of thromboxane A2 are vasoconstriction and platelet activation; therefore, suppression of thromboxane A2 synthesis would theoretically enhance myocardial preservation.

Our study has presented evidence that the increase in non-bicarbonate buffer capacity produced by the addition of imidazole enhances myocardial preservation during hypothermic cardioplegic arrest. However, the current preference for sanguineous cardioplegic solutions might preclude the use of such an additive to a crystalloid solution. Recent studies have demonstrated that efficacy of sanguineous cardioplegia is due to non-bicarbonate buffer capacity provided by the imidazole moieties of histidine contained in plasma proteins and erythrocytes (25,26). Since the blood utilized to make sanguineous cardioplegic solution is hemodiluted, the addition of imidazole, if not another non-bicarbonate buffer, to the crystalloid component should be considered. Clearly, more research should be conducted on the use of imidazole in cardioplegic solutions.

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TABLE 1: KREBS-HENSELEIT AND CARDIOPLEGIC SOLUTIONS

CONSTITUENTS & VALUES	KREBS-HENSELEIT SOLUTION	CARDIO-PLEGIC SOLUTION #1	CARDIO-PLEGIC SOLUTION #2	CARDIO-PLEGIC SOLUTION #3
K^+ (mEq/L)	5.9	20	20	20
Cl^- (mEq/L)	131.8	130.4	130.4	130.4
Ca^{++} (mmol/L)	5.0	1.2	1.2	1.2
Na^+ (mEq/L)	146.0	100	100	100
Mg^{++} (mmol/L)	3.9	16	16	16
HCO^- (mEq/L)	24.9	24	24	24
Dextrose (mg%)	109	180	180	180
Imidazole (mmol/L)	0	0	15	30
$H_2PO_4^-$ (mEq/L)	1.2	0	0	0
Pyruvate (mEq/L)	3.0	0	0	0
pH at 37°C	7.6	8.4	8.7	9.0
Non-Bicarbonate Buffer Capacity (slykes or mmol HCl neutralized per unit pH)	very low from $H_2PO_4^-$	0	14	28
Osmolarity (mOsm/L)	321.9	318.8	333.8	348.8

TABLE 2: HEMODYNAMIC DATA

PARAMETER	GROUP 1	GROUP 2	GROUP 3
% Difference myocardial oxygen consumption	-1.9 +/- 15.0	-10.23 +/- 1.22 P = 0.59	-15.5 +/- 12.9 P' = 0.51 P" = 0.69
% Difference heart rate	5.1 +/- 9.83	-20.82 +/- 6.39 P = 0.052	-10.92 +/- 3.31 P' = 0.15 P" = 0.20
% Difference coronary flow index	3.5 +/- 15.4	-11.72 +/- 2.31 P = 0.35	-18.9 +/- 10.2 P' = 0.25 P" = 0.51
% Difference developed pressure	19.4 +/- 21.9	-11.2 +/- 12.5 P = 0.25	-25.8 +/- 15.3 P' = 0.15 P" = 0.48
% Difference dP/dt	51.5 +/- 46.4	-18.1 +/- 15.4 P = 0.19	-27.4 +/- 15.5 P' = 0.14 P" = 0.68
% Exhibiting persistent arrhythmias	83%	33%	14%

Table 2: Hemodynamic Data
P denotes the P value of a pooled two sample t test between Groups 1 and 2. P' denotes the P value of a pooled two sample t test between Groups 1 and 3. P" denotes the P value of a pooled two sample t test between groups 2 and 3. An asterisk (*) denotes the statistical significance of a P value at less than the 0.05 level.

TABLE 3: BIOCHEMICAL DATA

PARAMETER	GROUP 1	GROUP 2	GROUP 3
% Difference heart weight	87.24 +/- 4.5	68.71 +/- 8.06 P = 0.077	65.77 +/- 5.67 P' = 0.014 * P" = 0.77
Pre-ischemic creatine phosphokinase	28.72 +/- 6.17	19.57 +/- 4.93 P = 0.27	6.15 +/- 3.52 P' = 0.0099 * P" = 0.05 *
Post-ischemic creatine phosphokinase (U/L/g)	80.68 +/- 5.58	103.3 +/- 26.7 P = 0.31	104.6 +/- 21.4 P' = 0.31 P" = 0.97
Pre-ischemic lactate dehydrogenase (U/L/g)	121.4 +/- 17.2	122.2 +/- 12.5 P = 0.97	92.4 +/- 22.5 P' = 0.33 P" = 0.28
Post-ischemic lactate dehydrogenase (U/L/g)	149.1 +/- 12.1	138.6 +/- 16.4 P = 0.62	111.5 +/- 24.5 P' = 0.20 P" = 0.38
pre-ischemic lactate (mmol/L/g)	0.2239 +/- 0.0219	0.2243 +/- 0.0400 P = 0.99	0.2409 +/- 0.0495 P' = 0.76 P" = 0.80
post-ischemic lactate (mmol/l/g)	0.3695 +/- 0.0545	0.6776 +/- 0.0965 P = 0.019 *	1.0114 +/- 0.0952 P' = 0.0001 * P" = 0.034 *

Table 3: Biochemical Data
P denotes the P value of a pooled two sample t test between Groups 1 and 2. P' denotes the P value of a pooled two sample t test between Groups 1 and 3. P" denotes the P value of a pooled two sample t test between groups 2 and 3. An asterisk (*) denotes the statistical significance of a P value at less than the 0.05 level.

TABLE 4: NORMAL INTRACELLULAR pH VALUES AND BUFFER RANGES OF 25 mmol IMIDAZOLE WITH RESPECT TO TEMPERATURE

Temperature (°C)	Intracellular pH	Imidazole Buffer Range
4	7.341	7.93 - 6.91
14	7.188	7.71 - 6.71
24	7.018	7.49 - 6.49
37	6.800	7.22 - 6.19

Table 4: Normal Intracellular pH Values and Buffer Ranges of 25 mmol Imidazole with Respect to Temperature
Jenkins EH. Unpublished data. The Ohio State University, 1990.

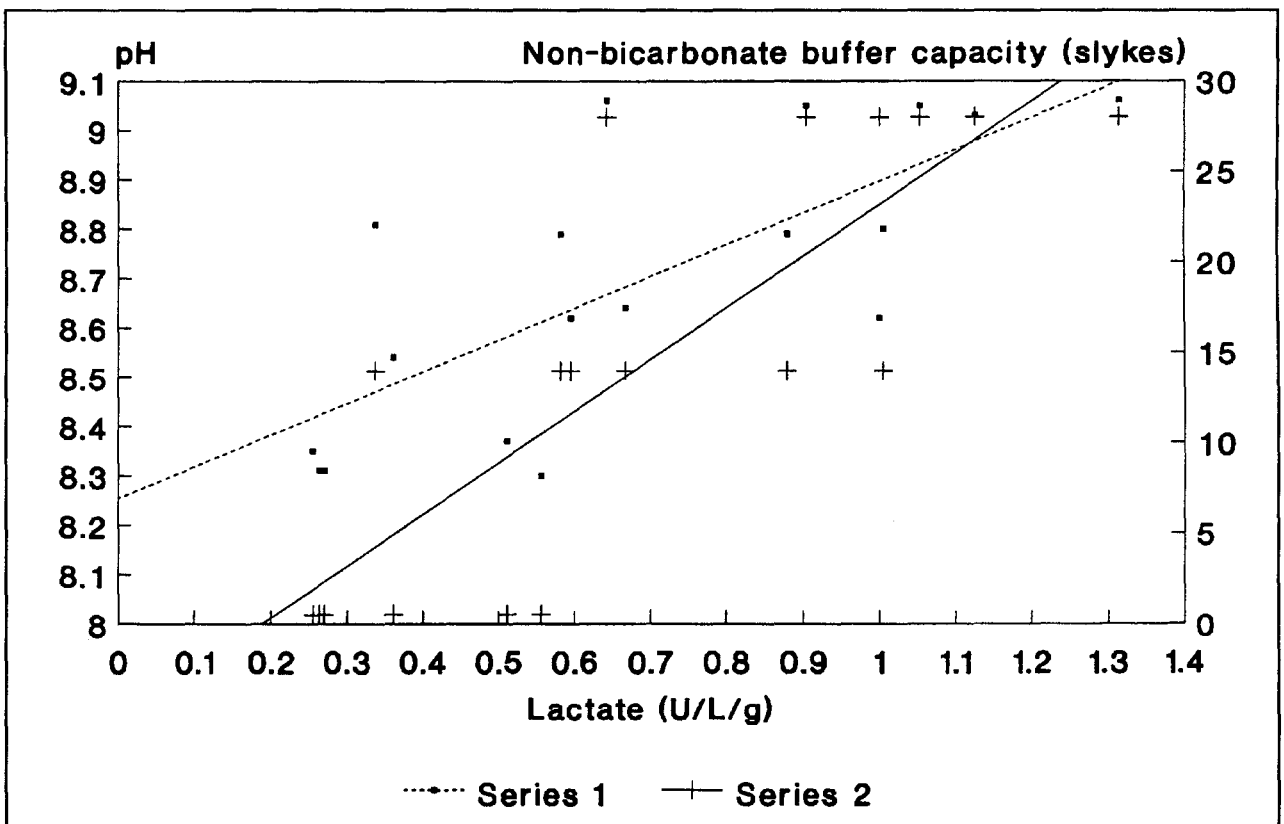

Figure 1: The pH (series 1) and non-bicarbonate buffer capacity (series 2) of the infused cardioplegic solutions are plotted against the immediate post-ischemic lactate.

Questions and Comments

Q. Can you comment a little bit on other alkalizing agents that are used besides bicarb, perhaps pharmahepis, and are you aware more so hepsis that Tham used clinically, not only of their use clinically but their toxicity, and also relate the toxicity of Imidazole in regard to its LD if you know that value?

A. OK. The most common non-bicarbonate buffer used in current solutions is Tham, I believe. Dr. Buckberg is responsible for that. I have read some studies that show that during profound hypothermia it's not as good as a hydrogen ion receptor as Imidazole is, and I'm certain there are other biological reactions with Imidazole, it is known to inhibit thromboxin synthesis so it would cause vasodilation and reduce platelet aggregation. The previous studies done with Imidazole didn't show any such results. I think the concentrations that they are giving in cardioplegia wouldn't matter to the systemic body; I don't think you would get a major vasodilatory response.

Q. I think you just answered that question but I was going to ask which affects platelet function? Did you look at platelet function in these studies?

A. No, we didn't. The Langendorf apparatus uses a crystalloid solution called Krebs-Henseleit, so there are no blood cells in the solution at any time, so we can't really say about platelets at all.

Q. I wonder if there would be a systemic effect after it is all absorbed and gets into the rest of the blood stream.

A. Well, I really don't have any data from the type of study we did. That is something that should be studied from an in vivo study. I think that would be the next step. I don't think that at the concentrations being used, systemically it would cause a vasodilatory response. But I can't be sure. We would have to study it first.

Q. There have been several institutions that have begun advocating using warm blood cardioplegia and normothermic continuous cardioplegia. If this does catch on as a trend, would this alleviate the need for alkalizing agents that are not present or non-indigenous alkalizing agents?

A. That's a good question. This is mostly used with hypothermic solutions. The conditions using warm cardioplegia would probably render it not necessary.