PROPOSED IMPROVEMENTS IN CARDIOPLEGIA

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ABSTRACT

Cardioplegic solutions are used to arrest and preserve hearts during cardiac bypass and donor heart storage for transplantation. Optimal efficacy of these solutions is required to ensure maximum recovery of the hearts.

The St Thomas' Hospital cardioplegic solution No 2 (ST) is a basic crystalloid solution with an electrolyte composition similar to blood, but with a high potassium concentration (16 mmol/l) to induce arrest. The addition of glucose to this solution to provide anaerobic energy has been disputed. Using the isolated perfused rat heart model, the inclusion of 11 mmol/l glucose in the cardioplegic solution was tested with an arrest period of three hours at 10°C. With an initial bolus of 10 ml of cardioplegia (single dose), followed by uninterrupted arrest, percentage recoveries of aortic output indicated little or no effect with the addition of glucose. Aortic output recovery with ST was 57.4 ± 5.2 % (mean ± sem) while with glucose (ST+G) the recovery was 65.9 ± 2.9 %. However, with multidose infusions of cardioplegic solution (6 ml every 30 minutes), recoveries increased to 74.6 ± 1.9 % (ST) ($p<0.05$) and 87.4 ± 1.9 % (ST+G) ($p<0.05$). The use of multidose cardioplegia proved to be beneficial, possibly because of washout of harmful end products of glycolysis and protons. The presence of glucose in this protocol was additionally advantageous (multidose ST vs ST+G - $p<0.05$), by providing essential anaerobic ATP.

The question arose as to whether oxygen is beneficial to the arrested hearts, and what the optimal pH is for the cardioplegic solution. This was tested with the ST+G cardioplegic solution used in the above multidose protocol. The cardioplegic solution was gassed either with 100% N_2 (anoxic, alkaline - pH 9), 100% O_2 (oxygenated, alkaline - pH 9), 95%N_2 5%CO_2 (anoxic, acidotic - pH 7), or 95%O_2 5%CO_2 (oxygenated, acidotic - pH 7). In alkaline cardioplegia the aortic output recovery was improved from 52.3 ± 2.7% (100% N_2) to 66.3 ± 2.8 % (100% O_2) ($p<0.05$) with oxygenation. In

acidotic cardioplegia, oxygenation again proved beneficial with recoveries of aortic output of 88.9 ± 3.7 % ($95\%O_2$ $5\%CO_2$) compared to 63.9 ± 2.8 % ($95\%N_2$ $5\%CO_2$) ($p<0.05$). When comparing the respective anoxic and oxygenated groups, acidotic cardioplegia proved to be more beneficial ($p<0.05$). The optimal results were obtained by oxygenation at a low pH ($95\%O_2$ $5\%CO_2$). Oxygenation switches the production of energy from glycolysis to oxidative phosphorylation, a far more efficient process. A low pH is thought to protect the heart against the entry of calcium.

These results were obtained using a multidose protocol model which can only be applied with cardiac bypass. With long term storage of donor hearts for transplantation, the use of an initial bolus of solution only is practical. The accumulated metabolites are therefore not washed out and must be dealt with in an alternative manner. Using the ST+G cardioplegia, we tested the effect of an increased buffering capacity by the addition of 50 mmol/l histidine. This may be especially beneficial in the presence of glucose, as enhanced glycolysis is associated with greater proton production. Osmotic space was made available by a reduction of 20 mmol/l in the NaCl concentration. As a control, sucrose (a non-metabolisable sugar) was added instead of histidine. There were no significant differences in the aortic recoveries of the control groups (ST+G - 61.0 ± 2.1 %; ST+G+sucrose - 57.2 ± 2.4%). However, with the addition of histidine, recoveries increased significantly (76.1 ± 3.4 %) ($p<0.05$ vs control groups). A higher concentration of histidine was tested (80 mmol/l) but this was found to reduce recoveries (54.7 ± 3.3 %). The increased buffering capacity was thought to prevent the large decrease in pH associated with ischaemia, and thereby protect the myocardium.

Conclusions

Although the rat and the human myocardium show distinct differences, various conclusions can be drawn from this work

1) 11 mmol/l glucose was advantageous in a multidose protocol

2) In a multidose protocol, the glucose-containing St Thomas' Hospital No 2 cardioplegic solution should be gassed with 95%O_2 5%CO_2.

3) A relatively acidotic pH is more beneficial to the arrested myocardium.

4) Histidine should be considered as an additive to cardioplegia used both with single dose and multidose protocols.

These alterations must be further tested on a larger animal model before being used on human patients.

I, Linda Mary King hereby declare that the work on which this thesis is based is original (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other University.

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L King

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PUBLICATIONS ARISING FROM THIS WORK

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UO von Oppell, LM King, EF du Toit, P Owen, B Reichart, LH Opie. Oxygenation of crystalloid cardioplegic solutions: induced pH shifts and their effect on postischaemic myocardial function. Abstract. Cardiovasc J S Afr, 1990:1 (Suppl 1); 1 (Presented at XVII Biennial Congress Southern Africa Cardiac Society. Bloemfontein, August 1990).

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UO von Oppell, LM King, EF du Toit, P Owen, B Reichart, LH Opie. Effect of oxygenation and consequent pH changes on the efficacy of St Thomas' Hospital cardioplegic solution. J Thorac Cardiovasc Surg 1991 (In Press).

UO von Oppell, EF du Toit, LM King, P Owen, B Reichart, LH Opie. St Thomas' Hospital cardioplegic solution: Beneficial effect of glucose and multidose reinfusions of cardioplegic solution. J Thorac Cardiovasc Surg 1991 (In Press).

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CONTENTS

I. AIMS AND HYPOTHESIS

The aim of this investigation was to improve on the formulation of a cardioplegic solution used in cardio-pulmonary bypass operations, thereby improving the recovery of arrested hearts. In extension, we wished to be able to prolong the duration of storage of donor hearts for transplantation, to enable long distance procurement of such hearts.

A cardioplegic solution must be adequately tested in the laboratory before being used clinically. We chose to evaluate alterations to the basic St Thomas' Hospital crystalloid cardioplegic solution No 2 (ST) (see Table 1). This is an internationally recognised solution formulated by David Hearse and colleagues (Hearse et al., 1981) which has been in use since 1981. Its ionic composition is similar to that of blood, with a high potassium concentration as the cardioplegic agent.

Much research has been done on cardioplegic solutions in recent years, with many varying reports as to the efficacies of different formulations. Many assumptions have been made using results which cannot be directly extrapolated to one's own model. Variations in ionic composition, temperature, substrate availability, number of administrations of solution, time of arrest, and species differences lead to confusion in the literature as to an optimal formulation. While the addition of a specific agent may be efficacious in one formulation, in another formulation with a different ionic composition and method of usage, the same agent may prove deleterious. We wished to follow a logical approach based on the principle of enhanced energy production by glycolysis and oxidative phosphorylation, taking into account the changes which occur during an ischaemic period.

The isolated working rat heart model (the Langendorff apparatus (1895) modified by Neely et al., 1967) was used to test the effects of modifications to this solution on the functional recovery of the hearts after an extended period of arrest. Protocols approximating both cardio-pulmonary bypass (intermittent flushing with cardioplegic solution - multidose)

and donor heart storage (initial dose of cardioplegic solution followed by simple storage - single dose) were used.

1) The inclusion of glucose in the ST was tested, as glycolytic ATP has been shown to be important to the ischaemic myocardium. The increased production of high energy phosphates with the addition of this energy substrate may improve recoveries.

2) The use of single dose and multidose protocols of administration of cardioplegic solution with and without glucose was compared, as glucose is thought to lead to the production of metabolites which may be harmful to the arrested heart if these accumulate. If, however, these are washed out by intermittent flushing with cardioplegic solution, this may be beneficial to the hearts.

3) Using a multidose protocol, which approximates to the clinical cardio-pulmonary bypass, we tested whether the presence of oxygen in the ST+Glucose cardioplegic solution was required in the presence of glucose, as this converts the usage of glucose from glycolysis to oxidative phosphorylation, a much more efficient pathway.

4) With oxygenation of a solution, the pH is altered. We tested whether a high or a low pH was more beneficial in the presence of oxygen in the ST+Glucose cardioplegic solution used in a multidose protocol. A low pH is thought to be protective during ischaemia, but with long term arrest, intracellular pH may decline to levels resulting in injury to the myocytes.

5) Using a single dose protocol of administration of cardioplegic solution, gassing of the solution is a relatively short term effect. As we wished to be able to extend the duration of arrest of donor hearts, we tested a more longterm alteration to the ST+Glucose solution, with the addition of histidine as a buffer to combat the increased proton production associated with the presence of glucose.

Table 1: Ionic composition (mmol/l) of modified Krebs-Henseleit buffer (K-H - pH given when gassed with 95%O_2 5%CO_2 as is normally done during perfusions) and St Thomas' Hospital cardioplegic solution No 1 (ST No1) and No 2 (ST No2) (from Ledingham et al., 1987 - ungassed solutions), with intracellular (IC) and extracellular (EC) values (from Opie, 1984) (In brackets - free ionised concentrations) (Osmolality - measured).

	IC	EC	K-H	ST No1	ST No2
Na^+	5-12 (6)	120-145	143	144	120
K^+	110-140 (80)	5	5	20	16
Mg^{2+}	15-17 (0.7)	1.2 (0.6)	1.2	16	16
Ca^{2+}	1-2 ($<10^{-6}$)	2.5 (1.25)	1.25	2.2	1.2
Cl^-	30	110-140	123	183	160
PO_4^{2-}	80	5	1.2	-	-
HCO_3^-	8-10	24-31	25	-	10
SO_4^{2-}	20	3	1.2	-	-
Glucose			11	-	-
Procaine hydrochloride				1	
pH (37°C)	7.4	7.4	7.4	5.5-7.0	7.8
Osmolality (mOsm/ kg H_2O)	282	282	290	300-320	285-300

II. BACKGROUND

i) Definition of cardioplegia

Cardioplegia - Paralysis of the heart. This, the dictionary definition, indicates the original aim of cardioplegia; to arrest the heart and prevent electromechanical activity. Arrest of the heart is required to allow surgery to be carried out in a bloodless field in the absence of mechanical movement.

However, an arrested heart is also made ischaemic, which is associated with myocardial damage and poor recovery on reperfusion. The more modern approach for a cardioplegic solution therefore places greater emphasis on maintenance of the integrity of the heart, or "myocardial preservation" (Bretschneider, 1980), during arrest, thereby ensuring maximal recovery upon reperfusion.

ii) Approaches to the development of a cardioplegic solution - historical review

There are many formulations of cardioplegic solutions which have been tested using different animal models, and different protocols of administration.

Melrose et al. (1955) introduced the concept of "elective cardiac arrest", using a solution of blood containing a high concentration of potassium citrate (2.5%). However, this proved to be deleterious to the myocardium (see Hearse et al., 1981) and the use of cardioplegic solutions was largely ignored in preference to other means of arrest, such as coronary perfusion and topical hypothermia. However, Bretschneider and others in Germany continued research in this field and developed the so-called "intracellular" cardioplegic solutions during the 1960's (see Bretschneider et al., 1975). The principle was to induce arrest by reducing the sodium and calcium concentrations, in an effort to inhibit the development of an ionic gradient across the membrane. This inhibits the expenditure of energy by decreasing electrophysiological and contractile activity.

In 1976, Hearse et al. published a study using the working rat heart model in which they tested various components of cardioplegic solutions previously used. A solution based on the Krebs-Henseleit buffer (see Table 1), with 12 mmol/l potassium, 16 mmol/l magnesium, 10 mmol/l adenosine triphosphate, 10 mmol/l creatine phosphate, and 1 mmol/l procaine, to be used at a temperature of about 30^{o}C, was formulated. The ionic composition of this first "St Thomas' Hospital cardioplegic solution" was therefore based on the ionic composition of blood, that is, a so-called "extracellular" solution. Over the following years the St Thomas' Hospital No 1 solution was fully tested and developed further (Hearse et al., 1978a; 1978b; Hearse et al., 1981) (see Table 1). This solution was used until 1981, when further alterations, with a control for pH with the inclusion of bicarbonate as a buffer, and reduced sodium for osmotic space, were made. This was called the St Thomas' Hospital No 2

solution (see Table 1), which has been found to be far better than the No 1 solution (Ledingham et al., 1987).

Buckberg reintroduced the concept of blood-based cardioplegic solutions in 1979, which may have many advantages over crystalloid solutions as there are many intrinsic substances of use to the arrested heart. There is an oxygen carrying vehicle (haemoglobin) which can also carry CO_2, good buffers (histidine, bicarbonate, phosphates), substrates, and the correct ionic composition (Ledingham et al., 1988). However, there are disadvantages associated with a blood based solution, including increasing viscosity at lower temperatures, which may block the vascular bed and prevent complete flushing of the heart (Hearse et al., 1981).

iii) Ischaemia

a) Ischaemic injury

Ischaemia is defined as developing "whenever the flow of arterial blood through the diseased vessels is reduced to a volume below that required by the myocardium for adequate function", and extended to "whenever the arterial blood flow is insufficient to provide enough oxygen to prevent intracellular respiration from shifting from the aerobic to the anaerobic form" (Jennings, 1970). This reduction in, or inadequacy of, blood flow leads to metabolic and contractile dysfunction because of a lack of oxygen and substrate supply, and of washout of metabolites. Ischaemia is an essentially reversible phenomenon depending on its severity and duration. If ischaemia is irreversible, this is infarction. Cardioplegic arrest represents the extreme, of global ischaemia, in which no flow exists.

Ischaemic injury is observed as changes in the cellular ultrastructure including membrane disruption, mitochondrial swelling, oedema and shortening of the sarcomeres, with an overall reduction in contractile function (Jennings and Ganote, 1976; Schaper et al., 1979; Tranum-Jensen et al., 1981). Contracture, or the so-called "stone heart" phenomenon, is a manifestation of severe ischaemic injury.

These changes in the myocardium are thought to be brought about by a depletion in energy stores (Neely et al., 1973; Jennings and Ganote, 1976; Jennings et al., 1983), accumulation of waste products (Neely et al., 1973; Neely and Grotyohann, 1984) with subsequent inhibition of glycolysis (Rovetto, et al., 1975; Opie, 1988; Jennings et al., 1989; Owen et al., 1990), membrane disruption (Schaper et al., 1979; Jennings et al., 1983; Corr et al., 1984; van der Vusse et al., 1989), intracellular acidosis (Cobbe and Poole-Wilson, 1980a, 1980b), intracellular Ca^{2+} accumulation (Clusin et al., 1983; Nayler et al., 1988; Opie, 1989a; Tani and Neely, 1989, 1990), free radical activity (Hess and Manson, 1984; Kako, 1987; Vandeplassche et al., 1990), and activation of catalytic enzymes (Corr et al., 1984; van der Vusse et al., 1989).

1) Energy production

Energy continues to be consumed at the normal rate with the onset of ischaemia. However, a reduction in the supply of substrates and inhibition of high energy phosphate formation pathways reduces the rate of replenishment of energy stores. Creatine phosphate (CP) in the initial stages of ischaemia acts as a source of energy for the formation of adenosine triphosphate (ATP), but the levels are rapidly depleted. Oxidative phosphorylation is dependent on the amount of oxygen available to the cells. With a reduction in blood flow, oxygen supply is diminished which inhibits oxidative phosphorylation, and stimulates glycolysis by the Pasteur effect. ATP production via anaerobic glycolysis is small (4 ATP's per molecule of glucose) when compared to oxidative phosphorylation (36 ATP's per molecule of glucose). However, in restricted oxidative phosphorylation, glycolysis becomes the major source of energy (Opie, 1990). Although exogenous substrates are no longer available with ischaemia, glycogen can be utilised by this pathway.However, increased glycolysis leads to the formation of lactate and, indirectly, an increase in intracellular proton concentration (Opie, 1989c). These products accumulate with no washout and inhibit the enzymes of the glycolytic pathway (Rovetto et al., 1975). ATP production in the myocardium is therefore reduced with time of ischaemia.

Changes in pH affect many of the processes in the cell (see below) while lactate accumulation in the cell inhibits glyceraldehyde-3-phosphate dehydrogenase activity (Rovetto et al., 1975), causes mitochondrial damage, increases the osmolar load of the cells, and has certain electrophysiological effects, including a shortening of the action potential duration (Marrannes et al., 1975; Saman and Opie, 1984).

ATP has a ubiquitous role in the cell, and a reduced level in the ischaemic myocardium is linked to many of the deleterious consequences of inhibition of flow. ATP is required for many cellular enzymatic reactions, including the ATP dependent Na^+/K^+ pump, which maintains the electrophysiological status of the cells. This pump which removes 3 Na^+ from the cell in

exchange for 2 K^+, is responsible for the consumption of a large amount of the available energy in the cell and is therefore highly dependent on ATP levels (Kléber, 1984).

Glycolytic ATP is thought to be especially important to the ischaemic myocardium. Jennings et al. (1989) found that a basal level of glycolysis in ischaemia is important, where its inhibition leads to rapid depletion in the reserves of high energy phosphates with consequent irreversible injury to the myocytes.

There is evidence for a functional compartmentalisation of ATP. Bricknell and Opie (1978) suggested that the ATP produced by glycolytic flux during ischaemia plays a role in preserving the integrity of the membranes by maintaining sarcolemmal pump activity, while ATP from oxidative phosphorylation is used for contractile function (Weiss and Hiltbrand, 1985; Weiss and Lamp, 1987). Glycolytic ATP is associated with inhibition of the ATP sensitive K^+ channel, and is thereby thought to reduce efflux of K^+ via this mechanism during ischaemia (Weiss and Lamp, 1987; Kantor et al., 1990). Glycolytic ATP is also thought to be involved in the control of intracellular Ca^{2+} levels with maintenance of the integrity of the cell membranes (Poole-Wilson et al., 1984), prevention of ischaemic contracture (Bricknell et al., 1981; Owen et al., 1990), inhibition of enzyme release (Hearse et al., 1976), and inhibition of free radical activity (Opie, 1989c). Glycolytic ATP may be available to maintain the phosphatidic acid cycle thereby preventing the accumulation of lysophospholipids which lead to membrane breakdown (Corr et al., 1984).

2) Energy levels

ATP levels have been used as indicators of the expected recovery of ischaemic hearts (Hearse et al., 1974; Nayler et al., 1979; Lazar et al., 1980). A critical limit on the level of ATP in the myocardium which determines the irreversibility of injury is thought to exist. However, the value of this limit is much debated (Opie, 1984).

Various reports as to the rate of decline of ATP levels in the ischaemic myocardium have been published. A reduction in ATP of 25% after 2 min, and of 70% by 20 min of low flow ischaemia in the isolated rat heart was found (Neely et al., 1973). CP levels decline more rapidly by about 70% after 2 min, acting as a source of energy for ATP formation (Neely et al., 1973). A comprehensive study by Edoute et al. (1983) shows a fairly linear drop in ATP levels with duration of ischaemia, which was in turn associated with a drop in function on reperfusion. CP levels were almost all severely depressed at the end of ischaemia.

However, more recent studies have found a lack of correlation of high energy phosphate levels at the end of ischaemia with the functional recoveries of the hearts on reperfusion (Neely and Grotyohann, 1984; Taegtmeyer et al., 1985; Saks et al., 1989; Steenburgen et al., 1990).

Taegtmeyer et al. (1985) found a reduction in ATP levels of 38% after 5 min, 56% after 10 min, and 79% after 20 min global ischaemia in isolated rat hearts. There was no functional recovery after 20 min ischaemia. CP levels dropped by 83% after 5 min, and by 88% after 10 min ischaemia. ATP levels were widely spread in correlation to recovery after 20 min ischaemia, whereas CP levels were grouped together. For longer periods of ischaemia, with a greater decrease in high energy phosphate levels, a better correlation was found but only with severe impairment of myocardial function.

Neely and Grotyohann (1984) found that ATP levels declined to about 85% of control values after 30 min ischaemia, but no further decreases with longer periods of ischaemia were observed (60 min). Little correlation with recovery of contractile function was found. By 90 min ischaemia, Jennings et al. (1989) found a drop in ATP levels to 3-4% of controls, with no recovery of contractile function.

In the dog heart, Reimer et al. (1981) found a depletion of ATP of 65% of control levels after 15 min severe myocardial ischaemia, although no necrosis was observed.

The free energy of hydrolysis derived from ATP in hypoxic hearts is reduced (Kammermeier et al., 1982), while a reduction in the efficiency of the conversion of the chemical energy from ATP to mechanical tension at low pH's (as found in ischaemic myocardium) is found (Takayasu et al., 1990). It is therefore not only a reduction in the amount of ATP during ischaemia which may be responsible for a depression in contractile function.

While a direct correlation of ATP with contractility after an ischaemic period does not necessarily hold, many of the changes occurring in the ischaemic myocardium are dependent on the availability of energy. The multifactorial effects of a decrease in ATP levels with ischaemia may combine to contribute to the reduction in functional recovery.

3) pH

The onset of ischaemia is associated with a rapid drop in pH (Cobbe and Poole-Wilson, 1980a). With global ischaemia, tissue pH falls from an initial value of 7.2 by 0.15 units (pH 7.05) by 5 min, 0.66 units (pH 6.54) by 15 min, and by as much as 1.4 units (pH 5.8) by 60 min (Cobbe and Poole-Wilson, 1980a). The initial decline in pH is thought to be due to an immediate increase in pCO_2 from usage of residual oxygen (Case et al., 1979), and subsequent acidosis is attributed mainly to the hydrolysis of ATP, with increased glycolysis and inhibition of oxidative phosphorylation. An increased glycolytic rate is associated with a decreasing pH because the rate of ATP production and the uptake of protons by oxidative phosphorylation is reduced compared to the rate of ATP hydrolysis (Gevers, 1977).

Acidosis in the normally functioning heart is associated with decreased contractility (Eisner et al. , 1982). A fall in pH_i of 0.2 units leads to a 50% decline in developed tension (Poole-Wilson, 1989). This has been attributed to the increased competition between H^+ and Ca^{2+} ions for binding sites (Berman et al., 1977; Langer, 1985; Pressler, 1987). Acidosis decreases the sensitivity of the troponin-tropomyosin complexes of the myofilaments to Ca^{2+} (Chapman, 1983; Barry et

al., 1987) and decreases the maximum tension developed at saturating levels of Ca^{2+} (Eisner et al., 1982; Langer, 1985). A reduction in the uptake and release of Ca^{2+} by the sarcoplasmic reticulum occurs at a low pH (Berman et al., 1977; Allen and Orchard, 1983), which is thought to reduce Ca^{2+} cycling essential for normal contractile function. Ca^{2+} binding to the membrane phospholipids is reduced (Langer, 1985) with release of Ca^{2+} into the cytosol. Cell to cell coupling is decreased with a reduction in junctional conductance (De Mello, 1982; Pressler, 1987).

However, in the hypoxic heart, acidosis (pH 6.8) has a protective effect resulting in improved recovery on reoxygenation (Bing et al., 1973). This is attributed to greater conservation of energy stores by the depressive effects of an acidotic pH on contractility and substrate transport, especially during the early stages of hypoxia. Nayler et al. (1979) also found that a mild respiratory acidosis is beneficial to an hypoxic heart, with preservation of mitochondrial activity and stores of ATP as well as a reduction in the rate of tension development. Prevention of Ca^{2+} accumulation in the cell and mitochondria is also found during arrest with a low pH (Yates and Dhalla, 1975; Nayler et al., 1979; Busselen, 1985; Nayler et al., 1988; Opie, 1989a), with preservation of ATP stores and restoration of oxidative phosphorylation on reperfusion. A reduction in sarcolemmal Ca^{2+} transport occurs after a fall in pH (Bielecki, 1969; Philipson et al., 1982) as pH affects the fluidity of the membranes and is thereby a deterrent to Ca^{2+} entry (Pressler, 1987). The Na^{+}/Ca^{2+} exchanger is also directly inhibited by acidosis in sarcolemmal vesicles isolated from cardiac cells (Philipson et al., 1982). The reduction in the ability of the sarcoplasmic reticulum (SR) to sequester and release Ca^{2+} with a reduced pH (Berman et al.,1977) may also help in the preservation of energy stores.

Thus the reduction in intracellular pH in the ischaemic myocardium may be a protective reaction initially. However, with prolonged ischaemia, intracellular pH may be reduced to

levels leading to cell necrosis with irreversible injury. This may be due to inhibition of the catalytic activity of many key enzymes and activation of proteases and lipases (Corr et al., 1984) leading to breakdown of the membranes. An intracellular proton accumulation may also lead to intracellular Na^+ overload via the Na^+/H^+ exchanger (Lazdunski et al., 1985; Kaila and Vaughan-Jones, 1987), an electroneutral reversible antiport with a 1:1 stoichiometry (Kaila and Vaughan-Jones, 1987). This may lead to subsequent Ca^{2+} influx via the Na^+/Ca^{2+} exchanger (Tani and Neely, 1990). However, during ischaemia, with a reduction in intracellular pH and subsequent extracellular equilibration, the Na^+/H^+ exchange is largely inhibited.

The levels of enzyme leakage are used as a diagnostic indicator of cellular membrane damage (Hearse et al., 1973; Hearse et al., 1975). Membrane integrity is very important in determining both the degree and extent of injury, as well as its reversibility. If a large proportion of the total membrane surface area in the myocardium is damaged, the cells will probably be irreversibly damaged, as large gaps in the membrane cannot easily be mended.

4) Calcium influx

Ca^{2+} plays a central role in the regulation of cellular functions (Lucchesi, 1989), including modulation of the rate of ATP utilisation, triggering of contractile activity, and maintenance of the integrity of the plasma membranes (Frank et al., 1977) and the intercalated discs (Rim et al., 1990). However, during ischaemia, there is an excess influx of Ca^{2+} (Kihara et al., 1989), and reduced ability of the cells to handle this increase.

Ca^{2+} is thought to enter ischaemic cells by a number of mechanisms. There is a drop in resting membrane potential with ischaemia, due to K^+ efflux, which may open the L - type Ca^{2+} channels. However, this means of Ca^{2+} entry is strongly debated (Fozzard and Makielski, 1985; Coetzee, 1988). Another means of Ca^{2+} entry which has been proposed is via the Na^+/Ca^{2+} exchange following intracellular Na^+ accumulation,

either from activation of the Na^+/H^+ exchange (Bers and Ellis, 1982; Lazdunski et al., 1985) or by inhibition of the ATP-dependent Na^+/K^+ pump by a reduction in ATP levels (Ellis and MacLeod, 1985 Kaila and Vaughan-Jones, 1987). The Na^+/Ca^{2+} antiport is thought to carry 3 Na^+ ions for each Ca^{2+} ion via a protein (Reuter and Seitz, 1968; Reeves and Sutko, 1979; Philipson, 1985; Eisner and Lederer, 1985; Kimura et al., 1986). The energy driving this exchange is provided by the concentration gradients of both ions, and the transmembrane potential (Eisner and Lederer, 1985). The exchange is therefore reversible, which can lead to the intracellular accumulation of Ca^{2+} when there is a high intracellular Na^+ concentration and a depolarisation of the membrane (Chapman, 1983; Lazdunski et al., 1985; Kaila and Vaughan-Jones, 1987; Tani and Neely, 1989; Vaughan-Jones et al., 1989).

A predominant mechanism for Ca^{2+} entry is thought to be diffusion through leaky membranes down its concentration gradient (Chapman et al., 1984; Nayler et al., 1988). The holes in the membrane formed during ischaemia are exacerbated by the increasing levels of free Ca^{2+} in the cell, which reduces the stability of the cellular membranes. Ca^{2+}-dependent phospholipases present in the membrane are activated by an increase of calcium (Rim et al., 1990). Lipid peroxidation occurs with the appearance of holes in the lipid bilayer through which intracellular enzymes and myoglobin are lost. Conformational changes of the membrane also occur with inhibition of sarcolemmal ATPase activity (Limbruno et al., 1989). Ca^{2+} sensitive proteases may be activated which also cause damage within the cell and to the cell membranes (Nayler et al., 1988; Rim et al., 1990).

Intracellular accumulation of free Ca^{2+} may also be due to reduced uptake by the SR and a lower buffering capacity of the cytosol. With a drop in ATP levels, Ca^{2+} sequestration from the cytosol is reduced (Smith and Allen, 1988) as Ca^{2+} pumps in the sarcoplasmic reticulum and sarcolemma are highly dependent on ATP (Carafoli, 1985). With ischaemic intracellular acidosis, the affinity of Ca^{2+} binding sites is

reduced and the Ca^{2+} buffering capacity of the cytosolic proteins is inhibited (Bers and Ellis, 1982; Kohomoto et al., 1990).

Ca^{2+} is accumulated in the mitochondria at the expense of ATP by the electron transport chain, using the proton gradient, or by ATP dependent processes (Pesaturo and Gwathmey, 1990). With extended ischaemia, calcium phosphate granules are deposited in the mitochondria (Jennings and Ganote, 1976). Ca^{2+} overload results in an impairment of mitochondrial function.

5) Potassium efflux

Potassium efflux and extracellular accumulation are known to be primary consequences of ischaemia (Kléber, 1983; 1984; Fozzard and Makielski, 1985). Resting membrane potential decreases soon after restriction of flow, and the amplitude and duration of the transmembrane action potentials are reduced (Kléber et al., 1978; Moréna et al., 1980; Kagiyama et al., 1982; Manning and Hearse, 1984).

K^+ loss from myocardial cells is attributed to a number of mechanisms including opening of the ATP dependent K^+ channel (Kantor et al., 1990). There is also a large efflux of K^+ down its concentration gradient. At rest, the membrane has a high permeability to K^+, resulting in a constant flux of K^+ out of the cell. The ATP dependent Na^+/K^+ pump acts to restore the gradient. However, when ATP is depleted in ischaemia, this pump is no longer active. Loss of K^+ and extracellular K^+ accumulation during hypoxia and ischaemia depolarises the membrane (Manning and Hearse, 1984), with resting membrane potentials of -50 mV recorded by late ischaemia (Kléber, 1983).

b) Reperfusion injury

Myocardial damage and poor heart function result both from ischaemia and the subsequent period of reperfusion (Opie, 1989b). Ischaemia is thought to predispose the myocardium to injury which is exacerbated on reperfusion, where the severity of reperfusion injury is thought to be directly related to the extent of ischaemic injury. There is a paradoxical need for

reperfusion of the ischaemic tissue, even though this appears to cause widespread damage.

Reperfusion damage may be apparent in myocardial swelling and a depression of ventricular function (Opie, 1989b; Laster et al., 1989). An inability of the post-ischaemic tissue to make efficient use of delivered O_2 (Laster et al., 1989), increased oedema upon reperfusion, with stretching of the membranes and mechanical abrasion, intracellular accumulation of Ca^{2+}, and increased oxygen free radical activity (Kao et al., 1986) are factors leading to permanent damage of the cells.

Pathological consequences of ischaemia include reperfusion arrhythmias. The phenomenon of "stunning", a reversible depression of function after an ischaemic period, has also been described (Braunwald and Kloner, 1982). Stunning may be a protective phenomenon, possibly allowing the heart a period of rest after the ischaemic insult. However, in the clinical situation, an immediate and complete restoration of mechanical functioning of the heart may be required.

1) Restoration of ATP levels

Levels of ATP on reperfusion are gradually restored although control levels are not reached for some time. A significant correlation of ATP content of hearts at the end of ischaemia with percentage recovery of work performed has been observed (Edoute et al., 1983). This was after differing periods of ischaemia. Reperfusion for 30 min led to an increase in ATP levels, although these did not reach preischaemic values. While ATP levels at the end of the ischaemic period must be above a certain level to ensure reversibility of damage, restoration of the mechanisms of ATP production on reperfusion, especially oxidative metabolism, may be more important (Reimer et al., 1981; Taegtmeyer et al., 1985; Laster et al., 1989; Saks et al., 1989).

During ischaemia, the precursors of ATP, including adenosine, inosine and hypoxanthine leak out of the cells. On reperfusion, these are washed out, which delays the restoration of ATP formation (Reimer et al., 1981). The

immediate recovery of ATP on reperfusion is therefore inhibited. Mitochondrial function is also thought to be disrupted during ischaemia and subsequently exacerbated on reperfusion (Edoute et al., 1983). Reperfusion is associated with a depression in mitochondrial oxidative function, and therefore reduced ATP production. Controversy exists as to whether mitochondrial damage occurs during ischaemia, or is exacerbated on reperfusion. Disruption to the compromised mitochondria, observed during ischaemia as swelling and formation of dense granules (Jennings and Ganote, 1976; Edoute et al., 1983) may be exacerbated by the Ca^{2+} influx observed on reperfusion (see below). The damage to the mitochondria may contribute to the delay in the restoration of ATP on reperfusion.

Laster et al. (1989) observed no absolute change in myocardial oxygen consumption in the post-ischaemic "stunned" myocardium but a depressed contractile function was observed. An increase in oxygen consumption per unit work was therefore found on reperfusion, indicating a decrease in the efficiency of energy consumption and usage, possibly associated with mitochondrial damage.

Alternatively, Saks et al. (1989) found that mitochondrial oxidative phosphorylation (in skinned myofibrils) was found to be tolerant to an ischaemic period which reduced contractile function. It was suggested that the decreased function is a result of disturbance of the contractile function or electromechanical coupling.

CP levels are immediately restored on reperfusion (Taegtmeyer et al., 1985) although creatine kinase and its coupling to oxidative phosphorylation are impaired with ischaemia (Saks et al., 1989).

2) Calcium overload

One of the major putative causes of reperfusion injury is an excessive influx of Ca^{2+} (Clusin et al., 1983; Nishioka et al., 1984; Poole-Wilson et al., 1984; Nayler et al., 1988). During ischaemia, membrane damage occurs (Corr et al., 1984),

together with an intracellular Na^+ overload, possibly following acidosis (Lazdunski et al., 1985). On reperfusion, these changes initiate an influx of Ca^{2+} in addition to that occurring during ischaemia, in excess of that which can be handled by the cytosolic Ca^{2+} buffering and uptake capacities (Kohomoto et al., 1990). Ca^{2+} uptake by the SR is still depressed on reperfusion (Krause et al., 1989) while there is an increased sarcolemmal permeability to Ca^{2+} (Nishioka et al., 1984). The excess Ca^{2+} is thought to be responsible for much of the reduced function on reperfusion, which may be due to a breakdown in excitation-contraction coupling (Krause and Hess, 1984; Krause et al., 1989; Limbruno et al., 1989).

With the massive influx of calcium on reperfusion, there is a need for increased uptake of Ca^{2+} by the SR by energy consuming processes, in the face of a depletion of energy stores. Additional Ca^{2+} overload of the mitochondria occurs, with the deposition of granules, as these organelles are a major buffer for this ion (Carafoli, 1985; McGuigan and Blatter, 1987). This depresses oxidative phosphorylation, while bands of contracture appear in the myocardium, decreasing energy stores further.

A major mechanism for the entry of Ca^{2+}, especially on reperfusion, is thought to be by increased activity of the Na^+/Ca^{2+} exchanger (Burt and Langer, 1982; Chapman et al., 1984; Chapman et al., 1986). On reperfusion with a relatively alkalotic perfusate (blood pH 7.4), the Na^+/H^+ exchanger may be activated, which leads to the protons being extruded, and pH returned to normal, but with a concomitant increase in intracellular Na^+.

Intracellular Na^+ accumulation is also associated with cellular swelling and oedema due to the retention of salt. Cl^- enters the cell because of the change in the electrochemical gradient. The Donnan equilibrium is upset, with loss of regulation of cell volume, and an increased vascular resistance. Disruption of the sarcolemmal membranes on reperfusion, decreased perfusion of the vascular bed and reduced removal of accumulated waste products result.

3) The calcium paradox

In the special case of an absence of extracellular Ca^{2+}, as occurs with the use of an calcium-free cardioplegic solution, re-exposure to a Ca^{2+}-containing perfusate results in the appearance of the calcium paradox (Zimmerman and Hulsmann, 1966; Bielecki, 1969; Yates and Dhalla, 1975; Chapman et al., 1984). This phenomenon is paradoxical as the heart requires Ca^{2+} for its normal function, but when reintroduced after a period of acalcaemic perfusion, results in a large depression in function and severe ultrastructural damage. Observed effects are a change in colour of the myocardium, a decrease in electromechanical activity with severe contracture, and an increase in the loss of LDH and other enzymes with changes in the cellular ultrastructure (Nayler et al., 1988; Hess and Manson, 1984; Yates and Dhalla, 1975). There are many similarities between this phenomenon and reperfusion after an ischaemic period.

When Ca^{2+} is removed from the extracellular space, the external lamina and glycocalyx of the sarcolemma separate, while swelling and margination of the mitochondria occur because of an increase in membrane fluidity (Frank et al., 1977; Langer, 1978). On reperfusion with a calcium containing perfusate, there is an excessive influx of Ca^{2+} because of damage to the sarcolemmal membranes, as well as other factors including intracellular Na^{+} overload (Hess and Manson, 1984). The formation of oxygen free radicals associated with reoxygenation may also disrupt the functioning of the membrane (McCord, 1985; Kako, 1987; Opie, 1989b). Free radical generation depresses the rate of Ca^{2+} uptake of the SR of the myocardium, and the Na^{+}/Ca^{2+} exchange and the Na^{+}/K^{+} ATP dependent pump may be also be affected (Kako, 1987).

While acidosis (Bielecki, 1969) and Mg^{2+} (Bhojani and Chapman, 1990) have been found to protect the heart against the Ca^{2+} paradox, alkalosis (Bers and Ellis, 1982), extracellular Na^{+} depletion and high extracellular K^{+} all increase Ca^{2+} influx (Torchiana et al., 1987). Oxygen free radical formation is also thought to be linked to the calcium paradox (Hearse et

al., 1978; Hess and Manson, 1984; Opie, 1989b), where an accumulation of intracellular Ca^{2+} may trigger the formation of these reactive molecules.

4) Free radical formation and the oxygen paradox

The oxygen paradox is observed on the reintroduction of oxygen after a period of hypoxia or ischaemia, the conditions of which predispose the tissue to the formation of free radicals. This phenomenon is thought to be mediated by the metabolism of molecular O_2.

Several mechanisms for the formation of oxygen free radicals have been proposed (Hess and Manson, 1984; McCord, 1987). These intermediates are normally produced by the cells from about 5% of available oxygen, but these are broken down by naturally occurring free radical scavengers (Hess and Manson, 1984). During ischaemia, these enzymes may be inhibited. Another possible mechanism has been proposed by McCord (1985; 1987). During the hypoxic or ischaemic phase, changes such as the accumulation of the breakdown products of ATP, including xanthine and hypoxanthine, and intracellular Ca^{2+} overload occur. Intracellular accumulation of Ca^{2+} is thought to activate a protease, possibly calpain, which converts the enzyme xanthine dehydrogenase to xanthine oxidase. In the presence of molecular oxygen, this enzyme will convert hypoxanthine to urate with the formation of free radicals. This process has been identified as a significant contributor to oxygen free radical formation in the heart, primarily in the capillary endothelial cells (Chambers et al., 1989)

Oxygen free radicals are highly reactive molecules which react with many compounds in the cell (McCord, 1985, 1987; Kako, 1987). This leads to denaturation of proteins (Prinsze et al., 1990) and peroxidation of phospholipids with subsequent membrane degeneration (Gutteridge and Halliwell, 1990). Loss of normal mitochondrial and SR function, altered membrane permeability, disorganisation of the myofibrils and disruption of the cellular transport processes occur depending on the duration of hypoxia (Gaudel and Duvelleroy, 1984; Miki et al., 1988; Menasché and Piwnica, 1989; Vandeplassche et al., 1990).

Free radicals may affect proteins by conformational changes in amino acid residues and in susceptibility to enzymes and denaturation (Kako, 1987).

The reversible decrease in contractile function evident on reperfusion, described as "stunning", may also be mediated by oxygen free radicals. Bolli et al. (1989) provided evidence of free radical-induced cellular damage responsible for stunning developing in the initial seconds after reperfusion. Free radical activity has also been associated with reperfusion arrhythmogenesis (Woodward and Zakaria, 1985 although this has been disputed (Coetzee et al., 1990). Membrane degeneration associated with free radical activity was found to be delayed, after the manifestation of early reperfusion arrhythmias (Coetzee et al., 1990). The interrelationship between free radical activity and Ca^{2+} has been strongly emphasised (Hearse et al., 1978; Hess and Manson, 1984; Opie, 1989b).

5) Reperfusion arrhythmias

These are a pathological consequence of ischaemia, the most extreme circumstance of which is ventricular fibrillation (VF), which leads to a critical decrease in cardiac output (Manning and Hearse, 1984), with increases in wall tension and energy consumption in the face of a depleted availability of oxygen. VF is a common clinical finding on reperfusion after cardio-pulmonary bypass, and its prevention is of critical importance (for review see Manning and Hearse, 1984).

Indices such as shorter refractory periods and uncoordinated depolarisation cause disruption of the normal heart rhythm with the resultant effect that the tissue is no longer in a functional syncytial state and cannot act as a cohesive unit. Alterations during ischaemia and on reperfusion including K^{+} efflux and extracellular accumulation, free radical activity, Ca^{2+} overload, inhibition of glycolytic activity (Bernier and Hearse, 1988; Kantor et al., 1990) and increases in cyclic adenosine monophosphate (cAMP) levels (Opie, 1982) are thought to contribute to arrhythmic activity on reperfusion.

iv) Objectives of a cardioplegic solution

The objectives of a cardioplegic solution are, according to Buckberg and Rosenkrantz (1987), "to stop the heart safely, create an environment for continued energy production, and counteract deleterious effects of ischaemia". Maximal recovery may be achieved not only by inhibition of extreme myocardial damage but also by avoiding all ischaemic injury. Cardioplegic solutions are therefore formulated on the basis of the changes occurring during ischaemia. Additional criteria for an improved solution include ease of administration, lack of complications during surgery, and ease of manipulation of the basic composition.

The basic requirements of a cardioplegic solution have been stipulated (from Buckberg, 1979; Bretschneider, 1980; Hearse et al., 1981; Buckberg and Rosenkrantz, 1987):

a) Immediate arrest

Arrest of the heart must be immediate, complete and reversible, without detrimental side effects. The heart must remain in an arrested state without intermittent recurrence of activity for the duration of the arrest period. The membrane must be stabilised and irreversible damage prevented.

b) Energy conservation

Energy must be conserved in order to counteract the adverse effects of ischaemia, as many functions are determined by the level of stored energy in the cell.

A fundamental approach when formulating an efficient cardioplegic solution is based on improved energy status of the heart. This may be by either increasing energy production (intracellular and extracellular sources); or by reduction of energy demand. The balance of these two factors is thought to be critical in determining the recovery of the hearts (Bretschneider, 1980).

Energy demand is brought about by

- basal metabolic rate
- continued electromechanical activity

- wall tension

Energy demand in an arrested heart may be reduced by a number of interacting factors.

- Hypothermia is conventionally used (Buckberg, 1979), which leads to a reduction in metabolic rate while also suppressing contractile and transmembrane ATPase activity (Fukumoto et al., 1990). All reactions and processes in the myocardial tissue are slowed down at low temperatures and the consumption of ATP is decreased.

- Electromechanical activity may also be inhibited by manipulation of the ionic composition of the cardioplegic solution. Usage of ATP by membrane pump mechanisms is consequently reduced.

- Wall tension can be reduced by inhibiting intracellular accumulation of ions such as Ca^{2+}, as this leads to contracture with increasing ATP consumption.

Energy production can be increased by

- the addition of substrates for anaerobic energy production. However, there are harmful end products associated with an increased rate of glycolysis, including lactate and protons. The accumulating end metabolites act to inhibit glycolysis, and have other harmful effects on the myocytes. The simple addition of substrate is therefore not always beneficial but may sometimes be deleterious. The relative contribution of these converse effects must be determined in the overall recovery of the hearts. Further alterations to the solution may be required to enhance the effect of the initial modification.

- increasing the supply of oxygen and consequently oxidative phosphorylation. This is a much more efficient energy pathway than glycolysis, without the accumulation of metabolites.

c) Adequate buffering

Buffering is required to prevent large changes in pH which are associated with ischaemia.

d) Osmolarity

Osmolarity must be maintained to prevent cell and tissue damage by oedema, especially on reperfusion.

e) Inhibition of reperfusion injury

On reperfusion of the heart, restoration of the ionic gradients must be immediate, and phenomena such as oedema and distension of the ventricles, ventricular fibrillation, ventricular collapse, and development of necrotic tissue must be avoided. Free radical activity and excess Ca^{2+} influx must be inhibited.

Reperfusion injury has been the focus of increasing interest in recent literature (Braunwald and Kloner, 1982, 1985; Jennings et al., 1985; Opie, 1989b). This may be mitigated in two ways; by developing a specific reperfusion solution; or by minimising ischaemic damage. The latter is the aspect of major concern in our investigation.

v) The St Thomas' Hospital cardioplegic solution No 2

a) Basis of formulation

The St Thomas' Hospital Cardioplegic solution No. 2 (ST) has a basic ionic composition (Table 1), with a proven record both in the laboratory and clinically. However, we felt that further changes could be made to this solution to optimise recovery of the hearts.

The ST cardioplegic solution has a high potassium concentration as the cardioplegic agent, and is used under hypothermic conditions. This leads to rapid arrest of the heart and substantially reduces metabolic rate. The solution is normally infused at a temperature of 4-10^{o}C, which decreases the metabolic rate without disrupting the cells due to the formation of ice crystals. The higher temperatures within this range have been shown to be more beneficial in preservation of hearts (Tyers et al., 1977; see Hearse et al., 1981 for discussion), after monitoring of various biochemical parameters including myocardial ATP content, and recovery after arrest. 10^{o}C and 15^{o}C resulted in the best recoveries, while 5^{o}C, 20^{o}C and 37^{o}C reduced function (Tyers et al., 1977). This has been corroborated in several other studies using different models (see Hearse et al., 1981).

The osmolality of the ST solution is maintained at about 300 mOsm/kg H_2O which is slightly above extracellular values (Table 1). The osmolality is maintained to prevent the development of intracellular oedema which is especially important in preventing reperfusion damage.

b) K^+ concentration

The ST solution has an optimal K^+ concentration of 16 mmol/l. A high K^+ concentration has been found to act synergistically with a decreased temperature in suppressing electrical activity in the cells (Hearse et al., 1975; Ferguson et al., 1986) The development of an action potential is prevented by an increased K^+ concentration as membrane potential is reduced. With 16 mmol/l K^+, the membrane potential is about -55 mV. At this potential, the Na^+ and Ca^{2+} channels are

inactivated. With a higher K^+ concentration, Ca^{2+} channels will be activated, which is undesirable.

A large K^+ concentration gradient and a relatively high permeability of the membrane to the ion during ischaemia cause efflux of K^+. Intracellular K^+ concentration must be maintained, usually with the expenditure of energy. With a high extracellular K^+ concentration, this energy expenditure is reduced.

c) Na^+ concentration

A concentration of 120 mmol/l Na^+ is used in the ST solution. A more reduced Na^+ concentration would prevent the development of an action potential, but too low an extracellular concentration may cause Ca^{2+} influx across the sarcolemma by activation of the Na^+/Ca^{2+} exchange mechanism, which is dependent on the gradients of Na^+ and Ca^{2+} across the cell membrane (Chapman et al., 1984; Eisner and Lederer, 1985). Na^+ must therefore be maintained at near normal levels. A dose response curve indicates that a concentration of 100-120 mmol/l is optimal for cardioplegic solutions (Jynge, 1980).

d) Ca^{2+} and Mg^{2+} concentration

Ca^{2+} must be present in a cardioplegic solution at a minimum concentration of 50 µmol/l, to prevent the calcium paradox phenomenon upon reperfusion of the heart (Hess and Manson, 1984). The ST solution uses a concentration of Ca^{2+} of 1.2 mmol/l which is similar to that usually found in the extracellular medium (Yamamoto et al., 1984; Ledingham et al., 1987). This relatively high concentration can be used because of the presence of a high Mg^{2+} concentration.

The triggering action of Ca^{2+} in excitation-contraction coupling of the myocardium with subsequent consumption of energy is inhibited by Mg^{2+} which competes with Ca^{2+} binding sites (Hearse et al., 1978a). Ca^{2+} entry is blocked and the Ca^{2+} ATPase of the SR is activated in the presence of Mg^{2+}, thereby increasing Ca^{2+} uptake. A concentration of 16 mmol/l is recommended for the ST solution (Hearse et al., 1978;

Torchiana et al., 1987; Geffin et al., 1989; Reynolds et al., 1989).

e) HCO_3^- concentration

The bicarbonate system ($NaHCO_3/H_2CO_3$) is a major buffer in blood although its capacity is fairly weak even at its pK of 6.35 (at 25°C). It plays an important role in maintaining pH in the face of changes in the CO_2 concentration. Carbon dioxide, freely permeable across the plasmalemma, determines the intra- and extracellular pH to a large extent. 10 mmol/l HCO_3^- is included in the ST solution, to buffer the effects of ischaemia on pH.

vi) Clinical models - in situ aortic cross clamping and ex vivo donor heart storage

There are two main strategies for the usage of a cardioplegic solution. In situ cardio-pulmonary bypass with subsequent aortic cross-clamping allows surgery to be performed in a bloodless field. Donor heart storage allows for harvesting of hearts for transplantation. In both cases, an extension of the time of arrest while ensuring maximum recovery is required.

There are slight differences in the principles involved in the above procedures (Table 2), where hearts in situ in aortic cross-clamping are in the presence of non-coronary collateral flow (blood flow from the surrounding tissue). This provides an external supply of substrates, but also washes out the cardioplegic solution and reduces hypothermia (Buckberg, 1979). This may or may not be advantageous. To combat these effects, the hearts can be intermittently flushed with cardioplegic solution during the arrest period. This may confer additional advantages to the arrested heart, such as provision of substrate, and removal of metabolites.

In donor heart storage, the environment is more consistently controlled. Donor heart storage can be of two kinds: continuous perfusion of cardioplegic solution at low pressures, or intermittent flushing; or simple hypothermic storage in a protective medium. There are advantages to both these methods. Low pressure perfusion and intermittent flushing of the heart, while possibly resulting in better recoveries, are impractical if the heart is to be transported over any distance. Simple immersion of the heart after a single flush of solution is more feasible although the recoveries are not always as good.

The choice of cardioplegic solution used in either of the above situations is made by consideration of factors including ease of administration, and possible complications which may arise from storage of the solution over long periods. Changes in the myocardium over time may differ depending on the protocol used, which may in turn determine the values of

variables such as pH and the degree of oxygenation of the cardioplegic solution.

Cardioplegic solutions for use during cardiac bypass have been well studied. Recently, there has been emphasis placed on solutions specifically for long term storage of donor hearts. These solutions have generally been developed for pulmonary or renal preservation (Baumgartner, 1990) and then applied to myocardial preservation. These solutions include Belzers's (or University of Wisconsin) solution (Belzer and Southard, 1988), the Euro-Collin's solution (Reitz et al., 1974) and the Sacks solution (Thomas et al., 1975). These solutions generally have a high K^+ (comparable to intracellular levels) and a low Na^+, with no Ca^{2+}. As yet few clinical studies have been made with these solutions. The ST solution has been proved with cardiac bypass, and its use may be extended for long term preservation by manipulation of the formulation.

Table 2: Comparison of in situ cardio-pulmonary bypass and ex vivo donor heart storage with simple immersion.

Cardio-pulmonary bypass	Donor heart storage
-Lowest attainable myocardial temperature 12-16^{o}C	4-10^{o}C
-Non coronary collateral flow washout of cardioplegia supply of substrate reduction in hypothermia	-Totally isolated in storage medium
-Relatively short term arrest period	-Extended period of arrest
-Diseased hearts	-Healthy hearts
-Handling of the heart	-Relatively little handling during storage
-Mechanical abrasion with flushing	-Ionic imbalance over time with no flushing
-Removal of waste products	-Possible build up of waste products with no flushing
-Substrates can be supplied intermittently	-Substrate depletion over time

III. METHODS

i) The isolated heart apparatus

The isolated working rat heart model was used to test the different cardioplegic solutions. The apparatus used was first described by Langendorff (1895), and modified by Neely et al. (1967). A diagram of the apparatus used is shown in Figure 1. It consists of essentially three different parts; the Langendorff unit for the initial perfusion and stabilisation of the heart in a retrograde mode; the working heart side for the measurement of indices of cardiac function with a preload of 20 cm H_2O and an afterload of 100 cm H_2O; and a storage unit allowing cardioplegic solution to be introduced to the heart via the aortic cannula. The temperature on the perfusion side was maintained at 37^{o}C, while the cardioplegic side was kept at 10^{o}C. This temperature has been cited as being optimal for cardioplegic solutions (Tyers et al., 1977; Hearse et al., 1981). Coronary effluent in the working mode was collected and recirculated as this has been shown to improve recoveries, by preventing a loss of protein from the perfusate. The recirculated solution was filtered before being reintroduced to the reservoir.

The aortic and left ventricular cannulas can be closed off using the three way taps, and removed from the circuit. This 'head' can then be placed in a reservoir for maintenance during arrest. Another set of cannulas can then be connected, to be removed in a similar fashion. This enables up to three experiments to be run concurrently. Maintenance of the hearts during arrest was set up so as to allow intermittent flushing with cold cardioplegic solution of three hearts simultaneously from a central vessel; or for simple storage at hypothermia.

Pressure measurements were taken via the aortic cannula and a Statham Gould P32ID transducer, and recorded on a Grass Model 79D polygraph (Grass Instrument Co., Quincy, Mass. USA).

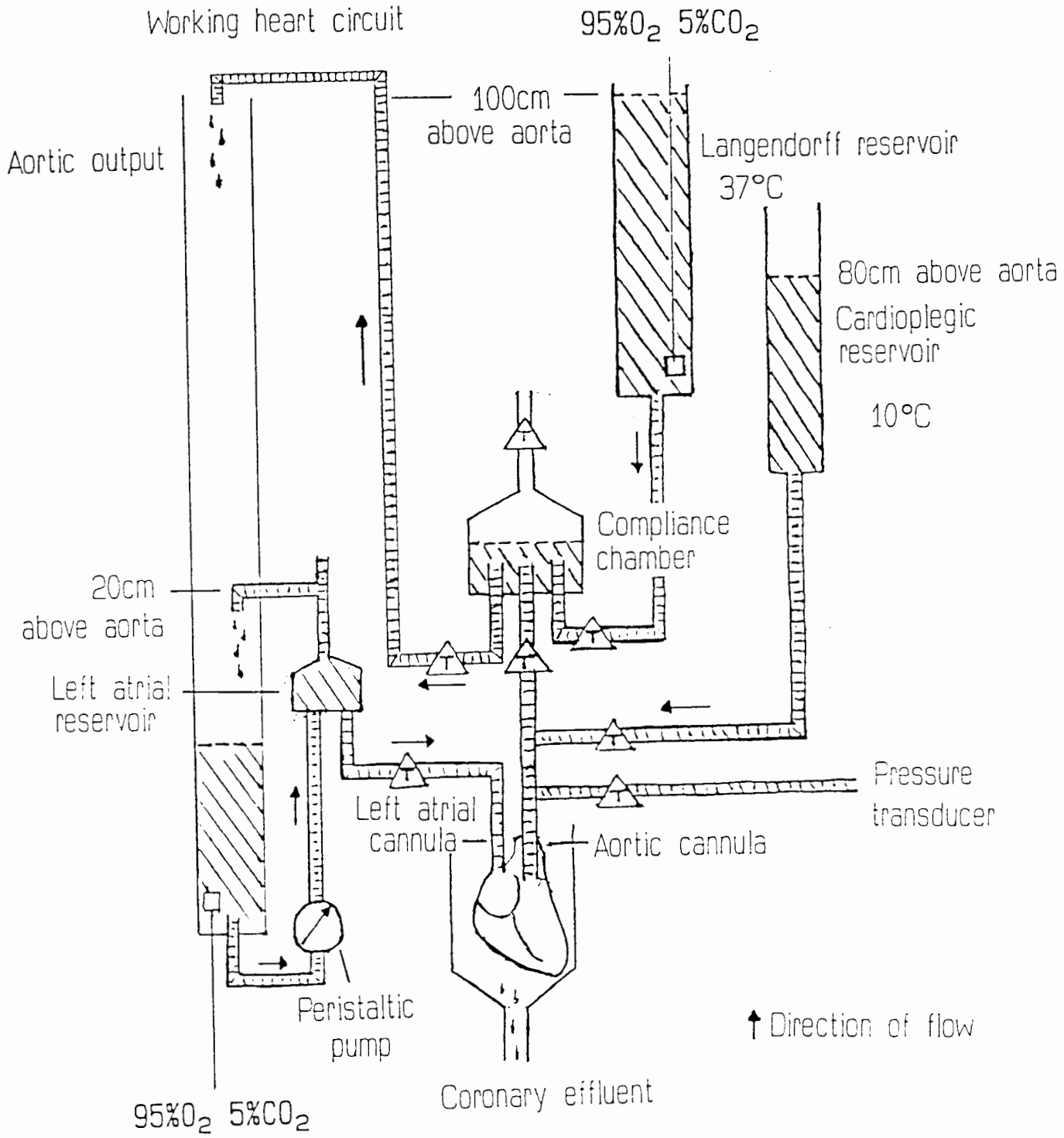

Fig. 1. The isolated working rat heart perfusion apparatus with cardioplegic reservoir. The perfusion solution is Krebs-Henseleit buffer, gassed with 95%O_2 5%CO_2 and maintained at 37^{o}C. The entire perfusion rig is water-jacketed to ensure that the temperature is maintained. Perfusion pressure on the Langendorff side is 100cm H_2O, and preload and afterload on the working heart side are 20cm H_2O and 100cm H_2O respectively. Cardioplegic solution, at a temperature of 10^{o}C, is introduced through the aortic cannula at a pressure of 80cm H_2O. Three way taps (T) can be closed off with removal of the cannulas as a separate unit to allow the hearts to be stored separately.

ii) Solutions

Modified Krebs-Henseleit buffer (mmol/l NaCl - 118.5, $NaHCO_3$ - 25, KCl - 4.75, KH_2PO_4 - 1.185, $MgSO_4$ - 1.19, $CaCl_2$ - 1.25, Glucose - 11), oxygenated with 95%O_2 5%CO_2, was used to perfuse the hearts at 37°C (Krebs and Henseleit, 1932). The solution was made up from stocks (50 ml/l of 692.30 g/l NaCl; 50 ml/l of 210.50 g/l $NaHCO_3$; 10 ml/l of 35.40 g/l KCl; 10 ml of 16.13 g/l KH_2PO_4; 10 ml of 29.33 g/l $MgSO_4.7H_2O$; 5 ml of 36.75 g/l $CaCl_2.2H_2O$). Before the addition of calcium chloride, the solution was gassed with 95%O_2 5%CO_2 to prevent the precipitation of calcium phosphate.

Cardioplegic solutions were made fresh each day, gassed with 95%O_2 5%CO_2 during preparation. All solutions were filtered through filters of 5.0 µm and 0.8 µm (Millipore Corporation, Bedford, MA, USA) to remove large particles. This is required to prevent vasoconstriction induced by particles larger than 10 µm in diameter to ensure adequate coronary flow on reperfusion of the heart (Hearse et al., 1985).

iii) Experimental procedure

Male Long-Evans rats were used, weighing between 280 and 420 grams. Diethyl ether was used to anaesthetise the animals and 200 i.u. of heparin injected into the exposed femoral vein.

The hearts were excised and arrested in cold Krebs - Henseleit buffer (about 4°C). The aorta was cannulated, and retrograde perfusion through the aorta immediately initiated. The coronary sinus was incised to allow drainage of the coronary circulation. The pulmonary veins were then cannulated. The heart was briefly perfused through the left atrium to ensure correct cannulation and then allowed to stabilise in the Langendorff mode for ten minutes, after which the system was switched over to perfusion in the working mode.

Functional measurements (see below) were taken at the two, five and ten minute intervals in the working heart mode. The hearts were then arrested by the introduction of 10 ml cold cardioplegic solution through the aortic cannula, after the perfusion lines had been closed.

The hearts were maintained in an arrested state for three hours. Clinical procedures were simulated in one of the following ways, depending on the protocol of the specific experiment:

- Simple storage (single dose)

The hearts were arrested with 10 ml cardioplegic solution and stored at 10°C for the three hour arrest period.

- Multiple dose infusions (multidose)

The hearts were initially arrested with 10 ml cold cardioplegic solution, and thereafter flushed with 6 ml cold cardioplegic solution every thirty minutes over the three hour arrest period, while stored at 10°C. The coronary effluent from each flush was collected.

After the three hour period of arrest, the hearts were reperfused in the Langendorff mode for 10 min. The coronary effluent was collected and the volume determined. Samples from the total volume of effluent were taken. This was followed by 10 minutes perfusion in the working mode, during which the functional measurements were repeated. The hearts were freeze-clamped at the end of the experiments using Wollenberg tongs, and the tissue subsequently freeze-dried.

iv) Functional measurements

Measurements in the working heart mode included

- Aortic output - the output through the aortic line per minute at a pressure of 100 cm H_2O, expressed as ml/min.

- Coronary flow - the output from the coronary circulation, expressed as ml/min.

- Heart rate - recorded via the pressure transducer, expressed as beats/minute.

- Aortic pressure - systolic and diastolic pressures recorded from the aortic pressure trace.

From the above measurements, other variables were calculated. These included:

- Cardiac output = Aortic output + Coronary flow (ml/min)

- Stroke volume = Cardiac output/Heart rate (ml)

Functional measurements obtained during the post-ischaemic working heart period after 20 min reperfusion (10 min non work + 10 min work) were expressed as a percentage of their individual pre-ischaemic control values taken at 10 min work.

v) Biochemical analyses

Samples of the post-ischaemic coronary effluent from the hearts when perfused in the Langendorff mode were collected, as was the 6 ml coronary effluent from each flush of cardioplegic solution. Simple biochemical analyses were performed on these samples, and on the freeze-dried heart tissue.

The results for the coronary effluent samples are expressed as per 6 ml sample reflecting total washout from the heart, while those from post-ischaemic reperfusion are expressed as per ml per minute, derived from the total volume collected in the 10 minute reperfusion period.

Results are expressed as per gram wet weight of the heart, which was determined from the body weight of the rat using the following relationship:

Wet Heart Weight = (Rat Wt + 75.18)/359.71

(derived from previous correlations for the laboratory strain - private communication - O. Bricknell)

a) Lactate dehydrogenase

Lactate dehydrogenase (LDH) is the enzyme responsible for the conversion of lactate to pyruvate during aerobic conditions, and the reverse reaction during hypoxia. There are five LDH isoenzymes, and the rate of release of these enzymes into the blood stream is an indication of the degree of myocardial injury (Hearse et al., 1973; Hearse and Humphrey, 1975).

The LDH concentrations of the coronary effluent samples were determined using the method according to Bergmeyer and Bernt (1963). Spectrophotometric measurements were made when the enzyme was activated in the presence of NADH, pyruvate, KH_2PO_4 and NaH_2PO_4 at a wavelength of 340 nm.

$$\text{Pyruvate} + NADH_2 \overset{LDH}{\leftrightarrow} \text{Lactate} + NAD^+$$

The following cocktail was made:

	Stock	Cocktail
KH_2PO_4	76 mmol/l	19.4 ml
$Na_2HPO_4.12H_2O$	183.4 mmol/l	79 ml
Pyruvate		32 µmol/l
NADH		21 µmol/l

The composition of the phosphate buffer gives a pH of 7.5.

0.5 ml sample was added to 1 ml cocktail and read at 340 nm (Beckman Model 42 Clinical Analyzer, Beckman Instruments Inc., Irvine, California). 10 readings were made at intervals of 10 seconds at 37°C and the mean difference in optical density calculated (δOD). The calculation was

$$\frac{(\delta OD)/min * 5053 * 0.419}{\text{heart weight}} * \frac{\text{total vol}}{\text{sample vol}} = \text{mUnits LDH/gm/ml}$$

where 5053 is the extinction coefficient for 1 mU/ml LDH at 25°C and 0.419 is the correction for the assay at 37°C.

The results for post-ischaemic LDH were expressed as milliunits per gram wet weight per minute (mU/gm/min). The results for the samples obtained with flushing were expressed as milliunits per gram wet weight per 6 ml flush (mU/gm/6 ml).

b) Lactate

The lactate concentration in the coronary effluent was determined by the method of Hohorst (1963), based on the reaction:

$$\text{Lactate} + NAD^{+} \overset{LDH}{\leftrightarrow} \text{Pyruvate} + NADH_2$$

The difference in the extinction at 340 nm was measured.

A cocktail mixture was made up with a buffer (160 mmol/l hydrazine, 2 mmol/l EDTA, 400 mmol/l glycine - pH 9.3 - 9.5 with 2N NaOH):

	Cocktail (Per Cuvette)
Buffer	1.5 ml
NAD 1% (w/v)	0.2 ml
Distilled H_2O	1.1 ml

0.2 ml sample was added to 2.8 ml cocktail per cuvette. Blanks and standards at the appropriate concentrations (1 mmol/l lactate standard) were run.

The absorbance at 340 nm was read (Beckman DU-7 Spectrophotometer, Beckman Instruments Inc., Irvine, California), after which 10 µl LDH was added to each cuvette, and mixed. The absorbance was measured after approximately 1 hour at 340 nm, and again every ten minutes until the values were stable. The difference in optical density (δOD) was determined. The calculations were

$$[\delta OD\ (\text{sample}) - \delta OD\ (\text{Blank})]/0.414 = \text{mmol Lactate/ml}$$

where 0.414 is the extinction coefficient for 200 µl of 1 mmol/l lactate. The results for post-ischaemic lactate were expressed as µmol per gram wet weight per minute (µmol/gm/min). The results for the samples obtained with flushing were expressed as µmol per gram wet weight per 6 ml flush (µmol/gm/6 ml).

c) Adenosine triphosphate and creatine phosphate

The ATP and CP contents of the hearts were determined from the freeze dried tissue. The perchloric acid (PCA) extraction method was used.

20-25 mg of powdered freeze-dried tissue was added to 1.2 ml of cold 5% PCA. The mixture was immediately homogenised for 15-30 secs while still on ice. The samples were then spun using a Sigma 202MK centrifuge at 5000 rpm for 10 min at 4°C. 1 ml of supernatant was placed in Eppendorf tubes, and the acid neutralised to a pH of 6.5 - 7.5 with the addition of neutralising solution (200 mmol/l Tris, 40% (w/v) KOH and saturated with KCl), using 5 µl Universal indicator. The sample was allowed to precipitate for 10 min, and then centrifuged for 5 min at 5000 rpm. The supernatant fractions were decanted and frozen until assayed.

The dilution factor due to neutralising was calculated:

$$F = [1.2 \text{ ml (PCA)} \times \text{total volume}]/\text{wt (g)}$$

where Total volume = neutralising volume + volume for neutralising (1 ml) + 5 µl Universal indicator

The ATP and CP of the samples from the heart tissue were determined according to the method of Lamprecht and Trautschold (1963) based on the breakdown of glucose to form glucose 6-phosphate (G 6-P):

$$\text{CP} + \text{ADP} \xrightarrow{\text{Creatine phosphokinase}} \text{ATP} + \text{creatine}$$

$$\text{ATP} + \text{Glucose} \xrightarrow{\text{Hexokinase}} \text{ADP} + \text{G 6-P}$$

$$\text{G 6-P} + \text{NADP} \xrightarrow{\text{Glucose 6-phosphate dehydrogenase}} \text{6-Phosphoglucono-}\delta\text{-lactone} + \text{NADPH}^+ + \text{H}^+$$

The following cocktail was made:

	Stock	Cocktail (Per cuvette)
$MgCl_2$	1 mmol/l	0.1 ml
Tris	0.2 mmol/l (pH 7.5)	1.0 ml
NADP	1% (w/v)	0.1 ml
Glucose	100 mmol/l	0.05 ml
H_2O		1.65 ml

0.1 ml sample and 5 µl G_6PDH (10 mg/ml) was added to 2.9 ml cocktail per cuvette. The extinction at 340 nm was read (Beckman DU-7 Spectrophotometer, Beckman Instruments Inc., Irvine, California). 5 µl hexokinase (10 mg/ml) was added to each cuvette, and the extinction at 340 nm read after ten minutes when stable.

The difference in optical density was obtained and used to calculate ATP concentration:

$$(\delta OD - \text{blank})/0.414 * F = \mu mol/gm \text{ dried weight}$$

For the determination of CP levels, 0.05 ml of 10 mmol/l ADP was added to the cuvette. After 10 min, a reading at 340 nm was taken for the baseline reading of CP. 10 µl Creatine phosphokinase (5 mg/ml) was added, and a reading taken after 30-60 min.

The above calculation was used.

High energy phosphate levels were expressed as µmol/gm fresh weight, where the results for dried weight were divided by a factor of 5.

Control levels of ATP and CP were obtained from hearts following the above protocol clamped after 10 min work before arrest.

vi) pH, pO_2, pCO_2

These variables were determined for the cardioplegic solutions and the effluent samples collected during arrest. An Instrumentation Laboratory System 1302 pH/Blood gas Analyzer (Milan, Italy) corrected for measurements at 10°C was used.

Oxygen content of the solutions was determined using the solubility coefficient (α) of oxygen in saline solution at 10°C equilibrated at a pO_2 of 760 mmHg:

$$\text{Oxygen content/ml} = \alpha * pO_2/760 \text{ mmHg}$$

where α = 37 ml O_2 per litre of solution (Altman and Dittmer, 1971).

The results were expressed as ml O_2/6 ml.

The oxygen uptake by the hearts during arrest per 6 ml flush of cardioplegic solution was calculated as:

$$O_2 \text{ uptake} = (pO_2 \text{ cardioplegic solution} - pO_2 \text{ coronary effluent}) * O_2 \text{ content}$$

The results were expressed as ml O_2/gm wet weight/6 ml.

The pH at different temperatures was measured to obtain Rosenthal correction factors for the different solutions.

With a reduction in temperature of a solution, there is an increase in pH and in the pO_2. The relationship between a change in temperature (from x°C to y°C) and pH is expressed as

$$pH_y = pH_x + (x-y) * R$$

where R = Rosenthal correction factor

= 0.017 (water)
= 0.0147 (blood)

vii) Titration curves and buffering capacities

Titration curves for the cardioplegic solutions were determined by adding 500 µl aliquots of 1N HCl to the solution kept at 10°C.

The buffering capacities for the solutions were obtained by determining the instantaneous (derivative) slope of the

titration curves. Buffering capacity was expressed as mmol HCl/l/pH.

viii) Statistical analyses

a) Exclusion criteria

In the pre-ischaemic control period in the working mode, the following values were used to discard hearts:

30 ml/min < Aortic output < 60 ml/min
12 ml/min < Coronary flow < 22 ml/min
Heart rate < 200 beats/min, or irregular rhythm

In the post-ischaemic period, any heart whose coronary flow increased significantly (50% greater than pre-ischaemic value) was excluded, because of the probability of a left atrial leak.

Extreme observations or "outliers" based only on post-ischaemic aortic output, were tested by Dixon's criteria for testing extreme observations in a single sample (Snedecor and Cochran, 1981). With this test, the extreme values of a sample size n, given in ascending order from 1..n, are tested according to the relationship

$$(X_n - X_{n-1})/(X_n - X_1) \text{ or } (X_2-X_1)/(X_n-X_1)$$

The levels for significance are read off a table of Dixon's criteria. A significance value of $p<0.05$ was used.

The entire data set of these hearts was then excluded from further analysis in order not to distort the mean by inflating the error variance due to major experimental errors. In each sample group, 1 to 2 hearts were excluded.

b) Determination of significance

Pre- and post-ischaemic functional values were compared for significance using a three-way analysis of variance. This test depends on an assumption of common variance amongst observations within each treatment group, which was not observed in this study. Therefore, in specific comparisons of interest, necessary adjustments for the violation of common variance was made. Pairwise comparisons of means using no

assumption of common variance was performed using t-statistics with a nominal level of significance set at $p<0.05$.

A consequence of multiple comparisons is that a greater degree of significance may be found than actually exists. However, the number of significant comparisons obtained was a large fraction of the total number of comparisons studied, too large to be explained by probability.

Multiple comparison t-tests were used to establish significance of differences in measured biochemical values during and after arrest. Values obtained during arrest were compared between groups and within groups for differences. The average of the variables measured for each group were compared, with means for all samples per group taken. Significance was taken at $p<0.05$.

All results are expressed as means ± standard error of the mean (SEM).

c) Curve fitting

Regression lines to determine the slopes of the pH/temperature curves, and the sigmoid titration curves were fitted using GraphPAD^{TM} computer software (ISI Software, Philadelphia, Penn., USA).

ix) Chemicals

Enzymes were obtained from Boehringer Mannheim GmbH, W. Germany. L-Histidine (free base) was obtained from Sigma Chemical Company, St Louis, MO, USA. Bretschneider's HTK cardioplegic solution was obtained from Dr. F. Köhler Chemie GmbH, Alsbach, W.Germany.

IV. RESULTS AND DISCUSSION

A. Glucose in the ST solution - single dose and multidose

a) Introduction

Energy levels in the ischaemic myocardium are thought to be a crucial determinant of recovery. The energy demands of the arrested heart depend mainly on maintenance of the ionic gradients and cellular structure, and myocardial wall tension (Buckberg, 1979). One means of maintaining energy levels in an arrested heart is to increase production via anaerobic glycolysis by adding glucose to the cardioplegic solution. Glucose is a naturally occurring substrate and is routinely included in the K-H buffer for perfusion of isolated hearts. It has many other beneficial effects which may aid ischaemic arrested hearts.

The inclusion of glucose in a cardioplegic solution has been disputed in the literature (Lolley et al., 1974; Hearse et al., 1978b; Buckberg, 1979; Conti and Kao, 1983; Guilbeau et al., 1984; de Wit et al., 1988). An increased rate of glycolysis is associated with an increased intracellular proton concentration and lactate accumulation which may be deleterious to the ischaemic cells (Neely et al., 1975). The adverse effect of glucose in a cardioplegic solution has also been attributed to the build up of the waste products of glycolysis which are not washed out of the cell (Hearse et al., 1978b).With the reduction in temperature of cardioplegic arrest, the expenditure of energy is decreased, and usage of intrinsic substrate stores may meet the reduced metabolic requirements. The deleterious effects of glucose inclusion may therefore outweigh the benefits under these circumstances.

However, intermittent flushing of the cardioplegic solution with the provision of a substrate at a low temperature may be desirable. Low flow ischaemia with washout of accumulated metabolites has been shown to be advantageous (Neely et al., 1975) and the use of multidose administrations of a cardioplegic solution may utilise this effect.

b) Experimental Procedure

11 mmol/l glucose was added to the basic ST solution as this is the concentration used in the modified K-H solution. This concentration in the perfused rat heart has been shown to be optimal for glucose transport into the cell, and subsequent oxidation (Opie et al., 1962).

Comparisons between the preservative qualities of the standard solution with and without glucose in a single dose protocol were made and compared to the effects of the solutions with multidose administrations.

c) Results

i) Single dose cardioplegia followed by 3 hrs global ischaemia at 10°C with St Thomas' No 2 versus St Thomas' No 2 + Glucose

a) Functional recoveries

Measurements taken for heart rate, aortic output and coronary flow during the working heart mode prior to arrest were compared for differences between groups. No significant differences were found (Table 3) (Aortic pressures are not shown as these did not reflect any differences between groups).

The percentage recovery of aortic output after 20 min reperfusion (10 min non-work followed by 10 min work) after arrest with the ST+Glucose cardioplegic solution was slightly greater than that after arrest with ST (65.9 ± 2.9 % compared to 57.4 ± 5.2 %) (Table 3). Cardiac output was similarly improved. These changes were not significant. There was, however, a significant increase in stroke volume with the inclusion of glucose (76.2 ± 3.2 % versus 58.1 ± 4.0 % - $p<0.01$). This may have been due to the combination of improved cardiac output and a lower heart rate with ST+Glucose. However, overall functional performance of the hearts was not greatly affected by the inclusion of glucose in the cardioplegic solution.

b) Post-ischaemic LDH washout

During the first ten minutes of reperfusion in the Langendorff mode, a significantly greater washout of LDH was seen with hearts arrested with ST+Glucose compared to those arrested with ST, with 66.4 ± 7.7 mU/gm/min and 37.7 ± 7.5 mU/gm/min respectively ($p<0.02$) (Table 4). LDH loss is thought to reflect membrane integrity (Hearse et al., 1973; Hearse et al., 1975). Glycolytic ATP has been associated with membrane preservation (Bricknell and Opie, 1978; Corr

Table 3: Means ± sem of functional measurements of hearts taken after 10 minutes work before (Pre-arrest) and after (Post-arrest) arrest, with percentages of functional recoveries. Comparison of hearts arrested with St Thomas' Hospital cardioplegic solution No. 2 without (ST) and with 11mmol/l glucose (ST+G). The hearts were then stored for 3 hours at 10°C (single dose) or flushed intermittently with 6ml cardioplegic solution (multidose).

		Aortic output (ml/min)	Cardiac output (ml/min)	Stroke volume (ml)
Single dose				
ST	Pre-arrest	47.0 ± 1.9	65.6 ± 2.7	0.24 ± 0.01
(n=7)	Post-arrest	27.4 ± 3.5	42.0 ± 4.1	0.14 ± 0.01
	% Recovery	57.4 ± 5.2 %	63.8 ± 4.9 %	58.1 ± 4.0 %
ST+G	Pre-arrest	43.5 ± 2.3	61.7 ± 2.1	0.23 ± 0.01
(n=10)	Post-arrest	28.7 ± 2.0	45.9 ± 2.6	0.18 ± 0.01
	% Recovery	65.9 ± 2.9 %	74.6 ± 3.8 %	76.2 ± 3.2 % *
Multidose				
ST	Pre-arrest	48.6 ± 2.0	66.4 ± 1.3	0.25 ± 0.02
(n=9)	Post-arrest	36.3 ± 2.0	53.2 ± 1.8	0.22 ± 0.02
	% Recovery	74.6 ± 1.9 % $	79.9 ± 1.6 % $	89.4 ± 2.6 % $
ST+G	Pre-arrest	46.3 ± 1.2	63.8 ± 1.5	0.21 ± 0.01
(n=8)	Post-arrest	40.5 ± 1.6	56.5 ± 1.6	0.20 ± 0.01
	% Recovery	87.4 ± 1.9 % #&	88.7 ± 2.2 % #&	93.8 ± 4.9 % #

* $p<0.01$ vs ST (single dose)
$ $p<0.05$ vs ST (single dose)
# $p<0.025$ vs ST+G (single dose)
& $p<0.025$ vs ST (multidose)

Table 4: Means ± sem of average washout of lactate dehydrogenase (LDH) and lactate by intermittent flushing during arrest, and during 10 minutes reperfusion in the Langendorff mode (Post-arrest). Comparison of hearts arrested with St Thomas' Hospital cardioplegic solution No. 2 without (ST) and with 11mmol/l glucose (ST+G) and stored for 3 hours at 10°C (single dose) or flushed intermittently with 6ml cardioplegic solution (multidose).

	LDH (mU/gm/6ml)	Post-arrest LDH (mU/gm/min)	Lactate (μmol/gm/6ml)	Post-arrest Lactate (μmol/gm/min)
Single dose				
ST (n=7)		37.7 ± 7.5		1.08 ± 0.32
ST+G (n=10)		66.4 ± 7.7 *		1.38 ± 0.24
Multidose				
ST (n=9)	72.6 ± 3.8	148.0 ± 18.1 *	1.87 ± 0.11	0.67 ± 0.08
ST+G (n=8)	82.2 ± 6.1	109.5 ± 18.8 #	2.49 ± 0.16 $	1.76 ± 0.32 &

* $p<0.02$ vs ST (single dose)
# $p<0.05$ vs ST+G (single dose)
$ $p<0.002$ vs ST (multidose)
& $p<0.03$ vs ST (multidose)

et al., 1984) and prevention of leakage of LDH (Hearse et al., 1976). However, the presence of glucose in the ST solution after single dose cardioplegia increased LDH washout.

c) Post-ischaemic lactate washout

Lactate efflux during the first ten minutes of reperfusion in the Langendorff mode showed no significant differences between the two groups indicating similar rates of glycolysis, although lactate efflux after arrest with ST+Glucose tended to be higher. Lactate was produced both during ischaemia and reperfusion, as both groups were reperfused with 11 mmol/l glucose in the KH solution.

d) High energy phosphate levels in tissue

Values of 3.91 ± 0.25 µmol ATP/gm fresh weight and 5.05 ± 0.45 µmol CP/gm fresh weight were obtained for control hearts clamped after the initial working period (Table 5).

The tissue ATP levels at the end of 20 min reperfusion were significantly depressed when compared to control ATP values, with 2.61 ± 0.37 µmol/gm fresh weight for ST and 2.73 ± 0.43 µmol/gm fresh weight for ST+Glucose ($p<0.04$) (Table 5). There was no difference between the ATP tissue values of the two experimental groups.

The levels of CP after arrest and reperfusion for both groups were not significantly different from control values or from each other (Table 5).

Table 5: Means ± sem of tissue levels of adenosine triphosphate (ATP) and creatine phosphate (CP) of control hearts clamped after 10 minutes work before arrest, and of arrested hearts after 20 minutes reperfusion. Comparison of hearts arrested with St Thomas' Hospital cardioplegic solution No. 2 without (ST) and with 11mmol/l glucose (ST+G) and stored for 3 hours at 10°C (single dose) or flushed intermittently with 6ml cardioplegic solution (multidose).

	ATP (μmol/gm)		CP (μmol/gm)
Control (n=6)	3.91 ± 0.25		5.05 ± 0.46
Single dose ST (n=7)	2.61 ± 0.37	*	4.52 ± 0.10
ST+G (n=10)	2.73 ± 0.14	*	3.85 ± 0.34
Multidose ST (n=9)	3.22 ± 0.14	*	4.99 ± 0.34
ST+G (n=8)	3.39 ± 0.08		4.90 ± 0.28

* $p<0.04$ vs control

ii) Multidose cardioplegia (initial arrest followed by intermittent infusions every 30 min over 3 hrs) with and without glucose

a) Functional recoveries

The effects of glucose in the ST cardioplegic solution using a multidose protocol over a 3 hour period were tested. The cardioplegic solutions were intermittently introduced at 30 min intervals.

Pre-ischaemic values of functional parameters were not significantly different between groups (Table 3).

ST+Glucose in a multidose protocol resulted in a greatly improved contractile performance on reperfusion when compared to ST. Aortic output and cardiac output were significantly greater ($p<0.025$), with an average of about 90% recovery of pre-ischaemic function for all parameters (Table 3).

b) LDH washout during and after arrest

During arrest, washout of LDH was slightly greater in the ST+Glucose multidose group, although there was a gradual decline over time in both groups (Figure 2). Average LDH washout showed no significant differences between groups (Table 4).

Post-ischaemic LDH washout was, however, higher in the ST multidose group although not significantly so (Table 4). Glucose may have had a slightly beneficial effect on membrane integrity on reperfusion.

c) Lactate efflux during and after arrest

The mean lactate efflux with each cardioplegic reinfusion did not differ significantly between the ST and ST+Glucose groups. This is shown in Figure 3. There was an initial high level of lactate washout in both groups, which was slightly greater with ST+Glucose. This was possibly due to a slightly higher initial glycolytic flux with glucose, before a build

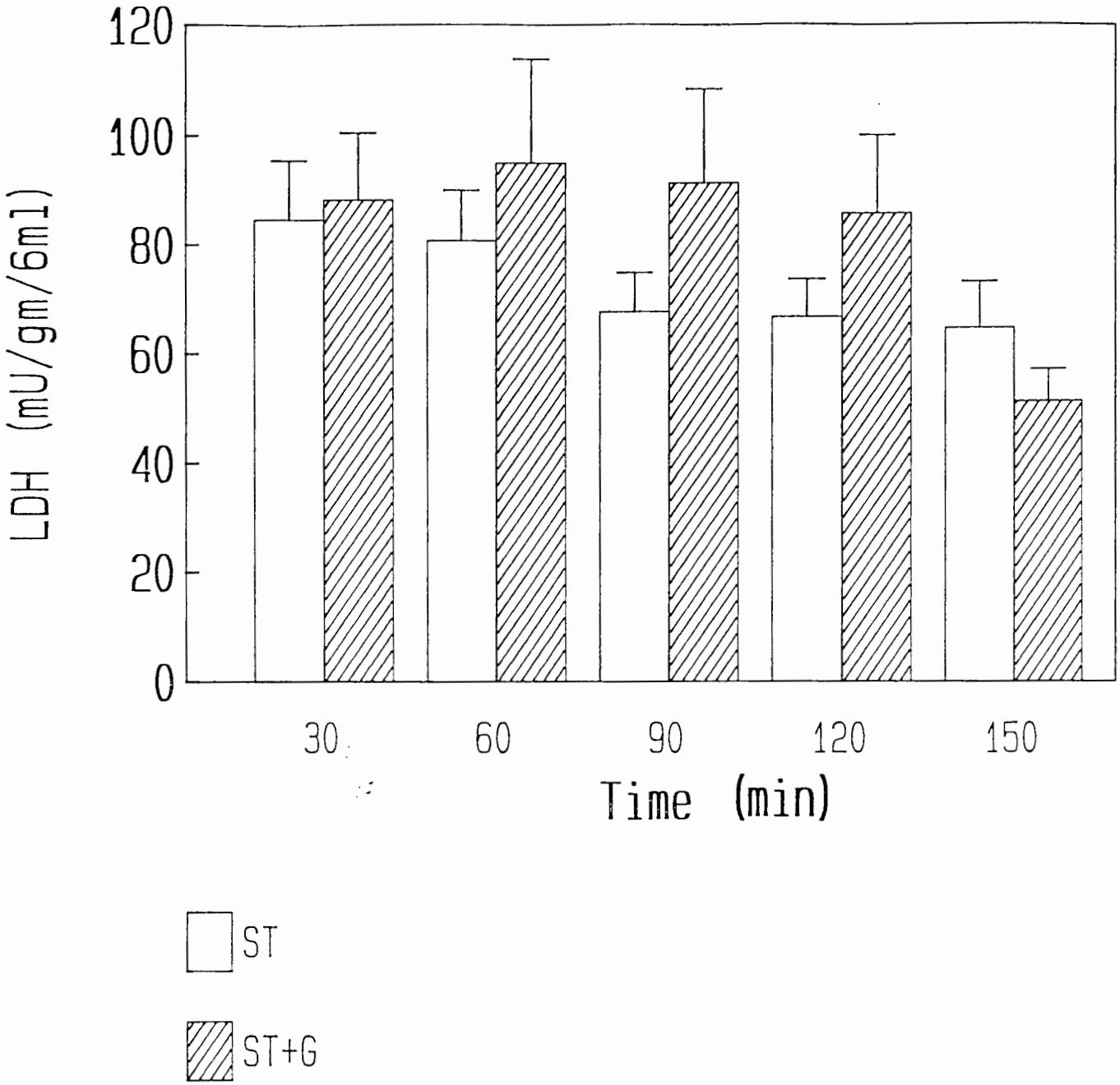

Fig. 2. Means ± sem of washout of lactate dehydrogenase (LDH) during arrest per 6ml flush of cardioplegic solution. Comparison of hearts arrested with St Thomas' Hospital cardioplegic solution No. 2 without (ST, n=9) and with 11 mmol/l glucose (ST+G, n=8). Arrested hearts were intermittently flushed with cardioplegic solution over 3 hours at 10°C.

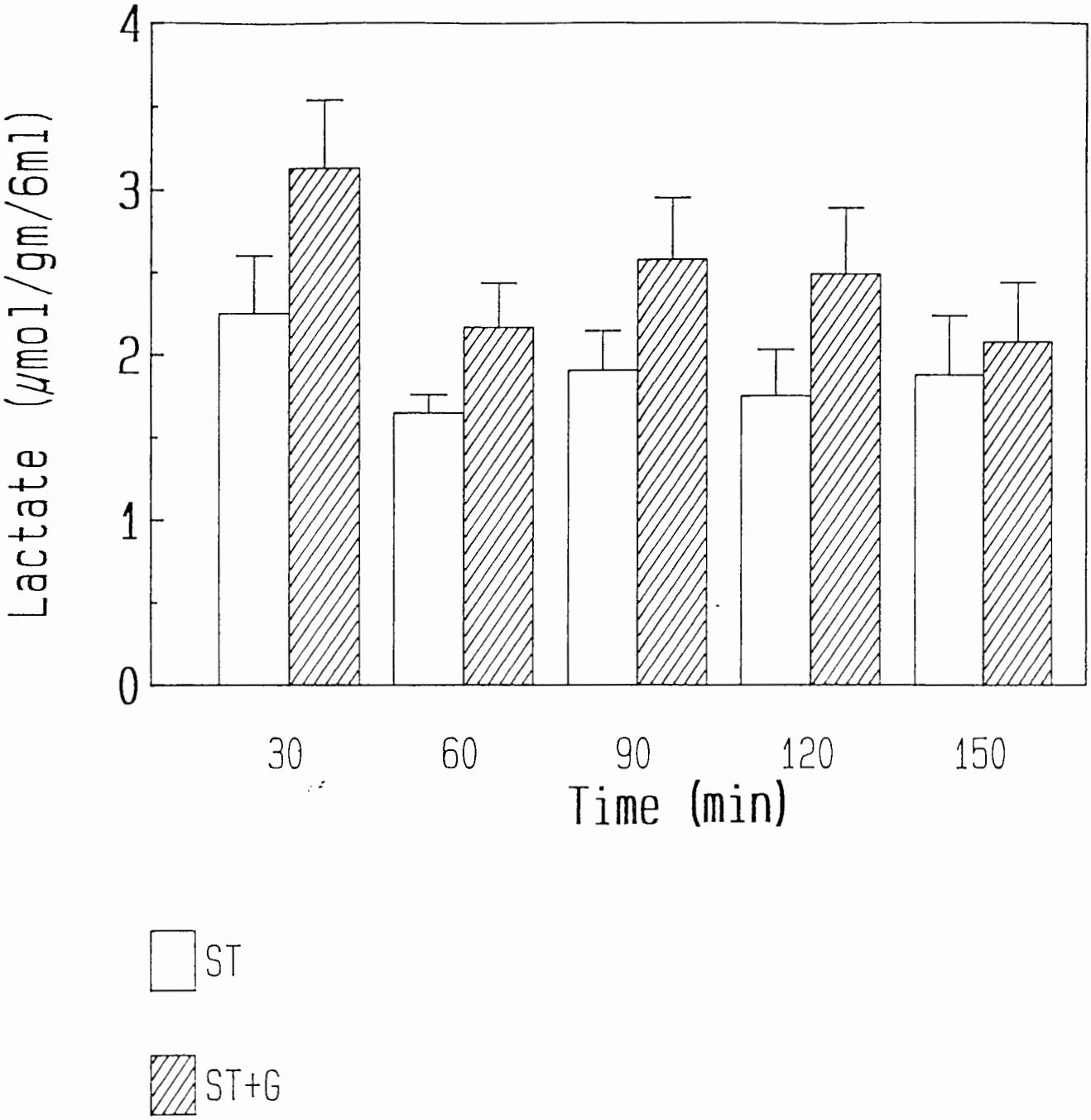

Fig. 3. Means ± sem of washout of lactate during arrest per 6ml flush of cardioplegic solution. Comparison of hearts arrested with St Thomas' Hospital cardioplegic solution No. 2 without (ST, n=9) and with 11 mmol/l glucose (ST+G, n=8). Arrested hearts were intermittently flushed with cardioplegic solution over 3 hours at 10°C.

up of inhibitory end products. The glycolytic rate appeared to remain fairly constant for the remainder of the ischaemic period with a tendency for greater lactate efflux with the glucose-containing cardioplegic solution. This was reflected in the average washout of lactate with 1.87 ± 0.11 µmol/gm/6ml for ST and 2.49 ± 0.16 µmol/gm/6ml for ST+Glucose ($p<0.002$) (Table 4).

Post-ischaemic lactate washout in the initial 10 min reperfusion in the Langendorff mode after multidose usage of ST+Glucose was high (1.76 ± 0.32 µmol/gm/min), more than double that after arrest with multidose ST (0.67 ± 0.08 µmol/gm/min) ($p<0.03$) (Table 4).

d) High energy phosphate levels in tissue

Similar levels of tissue ATP after 20 min reperfusion with multidose ST+Glucose was found compared to that with multidose ST (Table 5). However, in comparison with control values there was a significant decrease in the ST group ($p<0.04$) but not in the ST+Glucose group. CP was close to control values for all groups (Table 5).

iii) Comparison of multidose and single dose cardioplegia

Recovery of aortic output, cardiac output and stroke volume improved from single dose to multidose usage (Table 3) by at least 15-20% with both ST and ST+Glucose solutions. All were significant improvements ($p<0.05$).

There was a detrimental effect of multidose cardioplegia as shown in an increased washout of LDH on reperfusion compared to hearts with single dose cardioplegia (Table 4), possibly because of greater mechanical abrasion of the cells by intermittent reintroduction of fluid. However, the overall benefits of multidose cardioplegia were apparent, while the inclusion of glucose with this protocol had an additive effect on recovery of the arrested hearts. This may be attributed to prevention of the accumulation of harmful end products, thereby allowing more efficient metabolism of the glucose administered intermittently.

iv) Dose response curve during multidose cardioplegia

The inclusion of glucose in the ST solution was tested at various concentrations with multidose flushing. The dose response curve for aortic output and cardiac output thus obtained is illustrated in Figure 4. These curves indicate that a concentration of 8-12 mmol/l is optimal.

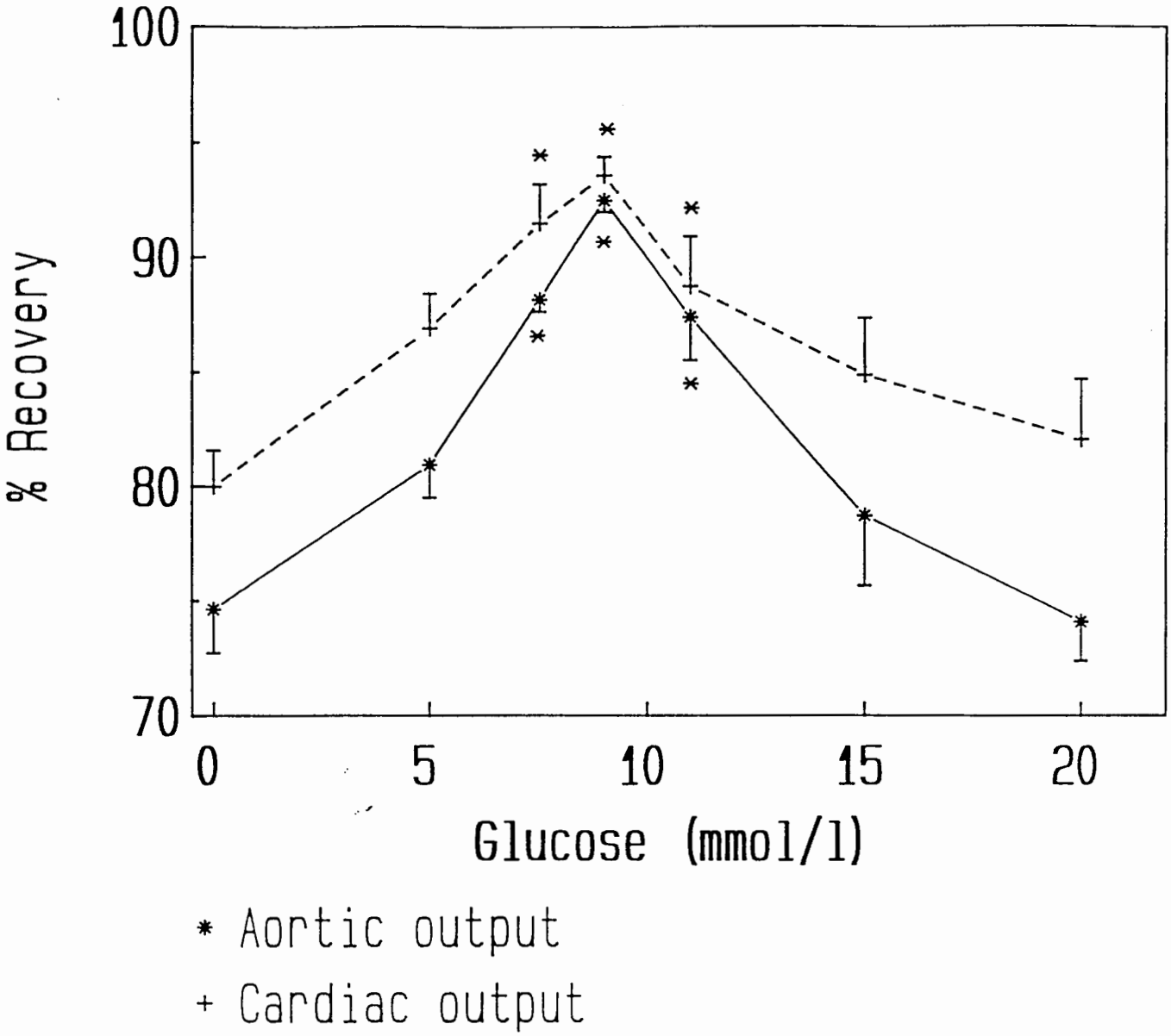

Fig. 4. Dose response curve for glucose added to the St Thomas' Hospital cardioplegic solution No. 2. Glucose concentrations of 0 mmol/l (n=9), 5 mmol/l (n=4), 7.5 mmol/l (n=4), 9 mmol/l (n=4), 11 mmol/l (n=8), 15 mmol/l (n=9) and 20 mmol/l (n=4) were compared. The arrested hearts were intermittently flushed for 3 hours at 10°C. Values for percentage recovery of aortic output and cardiac output are shown as means ± sem.

* $p<0.005$ vs 0 mmol/l, 20 mmol/l

d) Discussion

i) Role of glucose in ischaemia

The benefits of glucose in an ischaemic heart include enhanced anaerobic metabolism (Opie, 1989c), inhibition of ischaemic contracture (Owen et al., 1990), reduced K^+ efflux (Weiss and Lamp, 1987), decreased fluctuation in membrane potential with possible antiarrhythmic effects (Manning and Hearse, 1984; Bernier and Hearse, 1988), prevention of cellular swelling by exertion of an osmotic effect (Rink, 1984), preservation of membrane integrity (Bricknell and Opie, 1978), inhibition of enzyme release (Hearse et al., 1976), and action as a free radical scavenger (Hearse and Humphrey, 1975; Hess and Manson, 1984). When glucose is present during an ischaemic or arrest period, the glycolytic ATP produced may aid in the prevention of Ca^{2+} overload and contracture in the cells by enabling uptake by the ATP dependent Ca^{2+} pumps of the SR as well as prevention of Ca^{2+} overload by activation of the Na^+/K^+ pump (Bers and Ellis, 1982). Glucose can act as a free radical scavenger directly, as well as inhibiting the formation of free radicals by acting as a substrate for ATP production with prevention of Ca^{2+} overload, and consumption of the precursors of ATP.

Because of the associated benefits of glucose during ischaemia, the addition of this substrate to cardioplegic solutions has been proposed. However, it has been suggested that the stores of glycogen in the heart are sufficient for the supply of energy with a reduced metabolic rate (Lell and Buttner, 1983). Glycogen acts as a primary substrate for glycolysis, as it is the main storage form of energy in the cell, but its breakdown is inhibited by the accumulation of metabolites (Opie, 1989c).It has also been shown that in the absence of glucose, a high glycogenolytic flux is ineffective in preventing ischaemic contracture (Owen et al., 1990). It is postulated that this may be because glycogen is situated close to the SR, whereas glucose introduced to the cells is closer to the sarcolemma, where ATP is required for maintaining

homeostasis of the cell membrane. The metabolites of glycolysis can also be removed from the cytosol more easily.

ii) Glucose in cardioplegic solutions

The above results indicate that the inclusion of 11 mmol/l glucose in the ST solution when used in a single dose protocol is not disadvantageous to the heart, with an indication rather of slightly improved recovery. This may be related to an increased energy production as well as other actions of glucose. However, washout of lactate after arrest was similar in the two groups.

Upon cessation of flow in the arrested hearts, glycolysis is rapidly inhibited, with the accumulation of metabolites (Rovetto et al., 1975). The small amount of glucose taken up from the cardioplegic solution would not greatly affect the rate of energy production to a level resulting in increased functional recovery, whereas the slight increase in the accumulated waste products may have harmed the cells. This may have been reflected as membrane damage and subsequent enzyme loss, with a higher washout of LDH in the ST+Glucose group. The benefits of glucose may therefore not have been apparent under these conditions.

These results are in contrast to the results of Hearse et al. (1978b), which would appear to indicate that glucose in a cardioplegic solution is harmful to the arrested heart. A single infusion of a ST-like solution (mmol/l Na^+ 115, K^+ 16, Ca^{2+} 1.2, Mg^{2+} 16) with a greater bicarbonate concentration (25 mmol/l) and gassed with 95%O_2 5%CO_2 was used. Glucose was added at concentrations from 0-50 mmol/l. The hearts were maintained at a temperature of 28^{o}C for a period 70 minutes. A recovery of 59.2 ± 5.7% for aortic output with 11 mmol/l glucose, as opposed to 80.7 ± 2.4% without glucose was obtained. Similar trends for other functional parameters were observed. Further increases in the glucose concentration resulted in even lower recoveries. However, their study was conducted with hearts maintained at 28^{o}C as opposed to 10^{o}C. With a lower temperature over a longer time period, it would

seem that glucose has no detrimental effect and may, in fact, aid the heart. This may have important consequences when considering longer term preservation, where the benefits of glucose may be more apparent.

iii) Multidose cardioplegia and glucose

With the multidose protocol, glucose in the ST solution led to greatly improved recoveries. With intermittent flushing, there is removal of accumulated metabolites, which allows more efficient metabolism of glucose without the associated deleterious effects.

A regimen of multiple administrations of cardioplegic solution during cardio-pulmonary bypass has been found to be effective in other studies, with benefits including maintenance of arrest and hypothermia in the presence of noncoronary collateral flow, removal of harmful metabolites, replenishment of substrate, maintenance of an appropriate pH and prevention of oedema (Conti and Kao, 1983).

In the presence of glucose, multidose cardioplegia may be especially beneficial, by removing metabolites which inhibit glycolysis and ensuring an optimal intracellular pH. Neely et al. (1975) suggested that the harmful effects of ischaemia are due as much to the lack of washout of harmful products as to the lack of substrate as a correlation between coronary flow and rates of glycolysis was found in the ischaemic heart. Inhibition of glycolysis occurs as the flow rate is decreased, with accumulation of metabolites and a decrease in ATP and CP levels. The relationship between washout of metabolites and recovery is thus emphasised.

Other studies with multidose flushing have not clarified the role of glucose in a cardioplegic solution. Conti and Kao (1983) found that single dose administration of Tyer's cardioplegic solution (mmol/l Na+ 139, K+ 25, Ca^{2+} 0.9) at a temperature of $32^{o}C$ did not result in any measurable recovery, while multidose cardioplegia showed great improvements, both with and without glucose. However, Guilbeau et al. (1984) looked at the effect of single or intermittent perfusions of a

cardioplegic solution (mmol/l Na^+ 115, K^+ 16, Ca^{2+} 1.2) at 28°C with or without 27.8 mmol/l glucose. Multidose cardioplegic protection was found to reduce lactic acid accumulation and improve functional recoveries, although no differences were seen with the inclusion of glucose. De Wit et al. (1988) found that the addition of glucose to a cardioplegic solution gassed with 95%O_2 5%CO_2 did not increase the degree of recovery of the hearts. However, the amount of glucose added to the solution was 2.5% of glucose plus insulin. In the dose response curve on the optimal concentration of glucose, it can be seen that this high a concentration (approximately 45-50 mmol/l) is deleterious.

However, the use of a multidose protocol with the ST and ST+Glucose solutions increased the washout of LDH. The presence of glucose, with its putative role in preventing membrane damage, did not affect the washout of LDH in the multidose group to a marked degree, and was associated with increased loss of LDH in a single dose protocol.

LDH, which is lost from cells with membrane damage (Hearse et al., 1973; Hearse et al., 1975) is thought to accumulate in the interstitial spaces during ischaemia from which rapid washout occurs on reintroduction of fluid. Mechanical forces may add to the efflux of LDH, both from the interstitial space, and by damage to the cell membranes (Kao and Magovern, 1986). This may result in an increase in enzyme loss, and other deleterious effects associated with "leaky" membranes. The endothelial cells particularly may be damaged by this procedure as these are the primary barrier between the myocytes and the vascular system.

The use of a solution which has a lower colloid osmotic pressure than the intracellular and interstitial fluids may also increase LDH washout with multidose cardioplegia. There may be a continual removal of enzymes from the interstitium down an osmotic gradient allowing further leakage. With no maintenance, there is no gradient between the interstitium and the vascular system, slowing the rate of LDH washout. However, in the absence of washout of end products of metabolism, the

accumulated products may harm the cells and lead to reduced functional recovery. With the addition of glucose, there is greater accumulation of these end products, which may explain the greater washout of LDH on reperfusion after single dose arrest with ST+Glucose.

iv) Energy production with glucose

It is proposed that an increase in glycolytic ATP production as a result of the inclusion of glucose is beneficial, as a correlation between high energy phosphate content with recovery after an ischaemic period has been found (Neely et al., 1973; Hearse et al., 1974; Nayler et al., 1979; Bricknell et al., 1981; Lazar et al., 1980).

In our results, little correlation was observed between high energy phosphate measurements and recovery. However, these measurements reflect the ATP levels of the myocardium after 20 min reperfusion, by which time energy stores have been partially regenerated. Hence this measurement is not a reflection of the maintenance of high energy phosphate stores during arrest.

The total lactate content of the samples of cardioplegic solution flushed through the heart during arrest represents a minimum rate of about 5.61 μmol ATP/gm wet weight produced for ST per 30 min period (or 0.19 μmol ATP/gm wet weight/min) (6 mol ATP/2 mol lactate/mol glycogen in the absence of exogenous substrate). For the ST+Glucose group, this ranges between a minimum of about 4.98 μmol ATP/gm wet weight per 30 min (about 0.17 μmol ATP/gm wet weight/min) (only glucose used - 4 mol ATP/2 mol lactate/mol glucose) with a maximum of about 7.47 μmol ATP/gm wet weight per 30 min (about 0.25 μmol ATP/gm wet weight/min) (all from glycogen). Thus the inclusion of glucose did not seem to alter the turnover of this pathway.

The minimum rate of glycolysis which prevents the development of contracture in a model of low flow ischaemia would appear to be about 2 μmol/gm fresh weight/min (Owen et al., 1990). To maintain this rate, it is necessary to allow glycolysis to continue by the provision of glucose, and to prevent its

inhibition by the build up of waste products. This would be provided for by multiple infusions of cardioplegia during the arrest period. However, in the arrested heart at 10°C, the metabolic rate is much lower and the ATP thus produced appears to be sufficient to maintain cellular processes.

However, in estimating minimum rates of glycolysis and subsequent rate of ATP production from the washout of lactate during arrest, errors will occur because of several factors. A certain amount of the available glucose may have been utilised by the Krebs cycle and oxidative phosphorylation because there was a certain amount of oxygen available in the solution open to atmosphere (pO_2 approximately 150 mmHg). The production of ATP from this glucose will not be reflected as lactate. The amount of ATP derived from glucose is therefore underestimated. However, assuming equal rates of oxidative phosphorylation with both ST and ST+Glucose, any additional energy derived from glucose appears to confer a significant degree of protection by improving functional recoveries.

Not all the lactate is flushed out of the hearts each time and there may be an accumulation in the cells as well as in the interstitial fluid. Only 6ml cardioplegic solution is flushed through the heart with each reinfusion, and while the infusion pressure (80 cm H_2O) is thought to be sufficient to ensure perfusion of the whole vascular bed, the amount of solution flushed through may not remove all the accumulated lactate. The use of more solution to flush the heart may, however, be damaging because of abrasion and disturbance of the endothelial cells (Kao and Magovern, 1986).

Increased reperfusion lactate in the ST+Glucose group may be a reflection of either

1) higher tissue accumulation during ischaemia or
2) higher flux rates on reperfusion.

While the amount of ATP produced during arrest, and that present in the tissue after 20 min reperfusion may not show any significant trends, the levels of ATP measured may be an indication of the preservation of the mechanisms of ATP production. Lazar et al. (1980) suggests that the ischaemic

heart develops a defect in its oxidative metabolic pathways which determines the recovery of ATP production upon reperfusion. The mitochondria might be damaged by lactate accumulation, Ca^{2+} influx on reperfusion and free radical activity. In addition, there is a loss of nucleotides from the cells during ischaemia which are needed for the regeneration of ATP (Reimer et al., 1981). The presence of glucose may have improved the ability of the myocardium to regenerate its stores of ATP. While actual levels of ATP during and after ischaemia may not be critical, an improved recovery is correlated with slightly higher ATP levels.

CP levels are rapidly restored after an ischaemic period (Taegtmeyer et al., 1985) unless the mechanisms for regeneration are damaged, which is an indication of irreversible injury. All the hearts did recover to a certain extent, thus it was not expected that CP levels would be low after 20 min reperfusion. The measurements were not significantly different from control values.

v) Optimal glucose concentration of the ST solution

The results of the various studies of the inclusion of glucose in the ST and other cardioplegic solutions are conflicting, but it would appear that there is an optimum concentration of glucose, outside the range of which it is deleterious. This is also suggested by Bernier and Hearse (1988), in their studies on reperfusion of an ischaemic area with a glucose-containing solution. Outside of these concentrations, glucose may indeed be harmful to the heart, as found by Hearse et al. (1978b). This is corroborated by the dose response curve for glucose in the ST solution. An increasing glucose concentration may lead to excess intracellular proton and lactate accumulation, outweighing the advantageous effects of increased energy production. Glucose uptake into the cell is limited by transport rate across the membrane which is dependent upon the concentration. This rate is possibly saturated at excess concentrations, as may be other enzymatic reactions of the glycolytic pathway. The products of glycolysis accumulate with the initial high rate of the reaction at the onset of

ischaemia, and the enzymes cannot act to break down the accumulating glucose because the ATP pool is depleted.

vi) Conclusions

The addition of glucose to a solution used with a multidose protocol is recommended as this leads to greatly improved mechanical function on reperfusion of the isolated rat heart after 3 hrs arrest.

There were, however, variables in the use of the solution, which was gassed with 95%O_2 5%CO_2 when made up (to prevent precipitation of calcium phosphate), but then left open to atmosphere. The pO_2 and pH of the solution must have varied with time, and also with changes in temperature. These variables were not taken into consideration in these initial experiments and further studies were made to determine the exact contribution of these factors to the recoveries of the hearts. We wished to establish stable criteria for the use of the ST+Glucose solution with a multidose protocol, where the solution can be gassed, with possible improvements in recovery.

The efficacy of the glucose containing ST solution for use in a single dose protocol may possibly be increased by further changes in the composition. These may include the addition of a buffer to counter the effects of an increased proton production associated with an increased rate of glycolysis. The presence of glucose may also lead to improved long term preservation of donor hearts, where its action may be more pronounced.

B. Oxygenation and pH of the ST+Glucose solution with multidose cardioplegia

a) Introduction

i) Oxygenation of a cardioplegic solution

With cessation of coronary flow, oxygen supply to the mitochondria is suppressed, with a subsequent reduction in energy production. Although the metabolic rate is reduced with hypothermia and increased extracellular K^+, the supply of oxygen in a cardioplegic solution may be beneficial by increasing energy production. The O_2 imbalance which develops in the myocardium over the ischaemic period can be met in part by intermittent reinfusions of an oxygen containing solution, while the CO_2 produced will be removed. This will also enable more efficient usage of glucose present in the ST+Glucose cardioplegic solution.

In a solution open to atmosphere, the pO_2 is about 150 mmHg. With a single dose protocol, any oxygen provided will presumably be rapidly consumed. The question is whether, with intermittent reinfusions of the cardioplegic solution, a higher pO_2 would be more beneficial, and whether the myocytes do indeed utilise the additional oxygen. However, a disadvantage of a highly oxygenated solution with a multidose protocol may be an increased formation of oxygen free radicals at each reinfusion. The inclusion of oxygen at a high partial pressure is also associated with practical problems, where the simplest means of administration of a cardioplegic solution are preferred. Care must be taken to avoid the formation of gaseous emboli (Buckberg, 1979). The extra addition of oxygen in the face of these problems may not confer any additional protection to the myocardium.

ii) pH changes with gassing of a solution

Gassing with 100% O_2 in the presence of bicarbonate produces a marked increase in the pH as all the CO_2 in the solution is blown off. The change in pH thus elicited in the oxygenated solution may affect the recovery of the hearts. The presence

of glucose may also intensify the development of intracellular acidosis by increasing glycolytic flux (Gevers, 1977). The optimal pH of a cardioplegic solution under such circumstances must be determined.

Most surgeons maintain cardioplegic solutions at a pH of 7.8 at 10^{o}C, because of the presumed advantageous effects of alkalosis (Buckberg, 1979; Swan, 1984) which rest on extrapolation from the ectothermic strategy of acid-base management (Reeves and Malan, 1976; Swan, 1984). A large fall in intracellular pH is associated with long-term ischaemia (Cobbe and Poole-Wilson, 1980a), which is thought to be harmful to the tissue. However, experimental studies have shown the beneficial effect of a lower pH of a cardioplegic solution (Nugent et al., 1982; Bernard et al., 1985), linked to improved energy conservation.

The adjustment of pH by a change in pCO_2 is practically applicable because CO_2 is freely permeable through the cell membrane, has a rapid effect on intracellular pH, and the regulation of pCO_2 in solution is easy. CO_2 enters the cell rapidly, while the flux of H^+ and HCO_3^- is slow, thus respiratory acidosis exerts a greater effect than does metabolic acidosis (Cingolani et al., 1970; Nayler et al., 1979). In conjunction with other gases, this manipulation may increase the effectiveness of the ST+Glucose cardioplegic solution.

b) Expermental Procedure

The glucose-containing ST solution in a multidose protocol, was gassed with either 100% N_2 or 100% O_2 in order to determine the effect of oxygenation of a solution at a high pH. Oxygenation at a low pH was then tested, with the addition of 5%CO_2 (95%N_2 5%CO_2 and 95%O_2 5%CO_2 respectively).

c) Results

i) Multidose arrest with ST+Glucose gassed with 100% N_2 or 100% O_2

a) pH and pO_2 of the cardioplegic solutions

The effect of oxygenation of the ST+Glucose cardioplegic solution was tested by gassing with 100%N_2 or 100% O_2. This displaced all other gases in the solutions, and made the pH alkaline with high or low pO_2's. The pH's and pO_2's of the solutions are shown in Table 6. In comparison, the ST+Glucose solution open to atmosphere had relatively low pO_2's of about 150 - 400 mmHg and pH's ranging from 7.3 to 8.0, depending on the time of sampling after the initial gassing with 95%O_2 5%CO_2.

The pH of the 100% N_2 ST+Glucose solution was slightly lower than the 100% O_2 solution.

b) Functional recoveries

Pre-ischaemic control values were not significantly different. These are shown in Table 7, together with the values attained on reperfusion. (Aortic pressures are not shown as no significant changes were seen).

Percentage recoveries of aortic output and cardiac output obtained when the ST+Glucose solution was gassed with 100% O_2 were significantly greater than gassing with 100% N_2 ($p<0.05$), with an overall increase of about 15%, although the pH's of the cardioplegic solutions were similar (Table 7). The presence of a high pO_2 was beneficial compared to an absence of oxygen at a high pH.

c) pH of the coronary effluents

The pH's of the coronary effluents from intermittent flushing tended to equilibration with the arterial cardioplegic solutions over time, from initial extracellular pH's of about 7.4 (K-H). These trends are shown in Figure 5. Effluent pH is thought to reflect similar changes in intracellular pH as the proton gradient across the membrane is maintained by mechanisms including the Na^+/H^+ exchange, the H^+/lactate$^-$

Table 6: pH and gas compositions of the Krebs-Henseleit solution (K-H) gassed with 95%O_2 5%CO_2 at 37°C, and the St Thomas' Hospital cardioplegic solution No. 2 with glucose aerated (ST+G) and gassed with 100% N_2, 100% O_2, 95%N_2 5%CO_2 or 95%O_2 5%CO_2 at 10°C (n=6 for each solution).

	pH (mmHg)	pCO_2 (mmHg)	pO_2 (ml O_2/6ml)	O_2 content
K-H (37°C)	7.46 ± 0.01	31.3 ± 0.8	560 ± 9	0.164 ± 0.003
ST+G (10°C)	7.3 - 8.0	5 - 15	150 - 400	0.044 - 0.117
100% N_2 (10°C)	9.07 ± 0.10	3.0 ± 0.6	8 ± 2	0.002 ± 0.001
100% O_2 (10°C)	9.30 ± 0.08	3.1 ± 0.6	629 ± 11	0.184 ± 0.003
95%N_2 5%CO_2 (10°C)	6.98 ± 0.01	19.1 ± 1.9	8 ± 2	0.002 ± 0.001
95%O_2 5%CO_2 (10°C)	7.07 ± 0.02	16.6 ± 1.4	640 ± 26	0.187 ± 0.008

Table 7: Means ± sem of functional measurements of hearts taken after 10 minutes work before (Pre-arrest) and after (Post-arrest) arrest, with percentages of functional recoveries. Comparison of hearts arrested with St Thomas' Hospital cardioplegic solution No. 2 with 11mmol/l glucose gassed with 100% N_2, 100% O_2, 95%N_2 5%CO_2 or 95%O_2 5%CO_2. Arrested hearts were intermittently flushed with cardioplegic solution over 3 hours at 10°C.

		Aortic output (ml/min)	Cardiac output (ml/min)	Stroke volume (ml)
100% N_2 (n=6)	Pre-arrest	47.5 ± 3.9	64.5 ± 5.1	0.24 ± 0.04
	Post-arrest	24.8 ± 2.4	39.3 ± 4.2	0.14 ± 0.01
	% Recovery	52.3 ± 2.7 %	60.8 ± 3.6 %	59.91 ± 4.7 %
100% O_2 (n=8)	Pre-arrest	42.0 ± 2.2	60.4 ± 2.7	0.23 ± 0.01
	Post-arrest	27.8 ± 1.7	45.3 ± 2.5	0.17 ± 0.01
	% Recovery	66.3 ± 2.8 % *	75.3 ± 4.1 % *	74.73 ± 4.02 %
95%N_2 5%CO_2 (n=7)	Pre-arrest	39.6 ± 3.6	57.4 ± 3.9	0.21 ± 0.01
	Post-arrest	25.7 ± 3.2	43.4 ± 3.6	0.16 ± 0.01
	% Recovery	63.9 ± 2.8 % *	75.4 ± 3.3 % *	74.2 ± 2.7 % *
95%O_2 5%CO_2 (n=9)	Pre-arrest	43.1 ± 2.4	60.3 ± 2.5	0.23 ± 0.01
	Post-arrest	38.1 ± 2.1	53.6 ± 2.4	0.20 ± 0.01
	% Recovery	88.9 ± 3.7 % &#	88.9 ± 2.4 % &#	88.3 ± 5.1 % &#

* $p<0.05$ vs 100% N_2

& $p<0.05$ vs 95%N_2 5%CO_2

# $p<0.05$ vs 100% O_2

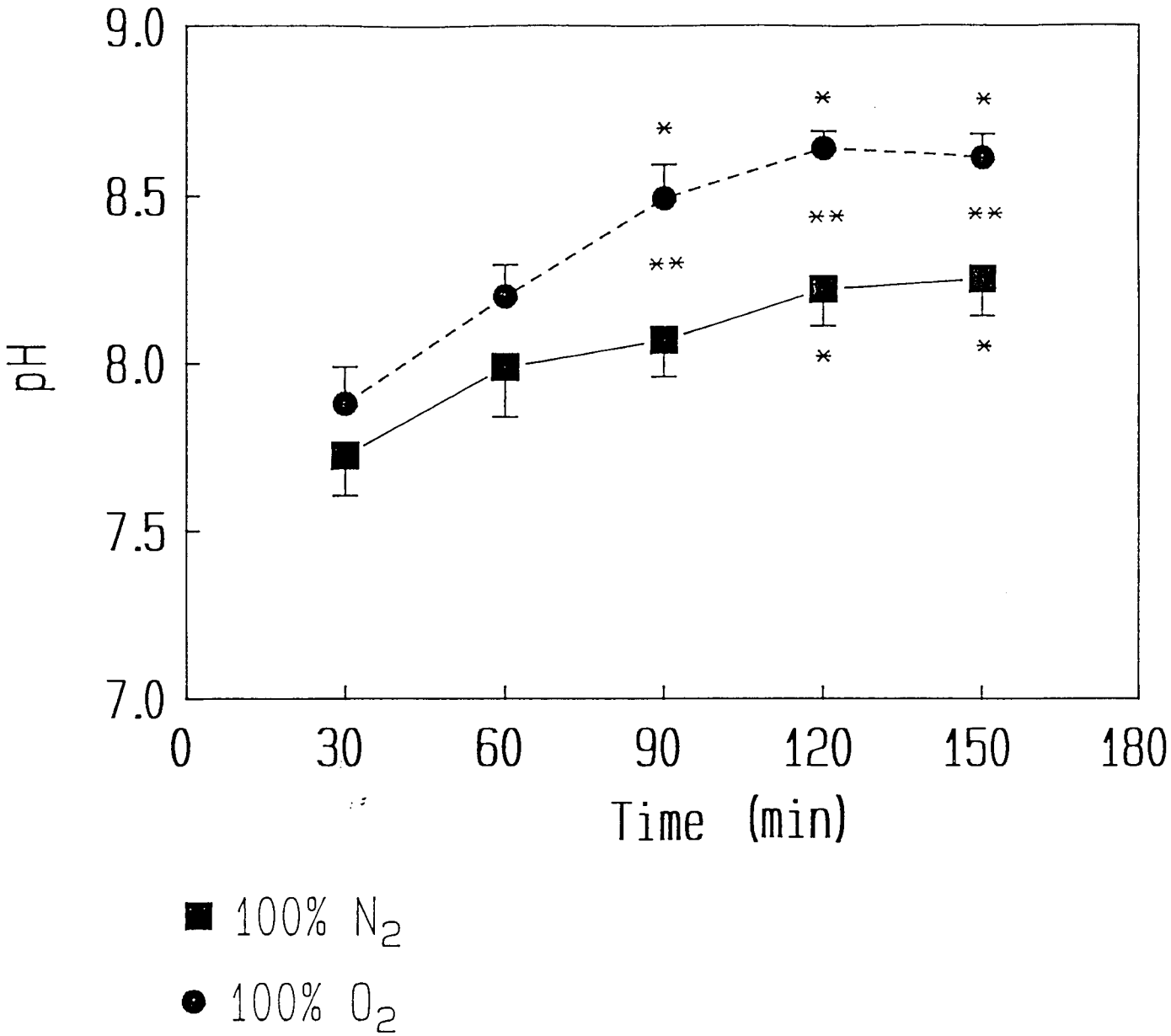

Fig. 5. Means ± sem of pH's of the coronary effluent samples during arrest. Comparison of hearts arrested with St Thomas' Hospital cardioplegic solution No. 2 with 11 mmol/l glucose gassed with 100% N_2 (pH 9.07 ± 0.1, n=6), or 100% O_2 (pH 9.3 ± 0.08, n=8). Arrested hearts were intermittently flushed with cardioplegic solution over 3 hours at 10°C.

* $p<0.02$ 100% N_2 vs 30 min

* $p<0.03$ 100% O_2 vs 30 min

** $p<0.02$ 100% N_2 vs 100% O_2

cotransport and the Cl^-/HCO_3^-exchange (Vaughan-Jones, 1988; Poole-Wilson, 1989). The average pH's of the effluents from the 100% O_2 group at 8.4 ± 0.1 were higher than with 100% N_2 at 8.1 ± 0.06, which corresponds to the difference between the pH of the arterial cardioplegic solutions (Table 6). During arrest, mean pH's were significantly different over the last three flushes ($p<0.02$). This may also have been an indication of reduced proton production by glycolysis, and increased proton consumption by oxidative phosphorylation with a high pO_2 in the cardioplegic solution.

d) LDH washout

Mean LDH content of the effluents showed greater washout with 100% O_2 during arrest compared to 100% N_2. These differences were significant at all times between the two groups ($p<0.04$) (except for the last flush because of a large variance) (Figure 6). There was a significant decrease in washout over time in the 100% N_2 group ($p<0.04$), but not with 100% O_2. Average washout was almost double with oxygenation, with 67.4 ± 4.7 mU/gm/6ml versus 34.0 ± 2.5 mU/gm/6ml ($p<0.001$) (Table 8).

Post-ischaemic washout of LDH was not significantly different between the two groups, although slightly higher with intermittent reoxygenation (Table 8).

e) Lactate washout

Average lactate washout during arrest with 100% N_2 was very high, at 3.7 ± 0.3 µmol/gm/6ml almost double that with 100% O_2 (2.0 ± 0.1 µmol/gm/6ml - $p<0.001$) (Table 8). The high lactate production with the 100%N_2 group is a result of the Pasteur effect in which glycolysis is stimulated in the absence of oxygen (Opie, 1989c). Significant differences in mean washout for each flush were only seen at 90 min, possibly because of a high variance in lactate measurements (Figure 7).

There was not a significant variation in lactate washout with time in either group, possibly due to an accumulation of lactate in the tissue without efficient washout (Figure 7).

Post-ischaemic lactate was no different between the two groups (Table 8).

Table 8: Means ± sem of average washout of lactate dehydrogenase (LDH) and lactate by intermittent flushing during arrest, and during 10 minutes reperfusion in the Langendorff mode (Post-arrest). Comparison of hearts arrested with St Thomas' Hospital cardioplegic solution No. 2 with 11mmol/l glucose gassed with 100% N_2, 100% O_2, 95%N_2 5%CO_2 or 95%O_2 5%CO_2. Arrested hearts were intermittently flushed with cardioplegic solution over 3 hours at 10°C.

	LDH (mU/gm/6ml)	Post-arrest LDH (mU/gm/min)	Lactate (μmol/gm/6ml)	Post-arrest Lactate (μmol/gm/min)
100% N_2 (n=6)	34.0 ± 2.5	36.2 ± 5.6	3.7 ± 0.3	0.59 ± 0.2
100% O_2 (n=8)	67.4 ± 4.7 *	46.5 ± 11.7	2.0 ± 0.1 *	0.57 ± 0.1
95%N_2 5%CO_2 (n=7)	56.3 ± 4.9 *	125.4 ± 12.3 *	1.6 ± 0.1 *	0.74 ± 0.18
95%O_2 5%CO_2 (n=9)	37.7 ± 2.6 &#	60.7 ± 6.7 &	1.3 ± 0.2 #	0.88 ± 0.24

* $p<0.001$ vs 100% N_2
& $p<0.002$ vs 95%N_2 5%CO_2
# $p<0.05$ vs 100% O_2

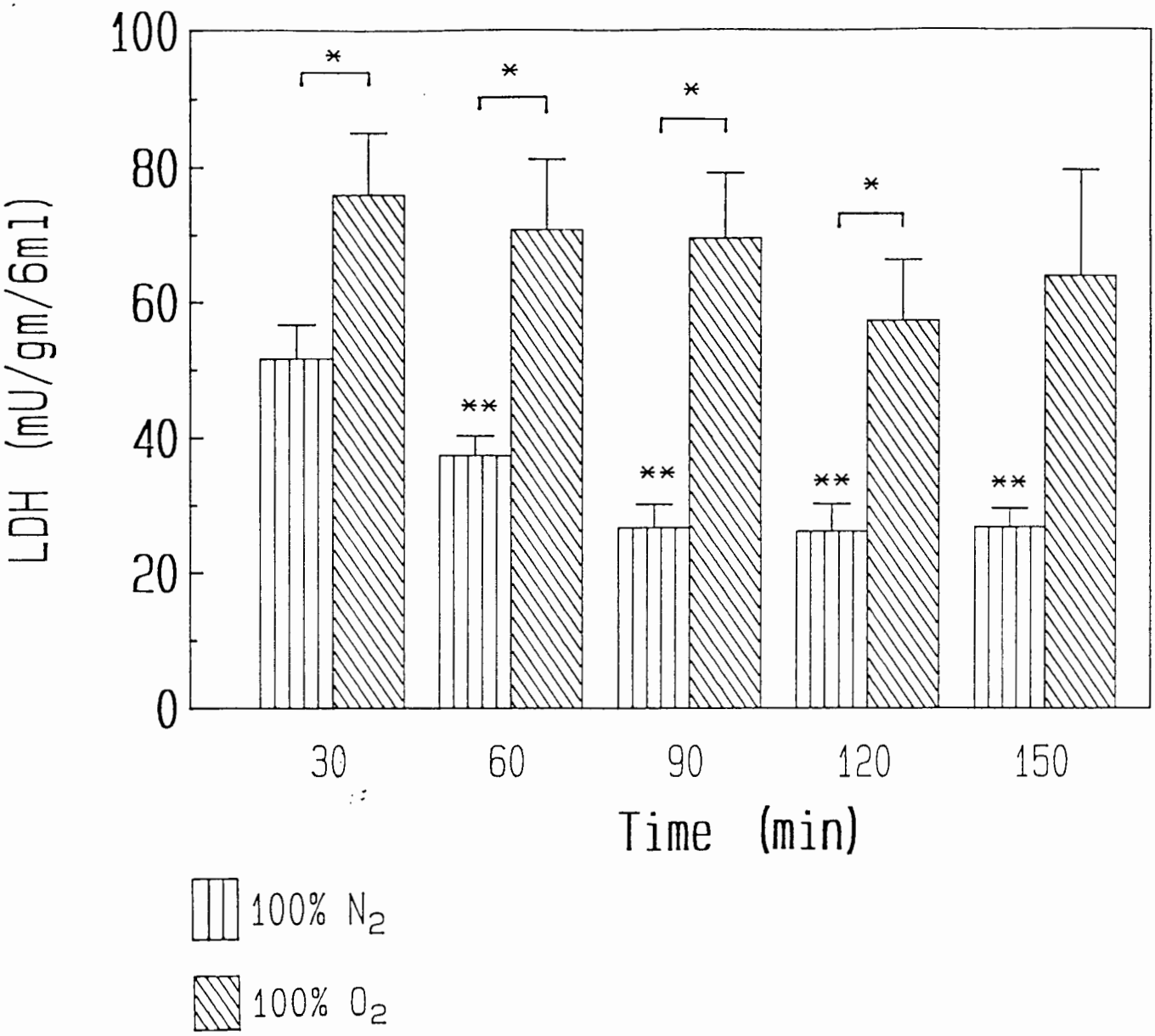

Fig. 6. Means ± sem of washout of lactate dehydrogenase (LDH) during arrest per 6ml flush of cardioplegic solution. Comparison of hearts arrested with St Thomas' Hospital cardioplegic solution No. 2 with 11 mmol/l glucose gassed with 100% N_2 (n=6), or 100% O_2 (n=8). Arrested hearts were intermittently flushed with cardioplegic solution over 3 hours at 10°C.

* $p<0.04$ 100% N_2 vs 100% O_2

** $p<0.04$ 100% N_2 vs 30 min

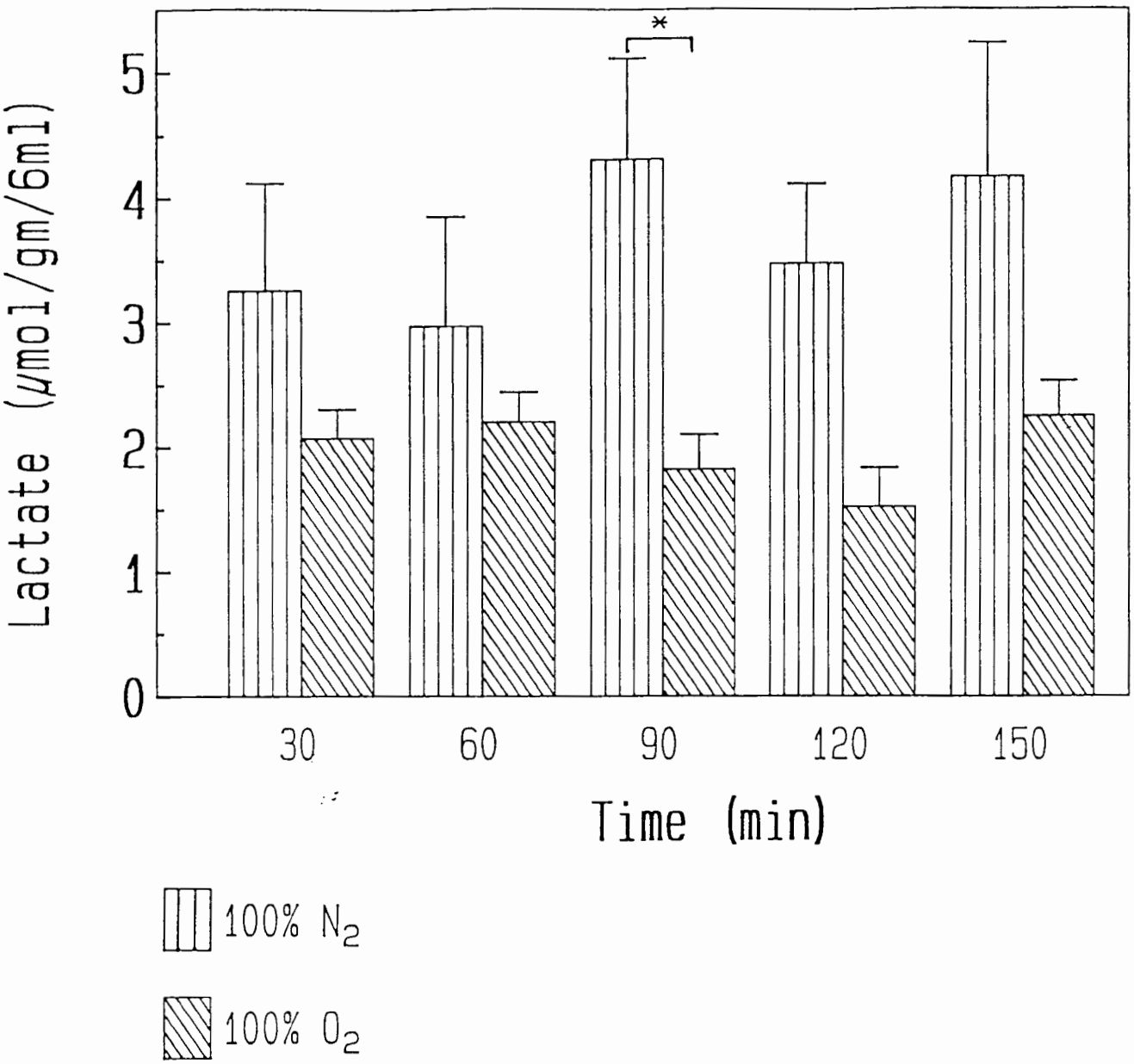

Fig. 7. Means ± sem of washout of lactate during arrest per 6ml flush of cardioplegic solution. Comparison of hearts arrested with St Thomas' Hospital cardioplegic solution No. 2 with 11 mmol/l glucose gassed with 100% N_2 (n=6), or 100% O_2 (n=8). Arrested hearts were intermittently flushed with cardioplegic solution over 3 hours at 10°C.

* $p<0.03$ 100% N_2 vs 100% O_2

f) Tissue high energy phosphates after reperfusion

The tissue ATP measurements for both groups were significantly less than control values ($p<0.03$) while no differences were seen between the two groups (Table 9). CP levels were no different compared to control levels or between groups (Table 9). There was no definite indication of improved conservation of energy with 100% O_2, although this must be looked at in the context of functional recovery.

Table 9: Means ± sem of tissue levels of adenosine triphosphate (ATP) and creatine phosphate (CP) of control hearts clamped after 10 minutes work before arrest, and of arrested hearts after 20 minutes reperfusion. Comparison of hearts arrested with St Thomas' Hospital cardioplegic solution No. 2 with 11mmol/l glucose gassed with 100% N_2, 100% O_2, 95%N_2 5%CO_2 or 95%O_2 5%CO_2. Arrested hearts were intermittently flushed with cardioplegic solution over 3 hours at 10°C.

	ATP (µmol/gm)		CP (µmol/gm)
Control (n=6)	3.91 ± 0.25		5.05 ± 0.46
100% N_2 (n=6)	2.08 ± 0.37	*	4.39 ± 0.62
100% O_2 (n=8)	2.48 ± 0.12	*	4.05 ± 0.29
95%N_2 5%CO_2 (n=7)	2.57 ± 0.13	*	4.67 ± 0.44
95%O_2 5%CO_2 (n=9)	2.66 ± 0.38	*	3.46 ± 0.70

* $p<0.03$ vs control

ii) Multidose arrest with ST+Glucose gassed with 95%N_2 5%CO_2 or 95%O_2 5%CO_2

a) pH and pO_2 of the cardioplegic solutions

To test the hypothesis of a beneficial effect of oxygenation at a decreased pH in a glucose-containing solution, the ST+Glucose solution was gassed with 95%N_2 5%CO_2 or 95%O_2 5%CO_2. 95%O_2 5%CO_2 was chosen as this is the gas composition used to aerate the K-H buffer during perfusion of an isolated heart. The presence of CO_2 reduces the pH to about 7.0 for both anoxic and highly oxygenated solutions (Table 6), although the pH for the oxygenated solutions were slightly higher. This may be due to the differences in the solubility coefficients for oxygen and nitrogen, which is reflected in a slightly higher pCO_2 in the 95%N_2 5%CO_2 group. The reduction in the amount of nitrogen dissolved in solution enables a greater amount of CO_2 to be present.

b) Functional recoveries

Pre-ischaemic control values for functional measurements were not significantly different (Table 7).

Gassing the ST+Glucose solution with 95%O_2 5%CO_2 resulted in overall recoveries of about 90%. These were significantly greater than recoveries with 95%N_2 5%CO_2 ($p<0.05$). The inclusion of oxygen in an acidotic solution was beneficial.

c) pH of coronary effluents

When the arrested hearts were flushed with ST+Glucose gassed with 95%N_2 5%CO_2 or 95%O_2 5%CO_2, the mean effluent pH's showed gradual decreases with time to about 7.1 (Figure 8). Average pH's for the effluents were very similar between the two groups, with 7.1 ± 0.01 for 95%N_2 5%CO_2, and 7.2 ± 0.02 for 95%O_2 5%CO_2, although initially pH for 95%O_2 5%CO_2 was significantly higher than for 95%N_2 5%CO_2 (7.3 ± 0.05 vs 7.16 ± 0.04, $p<0.05$). The trend for higher pH's with the

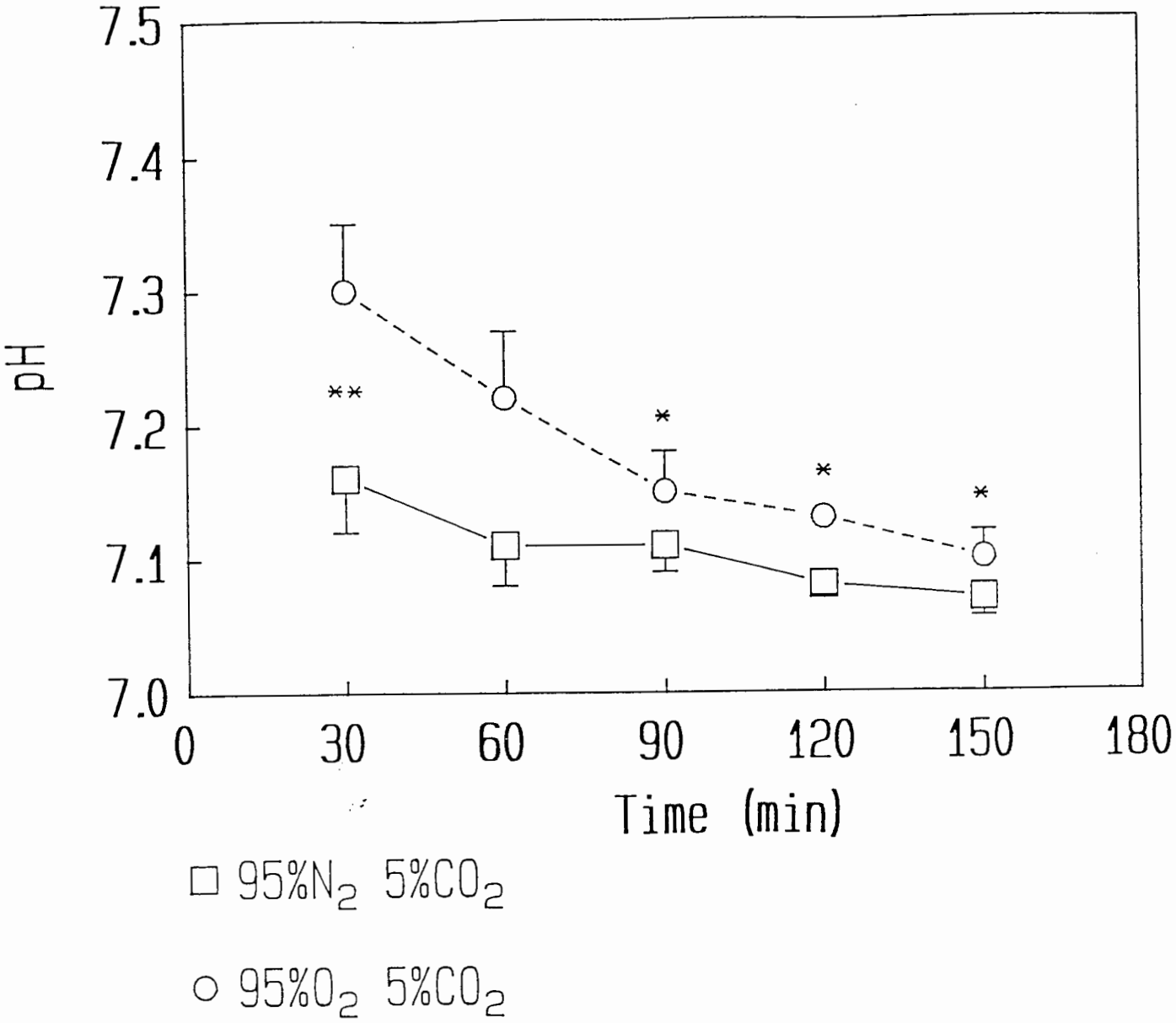

Fig. 8. Means ± sem of pH's of the coronary effluent samples during arrest. Comparison of hearts arrested with St Thomas' Hospital cardioplegic solution No. 2 with 11 mmol/l glucose gassed with $95\%N_2$ $5\%CO_2$ (pH 6.98 ± 0.01, n=7), or $95\%O_2$ $5\%CO_2$ (pH 7.07 ± 0.02, n=8). Arrested hearts were intermittently flushed with cardioplegic solution over 3 hours at $10^{o}C$.

* $p<0.05$ $95\%O_2$ $5\%CO_2$ vs 30 min

** $p<0.05$ $95\%N_2$ $5\%CO_2$ vs $95\%O_2$ $5\%CO_2$
All values were significantly different between low pH and high pH groups at each time ($p<0.01$).

oxygenated group may be due to consumption of protons by increased oxidative phosphorylation.

d) LDH washout.

Mean LDH washout during arrest showed a significant decline over time in hearts flushed with ST+Glucose gassed with 95%O_2 5%CO_2 ($p<0.04$) (Figure 9). A similar trend was observed in the 95%N_2 5%CO_2 group, although this was not significant (Figure 9). Average LDH washout during arrest was significantly lower in the presence of oxygen with 37.7 ± 2.6 mU/gm/6ml versus 56.3 ± 4.9 mU/gm/6ml for the anoxic group ($p<0.001$) (Table 8). The presence of oxygen thus reduced membrane damage associated with a low pH.

Post ischaemic loss of LDH was also greater with the anoxic acidic group, at 125.4 ± 12.3 mU/gm/min, compared to 60.7 ± 6.7 mU/gm/min with the 95%O_2 5%CO_2 group ($p<0.002$) (Table 8).

e) Lactate washout.

Average lactate washout during arrest was slightly lower with 95%O_2 5%CO_2 than with 95%N_2 5%CO_2 (Table 8). There was a significant decline in lactate washout with time in the 95%O_2 5%CO_2 group ($p<0.04$), although with 95%N_2 5%CO_2 there was a significant increase with time ($p<0.04$) (Figure 10). On reperfusion, lactate washout was marginally greater in the 95%O_2 5%CO_2 group.

f) High energy phosphate levels

The tissue ATP after reperfusion showed a slight increase in the 95%O_2 5%CO_2 group as against 95%N_2 5%CO_2 although this was not significant (Table 9). These were both significantly less than control values for ATP ($p<0.03$). CP levels did not differ significantly from control values or from each other (Table 9).

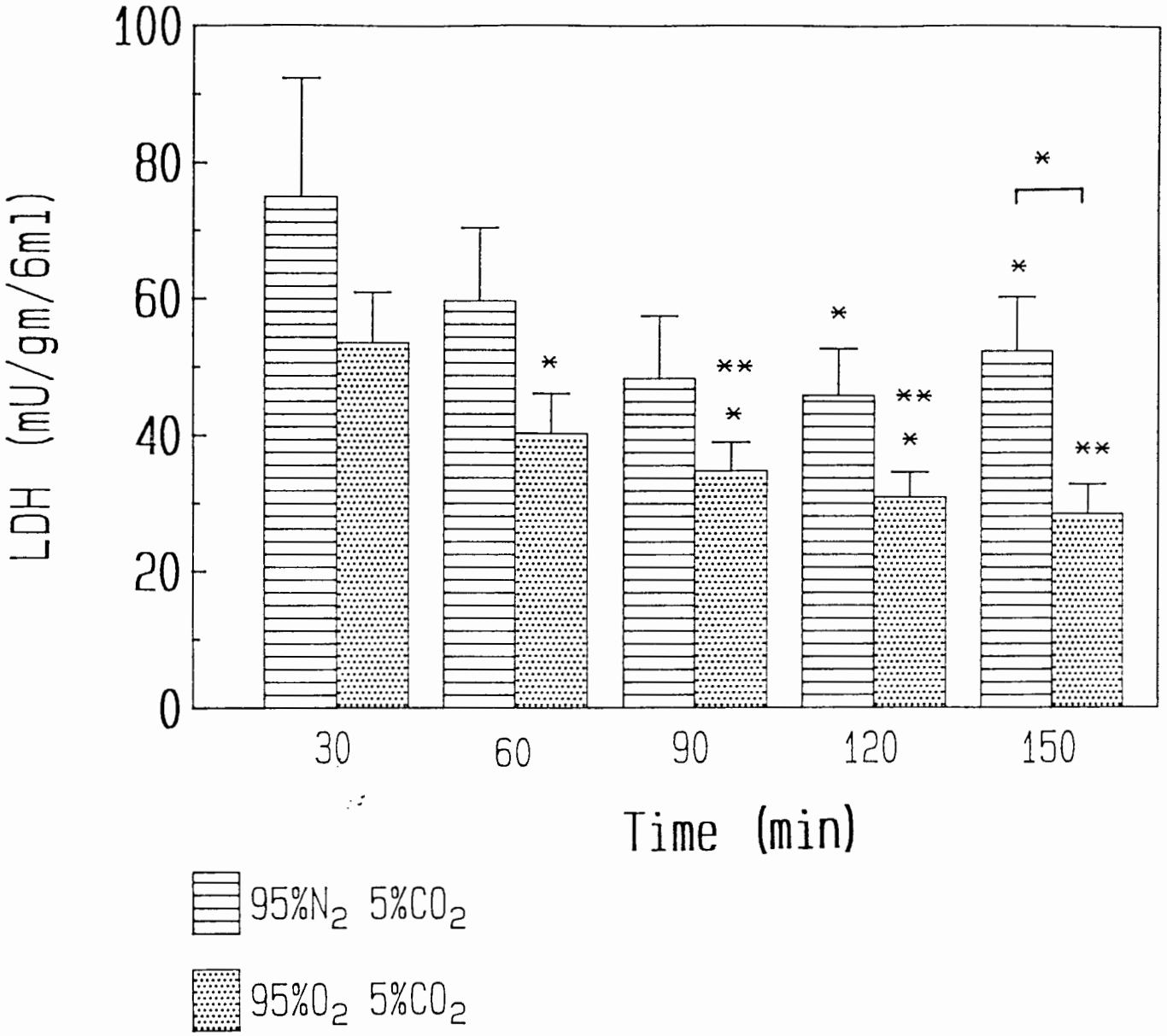

Fig. 9. Means ± sem of washout of lactate dehydrogenase (LDH) during arrest per 6ml flush of cardioplegic solution. Comparison of hearts arrested with St Thomas' Hospital cardioplegic solution No. 2 with 11 mmol/l glucose gassed with $95\%N_2$ $5\%CO_2$ (n=7), or $95\%O_2$ $5\%CO_2$ (n=8)Arrested hearts were intermittently flushed with cardioplegic solution over 3 hours at 10^{o}C.

** $p<0.04$ $95\%O_2$ $5\%CO_2$ vs 30 min

* $p<0.02$ $95\%N_2$ $5\%CO_2$ vs $95\%O_2$ $5\%CO_2$

* $p<0.03$ $95\%N_2$ $5\%CO_2$ vs 100% N_2

* $p<0.03$ $95\%O_2$ $5\%CO_2$ vs 100% O_2

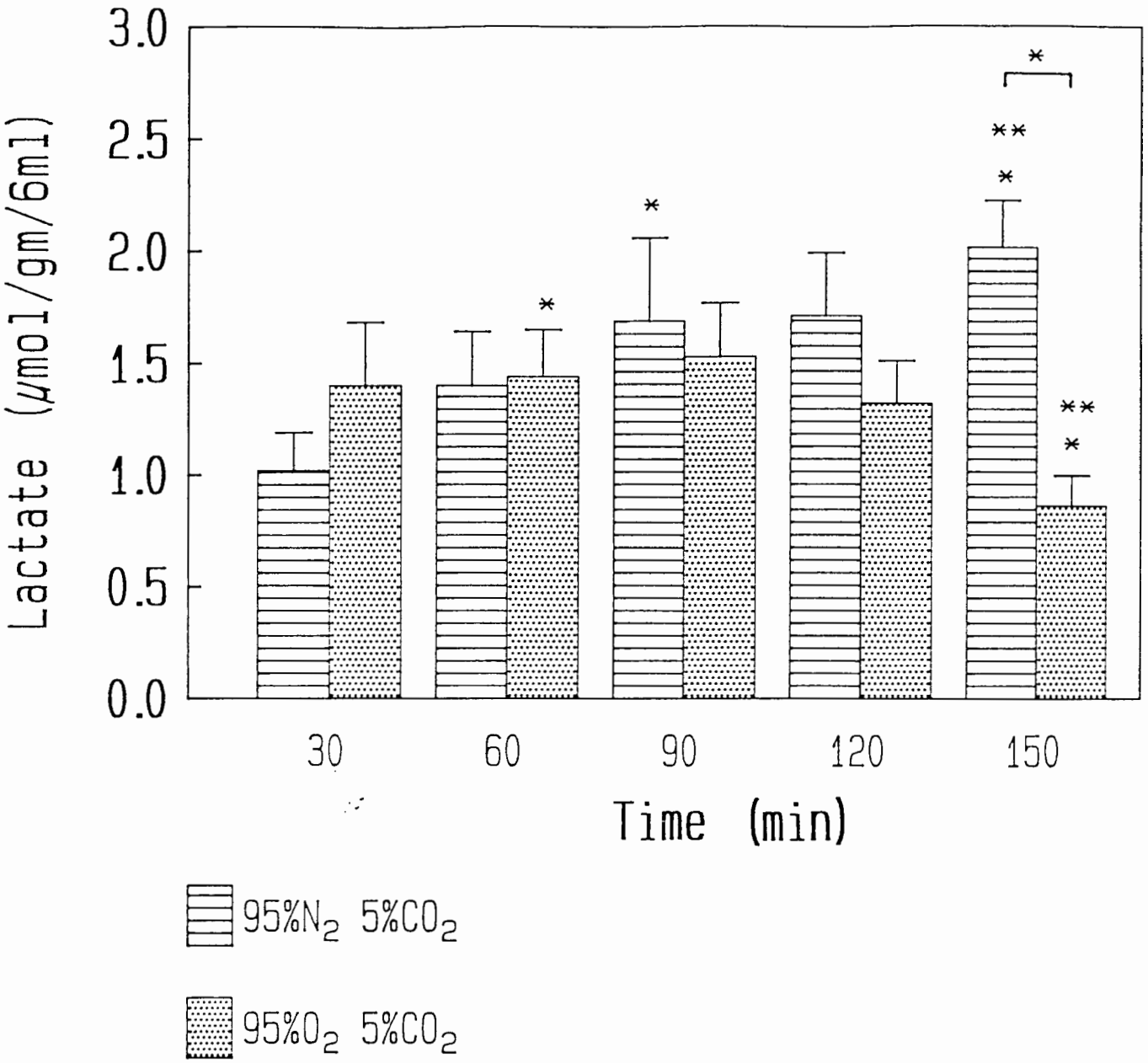

Fig. 10. Means ± sem of washout of lactate during arrest per 6ml flush of cardioplegic solution. Comparison of hearts arrested with St Thomas' Hospital cardioplegic solution No. 2 with 11 mmol/l glucose gassed with $95\%N_2$ $5\%CO_2$ (n=7), or $95\%O_2$ $5\%CO_2$ (n=8). Arrested hearts were intermittently flushed with cardioplegic solution over 3 hours at $10^{o}C$.

** $p<0.04$ $95\%O_2$ $5\%CO_2$ vs 60, 90 min
** $p<0.03$ $95\%N_2$ $5\%CO_2$ vs 30 min

* $p<0.001$ $95\%N_2$ $5\%CO_2$ vs $95\%O_2$ $5\%CO_2$

* $p<0.04$ $95\%N_2$ $5\%CO_2$ vs 100% N_2
* $p<0.03$ $95\%O_2$ $5\%CO_2$ vs 100% O_2

iii) Comparison of oxygenation with and without 5%CO_2

a) Functional recoveries

The presence of 5%CO_2 in the cardioplegic solution with both anoxic and oxygenated hearts improved functional recovery (Table 7). The improvement seen with anoxic hearts was about 10-15% in aortic output ($p<0.05$). There was also a significant improvement in recovery between the two oxygenated groups with a decrease in pH. Optimal recoveries were attained when gassing the ST+Glucose solution with 95%O_2 5%CO_2.

b) LDH efflux during and after arrest

There was a significant difference in the mean washout of LDH during arrest between the two nitrogen groups especially at the later flushes ($p<0.03$) (Figures 6 and 9). This was reflected in the average efflux, with 56.3 ± 4.9 mU/gm/6ml at the lower pH, compared to the anoxic alkaline solution, with 34.0 ± 2.5 mU/gm/6ml ($p<0.001$) (Table 8). Post-ischaemic LDH efflux was also significantly greater with the inclusion of CO_2, at a level about three times higher ($p<0.001$) (Table 8). A harmful effect of a lower pH on membrane integrity was observed.

Oxygenation with a high pH was also associated with increased membrane damage. However, with oxygenation at a low pH, LDH release was significantly reduced, both versus 100% O_2 ($p<0.05$) and 95%N_2 5%CO_2 ($p<0.002$) (Table 8). A low pH will inhibit Ca^{2+} entry and free radical activity, while stimulation of oxidative phosphorylation may further protect the heart by increasing energy levels during ischaemia.

c) Lactate washout during and after arrest

The average lactate washout during arrest of the 95%N_2 group and both O_2 groups were significantly lower than that of the 100% N_2 group, by about half ($p<0.05$) (Figures 7 and 10). Washout in these three groups was similar. Post-ischaemic lactates did not show any significant differences between the groups (Table 8). There was a tendency for slightly greater washout on reperfusion with the lower pH's, possibly linked to extrusion of lactate together with protons on reperfusion with

a relatively alkalotic solution (K-H pH 7.4). Preservation of glycolytic pathways with a low extracellular pH is possibly indicated.

d) Oxygen uptake

The pO_2 of the cardioplegic solutions was similar (Table 6) although the mean oxygen uptake by the hearts per 6 ml flush was greater in the presence of CO_2 (Figure 11) where average uptake with 100% O_2 was 0.0758 ± 0.002 ml O_2/gm/6ml and with 95%O_2 5%CO_2 was 0.0835 ± 0.003 ml O_2/gm/6ml ($p<0.05$). (The O_2 uptake of the nitrogen groups could not be determined because of the low pO_2 of the effluent which therefore picked up oxygen from the atmosphere very rapidly). There was thus greater oxidative phosphorylation at the lower pH, with enhanced energy production. A significant decline in oxygen uptake over time was seen in both groups, until equivalent values were reached.

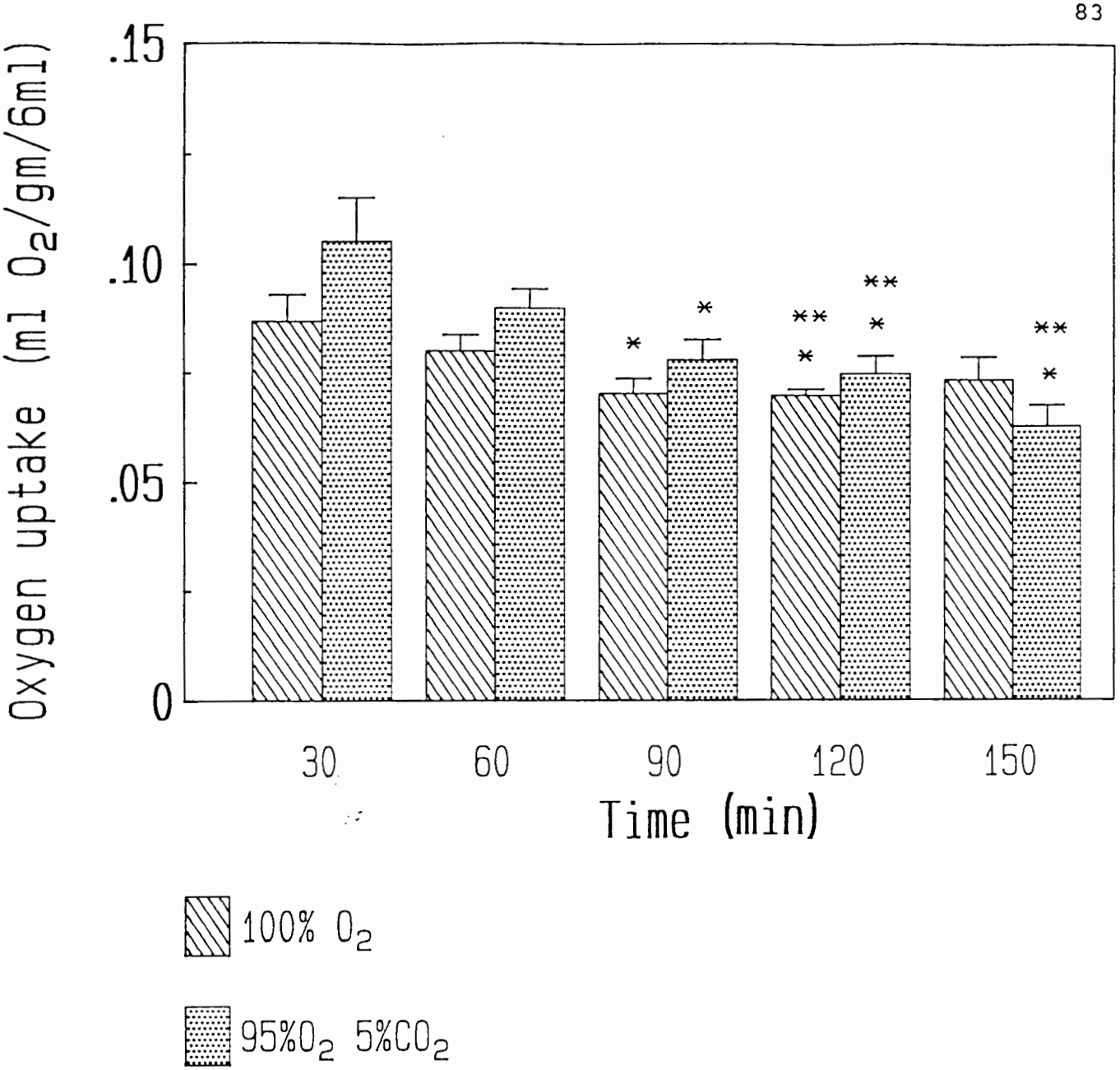

Fig. 11. Means ± sem of oxygen uptake during arrest per 6ml flush of cardioplegic solution. Comparison of hearts arrested with St Thomas' Hospital cardioplegic solution No. 2 with 11 mmol/l glucose gassed with 100% O_2 (0.184 ml O_2/6ml, n=6), or 95%O_2 5%CO_2 (0.187 ml O_2/6ml, n=7). Arrested hearts were intermittently flushed with cardioplegic solution over 3 hours at 10°C.

* $p<0.04$ 100% O_2 vs 30 min
** $p<0.03$ 100% O_2 vs 60 min

* $p<0.04$ 95%O_2 5%CO_2 vs 30 min
** $p<0.04$ 95%O_2 5%CO_2 vs 60 min

iv) Properties of the ST+Glucose cardioplegic solution

a) Temperature, pH and oxygenation

The ST+Glucose solution was either open to atmosphere, or gassed with 100% O_2 or 95%O_2 5%CO_2. Similar slopes for the non-gassed and 100% O_2 solutions were obtained, although the pH's were different (Figure 12). However, in the presence of CO_2, there was little change in pH with temperature.

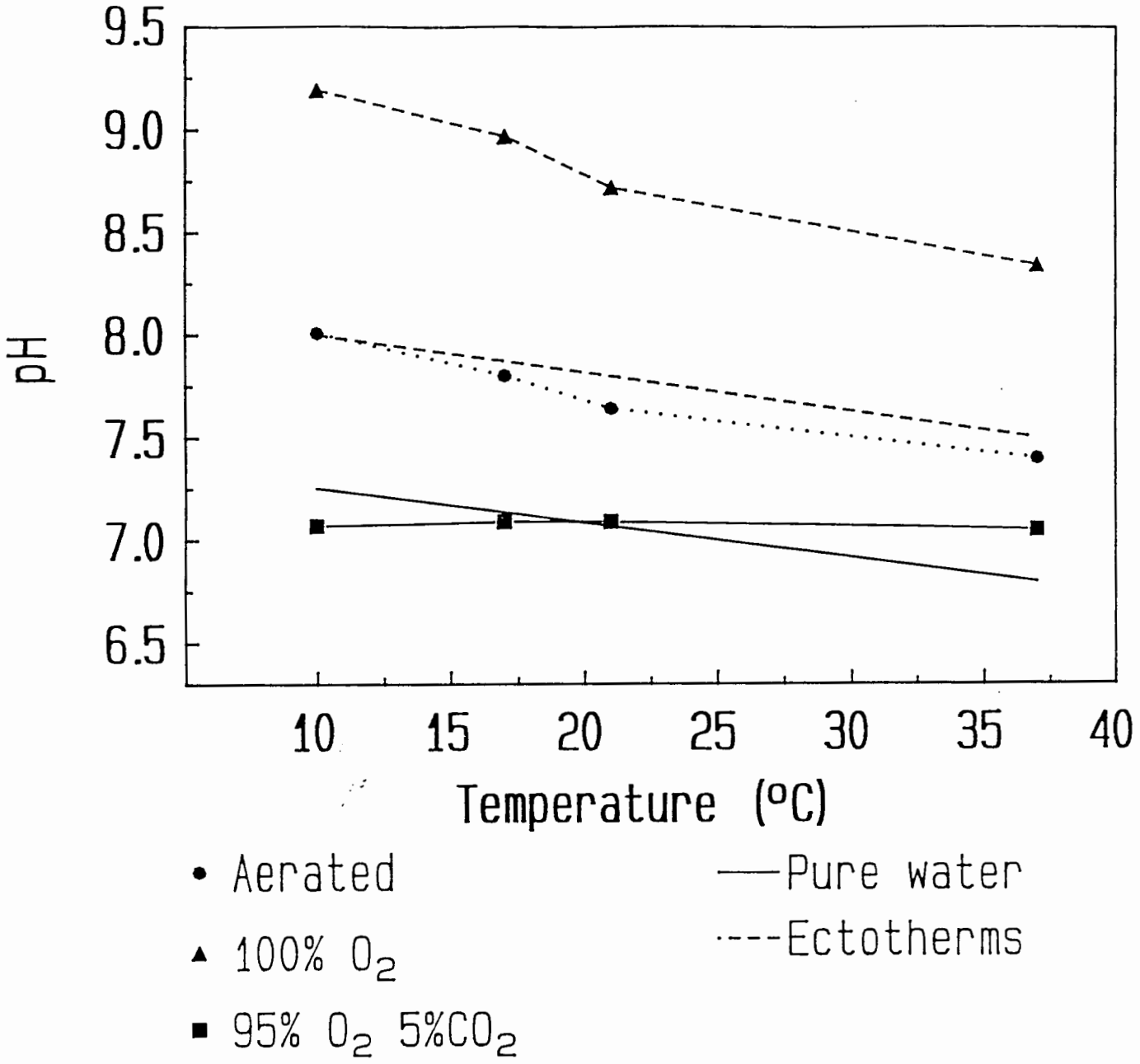

Fig. 12. Change in pH with temperature of the St Thomas' Hospital cardioplegic solution No. 2 open to atmosphere (aerated), or gassed with 100% O_2 or 95%O_2 5%CO_2. Also shown are curves for pure water and ectotherm tissue.
Slopes of the curves give the Rosenthal correction factors.

ST+G	Aerated	-0.022	Water	-0.017
	100% O_2	-0.031	Ectotherm	-0.019
	95%O_2 5%CO_2	-0.001		

b) Titration curves and buffering capacities

The titration curves for the ST+Glucose solution open to atmosphere, or gassed with 100% O_2 or 95%O_2 5%CO_2, were determined at 10°C (Figure 13). There was a shift in the curves dependent on the CO_2 concentration. The pK's for the solutions were about 6.35 to 6.40 at 10°C.

The buffering capacities for ST+Glucose determined from the titration curves (Figure 14) show that the range of buffering was maximal at a value of about -11 mmol HCl/l/pH at a pH of 6.35.

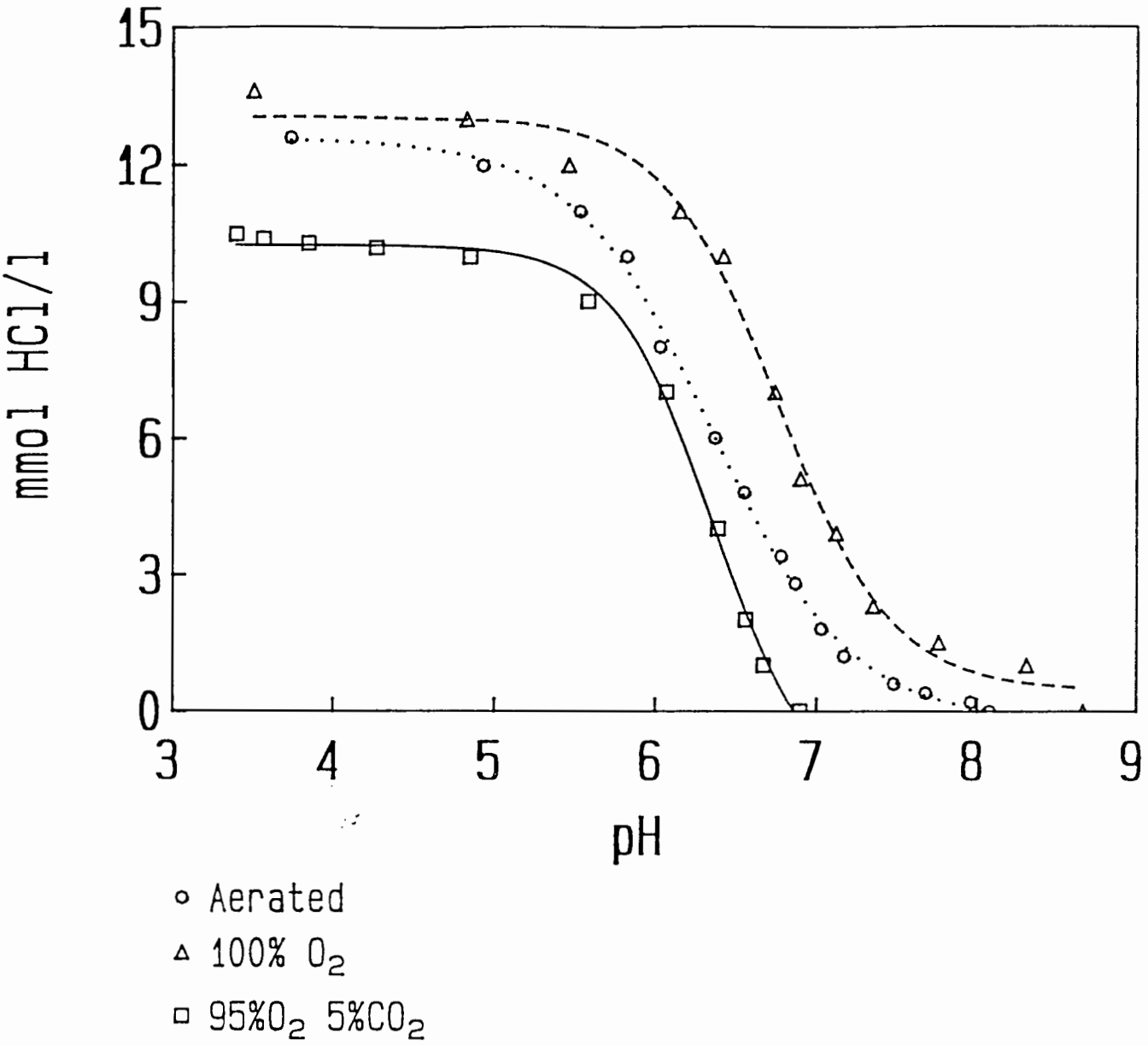

Fig. 13. Titration curves of the St Thomas' Hospital cardioplegic solution No. 2 gassed on preparation with 95%O_2 5%CO_2 and open to atmosphere (aerated), or continually gassed with 100% O_2 or 95%O_2 5%CO_2. The solutions were kept at 10°C. The inflection points of the curves give the pK for the solution, at 6.4. Increases in the CO_2 content lead to a downward shift in the curve.

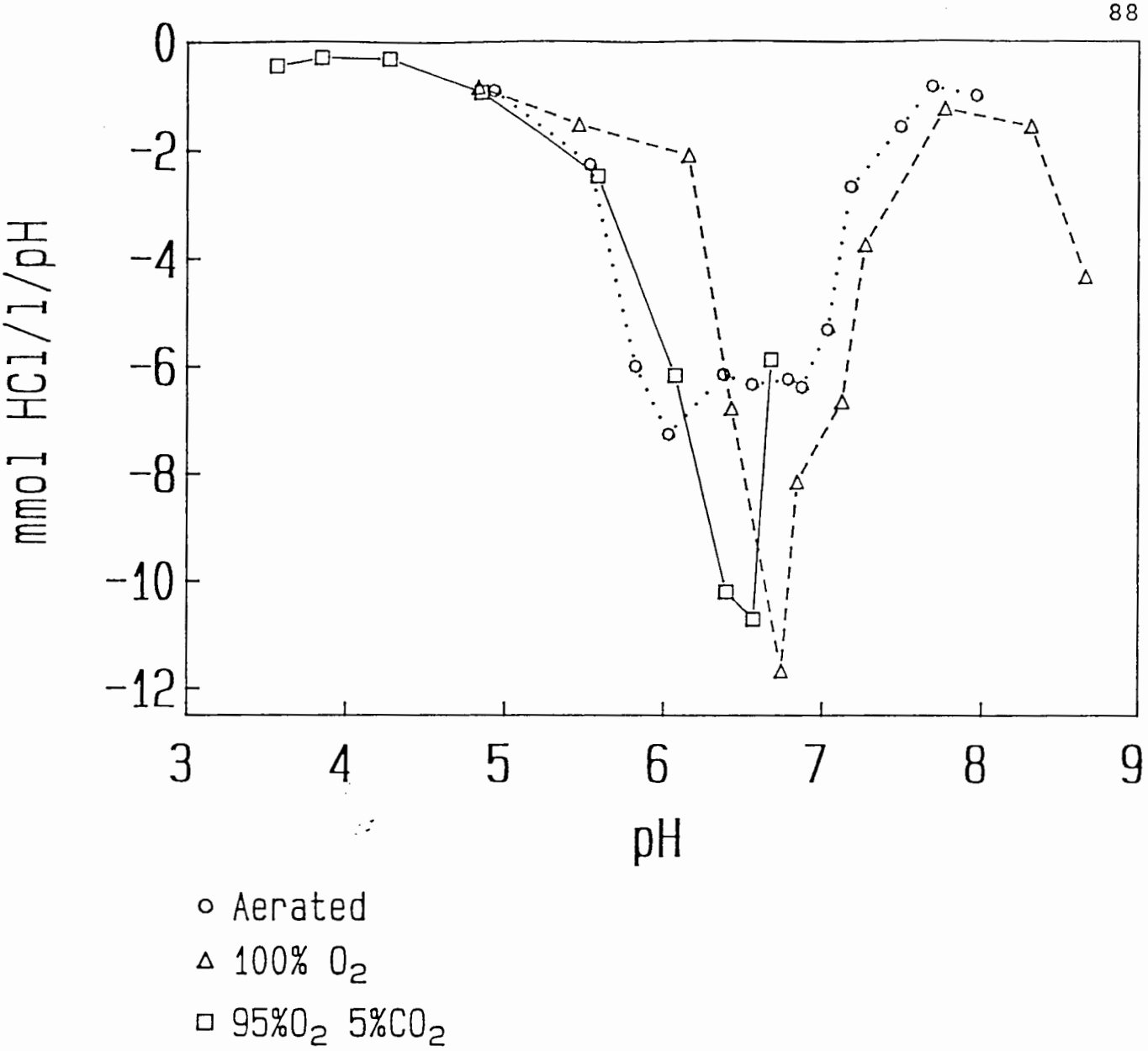

Fig. 14. Buffering capacity of the St Thomas' Hospital cardioplegic solution No. 2 gassed on preparation with 95%O_2 5%CO_2 and thereafter left open to atmosphere (aerated), or continually gassed with 100% O_2 or 95%O_2 5%CO_2. The solutions were kept at 10°C. Buffering capacity is expressed as the instantaneous (derivative) slope of the titration curves (Fig. 13).

d) Discussion

i) Oxygenation of the ST+Glucose solution

The addition of oxygen to the ST+Glucose cardioplegic solution used in a multidose protocol increased functional recovery compared to anoxic solutions. This may be related to enhanced energy production by oxidative phosphorylation during arrest and improved mitochondrial function on reperfusion.

The premise that an increase in glycolytic rate is of benefit to the arrested heart seems to be countered by the results of this experiment, as increased lactate output in the 100% N_2 group did not lead to improved recovery of the hearts. Intracellular accumulation of lactate has been shown to have deleterious effects on the cells (Neely and Grotyohann, 1984; Hagberg, 1985; Opie, 1990). The high rates of glycolysis as shown by the high lactate washout with the 100% N_2 group may also contribute to developing acidosis, which is shown by a greater washout of protons compared to the 100% O_2 group. However, the washout of lactate does not necessarily reflect the rate of glycolysis, as there may have been an accumulation of lactate in the cells. The instantaneous rates of glycolysis may have shown different trends. Lactate may have also been consumed by oxidative phosphorylation with 100% O_2 present, and the two groups may in fact have shown similar rates of glycolysis.

An accumulation of protons in the cells is associated with deleterious effects. However, with a high arterial pH intermittently reintroduced, the protons may be extruded down the restored concentration gradient, possibly in exchange for Na^+ with removal of inhibition of the Na^+/H^+ exchanger. This may allow the entry of Ca^{2+} by the Na^+/Ca^{2+} exchange.

In the presence of oxygen, an increased lactate may be beneficial as it can be used as an independent source of energy by the Krebs cycle, thus reducing lactate efflux. However, when gassing the cardioplegic solution with 100% O_2, glycolysis may also be inhibited by this metabolite, although the stimuli of cessation of coronary flow and a high pH are

fairly strong. Relative inhibition of glycolysis will reduce lactate and intracellular proton build up, while increased oxidative phosphorylation will consume protons and lactate. The pH of the coronary effluents reflects this, as the pH's for the 100% O_2 group were higher than with 100% N_2. However, the arterial pH of the latter solution tended to be lower than with the former. This may be due to the different solubility coefficients of nitrogen (16.9 ml O_2/l solution at 15^{o}C) and oxygen (37 ml O_2/l) resulting in slightly greater pCO_2's with the former, although the measured pCO_2's for the solutions were similar. This may have led to a slight bias in the effluent pH's but the overall trend is still significant.

With oxygenation, substrates are utilised in a more efficient way by oxidative phosphorylation than by glycolysis, with more ATP produced per mole glucose. In addition, the Krebs cycle utilises pyruvate thereby limiting the production of lactate and protons (Opie, 1989c). In the presence of glucose, energy production in the arrested heart may then be optimal since both glycolysis and oxidative phosphorylation are active without excessive build up of lactate and protons.

The beneficial effect of oxygenation of cardioplegic solutions is well documented (Bodenhamer et al., 1983; Guyton et al., 1985; Coetzee et al., 1986; Boggs et al., 1987; Hendren et al., 1987; de Wit et al., 1988; Ledingham et al., 1988), and supported by the results of these experiments. However, other studies have used different protocols and different solutions to test the effect of oxygenation.

Bodenhamer et al. (1983) used a hypothermic (4^{o}C) potassium crystalloid cardioplegic solution (mmol/l Na+ 118, K+ 27, pH 7.4) over a 4 hour arrest period with reinfusions every 20 min. Oxygenation with 100% O_2 as opposed to simple aeration (open to atmosphere) of the cardioplegic solution was beneficial. Preservation of high energy phosphate stores was greatly improved in the presence of a high pO_2. Guyton et al. (1985) also found a beneficial effect with an oxygenated (100% O_2) crystalloid cardioplegic solution (mmol/l - Na^+ 160, K^+ 30, Ca^{2+} 0) infused intermittently.

Boggs et al. (1987) used the isolated rat heart and found a salutary effect of oxygenation with 98%O_2 2%CO_2 (pH ± 7.4) in a hyperkalaemic cardioplegic solution (mmol/l - Na^+ 110, K^+ 20, Mg^{2+} 0, glucose 28) (multidose protocol at 4°C over 2 hrs).

The beneficial effects of both ST No 1 and No 2 solutions are increased when oxygenated with 100% O_2, at a temperature of 20°C (pO_2 320-560 mmHg, pH 8.2) as against aeration (pO_2 greater than 150 mmHg, pH 7.9) (Ledingham et al., 1988). This study was conducted with multidose cardioplegia at a temperature of 20°C in the isolated working rat heart.

However, when comparing the recoveries attained with 100% O_2 in this study, versus those observed in the initial studies with aerated ST+Glucose (see above), a reduction in function with the former solution was seen. The discrepancy between these results and those of Ledingham et al. (1988) may be due to the differences in pH of the solutions [pH 7.4-7.8 ST+Glucose (aerated) versus pH 9.3 with 100% O_2; and pH 7.9 ST (aerated) versus pH 8.2 (100% O_2)(Ledingham et al., 1988)]. A much larger change in the pH between the different solutions was observed in the present series of experiments, possibly due to the differences in temperature of the solutions (10°C versus 20°C). The intermittent reintroduction of the highly alkaline solution with a high pO_2 may have led to the formation of oxygen free radicals, exacerbating the membrane damage linked to ischaemia and multidose cardioplegia, and increasing reperfusion injury. These effects may reduce the functional recovery despite an improved energy production. An additional variable therefore to be taken into account with oxygenation of the solution was the change in pH observed. A comparison of a high and a low pH for cardioplegic solutions was then made, either without or with oxygenation.

ii) pH of the ST+Glucose solutions

a) Acidotic solutions

The use of an acidotic anoxic solution resulted in an improvement in functional recovery as opposed to an anoxic

solution with a high pH. There was a decrease in lactate production, due to inhibition of glycolysis by a low pH.

However, with the acidotic anoxic solution, there was an increase in LDH washout during and after arrest, indicating a harmful effect of a low pH on cell membranes. This was negated by the improved functional recovery. There was also an increasing washout of lactate in the 95%N_2 5%CO_2 group. This is conflicting as there was a decrease in the effluent pH over time, although a low pH is associated with inhibition of glycolysis. However, the stimulus of no flow and a lack of oxygen on glycolysis appears to be greater than the inhibitory effect of the low pH over time. The presence of glucose may also have stimulated glycolysis under these conditions. An accumulation of lactate in the myocardium was possibly found in this group.

Perfused hypothermic hearts function more efficiently with an alkaline solution. A rapid decline in contractility is observed in the perfused rat heart in response to increased CO_2 (Poole-Wilson and Langer, 1975) with a reduction in pH from 7.4 to 6.8. However, arrested hearts have been shown to respond better to acidotic cardioplegic solutions (Nugent et al., 1982; Bernard et al., 1985). This was substantiated by the above experimental results. The use of a hyperkalaemic cardioplegic solution (K^+ 25 mmol/l) titrated with bicarbonate (Nugent et al., 1982) indicated that an alkaline solution (pH 7.70) had a significantly depressive effect on postarrest left ventricular function, compared to an acidotic solution (pH 7.10). A decreased O_2 consumption was observed with pH 7.1, indicating greater conservation of energy. A study by Bernard et al. (1985), using the NMR technique to determine intracellular pH in perfused hearts, substantiates the hypothesis of an increase in recovery with a relatively acidotic, Ca^{2+}-containing cardioplegic solution.

The mechanisms for energy production on reperfusion of the heart may be better preserved with an acidotic solution, which will enhance the ability of the tissue to recover. The stores of ATP may also be increased because of inhibition of

contractile activity during arrest, with interaction of factors including hypothermia, the increased K^+ concentration and inhibition of activation of contraction by the low pH. Measurement of ATP levels at the end of ischaemia would help to elucidate this point.

b) Use of alkaline cardioplegic solutions

In contradiction to the above findings, several researchers recommend the use of alkalotic cardioplegic solutions. Recordings of intramyocardial pH of human hearts during arrest when intermittently flushed with an acalcaemic cardioplegic solution (pH 7.8) at 4°C, indicate that maintenance of mean pH during the aortic cross clamp period is well correlated with recovery (Khuri et al., 1983). A myocardial pH of 7.4 was associated with maximum recoveries, while pH's of 6.7 gave minimum recoveries. Prevention of acidosis during a cardioplegic arrest period was thought to be beneficial.

Takach et al. (1986) also measured intramyocardial pH during cardioplegic arrest. A pH of 6.8 was used as an end point, at which recovery of all the hearts was found to correlate at a low level. The time taken to reach this point was used as an index of efficacy of the solutions. Hypothermia was deemed to be advantageous by slowing the decline in pH. The convention thus established was that a low pH allowed to develop in the arrested heart is detrimental to its recovery. The use of an alkalotic solution was therefore advocated.

Another argument for the use of an alkalotic cardioplegic solution is the relationship of temperature and pH. A drop in the temperature of a solution is associated with an increase in pH (White, 1981), as there is greater binding of protons with anions. Ectotherms maintain the pH of their body fluids parallel to the neutral point of water by decreasing the pCO_2 with changes in temperature (Reeves and Malan, 1976). This strategy preserves the charge state of proteins, maintaining enzyme activity (Reeves and Malan, 1976). In extrapolation to cardioplegic solutions, Swan (1984) and others recommend a pH of 7.8, in a fully oxygenated solution used at 4-8°C. This strategy has been commonly applied by cardiac surgeons.

However, hibernating animals maintain a relatively low arterial pH of about 7.4 with decreasing temperatures (White, 1981). This is linked to preservation of energy stores. Metabolic activity is decreased both by the drop in body temperature to about 5°C, and proton inactivation of the enzymes with subsequent conservation of energy.

Becker et al. (1981) looked at the relationship between pH, pCO_2 and temperature of cardioplegic solutions in higher mammals (puppies). The pH of the cardioplegic solution was maintained either at 7.4 (physiological pH at 37°C) by maintaining a constant pCO_2 of 40 mmHg; or respiratory alkalosis was induced by decreasing the pCO_2 to 10 ± 4 mmHg at 17°C, to bring about pH values as would be found in the ectotherm. The pH was thereby increased to 7.85. The results showed better recovery of cardiac output with the pH-adjusted group, with a decreased lactic acidosis and prevention of ventricular fibrillation. This study used blood cardioplegia, and multidose administrations, with total body cooling. This should not be extrapolated to crystalloid solutions, because of the effect of oxygen and different buffering capacities of blood.

In our studies, a comparatively low extracellular pH and presumably a decreased intracellular pH led to better recovery of the hearts. The drop in pH associated by others with decreased recovery may be due to lower calcium concentrations used, or the absence of O_2. There may also be a critical limit on acidosis. The presence of glucose may also affect the results obtained with an increase in the glycolytic rate affecting intracellular proton levels.

c) Limit of extracellular acidosis

While a mild acidosis appears to be beneficial, in our experiments the effluent pH's did not drop below 7.00, which may be above a certain critical limit (Bernard et al, 1985). A severe acidosis of less than 6.6 leads to cellular necrosis, with activation of lipases and proteases, whereas decreasing extracellular pH only to 7.00 may be advantageous. This approximates the intracellular pH of a normal myocardial cell

(Roos and Boron, 1981). The effect of pH on phospholipase activation may be counteracted by the beneficial effect of acidosis on Ca^{2+} entry and activity. This may account for the high efflux of LDH during and after arrest with the use of the acidotic solution (95%N_2 5%CO_2), but an increased recovery of the myocardium despite this.

The optimal pH of a cardioplegic solution may depend on the temperatures involved as well as the solution used. The species tested may also be a determining factor.

d) Ca^{2+} entry on reperfusion

Acidosis is thought to be protective because of inhibition of Ca^{2+} binding, and preservation of energy stores (see Introduction). This is of advantage in cardioplegia, where a reduced Ca^{2+} influx and decreased ATP consumption is desired. Moderate acidosis also appears to be beneficial upon reperfusion of the heart after ischaemia, as well as after hypoxia (Kitakaze´ et al., 1988), as this prevents Ca^{2+} overload on reperfusion, one of the major putative causes of damage associated with an ischaemic period.

A possible mechanism for the prevention of Ca^{2+} overload on reperfusion involves the Na^+/H^+ and the Na^+/Ca^{2+} exchangers.

The activity of the Na^+/H^+ exchanger is dependent on the proton gradient. Its activity is enhanced at acidic pH's but is almost nil at normal and alkalotic pHi's. (Lazdunski et al., 1985; Kaila and Vaughan-Jones, 1987). When the pH_i is lower than pH_o, protons cross the membrane into the extracellular space, leading to an intracellular Na^+ accumulation. During ischaemia, intracellular acidosis develops. There is thus a proton gradient, leading to extrusion of protons via the Na^+/H^+ exchange. However, extracellular pH is then reduced with a lack of coronary flow, which causes inhibition of the exchanger (Lazdunski et al., 1985). With inhibition of the $Na^+/H+$ exchange in sheep Purkinje fibres, there is a decrease in Na^+ accumulation in the cell, and therefore prevention of Ca^{2+} influx via the Na^+/Ca^{2+} exchange (Vaughan-Jones et al., 1989). Thus, in the

presence of a low extracellular Na^+ or low pH_o, contractility is decreased.

On reperfusion, however, with the reintroduction of a relatively alkalotic solution (usually pH 7.4), the gradient is increased causing efflux of protons in exchange for Na^+. This alteration in the Na^+ gradient in turn activates the Na^+/Ca^{2+} exchange with the subsequent influx of Ca^{2+}.

With the use of an alkalotic cardioplegic solution introduced at intermittent intervals, the Na^+/H^+ exchanger may be intermittently reactivated, leading to the accumulation of Na^+ and Ca^{2+} in the cell. With the use of an acidotic cardioplegic solution, however, the Na^+/H^+ exchanger will be inhibited because of a reduced proton gradient. Thus an acidotic cardioplegic solution is proposed to be beneficial by inhibition of Ca^{2+} entry, as well as suppression of Ca^{2+} activity in the cytosol with a high proton concentration.

In contradiction to the above hypothesis, reperfusion after arrest with the acidotic anoxic solution resulted in a large washout of LDH. While this does not seem to be reflected in the recovery of the hearts, the cell membranes may be vulnerable to reperfusion with the alkalotic K-H solution, with Ca^{2+} entry no longer inhibited. The amount of Ca^{2+} entering on reperfusion will, however, be reduced if the membranes are not damaged during the arrest period.

The use of an acalcaemic solution is associated with the Ca^{2+} paradox on reinfusion of a Ca^{2+} containing perfusate (Ruigrok et al., 1983). This effect is inhibited by a low pH. Even though sufficient Ca^{2+} is present in the ST solution to prevent the appearance of this phenomenon (more than 50 µmol/l), the benefit of an acidotic solution is still apparent.

iii) pH, oxygenation and glycolysis

a) Beneficial effects of ST+Glucose gassed with 95%O_2 5%CO_2

The use of a low pH in a highly oxygenated solution proved to be the best combination, with high functional recoveries.

Hendren et al. (1987) also determined the effect of oxygenation and alterations in $\%CO_2$ of an acalcaemic bicarbonate-containing cardioplegic solution using the Langendorff isolated rat heart model. However, this solution was acalcaemic. The solution was used at a temperature of $8^{o}C$ with multidose infusions. The calcium paradox was found upon reperfusion with the 100% O_2 solution where the inclusion of O_2 at a high partial pressure drives the pH upward by blowing off the CO_2. A decreased pH with the acalcaemic solution was found to increase recovery of the hearts, with maximal recovery with a pH of 7.11 (5% CO_2). Using the calcium containing ST+Glucose solution, a similar value for pH was found to be optimal.

LDH washout was reduced as was lactate production and presumably intracellular proton accumulation, although this was not reflected in the effluent pH's. With oxygen present, lactate can be used as a substrate, while glycolysis is also inhibited, both by the presence of oxygen, and an intracellular accumulation of protons (Rovetto et al., 1975). This will reduce washout of lactate. However, buffering and membrane transport of protons also contribute to reducing changes in pH. Intermittent washout may reduce a build up of protons, and slow the development of intracellular acidosis.

Post-ischaemic lactate was relatively high, indicating a fair rate of glycolytic ATP production on reperfusion, although during arrest, gassing with $95\%O_2$ $5\%CO_2$ was lowest of all groups. There was also increased uptake of oxygen with a lower pH, in the presence of a high pO_2. Thus although levels of high energy phosphates 20 min after reperfusion did not show differences between groups corresponding to recovery, the indications are that increased availability of energy substrate during arrest is good, and results in better energy production on reperfusion.

The benefits of maintaining ATP production during ischaemia, and preserving the mechanisms for the production of ATP upon reperfusion, may act together to increase the beneficial effect observed with $95\%O_2$ $5\%CO_2$ in the presence of glucose

with intermittent flushing. While the inclusion of glucose in the ST solution may affect myocardial pH by increasing the glycolytic rate, thereby increasing the gradient for the efflux of protons, and subsequent intracellular Ca^{2+} accumulation, this is inhibited by the use of a cardioplegic solution with a low pH. With oxygen present, the rate of glycolysis will be reduced with a decline in the rate of acidosis, while energy production is also more efficient. Intermittent washout of accumulated protons and other metabolic waste products is also beneficial to the arrested hearts. Oxygenation and multidose delivery, together with hypothermia, are thought also to protect the heart against the calcium paradox.

While the recoveries attained with 95%O_2 5%CO_2 were better than those of the other groups in this series of experiments, when compared with the initial studies using the ST+Glucose solution intermittently (Table 3), no significant difference in recovery was seen between the two groups. Recoveries in both groups were in the region of 88-90%. However, in the initial studies it must be emphasised that the solutions were gassed and did contain oxygen and carbon dioxide. However, the exact value of these variables was not determined. A direct comparison of these two series of results cannot therefore be made.

b) Oxygen uptake

The oxygen tension gradient is the main determinant of the amount of O_2 which can be taken up, as shown by comparison between an aerated and an oxygenated solution (Bodenhamer et al., 1983). The dissociation curve of a crystalloid solution is linear and all O_2 in such a solution can be released (Digerness et al., 1981).

A comparison of oxygenated blood and crystalloid cardioplegia by Digerness et al. (1981) indicates that the amount of oxygen available at 10°C and 20°C is much the same for the two solutions. Blood had an oxygen availability of 3.61 ml O_2/100 ml and 5.08 ml O_2/100 ml respectively for the two temperatures, while the crystalloid cardioplegia cited had

oxygen contents of 4.06 ml O_2/100 ml and 4.00 ml O_2/100 ml respectively. In comparison, the ST+Glucose solution contained 3.07 ml O_2/100 ml (100% O_2), and 3.12 ml O_2/100 ml (95%O_2 5%CO_2) at 10°C.

In the arrested dog heart, oxygen consumption has been measured at a basal rate of 0.3 ml/100 gm/min at 22°C (Buckberg et al., 1977), 0.27 ml/100 gm/min at 15°C and 0.13 ml/100 gm/min at 5°C (Bretschneider et al., 1975). By regression of this curve, the oxygen consumption at 10°C is about 0.19 ml O_2/100 gm/min, or 1.9 µl O_2/gm/min. The metabolic rates of dog and man can be more closely compared than rat and man, and presumably a similar oxygen uptake in the arrested heart exists for the two species. The consumption of the rat heart has been assumed to be twice that of dogs (Ledingham et al., 1988).

Our results indicate that the uptake of oxygen at 10°C from the oxygenated ST+Glucose solution at a pH of 7 is about 40% greater in the rat heart than that in the dog heart. The oxygen consumption of the arrested rat hearts was about 2.52 µl O_2/gm/min (100% O_2) and 2.78 µl O_2/gm/min (95%O_2 5%CO_2) at 10°C (amount taken up from the cardioplegic solution - however, there may also be diffusion from the atmosphere). Oxygen uptake was about 40-45% of that supplied by the cardioplegic solution. Sufficient provision of oxygen in the gassed crystalloid cardioplegic solution therefore exists to satisfy the needs of the arrested human heart. Uptake of O_2 is enhanced at the lower pH and this may be sufficient to prevent the so-called "oxygen debt", especially with the lowered metabolic rate at hypothermia.

The use of an oxygenated crystalloid cardioplegic solution at low temperatures may be more beneficial than blood because of the effect of temperature on the O_2-haemoglobin dissociation curve, where a left shift occurs (Kanter et al., 1981). Release of oxygen from haemoglobin is reduced as the temperature is decreased and the oxygen taken up by the cells must be from that dissolved in solution.

Oxygenation of a crystalloid cardioplegic solution, as opposed to the use of oxygenated blood cardioplegia, is a safe and effective way of delivering oxygen, the supply of which is of benefit to the heart.

The greater O_2 uptake by the 95%O_2 5%CO_2 group is indicative of a higher metabolic rate resulting in increased ATP production by oxidative phosphorylation. The glucose provided in the solution is therefore efficiently metabolised, without excess accumulation of harmful metabolites. The addition of this ATP together with glycolytically derived ATP appears to be beneficial to the arrested hearts.

There was a decline in oxygen uptake over time in the oxygenated groups, which may decrease further if the time of arrest is extended. This may have a crucial role in determining the recovery of the hearts. With a decreased oxygen uptake, glycolysis may then increase, with the harmful effects associated with increased intracellular proton concentration. The pH gradient will be increased, leading to intracellular Ca^{2+} accumulation.

c) Free radicals scavengers in a cardioplegic solution

Gassing the ST+Glucose cardioplegic solution with 100% O_2, while resulting in improved recoveries compared to the alkaline anoxic solution, indicated a negative effect with a high LDH washout during arrest, with reduced recoveries when compared to the aerated ST+Glucose solution (see above). This effect may have been due to the formation of oxygen free radicals whenever the oxygen was reintroduced with intermittent flushing, as the preceding ischaemic period is thought to predispose the tissue to the formation of these molecules. This phenomenon may be combated in several ways, including the use of free radicals scavengers in the cardioplegic solution. Enzymatic free radical scavengers have been added to cardioplegic solutions in order to prevent the accumulation of these molecules during and after arrest (Halliwell, 1989; Jurmann et al., 1988; Gharagozloo et al., 1988; Chambers et al., 1989).

Glucose is also thought to have a direct free radical scavenging activity (Hearse and Humphrey, 1975). Its presence during hypoxia significantly reduces free radical activity on the reintroduction of oxygen (Hess et al., 1983). This may also be due to an increased rate of anaerobic glycolysis, with consumption of the precursors of ATP, thereby inhibiting the predisposition of the myocardium to the formation of free radicals. This indicates the importance of maintaining substrate levels and preventing the susceptibility of the heart to the initiation of the oxygen paradox.

Casale et al. (1984) demonstrated that multidose administrations of a highly oxygenated perfluorocarbon (PFC) cardioplegic solution (pO_2 = 590 mmHg, 17^{o}C) prevented the development of oxygen free radical perfusion injury, as against a PFC solution with only 150 mmHg oxygen. Maintenance of high oxygen in the tissue by the cardioplegic solution was therefore found to be beneficial. The addition of free radical scavengers in the highly oxygenated PFC solution did not increase the recovery of the hearts, although there was an improvement when they were added to the low pO_2 solution. However, the solution used in this study was Fluosol - DA, which has a different ionic composition to the ST No 2 solution. With oxygenation of the ST No 2 solution, free radical activity may have been present with a high pH, but was indeed reduced with a low pH and oxygenation. Increased energy production by oxidative phosphorylation may also play a role in inhibiting free radical formation while the prevention of intracellular Ca^{2+} accumulation by a low pH may also reduce the predisposition of the myocardium to the oxygen paradox. The ST is cheaper and easier to use than the perfluorocarbon solution, and when gassed with 95%O_2 5%CO_2, resulted in very good recoveries. There does not seem to be any need for the use of the PFC solution.

iv) Disadvantages of oxygenation

There are practical disadvantages associated with the addition of oxygen to a solution, especially at a high partial pressure. Gassing of the cold cardioplegic solution at a high

pO_2 leads to the gas coming out of solution at warmer temperatures. Bubbles were seen to form in the intervals between flushes in the tubing leading from the vessel storing the cardioplegic solution at $10^{o}C$, as the tubing was not kept cold. These bubbles can lead to the formation of emboli in the coronary vasculature which predispose to myocardial damage.

This problem may be avoided by gassing the solution at warmer temperatures than that at which it is to be used, although a reduction in oxygen content would result. The temperature of the solution infused into the hearts with a high pO_2 may also be altered.

pH of a solution is determined by the pCO_2 according to a derivation of the Henderson-Hasselbalch equation

$$pH = pK + \log [HCO_3^-]/0.03 \times pCO_2$$

where 0.03 is the Bunsen solubility coefficient of CO_2. The pCO_2 is in turn affected by the degree of oxygenation of a solution (Table 6). The changes in pH with different gassing over the range of temperatures at which the cardioplegic solution may be used were determined. The Rosenthal correction factors for the different solutions were obtained from the slopes of the graphs. The pH of the solution gassed with $95\%O_2$ $5\%CO_2$ was stable over the range of temperatures measured. As temperature decreases, there is an increase in the solubility of CO_2, and therefore an increased content in the solution which is constantly bubbled. The pH is thus reduced by this increase in CO_2 despite the effect of temperature on the dissociation of the protons. There was thus greater stability of pH with a change in temperature. This is a valuable property of the solution, especially considering the changes in temperature which occur in the use of a solution during an operation. This may allow a certain flexibility in the temperature at which the hearts are maintained. A practical advantage of this would be prevention of gaseous emboli.

An alternative would be to reduce the O_2 partial pressure obtained. The uptake of oxygen from the solution by our hearts accounted for only 40%-45% of that provided, although uptake

may be dependent on the gradient of oxygen. In our studies, the pO_2's of the oxygenated solutions kept at 10°C were very high, in the region of 600-650 mmHg. While this was beneficial to the hearts, especially at a low pH, the pO_2's of the ST+Glucose solution open to atmosphere (see above) were lower, ranging from about 150-400 mmHg, but the results were similar to those attained above.

Extrapolation from the work of de Wit et al. (1988) suggests that a partial pressure of at least 450 mmHg is required. The effects of differences in oxygen tension of a cardioplegic solution (mmol/l - Na^+ 130, K^+ 30, Ca^2 0, glucose 0, pH 7.4) were investigated. 5% CO_2 was used together with 30%, 60% and 95% O_2, and the balance made up with nitrogen. The partial pressures of oxygen were 193, 308 and 473 mmHg respectively. The temperature of the hearts was maintained at 20°C, and multiple infusions of the solution were administered over an arrest period of 5 hours. Oxygenation improved the functional recoveries in all cases, but maximal recovery was seen with 95% O_2. The addition of glucose and insulin to the solution with 95% O_2 had no effect on functional recovery. The solution used in this study was possibly inferior to the ST solution, especially as no calcium was included. The temperature used in these studies was higher than that in our experiments, which affects the oxygen content of the solutions. The optimal pO_2 for a cardioplegic solution therefore still remains open to investigation.

The use of an oxygenated solution at a warmer temperature may also help to prevent emboli formation. Coetzee et al. (1986) determined the effect of oxygenation in a cardioplegic solution (mmol/l - Na^+ 130, K^+ 30, HCO_3^- 28, Ca^{2+} 0, pH 7.4) at different temperatures using the working rat heart model. Nonoxygenated (pO_2 117 mmHg) and oxygenated solutions (pO_2 440 mmHg) were compared at 4°C and 20°C. After 2 hours arrest, better preservation of ATP was observed for the oxygenated cardioplegic solution group at the lower temperature, but no differences in functional recovery were observed. At 20°C, oxygenation of the solution was found to increase high energy

phosphate levels and functional recovery with a significant correlation between these variables. Temperature of the cardioplegic solution is therefore important when considering the inclusion of O_2.

v) Conclusions

The provision of oxygen at high partial pressures during multidose cardioplegia is feasible in the face of the above problems, and of significant benefit to the hearts. The question does remain as to whether such a high pO_2 is required, as when comparing this series of experiments with the initial study, the results attained with the ST+G (aerated) and when gassed with $95\%O_2$ $5\%CO_2$ are similar. However, it must be stressed that the former solution did contain oxygen at variable partial pressures, while the pH ranged from 7.4 - 8.0. Thus there is a large degree of variation in the solution used. It was decided to try and clarify the situation by determining the more advantageous range for these variables. This study attempts to give the direction for further research into the exact value of these variables. Emphasis is also placed on the beneficial effect of gassing the solution. This is not often done in the clinical situation, where the solutions used are commercially manufactured and stored in sterile, non-gassed bottles. The advantages of gassing the solution, preferably with $95\%O_2$ $5\%CO_2$, are stressed.

Although these results indicate the beneficial effects of oxygenation with $95\%O_2$ $5\%CO_2$ of the ST+G solution used intermittently during arrest, when considering storage of transplanted hearts, a single dose of cardioplegic solution is supplied, and the heart is then stored hypothermically. Further oxygen is not provided during arrest. Other, more long term, strategies must be investigated to improve the preservation of stored hearts, although results of the above experiments may be applied

The buffering capacity of the ST+Glucose solution was not very high and may perhaps be increased to allow better preservation of hearts with long term storage.

C. Buffering of the ST+Glucose solution with single dose cardioplegia

a) Introduction

The provision of glucose as a substrate in the ST solution used with a single dose protocol was not deleterious. However, an increased glycolytic flux may compound the intracellular acidosis which develops during an ischaemic period (Tsien, 1976; Case et al., 1979; Cobbe and Poole-Wilson, 1980a). While a small reduction in pH may be protective to a degree (Bing et al., 1973; Nayler et al., 1979), extreme acidosis is associated with cellular damage and necrosis. This may counter any positive effect of elevated glucose on energy production during the early arrest period, especially if the duration of arrest is extended. While intermittent flushing removes accumulated metabolites, with long term preservation of donor hearts, this is impractical. An alternative is improved buffering of the protons, either in the cell or in the extracellular medium, which may inhibit the development of extreme acidosis and associated injury to the cells and thereby extend the duration of arrest. Prevention of extreme acidosis may reduce the inhibition of glycolysis, thereby enhancing energy production via this pathway.

The choice of buffer to add to a cardioplegic solution is important. The buffer must be physiological with a high buffering capacity in the critical pH range required for optimal preservation of the arrested myocardium (Bretschneider et al., 1984). Histidine is an important physiological buffer, and has a strong buffering capacity. It has been used by Bretschneider's group in Germany in the formulation of their HTK cardioplegic solution (Bretschneider et al., 1984). At a concentration of 198 mmol/l, this is the major component of their so-called "intracellular" solution, which has a low Na^+ concentration to prevent development of an action potential, and no Ca^{2+} to suppress contraction of the myofibrils (Bretschneider et al., 1975). These reductions in Na^+ and Ca^{2+} also create the osmotic space for the addition of histidine. The rationale for the high buffering capacity is to improve

the metabolic energy gain by preventing inhibition of glycolysis (Bretschneider et al., 1984). The criteria used for determining the effectiveness of the HTK solutions is the duration of arrest until the ATP levels reach a minimum of 4 µmol ATP/g wet weight. This time is extended by the use of a highly buffered solution. As this reflects our aim of extending the time of arrest of donor hearts for transplantation, we wished to test this principle of increased buffering capacity in the isolated working heart model with a single initial dose of cardioplegic solution.

The addition of histidine in a multidose protocol may also be beneficial but with the present model, recoveries of 87% aortic output for multidose ST+Glucose, and 89% when gassed with 95%O_2 5%CO_2 could not really be improved upon. In order to see any significant effects with histidine, we used a single dose protocol, especially as buffering may be a long term strategy. The aim of our study was to improve preservation of donor hearts for long distance procurement. It was therefore felt that the single dose protocol was appropriate.

b) Experimental Procedure

The inclusion of a buffer in significant amounts increases the osmolarity of the cardioplegic solution (Bretschneider et al., 1984). As we wished to add 50 mmol/l histidine, the same amount of sucrose was added to the ST+Glucose solution for a separate control group, to test the effect of an increase in osmolarity on recoveries of hearts after 3 hours arrest at 10°C. In addition, the Na^+ concentration was reduced by 20 mmol/l to increase osmotic space, and tested with the addition of 50 mmol/l sucrose to the solution. This solution would then have a similar osmolarity to the normal ST+Glucose solution. Sucrose increases the osmolarity of the solution without any physiological effects. The use of 100 mmol/l Na^+ has been shown to have no effect on recoveries of the hearts (Jynge, 1980). The compositions of the solutions tested are shown in Table 10, together with the calculated osmolarities.

Histidine was added at two concentrations (50 and 80 mmol/l) to the ST+Glucose solution with a reduced Na^+ concentration, to obtain a dose response curve.

The presence of a strong buffer may alter the optimal pH of the cardioplegic solution. With a protocol of intermittent washout, a more acidotic solution appears to beneficial, as intra- and extracellular pH can be maintained at a fairly critical level. However, without intermittent flushing, a higher pH of the cardioplegic solution may be more beneficial, as a lower pH will precipitate the development of extreme acidosis. The pH of the histidine-containing solution was altered by removing the bicarbonate, inducing metabolic acidosis.

The Bretschneider's HTK solution was compared to the histidine-containing ST+Glucose solution in the present model, in order to determine the relative efficacies of these two highly buffered solutions.

Table 10: Alterations to the St Thomas' Hospital cardioplegic solution No. 2 with 11mmol/l glucose (ST+G) tested in a protocol of a single initial dose of cardioplegic solution, with hearts stored for 3 hours at 10°C. Changes in calculated osmolarity (Osm), and sodium, histidine (Hist) and bicarbonate concentrations.

		ST+G	Decr Na^+	Incr Osm	Hist 50	Hist 80	Hist 50, No HCO_3^-
Na^+	(mmol/l)	120	100	120	100	100	100
K^+	(mmol/l)	16	16	16	16	16	16
HCO_3^-	(mmol/l)	10	10	10	10	10	-
Mg^{2+}	(mmol/l)	16	16	16	16	16	16
Ca^{2+}	(mmol/l)	1.2	1.2	1.2	1.2	1.2	1.2
Cl^-	(mmol/l)	160.4	140.4	160.4	140.4	140.4	150.4
Sucrose	(mmol/l)	-	50	50	-	-	-
Hist	(mmol/l)	-	-	-	50	80	50
Osm	(mOsm/l)	334.6	344.6	384.6	344.6	374.6	344.6
pH	(10°C)	7.4	7.6	7.6	7.3	7.3	7.05
pO_2	(10°C)	198			208		238

c) Results

i) Effect of increased osmolarity and decreased Na^+ concentration of the ST+Glucose solution used with a single dose

Pre-ischaemic control values were compared between groups, and no significant differences were found. The functional recoveries obtained by adding 50 mmol/l sucrose to the ST+Glucose solution showed no differences from the standard solution, whereas decreasing the Na^+ concentration with osmolarity maintained had a slightly negative effect on overall recovery, although of no significance. Percentage recoveries for these groups were in the range of 55-65% (Table 11). There was a significant difference in cardiac output between the increased osmolarity and the decreased sodium group (75.9 ± 3.7 % vs 67.1 ± 2.0 % respectively, $p<0.05$). Coronary flows on reperfusion were slightly greater in the increased osmolarity group (14.5 ± 0.9 ml/min) compared to the control group (12.8 ± 1.3 ml/min) (Table 12). This effect may have been due to lower prearrest values in the increased osmolarity group, although these were not significant. The results may not indicate any real difference between the groups.

LDH washout on reperfusion after use of the increased osmolarity ST+Glucose cardioplegic solution was significantly increased compared to that with the standard solution, with 103.6 ± 7.1 mU/gm/min compared to 66.7 ± 7.5 mU/gm/min ($p<0.002$) (Table 12). This effect was not seen with the decreased Na^+ group. Lactate production was highest in the former group as well (Table 12). These factors suggest that a higher osmolarity and a high Na^+ have side effects which negate the slightly higher recoveries obtained. ATP's were significantly reduced compared to control levels ($p<0.02$). CP's were also lower than control levels, but not significantly so (Table 13).

It was felt that enough osmotic space was made available by the removal of 20 mmol/l Na^+ without compromising the

recoveries of the hearts. This was therefore used as the control solution.

Table 11: Means ± sem of functional measurements of hearts taken after 10 minutes work before (Pre-arrest) and after (Post-arrest) arrest, with percentages of functional recoveries. Comparison of hearts arrested with St Thomas' Hospital cardioplegic solution No. 2 with 11mmol/l glucose (ST+G), and with alterations in sodium concentration and osmolarity. The arrested hearts were stored for 3 hours at 10°C.

		Aortic output (ml/min)	Cardiac output (ml/min)	Stroke volume (ml)
ST+G (n=9)	Pre-arrest	49.1 ± 3.1	68.3 ± 4.1	0.26 ± 0.01
	Post-arrest	30.0 ± 2.2	47.9 ± 2.8	0.18 ± 0.01
	% Recovery	61.0 ± 2.1 %	70.5 ± 2.2 %	68.9 ± 3.8 %
Decr Na^+ (n=7)	Pre-arrest	53.3 ± 3.4	70.9 ± 3.9	0.25 ± 0.01
	Post-arrest	30.1 ± 1.7	47.1 ± 2.2	0.17 ± 0.01
	% Recovery	57.2 ± 2.4 %	67.1 ± 2.0 %	69.7 ± 6.6 %
Incr Osm (n=4)	Pre-arrest	45.5 ± 3.4	62.7 ± 3.6	0.27 ± 0.01
	Post-arrest	30.5 ± 4.9	48.0 ± 4.6	0.19 ± 0.02
	% Recovery	65.5 ± 5.8 %	75.9 ± 3.7 % *	71.3 ± 6.0 %

* $p<0.05$ vs Decr Na^+

Table 12: Means ± sem of coronary flow (CF), and average washout of lactate dehydrogenase (LDH) and lactate during 10 minutes reperfusion in the Langendorff mode (Post-arrest). Comparison of hearts arrested with St Thomas' Hospital cardioplegic solution No. 2 with 11mmol/l glucose (ST+G), and with alterations in sodium concentration and osmolarity. The arrested hearts were stored for 3 hours at 10°C.

	CF (ml/min)	Post-arrest LDH (mU/gm/min)	Post-arrest Lactate (μmol/gm/min)
ST+G (n=9)	12.8 ± 1.3	66.7 ± 7.5	1.7 ± 0.5
Decr Na^+ (n=7)	12.8 ± 0.7	79.1 ± 12.3	1.5 ± 0.4
Incr Osm (n=4)	14.5 ± 0.9	103.6 ± 7.1 *	2.2 ± 0.7

* $p<0.002$ vs ST+G

Table 13: Means ± sem of tissue levels of adenosine triphosphate (ATP) and creatine phosphate (CP) of control hearts clamped after 10 minutes work before arrest, and of arrested hearts after 20 minutes reperfusion. Comparison of hearts arrested with St Thomas' Hospital cardioplegic solution No. 2 with 11mmol/l glucose (ST+G), and with alterations in sodium concentration and osmolarity. The arrested hearts were stored for 3 hours at 10°C.

	ATP (μmol/gm)	CP (μmol/gm)
Control (n=6)	3.9 ± 0.3	5.1 ± 0.5
ST+G (n=9)	2.3 ± 0.1 *	4.2 ± 0.2
Incr Osm (n=4)	2.5 ± 0.1 *	3.9 ± 0.6

* $p<0.02$ vs control

ii) The addition of histidine to the ST+Glucose solution

Histidine was added at a concentration of 50 mmol/l to the ST+Glucose solution with a reduced Na^+ concentration. This led to a marked improvement of recovery after three hours single dose arrest compared to both the normal ST+Glucose solution and that with altered osmolarity and Na^+ levels, with percentage recoveries of aortic output of 76.1 ± 3.4 % ($p<0.05$) (Table 14).

Measurement of the coronary flows on reperfusion showed no change with the presence of 50 mmol/l histidine compared to both control groups (Table 15). LDH washout on reperfusion was reduced with the addition of 50 mmol/l histidine to the ST+Glucose solution, at 49.5 ± 3.9 mU/gm/ml (Table 15), compared to the control solutions.

Lactate efflux on reperfusion was slightly increased in the presence of histidine (Table 15). However, ATP and CP levels were no different from those with the other cardioplegic solutions (Table 16). The level of ATP was significantly lower than in pre-ischaemic heart tissue.

A higher concentration of histidine (80 mmol/l) was tested to see if functional recoveries could be improved upon, but the opposite effect was seen (Table 14). A depressed recovery of function of only 54.7 ± 3.3 % for aortic output resulted, even lower than control values, especially compared to the increased osmolarity solution (65.5 ± 5.8 %) (Table 11), which had a similar osmolarity. The 80 mmol/l histidine solution resulted in a 2.5 fold increase in LDH output against the 50 mmol/l histidine solution (129.1 ± 23.5 mU/gm/ml) ($p<0.05$) (Table 15), and a decline in lactate output of 50% (Table 15). Coronary flow was, however, slightly increased versus the 50 mmol/l histidine group (15.4 ± 0.8 ml/min versus 13.7 ± 0.4 ml/min) (Table 15). Tissue ATP and CP levels showed no differences between the two groups although ATP was significantly lower than control values in both groups (Table 16).

The more beneficial concentration of histidine in the ST+Glucose cardioplegic solution was found to be around 50 mmol/l.

Table 14: Means ± sem of functional measurements of hearts taken after 10 minutes work before (Pre-arrest) and after (Post-arrest) arrest, with percentages of functional recoveries. Comparison of hearts arrested with St Thomas' Hospital cardioplegic solution No. 2 with 11mmol/l glucose with a decrease in sodium concentration with the addition of 50mmol/l (Hist 50) or 80mmol/l histidine (Hist 80), and the addition of 50mmol/l histidine with the removal of HCO_3^- (Hist 50, No HCO_3^-). The arrested hearts were stored for 3 hours at 10°C.

		Aortic output (ml/min)	Cardiac output (ml/min)	Stroke volume (ml)
Hist 50 (n=8)	Pre-arrest	46.5 ± 3.1	62.5 ± 3.4	0.26 ± 0.01
	Post-arrest	35.0 ± 2.2	50.5 ± 2.2	0.21 ± 0.01
	% Recovery	76.1 ± 3.4 % &$	82.0 ± 4.2 % &$	79.3 ± 3.2 % &
Hist 80 (n=7)	Pre-arrest	44.9 ± 1.9	66.3 ± 1.9	0.25 ± 0.01
	Post-arrest	24.6 ± 1.9	45.1 ± 1.3	0.16 ± 0.01
	% Recovery	54.7 ± 3.3 % *	68.2 ± 1.6 % *	65.3 ± 3.4 % *
Hist 50 No HCO_3^- (n=6)	Pre-arrest	53.2 ± 3.0	76.7 ± 2.2	0.25 ± 0.01
	Post-arrest	28.5 ± 3.3	48.2 ± 3.6	0.15 ± 0.01
	% Recovery	52.7 ± 4.0 % *	62.3 ± 3.2 % *	60.2 ± 2.9 % *

& $p<0.05$ vs ST+G $ $p<0.01$ vs Decr Na^+ * $p<0.01$ vs Hist 50

Table 15: Means ± sem of coronary flow (CF), and average washout of lactate dehydrogenase (LDH) and lactate during 10 minutes reperfusion in the Langendorff mode (Post-arrest). Comparison of hearts arrested with St Thomas' Hospital cardioplegic solution No. 2 with 11mmol/l glucose with a decrease in sodium concentration with the addition of 50mmol/l (Hist 50) or 80mmol/l histidine (Hist 80), and the addition of 50mmol/l histidine with the removal of HCO_3^- (Hist 50, No HCO_3^-). The arrested hearts were stored for 3 hours at 10°C.

	CF (ml/min)	Post-arrest LDH (mU/gm/min)	Post-arrest Lactate (μmol/gm/min)
Hist 50 (n=8)	13.7 ± 0.4	49.5 ± 3.9	2.0 ± 0.4
Hist 80 (n=7)	15.4 ± 0.8 &	129.1 ± 23.5 #	1.1 ± 0.4
Hist 50 No HCO_3^- (n=6)	16.9 ± 0.7 #*&	97.1 ± 22.8	1.0 ± 0.5

& $p<0.02$ vs Decr Na^+
# $p<0.05$ vs Hist 50
$ $p<0.03$ vs Hist 80
* $p<0.04$ vs ST+G

Table 16: Means ± sem of tissue levels of adenosine triphosphate (ATP) and creatine phosphate (CP) of control hearts clamped after 10 minutes work before arrest, and of arrested hearts after 20 minutes reperfusion. Comparison of hearts arrested with St Thomas' Hospital cardioplegic solution No. 2 with 11mmol/l glucose with a decrease in sodium concentration with the addition of 50mmol/l (Hist 50) or 80mmol/l histidine (Hist 80), and the addition of 50mmol/l histidine with the removal of HCO_3^- (Hist 50, No HCO_3^-). The arrested hearts were stored for 3 hours at 10°C.

	ATP (µmol/gm)		CP (µmol/gm)
Control (n=6)	3.9 ± 0.3		5.1 ± 0.5
Hist 50 (n=8)	2.3 ± 0.2	*	3.5 ± 0.5
Hist 80 (n=7)	2.3 ± 0.1	*	4.4 ± 0.3
Hist 50 No HCO_3^- (n=6)	2.7 ± 0.2	*	4.2 ± 0.1

* $p<0.02$ vs control

iii) Removal of HCO_3^- from the histidine-containing ST+Glucose solution

The effect of a lower pH in the presence of histidine was tested by removing the HCO_3^- from the solution, which leads to a metabolic acidosis. The pH of the ST+Glucose solution with histidine but lacking HCO_3^-, gassed with 95%O_2 5%CO_2 on preparation, was 7.05 ± 0.02, compared to the pH of the 50 mmol/l histidine solution, of 7.3 ± 0.02, and the ST+Glucose control solution, of 7.4 ± 0.1 (Table 10).

The recovery of the hearts with this solution was seen to decrease to 52.7 ± 4.0 % for aortic output ($p<0.01$ versus 50 mmol/l histidine group) (Table 14). The least lactate was produced with the 0 mmol/l HCO_3^- group, and a relatively high LDH efflux was present (97.1 ± 22.8 mU/gm/min) (Table 15). This corresponded to a high coronary flow which was significantly greater than those of the 50 mmol/l histidine and the increased osmolarity, standard Na^+ groups (16.9 ± 0.7 ml/min) (Table 15).

ATP and CP levels were not significantly different against the other groups or against control values (Table 16). The ATP was the highest of all values attained in the above experiments, but was correlated with the lowest recovery, as were the relatively high CP levels.

iv) Properties of the histidine-containing ST+Glucose solution

a) pH and temperature curves

The change in pH with a reduction in temperature of the 50 mmol/l histidine solution was parallel to that of blood, with Rosenthal correction factors of -0.0143 and -0.0147 respectively (Figure 15).

b) Titration curves and buffering capacities

Titration curves for the standard ST+Glucose solution, and the ST+Glucose solutions with 50 mmol/l histidine, 80 mmol/l histidine or 50 mmol/l histidine, 0 mmol/l HCO_3^- were determined, and the derivatives for these curves calculated. These are shown in Figures 16 and 17. The maximum buffering capacity with 80 mmol/l histidine was found to be -50 mmol HCl/l/pH, and -30 mmol HCl/l/pH with 50 mmol/l histidine and 50 mmol/l histidine/No HCO_3^- at pH's from 6.1 to 6.3. At pH 7, the buffering capacity of the 50 mmol/l histidine containing solution was still relatively high, at -10 mmol HCl/l/pH, while that of the ST+Glucose solution was about -4 mmol HCl/l/pH. The histidine-containing solution was therefore a much better buffer at physiological ranges of pH.

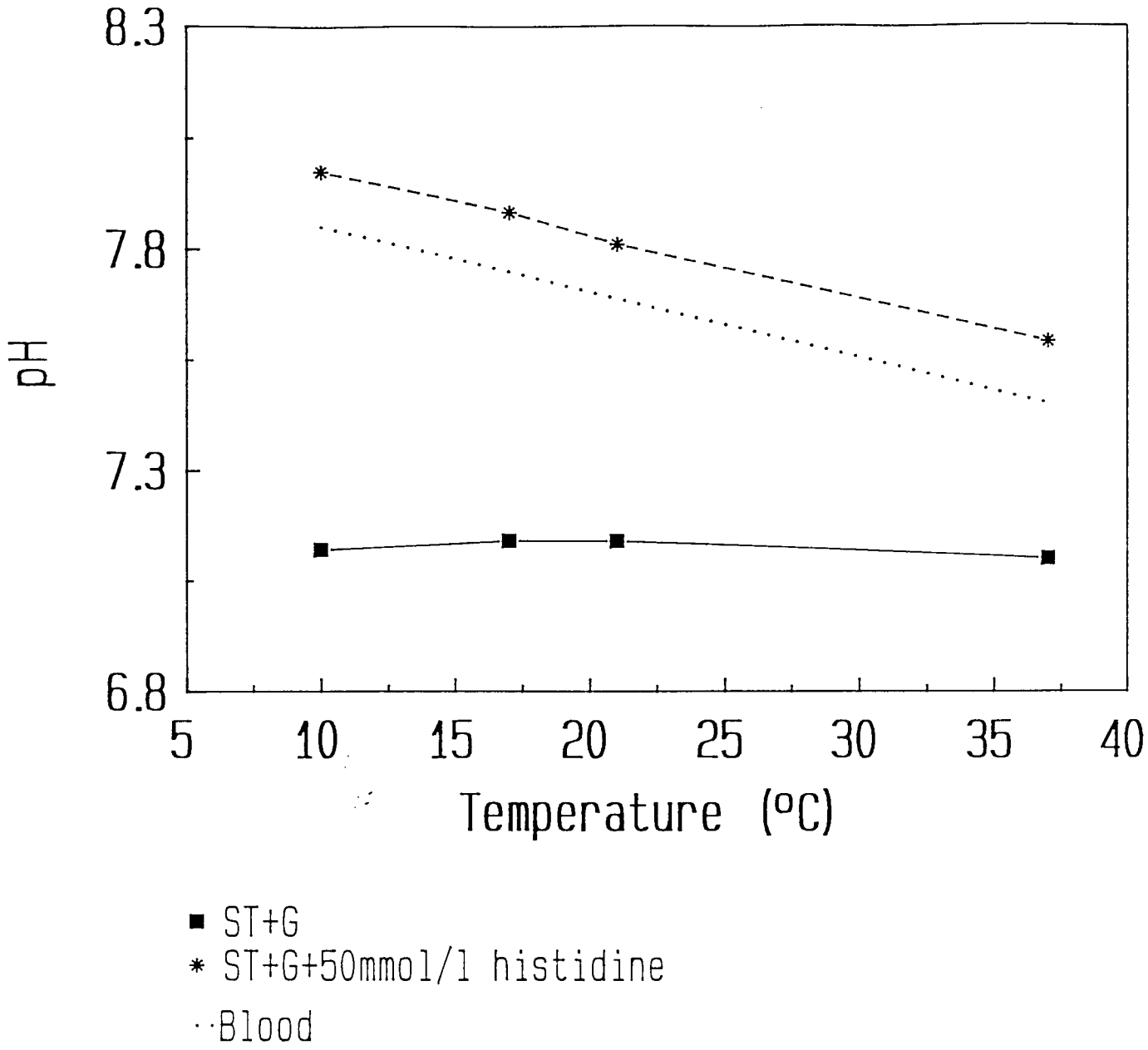

Fig. 15. Change in pH with temperature of the St Thomas' Hospital cardioplegic solution No. 2 with 11 mmol/l glucose (ST+G), and with the addition of 50 mmol/l histidine, compared to blood. The solutions were continually gassed with 95%O_2 5%CO_2.
Slopes of the curves give the Rosenthal correction factors.

ST+G	-0.0220
ST+G+50 mmol/l Histidine	-0.0143
Blood	-0.0147

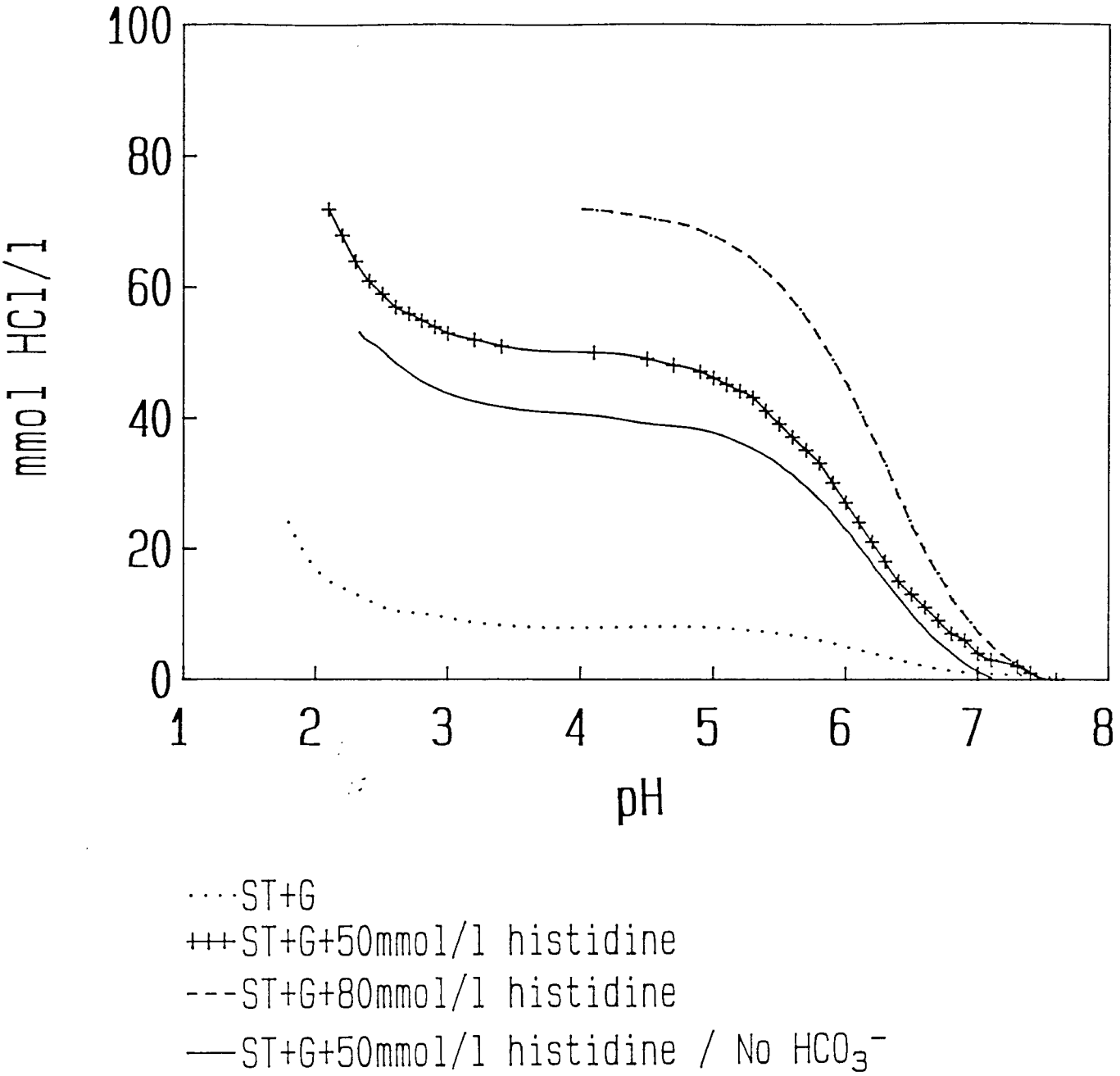

Fig. 16. Titration curves of the St Thomas' Hospital cardioplegic solution No. 2 with 11 mmol/l glucose (ST+G), and with the addition of 50 mmol/l or 80 mmol/l histidine, and the addition of 50 mmol/l histidine in the absence of HCO_3^-. The solutions were continually gassed with 95%O_2 5%CO_2 at room temperature. The inflection points give the pK's for the solutions.

ST+G	6.2
ST+G+50 mmol/l Histidine	6.1
ST+G+80 mmol/l Histidine	6.3
ST+G+50 mmol/l Histidine/No HCO_3^-	6.2

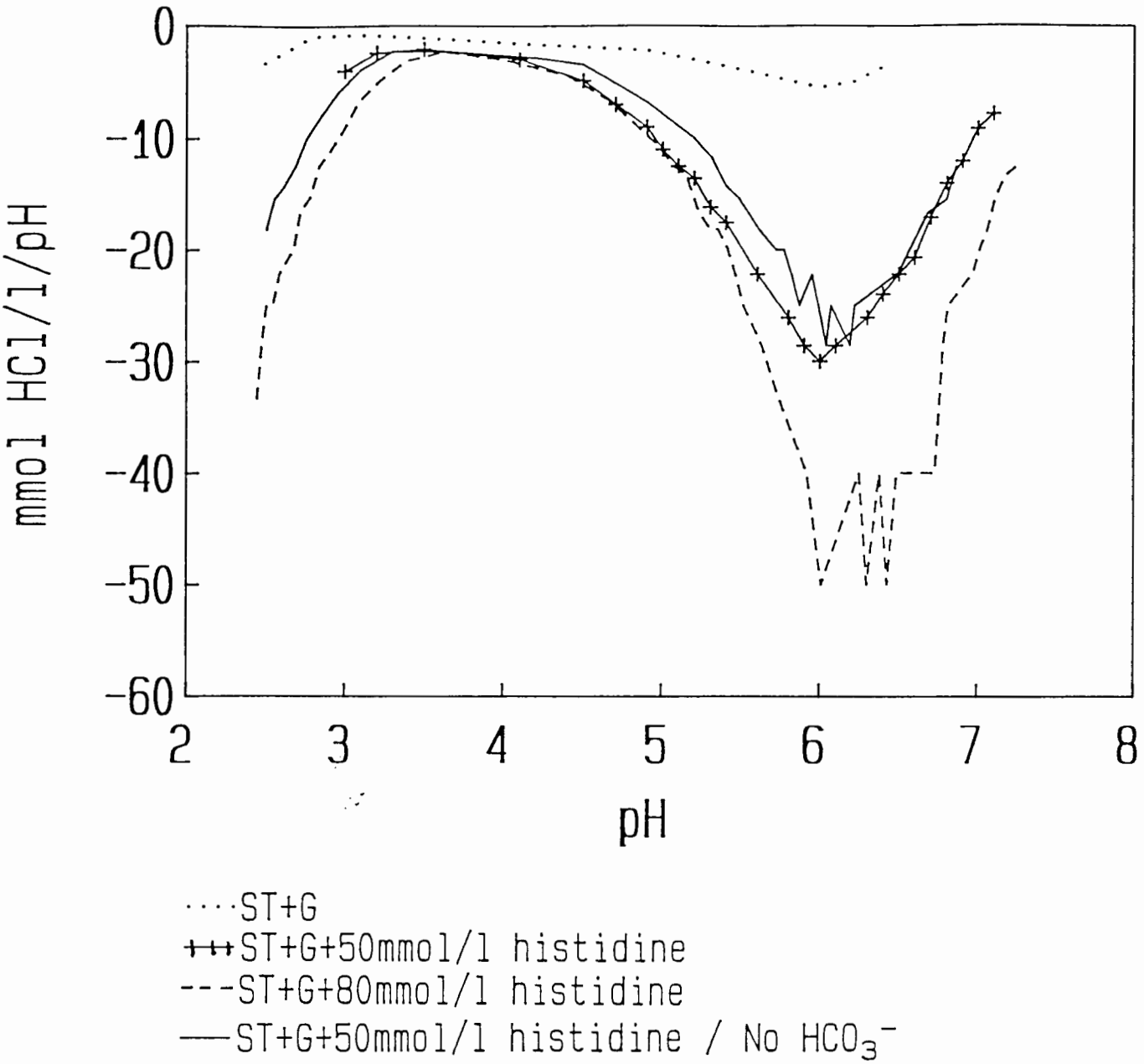

Fig. 17. Buffering capacities of the St Thomas' Hospital cardioplegic solution No. 2 with 11 mmol/l glucose (ST+G) gassed with 95%O_2 5%CO_2, and with the addition of 50 mmol/l and 80 mmol/l histidine, and the addition of 50 mmol/l histidine in the absence of HCO_3^-. The solutions were continually gassed with 95%O_2 5%CO_2 at room temperature. Buffering capacity is expressed as the instantaneous (derivative) slope of the titration curves (Fig. 16).

v) Bretschneider's HTK cardioplegic solution versus the ST+Glucose and the ST+Glucose+histidine solution

Bretschneider's HTK cardioplegic solution was used to arrest the hearts, which were then stored for 3 hrs at 10°C. The composition of this solution is shown in Table 17. Functional recoveries were comparable to those with the ST+Glucose solution (55.2 ± 3.4 % aortic output) (Table 18). However, LDH efflux was greater than the standard ST+Glucose solution (110.6 ± 15.6 mU/gm/min) ($p<0.02$) (Table 19). Lactate output was similar in the two groups (Table 19). The ST+Glucose solution was slightly better than Bretschneider's solution in the isolated working rat heart model. This may be because of species differences, where Bretschneider and colleagues have used the in vivo dog heart model, although it is suggested that the Bretschneider's solution is not as good as the ST+Glucose solution, as its composition follows that of an "intracellular" solution.

When compared to the 50 mmol/l histidine ST+Glucose solution, the recoveries using Bretschneider's solution were found to be significantly lower ($p<0.05$). Lactate output was lower than with 50 mmol/l histidine, while LDH washout was significantly higher ($p<0.001$). Thus with an increased buffering capacity, ST+Glucose+50 mmol/l histidine was markedly better than Bretschneider's HTK solution.

Table 17: Composition of St Thomas' Hospital cardioplegic solution No. 2 with 11mmol/l glucose and 50mmol/l histidine (ST+G+histidine) and Bretschneider's HTK solution (in mmol/l) (Osmolarity - calculated).

	ST+G+Histidine	HTK
Na^+	100	15
K^+	16	10
Mg^{2+}	16	4
Ca^{2+}	1.2	-
Cl^-	140.4	50
HCO_3^-	10	-
Glucose	11	-
Histidine	50	198
Mannitol	-	30
Tryptophan	-	2
2-oxyglutarate	-	1
pH (37°C)	7.8	7.4
(10°C)	7.2	7.0
Osm (mOsm/l)	344.6	314

Table 18: Means ± sem of functional measurements of hearts taken after 10 minutes work before (Pre-arrest) and after (Post-arrest) arrest, with percentages of functional recoveries. Hearts were arrested with Bretschneider's HTK solution and stored for 3 hours at 10°C.

		Aortic output (ml/min)	Cardiac output (ml/min)	Stroke volume (ml)
HTK (n=7)	Pre-arrest	51.1 ± 2.6	70.8 ± 2.6	0.25 ± 0.01
	Post-arrest	28.1 ± 1.9	49.0 ± 1.4	0.16 ± 0.01
	% Recovery	55.2 ± 3.4 % *	69.8 ± 3.4 % *	66.0 ± 3.1 % *

* $p<0.05$ vs Hist 50

Table 19: Means ± sem of coronary flow (CF), and average washout of lactate dehydrogenase (LDH) and lactate during 10 minutes reperfusion in the Langendorff mode (Post-arrest), and of tissue levels of adenosine triphosphate (ATP) and creatine phosphate (CP) of hearts after 20 minutes reperfusion. Hearts were arrested with Bretschneider's HTK solution and stored for 3 hour at 10°C.

	CF (ml/min)	Post-arrest LDH (mU/gm/ml)	Post-arrest Lactate (µmol/gm/min)	ATP (µmol/gm)	CP (µmol/gm)
HTK (n=7)	10.6 ± 1.9	110.6 ± 15.6 $&	0.9 ± 0.5	1.8 ± 0.03 *	4.6 ± 0.2

$ $p<0.02$ vs ST+G
& $p<0.001$ vs Hist 50 Decr Na^+
* $p<0.03$ vs control

vi) Titration curve and buffering capacity of Bretschneider's solution

The titration curve (Figure 18) and buffering capacity (Figure 19) for the Bretschneider's solution was compared to the ST+Glucose with 50 mmol/l histidine solution. Because of the high histidine concentration in the Bretschneider's solution, the buffering capacity is very large, -40 - -110 mmol HCl/pH.

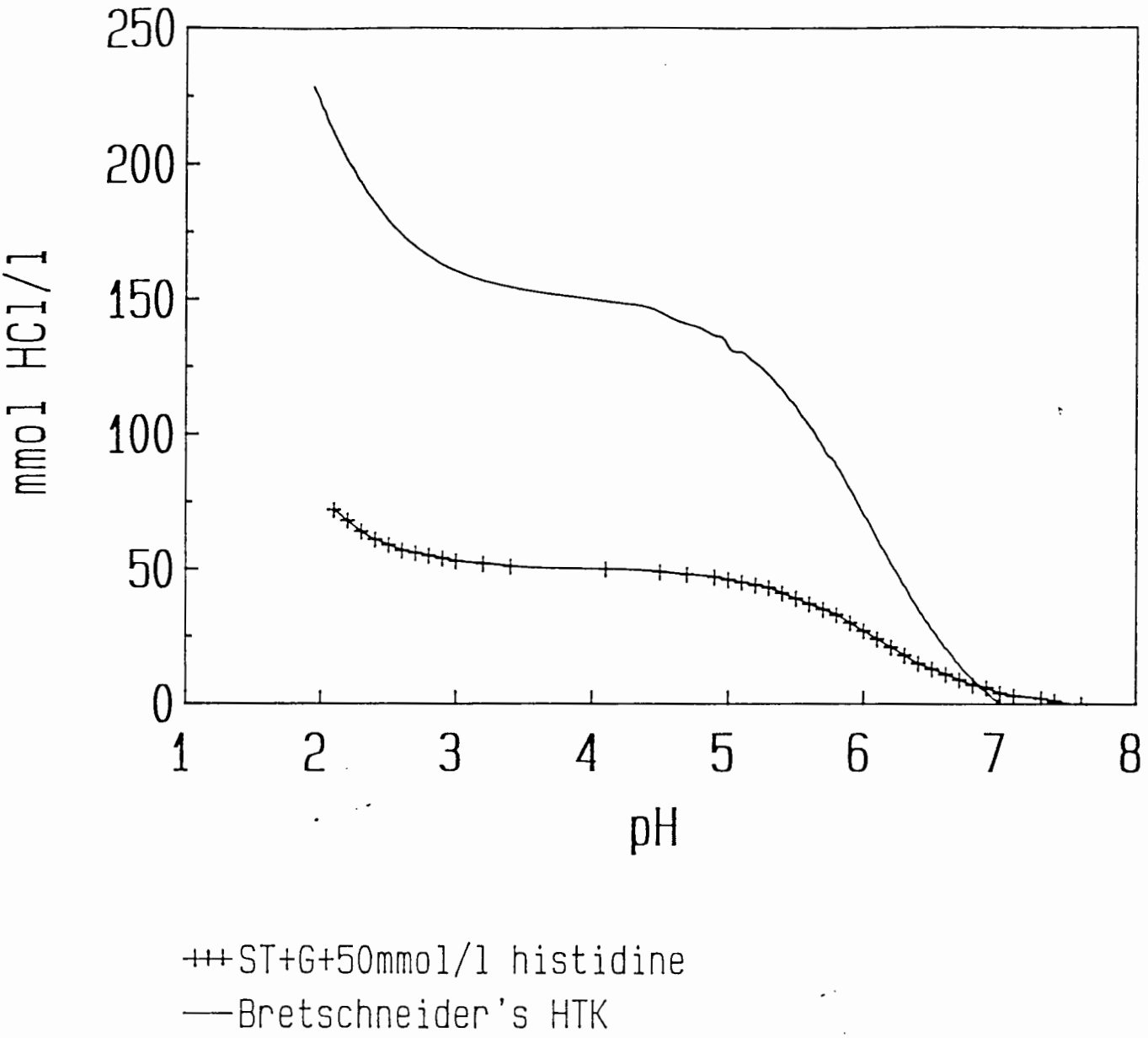

Fig. 18. Titration curves of the St Thomas' Hospital cardioplegic solution No. 2 with 11 mmol/l glucose and 50 mmol/l histidine, and Bretschneider's HTK solution. The solutions were continually gassed with 95%O_2 5%CO_2 at room temperature. The inflection points of the curves give the pK's for the solutions.

ST+G+50 mmol/l Histidine	6.1
Bretschneider's HTK	6.1

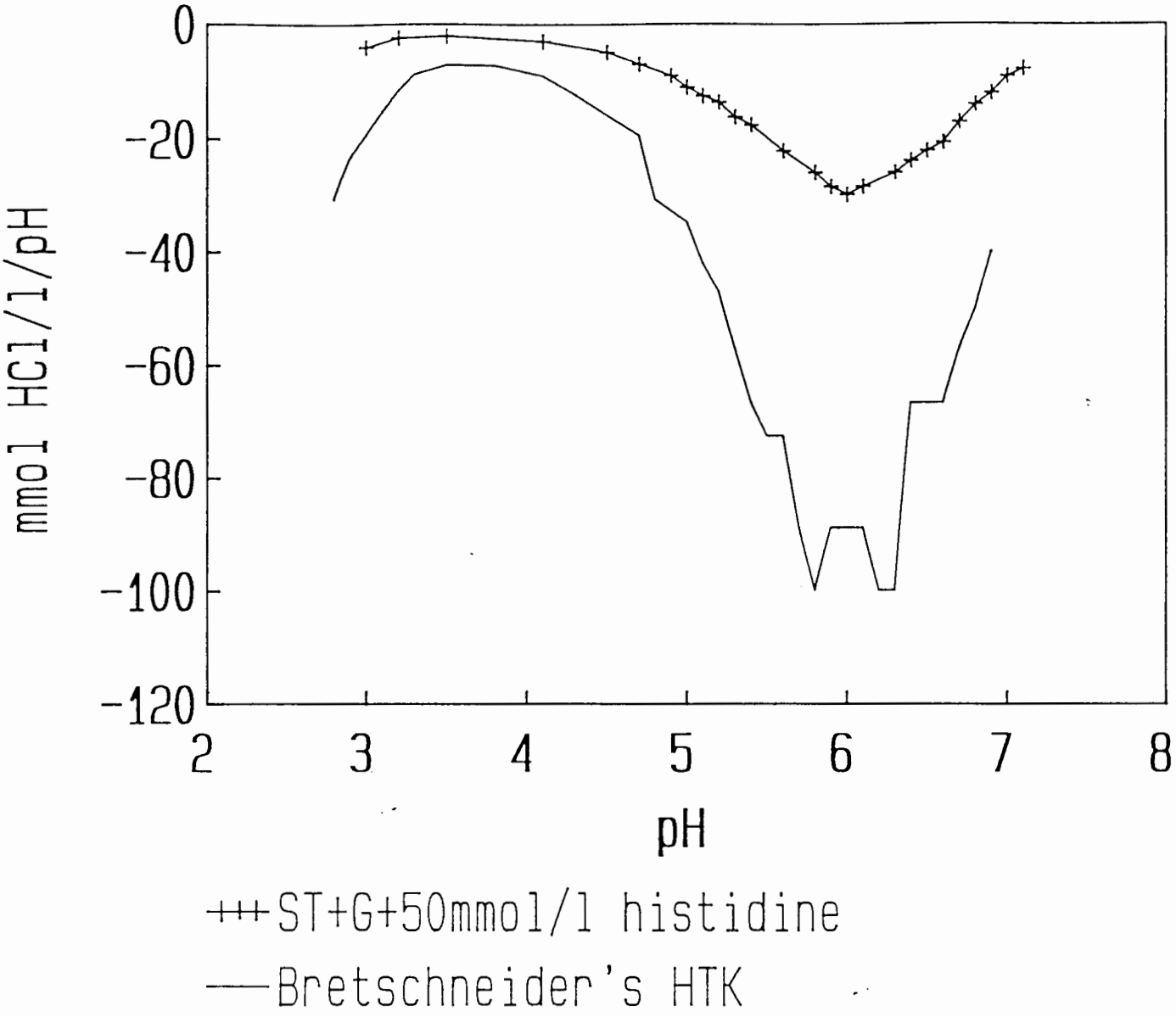

Fig. 19. Buffering capacities of the St Thomas' Hospital cardioplegic solution No. 2 with 11 mmol/l glucose and 50 mmol/l histidine, and Bretschneider's HTK solution. Buffering capacity is expressed as the instantaneous (derivative) slope of the titration curves (Fig. 18).

d) Discussion

i) Histidine

Histidine is an amino acid with an imidazole side chain group which confers strong buffering properties at physiological pH's. The structure of histidine is shown in Figure 20. As the temperature decreases, it is the imidazole moiety which is physiologically significant as it acts as a buffer with characteristics of a shift to alkalinity with hypothermia (Reeves et al., 1976). Although histidine is an amphoteric molecule, as are other amino acids, it is the pK of the imidazole moiety which is closest to physiologically important ranges of pH. The pK of this group changes with temperature in almost the same way as pK_w (the dissociation constant of water), allowing histidine to retain its buffering capacity at all temperatures (Swan, 1984).

Histidine is found in great abundance in haemoglobin where it is responsible for the transmission of structural changes in the heme group on the binding of oxygen (Metzler, 1977). Histidine residues are thought to be responsible for the Bohr effect, where the presence of H^+ and CO_2 promote the release of O_2 from the haemoglobin molecule. Histidine is also present in other blood proteins, where, as the principal titratable group, its buffering capacity is expressed. The total concentration in blood is about 30 mmol/l (Swan, 1984).

Histidine in muscle is usually present as a dipeptide carnosine (ß-alanyl-L-histidine), or the bulk are in peptide linkage in proteins. Total buffering by histidine in striated muscle has been calculated at 80% of the total capacity (Abe et al., 1985).

ii) Myocardial buffering

During ischaemia, the cytosol becomes acidic because of increased glycolysis and inhibition of oxidative phosphorylation (Rovetto et al., 1975). The cell has various mechanisms to reduce the intracellular acid load. The primary mechanisms include intracellular buffering, which has been estimated to be about 50-70 mmol HCl/l/pH for the rat

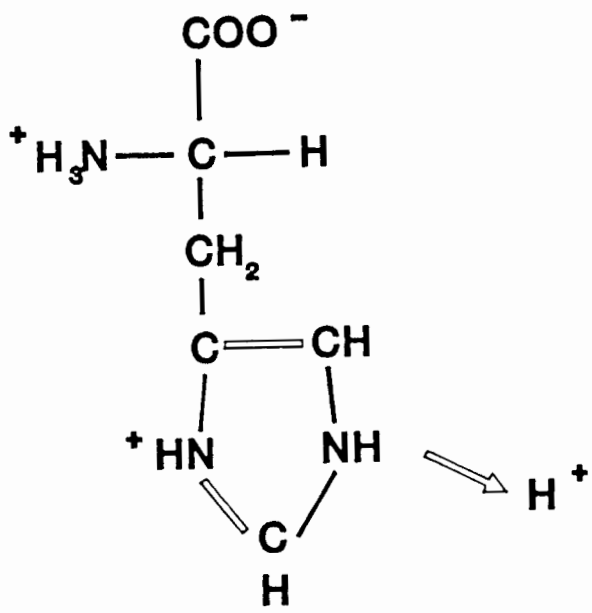

Fig. 20. Structure of histidine showing imidazole group with the basic site which can accept a proton to form a conjugate acid. The histidine residue is found in the active sites of many enzymes because of this R group.

ventricle cells (Roos and Boron, 1981). Intracellular hydrogen ions are buffered by phosphates, mitochondria, the sarcoplasmic reticulum, and the histidine residues of the proteins (Poole-Wilson, 1989). However, these mechanisms are not always sufficient to deal with the excess protons produced. These protons can be extruded together with lactate, or in exchange for sodium, thought to be a major mechanism of pH regulation (Vaughan-Jones, 1988).

Correct buffering is important in maintaining Donnan ratios across the cell membrane as well as cell volume (Rink, 1984), and may determine the degree of oedema on reperfusion of the arrested hearts (White, 1981). The presence of large intracellular impermeant anions leads to a greater intracellular buffering capacity compared to the extracellular space (Roos and Boron, 1981; Rink, 1984). This increase the intracellular pH as calculated from the Donnan equilibrium, from pH_i of 5.7 to approximately 7.0, thereby reducing the pH gradient, with pH_o of 7.4 (Roos and Boron, 1981). A large increase in intracellular acidosis affects the valency of these large impermeant solutes, which in turn affects the Cl^- and K^+ fluxes across the cell membrane (Rink, 1984). If Cl^- enters the cell down its concentration gradient following K^+ loss, especially in ischaemia, this will draw in both Na^+ and water, with cellular swelling. Cl^- is also exchanged for HCO_3^- across the membrane, which may increase intracellular acidosis (Vaughan-Jones, 1988).

iii) Buffering during cardioplegic arrest

The addition of 50 mmol/l histidine to the ST+Glucose solution was advantageous to the hearts arrested with a single dose of cardioplegic solution. The recoveries of the hearts after three hours arrest at 10°C were significantly increased, while LDH washout on reperfusion was reduced and lactate efflux was relatively higher.

The addition of a buffer to a cardioplegic solution has been suggested to prevent a decline in pH (Vander Woude et al, 1985), with reduced inhibition of glycolysis (Bretschneider,

1980) and other enzymatic pathways, as enzymes are highly dependent on pH for their optimal activity. Preservation of myocardial ATP stores has been found (del Nido et al, 1985) with increased metabolic efficiency and greater energy production per mole of substrate utilised.

Choice of a buffer to add to a cardioplegic solution rests on the characteristics of that buffer. The optimal buffering power, which is usually at its pK, must be within the physiological range of pH, and preferably close to that considered ideal for cardioplegic arrest. A lower pH than would normally be present in the extracellular solution has been found to be beneficial (Bernard et al., 1985), and a buffer with a pK in this range would be best. The pK's and changes in pH with a change in temperature for various physiological buffers are shown in Table 20.

Kresh et al. (1987) determined the relative buffering powers of bicarbonate and tromethamine-based (THAM) crystalloid cardioplegic solutions, and a histidine-buffered solution (Bretschneider's HTK - 198 mmol/l histidine). The values obtained are shown in Table 21. The buffering power of the histidine-containing buffer was far greater than the bicarbonate or THAM cardioplegia. Thus protein buffers may be more beneficial than the bicarbonate buffer system, especially as the buffering power of homogenised myocardial tissue at -40 mmol HCl/l/pH is closer to that of histidine and maximal at similar pH's (Kresh et al., 1987). Histidine is therefore a more physiological buffer.

Table 20: Characteristics of physiological buffers compared to blood and water - pK's and coefficient of change in pH with change in temperature (Rosenthal correction factors).

	pK ($25^{o}C$)	$\delta pH/\delta^{o}C$
Histidine		
- $\alpha COOH$	1.82	
- αNH^{+}	9.17	
- Imidazole	6.0	
- total	6.7	-0.022
Bicarbonate	6.35	-0.005- -0.010
Phosphate	6.9	-0.003
Blood ($37^{o}C$)	7.4	-0.0147
Water		-0.017

Table 21: Range of buffering capacities at $27^{o}C$ of cardioplegic solutions with different buffers at physiological pH's (pH 6-8) (maximum buffering capacity is at the pK for the buffer - see table 20). The buffering capacity of a myocardial homogenate and ventricular cells is shown.

Buffer	Concentration (mmol/l)	Buffering capacity (mmol HCl/l/pH)	Reference
Bicarbonate	27	-2 - -15	a
Tromethamine	27	-1 - -15	a
Histidine	161	-25 - -60	a
Blood		-30	a
Myocardial homogenate		-40	a
Intracellular		-50 - -70	b

a Kresh et al., 1987
b Roos and Boron, 1981

The buffer added to the cardioplegic solution may act intra- or extracellularly, depending on its ability to be taken into the cells. The addition of phosphate buffer in the extracellular medium has been shown to increase proton flux by up to fourfold (Roos and Boron, 1981). Vanheel et al. (1985) showed that buffering of the superfusate of an isolated papillary muscle preparation prevents intracellular acidosis by reduction of surface acidification. With a buffer in the extracellular medium, protons will be extruded together with lactate, resulting in a greater efflux of lactate with a higher buffering capacity. With 50 mmol/l histidine in the ST+Glucose solution, lactate efflux on reperfusion was increased compared to the control groups, possibly by this mechanism. If the buffer enters the cells, intracellular buffering will affect intracellular pH more directly, and inhibit proton flux across the membrane. This may reduce subsequent intracellular Na^+ overload, which has been linked to Ca^{2+} influx on reperfusion. However, the two mechanisms of the action of a buffer are contradictory. Whether a buffer is taken up into the cells or not may determine the mechanism of its action. Histidine has been shown to be transported into the hepatocyte via a Na^+-dependent (System N) and a Na^+-independent (System L) carrier mechanism (Kilberg et al., 1980; Christensen, 1984; Leonardi et al., 1988). This may also occur in the myocyte, although the rate of uptake is possibly reduced because of differences between liver and heart metabolism. This rate will also be substantially reduced by a decrease in temperature and inhibition of metabolism and a decrease in the Na^+ gradient. With time there will be increased intracellular proton accumulation, but the rate of histidine entry into the cell, while slow, may be enough to combat the development of an extreme acidosis, and the possible build up of a proton gradient. A mild intracellular acidosis would appear to be beneficial, but not too extreme (see above). A slower rate of entry of histidine into the cell, instead of a rapid influx, will also inhibit the development of cellular oedema by the osmotic movement of water. These long term effects may be especially beneficial

when there is no intermittent washout of accumulating metabolites.

iv) Bretschneider's solution versus ST+G+histidine

A comparison of multidose usage of a crystalloid bicarbonate containing cardioplegic solution, blood cardioplegia, and Bretschneider's solution buffered with histidine, was made (Tait et al., 1982). The greater buffering capacity of histidine was advantageous in increasing the glycolytic rate and preventing a drop in pH. A greater washout of lactate with the Bretschneider's solution was found. The use of a crystalloid cardioplegic solution with low buffering capacity was found to be unable to prevent progressive tissue acidosis to about 6.6 after 3 hrs arrest, while Bretschneider's solution and blood cardioplegia prevented a reduction in tissue pH below 7.2. With the higher tissue pH maintained at about 7.2, glycolysis is not greatly inhibited, while increased extracellular buffering will also increase the flux of lactate and protons across the membrane by maintaining the pH gradient (Vaughan-Jones, 1988).

Several disadvantages of the Bretschneider's solution have been observed. Bretschneider's solution proved the least effective in inducing and maintaining electromechanical arrest (Tait et al., 1982) while the lack of calcium is thought to predispose to the Ca^{2+} paradox (Ruigrok et al., 1983). While a lower temperature is thought to inhibit the appearance of the Ca^{2+} paradox when using this solution (Bretschneider et al., 1984), the addition of 50 µmol/l Ca^{2+} to Bretschneider's solution was found to improve recovery (Ruigrok et al., 1983), possibly by depressing excess Ca^{2+} influx on reperfusion. Although the use of a low temperature may inhibit the occurrence of this phenomenon, it does limit the use of the solution.

In comparison to the ST+G solution, Bretschneider's formulation was as effective in the isolated rat heart model. However, when the ST+G solution was additionally buffered with histidine, the latter was far more efficacious. Intracellular

solutions do not appear to work well in the rat heart (unpublished observations), and the difference in animal models used, between this and other studies, may account for the differing observations. However it is believed that the extracellular formulation of the ST solution is better than the intracellular formulations, as there is less manipulation of the ionic gradients and less perturbation to the myocardium.

v) Concentration of histidine in the ST+Glucose cardioplegic solution

A higher histidine concentration of 80 mmol/l in the ST+Glucose cardioplegic solution was found to depress recoveries to below those found with the standard ST+Glucose solution. This may have been due to an osmotic effect, although this was tested for with the solution containing 50 mmol/l sucrose and 120 mmol/l Na^+, which had a slightly higher osmolarity, but better recoveries. These results are unexplained as the Bretschneider's solution uses a very high histidine concentration of 198 mmol/l. Del Nido et al. (1985) also found a beneficial effect of 195 mmol/l histidine in a low Na^+, high glucose (83 mmol/l) solution with a relatively low K^+ (10 mmol/l) with a high pH of 7.8 at 27°C. HCO_3^- was not included. Haemodynamic and histopathological assessments of recovery indicated reduced necrosis with improved contractile recovery, and better preservation of high energy phosphate stores with buffering. However, with a high K^+ (30 mmol/l) and buffering, recoveries were reduced compared to those of the unbuffered solutions, either with 10 mmol/l or 30 mmol/l K^+. The explanation for these findings was that with the high pH of the solution, a depolarisation of the resting membrane potential with a high K^+ and the absence of Ca^{2+} led to the Ca^{2+} paradox phenomenon. The interaction of the various ionic components of the cardioplegic solution was emphasised, and may explain the variability in results with differing extracellular buffering capacities.

Bretschneider's solution and that used by Del Nido et al. (1985) both have a low Na^+ concentration and no Ca^{2+}, compared

to the high Na^+, Ca^{2+} containing ST+Glucose solution. Histidine transport into the cell is partially dependent on the Na^+ gradient, and its entry may be inhibited with a low extracellular Na^+. This may prevent excess influx of a large molecule into the cytosol with osmotic effects. The benefits of increased extracellular and intracellular buffering with a higher histidine concentration of 80 mmol/l may be outweighed by these effects. The results obtained with similar osmolarities with the sucrose containing solution may be because sucrose does not enter the cells, and therefore will not increase cellular oedema. However, this is contradicted by the results attained on reperfusion of the hearts, where coronary flow was significantly increased with the higher histidine concentration. There was also a significant increase in LDH efflux in the ST+Glucose+80 mmol/l histidine group compared to the 50 mmol/l histidine group. The sucrose solution with standard Na^+ concentration also showed a higher LDH efflux and coronary flow compared to the ST+Glucose solution. An increased extracellular osmolarity will draw out fluid from the cells, increasing coronary flow because of shrinking of the cells. However, an increased intracellular oedema may also lead to an increased coronary flow on reperfusion because of breakage of cell membranes, especially of the endothelial cells. This was seen with an increased efflux of LDH in the 80 mmol/l histidine group, and may have led to increased coronary flow on reperfusion, and an overall reduction in function.

vi) Bicarbonate buffer system versus histidine

Vander Woude et al. (1985) examined the effect of imidazole (25 mmol/l) (pK 6.7 at 37°C) in an acalcaemic cardioplegic solution with a high Na^+ and high K^+. This was compared to a HCO_3^- containing solution (25 mmol/l). The benefits of imidazole buffering were observed in increased functional recovery, and a higher pH of the coronary effluents with intermittent flushing. Vander Woude et al. (1985) wanted to exclude the effects of changes in pCO_2 on the bicarbonate buffer system. Imidazole buffering is not dependent on the

pCO_2 of the solution. The level of CO_2 in an ischaemic heart changes with time, altering the bicarbonate equilibrium, leading to acidosis. With longterm arrest, CO_2 will be affected which will alter the buffering effect of the bicarbonate system. In addition, the pK of the bicarbonate system is 6.35 at 25°C (see Table 20) which is below that of optimal intracellular pH during arrest. These results indicate that HCO_3^- in a cardioplegic solution is not necessary in the presence of a stronger buffer.

However, in our results, an absence of HCO_3^- in the histidine containing ST+Glucose solution reduced recoveries, stressing the importance of this buffer. While buffering capacity is increased with histidine, the presence of HCO_3^- may be required to prevent acidosis by accumulating CO_2. Oxygen is present in the cardioplegic solution at a pO_2 of about 150 mmHg (atmospheric pO_2) as it is open to atmosphere. There is also uptake of oxygen from the atmosphere by the heart suspended during arrest. This oxygen will be metabolised with the production of CO_2.

vii) pH and histidine

Bernard et al. (1985) examined the effect of different buffers in a crystalloid cardioplegic solution (mmol/l Na^+ 100, K^+ 4, Ca^{2+} 0.25, Mg^{2+} 13). Glutamate (20 mmol/l), Tris (47.5 mmol/l), bicarbonate (20 mmol/l) or histidine (64.4 mmol/l) were added. The solutions were titrated to pH's of 7.00 and 7.4 at 20°C. In control hearts, treated only with hypothermia (15°C), a drop in pH_i was found with time of arrest (2 hrs) from 7.1 to 6.13. With a pH of 7.4, bicarbonate in the cardioplegic solution was found to cause an increase in pH_i over time to 7.32 with a multidose protocol. Histidine prevented a change in pH_i from control values, while resulting in fastest recovery of function on reperfusion. No effect on high energy phosphate preservation was seen, with all hearts showing a decrease.

However, with the solutions titrated to pH 7.00, histidine caused a severe depletion in high energy phosphates at the end

of arrest, with a poorer recovery in aortic flow compared to the other buffers. Thus a higher pH for the histidine-containing solution is better.

This was indirectly corroborated in the present study, as a decreased pH with 50 mmol/l histidine with the induction of metabolic acidosis by the removal of HCO_3^- reduced functional recovery. The pH of the 50 mmol/l histidine solution was higher than that found to be optimal for multidose usage.

With intermittent flushing of the heart, harmful metabolites are removed, and a decreased pH was advantageous to the myocardium (see above). The advantage of a relatively low pH would be to decrease enzyme activity and metabolic energy expenditure. However, if the metabolites are not removed, acidosis may increase to the extent that it becomes injurious. If the initial pH of the extracellular space is high, this may slow down the decline in pH by providing a larger gradient for proton efflux, while the increased buffering capacity with the addition of histidine also inhibits the development of acidosis (Tait et al., 1982; Bernard et al., 1985).

The effects of histidine on glycolysis were observed in the reasonably high levels of lactate washout on reperfusion, indicating that inhibition of glycolysis by the accumulation of protons is decreased. Energy is possibly conserved with the inclusion of histidine as the pH is maintained at mildly acidotic levels.

viii) Histidine as an oxygen quencher

Protection of the membranes appears to be a major effect of histidine in preservation of the arrested hearts, as LDH loss on reperfusion was lowest with 50 mmol/l histidine in the ST+Glucose solution. Histidine is a specific quencher of singlet oxygen and may thereby limit oxygen-induced cell damage on reperfusion (Gaudel and Duvelleroy, 1984). Singlet oxygen fragments the lipid hydroperoxides and reacts with unsaturated lipids of biomembranes, which may impair transmembrane ion movements. It is also associated with protein damage (Prinsze et al., 1990) leading to inactivation

of enzymes, increased susceptibility to proteolytic degradation, and modification of amino acid residues. Singlet oxygen is a prominent feature of the toxicity of molecular oxygen. The preservative effect of histidine is greater at the onset of reoxygenation than 10 min after, as it is suggested that a burst of free radical production occurs on reintroduction of O_2 (Bolli et al., 1989).

ix) Conclusions

The single dose protocol in the isolated working rat heart model is comparable to long term preservation of hearts for transplantation. When using the ST+Glucose+50 mmol/l histidine solution, recoveries of about 76% aortic output after three hours arrest were obtained, compared to 61% with the standard ST+Glucose solution and 57% with ST. With longer periods of arrest, the inclusion of histidine may be of even more importance, as this may be a long term strategy. The inclusion of histidine in a multipurpose solution for use in both cardio-pulmonary bypass and donor heart storage must be investigated, although the recoveries attained with gassing the ST+Glucose solution with $95\%O_2$ $5\%CO_2$ cannot really be improved upon.

V. CONCLUSIONS

i) Clinical recommendations

The aim of the study was to improve functional recovery of hearts after a period of arrest using a crystalloid cardioplegic solution. The results must be therefore be seen in the light of the clinical implications. The solutions devised on the basis of improved energy conservation were shown to lead to better functional recovery of the arrested hearts. Two possible alternatives were tested, and found to be applicable to different clinical usages.

It is suggested that
a) 11 mmol/l glucose is added to the ST No 2 solution
b) when used with intermittent flushing, the solution is gassed with 95%O_2 5%CO_2
c) the addition of histidine to the glucose-containing solution should be considered, especially with long term storage.

The provision of 11 mmol/l glucose in the ST No 2 cardioplegic solution resulted in significantly improved recoveries of arrested hearts when this effect was enhanced by intermittent removal of harmful metabolites. The presence of oxygen was found to be important at a low extracellular pH. However, with long term storage, a more stable mechanism for the inhibition of proton and lactate accumulation was found to be advantageous, with the addition of histidine as a buffer.

The approach of increased energy production would seem to be valid on the basis of the above results, especially when this is optimised by use of various strategies to enhance energy conservation and production. This was the primary rationale behind the alterations to the ST solution that were made although other advantages were also seen such as preservation of the membranes. Further alterations to the solution based on this approach may lead to better long-term preservation of hearts for transplantation. However, direct clinical extrapolation from these studies is inadvisable for several

reasons, including species differences, and limitations in the technique used.

In extrapolating from these results, the variables used must be considered. The solutions were tested under specific conditions, and it is only under these circumstances that the use of these solutions can be fully recommended. Variables including temperature of the myocardium, and of the cardioplegic solution, duration of arrest, intervals between flushes, gas solutions used, and the extent of gassing must be maintained.

ii) Suggested strategies for the improvement of the ST solution

The results obtained from oxygenation of the ST+Glucose cardioplegic solution, with a relatively low pH, and used in a multidose protocol of administration are possibly as good as can be obtained in the present model. This solution is simple to make and to administer with few disadvantages. However, additional changes to the solution for use with intermittent flushing cannot really be tested in our model, because of the high percentage recoveries attained.

In the model of single dose administration of cardioplegic solution to the isolated rat heart, the presence of histidine was found to be advantageous. However, recoveries were still significantly depressed compared to pre-ischaemic control values. There may be additional advantages conferred, for example, by the addition of agents including additional substrates (Bernard et al., 1985; Rousou et al., 1986; Robinson et al., 1987), free radical scavengers (Gharagozloo et al., 1988; Jurmann et al., 1988; Chambers et al., 1989), channel blocking agents, oxygen carrying vehicles (Kanter et al., 1981; Novick et al., 1985; Rousou et al., 1986; Tabayashi et al., 1988), and those which increase colloid and oncotic pressure (London et al., 1989; Marelli et al., 1989). A review of the literature listing the various possible interventions tested is given by McGoon (1985).

Consideration of a different model for testing the various alterations to the solution suggested here must also be made.

iii) Choice of model - advantages and precautions

a) Species differences

The choice of species used to test a cardioplegic solution has been debated (Bretschneider, 1980; Galiñanes and Hearse, 1990). While dogs are recommended by Bretschneider (1980), these animals are expensive and cannot be used in a series of experiments requiring large numbers of animals. The use of the rat model offers several advantages including availability of animals, simple experimental procedures without the need for sterile clinical conditions, reproducibility of experiments, and use of an isolated organ perfusion system with direct measurement of indices of function. For these reasons, in our experiments requiring large numbers of animals, we used the isolated rat model to test alterations to our solutions. These studies give an indication of the efficacy of the various formulations. However, there are differences between the rat and other animal species which must be taken into account. Because of these and other factors, the solutions must be tested in animal models which more closely resemble the human myocardium, with conditions similar to those in the operating theatre before extrapolating results to the clinical situation.

1) Myocardial structure

The rat myocardium is different in many respects from that of the human. Fundamental differences include metabolic rate, contractile performance, enzymatic functioning, and electrophysiological characteristics (Bretschneider, 1980; Galiñanes and Hearse, 1990). The rat has a high metabolic rate inversely correlated to body weight, and a consequently higher heart rate. This is reflected in more rapid enzymatic activities especially of the oxidative phosphorylation pathways in the myocardium, (Blank et al., 1989). The rat has a characteristic short ventricular action potential with an attenuated plateau, a high intracellular Na^+ content, and an excitation-contraction coupling mechanism unusually dependent on Ca^{2+} (Galiñanes and Hearse, 1990).

The susceptibility to a period of ischaemia is also different between species. The effectiveness of the different cardioplegic solutions may differ within a species, as well as between species. These differences were observed by Galiñanes and Hearse (1990) who looked at the effect of cardioplegic arrest using the ST No 2 cardioplegic solution, on the recovery of hearts from different species of small animals, including the rat, guinea pig, rabbit and others. The guinea pig was found to be most susceptible to a period of ischaemia, while the rabbit was the least. The rat was intermediate between these extremes. Correlation of functional recovery with metabolite content was also species dependent. The use of a species which is vulnerable to ischaemia with a margin for improvement of recovery allows a safety factor when extrapolating to human patients.

2) Size of heart

Adequate distribution of the cardioplegic solution may to a large extent determine its efficacy. The ratio of perfusion to heart weight of the rat heart is 3 to 4 times that of the larger hearts, related to differences in metabolic rate (Bretschneider, 1980). The volume of cardioplegic solution flushed through the heart depends on the size of the heart. However, in larger hearts, there may be regions which are underperfused with each flush, which may then become necrotic over an extended period of arrest.

The rat heart has a large surface to volume ratio, with smaller distances for diffusion, allowing considerably greater uptake of oxygen from the atmosphere in comparison to larger hearts. These differences may affect the relative recoveries of different species with different cardioplegic solutions.

The size of the rat hearts also prevents the taking of biopsy samples for biochemical evaluations during and after the arrest period.

b) Isolated organ perfusions versus in vivo models

1) Isolated heart perfusions

Use of an isolated organ is associated with problems not found in vivo. When removing the heart, it may be damaged in some way. The heart is also not perfused for a short period before being cannulated. Factors including ischaemic preconditioning, usage of endogenous glycogen stores and areas of regional ischaemia may all affect heart function. It is difficult to control for these factors, and this results in a large variation in the function of the isolated perfused hearts. While exclusion criteria are used to discard hearts which do not initially perform within a normal range and each heart acts as its own control in the expression of the results, there is still a possible rundown in function over time in the isolated working heart, which may differ depending on initial function. While reperfused hearts are also discarded using exclusion criteria, there is still a large degree of variation, which may mask what is actually happening. The use of a model which more nearly replicates that of the clinical situation is recommended, such as in vivo recording of heart function.

2) External factors

Conditions in the laboratory, and in an isolated organ, can be much more easily controlled than in the theatre. The temperature of the myocardium can be kept much more constant, without interference from non-coronary collateral flow, and the difficulty in thermally isolating the in situ heart from the rest of the body. Temperature regulation in a donor heart is also difficult as hearts are normally immersed in a container of solution, which is then surrounded by ice. The temperature of the myocardium may fall to levels causing ice crystal formation, and subsequent injury to the cells.

3) State of the heart

The hearts used in the experiments are from young, healthy animals. In cardio-pulmonary bypass, the hearts are in a

diseased or weakened state with different biochemical function. This may alter the effect of the solution.

4) Perfusion - blood versus Krebs-Henseleit buffer

Another variable which must be considered in the isolated organ model is the perfusate used. A crystalloid solution does not contain elements of blood such as neutrophils which may affect the degree of reperfusion injury of the hearts, as these are associated with a large free radical activity (Reimer et al., 1989). There are other components in the blood which also affect performance. In the isolated heart, the coronary flow is much higher than in vivo because of the absence of haemoglobin in the crystalloid solution. The oxygen content of the solution is less than that of oxygenated blood because of the lower oxygen carrying capacity. This results in a higher coronary flow in the isolated heart, and possibly reduces oxygen delivery to the myocardial cells.

With a protein-free saline solution, water escapes from the vascular bed over time, leading to interstitial oedema. With an experiment of long duration, this may alter heart function significantly over time, leading to a bias in the functional recoveries of arrested hearts. Whole blood prevents oedema developing and decreases the interstitial space.

Whole blood is difficult to use in an isolated perfusion system because of foaming, erythrocyte aggregations, clotting, protein precipitation, haemolysis and precipitation of erythrocyte ghosts. If blood is to be used, a model using a donor animal for perfusion of the heart, or an in vivo model, both with larger animals, is more advisable.

5) Duration of experiments

The time period allowed for recovery of the isolated organ is of necessity very short, in terms of minutes and hours, as there is rundown in function over time. The long term effects of heart preservation and subsequent recovery, in terms of days, cannot be adequately assessed. Post operative survival cannot be investigated. While the initial recoveries on reperfusion may be an indication of the efficacy of a

solution, the phenomenon of stunning is also present. While stunning may be regarded as deleterious to the patient, the heart may require a certain time period to recover from the ischaemic insult. The period of stunning may therefore be a protective mechanism, allowing conservation of energy in the initial reperfusion period. The clinical implications of stunning remain to be determined. The rate of recovery after cardioplegic arrest may differ with different solutions, but the absolute recovery may also differ.

c) Determinants of heart function

The aim of cardioplegia is to slow the rate of development of ischaemic injury. The extent of the injury can be assessed by measurement of contractile function, enzyme leakage, tissue metabolites, ultrastructure and electrophysiology. These show the directionality of injury, sometimes with a good correlation with recovery.

However, the functional indices and biochemical parameters used to determine recovery of heart function may also affect interpretation of results. The best possible index of function must be chosen to allow as accurate a reflection of recovery on reperfusion as possible to be made. This must be correlated with what is required in the clinical situation, where a good pressure may be needed although not at the expense of increased energy expenditure. Galiñanes and Hearse (1990) in their study of the effects of ischaemia emphasised the use of the optimal index of function and metabolic recovery. Factors such as the bias in cardiac output due to the exaggerated coronary flow in the isolated organ perfused with a crystalloid solution as compared to sanguinous perfusion must be taken into account.

The results of this study correspond with our findings. Although there was some correlation of functional recovery with metabolic indices, the latter were not always indicative of the degree of recovery. Other indices of contractile heart function, as well as measurement of other biochemical variables, may give a more precise indication of the efficacy of the solution, and allow better extrapolation to the human

heart. The correlation of release of LDH during and after arrest is particularly indicative of a lack of correlation with recovery. Different factors led to differences in the amount of LDH washout, with little correspondence with the degree of recovery. These factors are discussed in the relevant sections of the results and discussions. As an absolute indicator of possible recovery, LDH could not be used. However, this index is a useful determinant of where injury may be occurring, and whether these effects have a large role to play in the recoveries of the hearts.

d) Improvement of the model

There are several models which have been used to test cardioplegic solutions. The isolated perfused rat heart model has many advantages including ease of procedure and enabling a large number of experiments to be done. There are several possible ways in which the model can be improved and its use extended:

1) Measurement of biochemical parameters including tissue ATP, CP and lactate could be made immediately after arrest, before reperfusion. This is a more sensitive indication of maintenance of high energy phosphates during arrest, and possibly of the degree of preservation engendered by the cardioplegic solution.

2) More extensive biochemical studies can be made on the myocardial tissue to determine the effects of the cardioplegic solutions on various processes in the myocytes. This would give a more complete picture of the changes which occur.

3) Electron micrograph studies can be made to determine changes to the myocardial structure with the cardioplegic solution.

4) The use of diseased or compromised hearts may also affect the formulation of an optimal cardioplegic solution, as these hearts may undergo various alterations in their biochemical processes which alter their response to the solutions. Hearts from older animals, hypertensive or diabetic rats, and models with coronary ligation and various other interventions, can be

studied. These hearts may not recover as well as the normal healthy hearts, allowing room for more exploration of the effects of a cardioplegic solution.

5) Reperfusion solutions can also be looked at with this model. Reperfusion injury is implicated as one of the major causes of damage associated with ischaemia and reperfusion. This is an important alternative to improving on the formulation of cardioplegic solutions as the best protection possible during arrest cannot necessarily prevent reperfusion injury. This may be especially important with the more long term preservation of donor hearts for transplantation.

6) The use of blood as the perfusate before and after arrest may improve results as this alters the functioning of the hearts. However, this necessitates the use of a far more complicated model and the use of a rat which has a small heart is not necessarily possible. The use of larger animals is thus more advisable if blood is to be used.

Larger animal models:

1) The use of larger animals with correspondingly larger hearts facilitates experiments where more complex procedures are to be performed. This enables a model which more closely approximates the clinical situation to be used. An animal with a similar vasculature and general myocardial structure to that of the human is desirable. Such models including the baboon, dog, and pig have been used extensively in this field of research. However, the use of a slightly smaller animal such as the rabbit may also be used to obtain adequate answers. The use of this animal allows perfusion with blood, and in vivo model.

2) The use of blood as the perfusate reduces many of the factors which are brought about by the use of an asanguinous perfusate. Blood may be used with a para-corporeal perfusion, where the heart is removed from the body but is still perfused by the animal. This allows good measurement of the functional parameters of the heart. An in vivo model with blood perfusion

is also good but the functional measurements obtained are not as accurate.

3) An in vivo model allows more long term experiments to be performed, with recovery of the animal after arrest and reperfusion. The long term effects of the solution can then be studied.

Other approaches which may be used include the use of cultures of the various cell populations, and investigation into the effects of the different solutions on the survival of these cells. The electrophysiological changes elicited can be studied using single cells. Measurement of changes in resting membrane potential and subsequent Ca^{2+} fluxes across the sarcolemma can be made.

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