MYOCARDIAL PROTECTION

DURING

CARDIAC SURGERY

PhD THESIS

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PROLOGUE

ORGANIZATION OF THE THESIS

This thesis deals with myocardial protection during open-heart surgery. The chapters of this thesis are based on experimental work published in eight papers, as well as additional unpublished data. These studies are discussed in chapters 1 - 7. However, the more detailed experimental protocols and results for the different experiments are presented in Appendix A (published data), and Appendix B (unpublished data) in order to not interrupt the flow of the text.

Chapter 1 provides an introductory overview of both the history and clinical practice of myocardial protection today. Although the experimental studies presented in this thesis primarily concern the effect of changes in the composition of crystalloid cardioplegic solutions, a number of other factors can effect the adequacy of myocardial preservation in the clinical situation. It is thus essential that cardiac surgeons be aware of all the variables that can alter myocardial recovery following cardiac surgery. Experimental studies comparing single or multiple doses of cardioplegia administration (Appendix A-2) are also discussed in this section.
The importance of a complete overall strategy of "myocardial protection" is emphasized.

Chapter 2 discusses the models used in this thesis to evaluate cardioplegic solutions, and their limitations. The first model used was the isolated working rat heart. This was used to evaluate the addition of glucose to the St Thomas' Hospital No 2 cardioplegic solution, in either a single dose or multiple dose protocol. The results of which are presented in appendix A-2. This model was also used to study the effects of oxygenating a modified St Thomas' cardioplegic solution containing glucose (11 mmol/L) with either 95 % O_2 5 % CO_2 or alternatively 100 % O_2, and the effects of including perfluorocarbons (appendices A-3 and A-4). Finally, it was used to evaluate the effect of altering the concentration of sodium and osmolality (appendix B-3), chloride

(appendix B-4), pH (appendix B-2), the addition of the buffer histidine (appendix B-5), and colloid (appendix B-7) to a modified St Thomas' cardioplegic solution.
The in vivo primate model, was used to evaluate the efficacy of cardioplegic solutions used in South Africa (appendix A-1). This model was also used to evaluate the effect of glucose (appendix B-1) and histidine (appendix B-6 & B-9) when included in the St Thomas' cardioplegic solution.
A third model was developed to enable us to study the effects of cardioplegic solutions on the endothelium. The results of these studies are presented in appendices A-5, and A-6.

Chapter 3 reviews the composition of cardioplegic solutions used to provide myocardial protection during cardiac surgery. The three primary types of cardioplegic solutions; crystalloid - either extracellular or intracellular electrolyte equivalent formulations, and blood cardioplegic solutions are discussed. In addition, solutions used to preserve the isolated heart for transplantation are also discussed.

Chapter 4 reviews techniques and cadioplegic solutions used to protect the myocardium during clinical open-heart surgery in South Africa. Cardioplegic solutions used in South Africa in 1988 were compared with the St Thomas' Hospital No 2 cardioplegic solution (appendix A-1). The results of a second survey in 1991, of techniques and cardioplegic solutions used for myocardial protection currently, are also presented.

Chapter 5 discusses the results of experimental modifications to the composition of the St Thomas' Hospital No 2 cardioplegic solution (appendices A-2 to A-4, B-1 to B-7, B-9), done in both the isolated rat and in vivo primate models.

Chapter 6 is divided into two parts. The first section reviews the importance of the vascular endothelium to the cardiovascular surgeon. Thereafter, the results of experimental studies on the effects of cardioplegic solutions on the endothelium (appendices A-5 & A-6) are discussed.

Chapter 7 discusses the practicalities of delivering cardioplegic solutions in the clinical situation. The results of our studies on the methods of oxygenating commercially available cardioplegic solutions are discussed (appendix A-7). In addition, we examine the potentially detrimental effect of rewarming of the cardioplegic solution contained in the delivery line leading to the operating table (appendix A-8), and how perfusion pressure should be monitored during infusion of cardioplegic solutions (Appendix B-8).

Chapter 8 is a summary of the thesis.

ABBREVIATIONS USED IN THE TEXT

Units of Measurement:

^{o}C	degrees Celsius
cm	centimeter
δ	delta (change in)
g	gravitational force
gm	gram
h	hour
kg	kilogram
kPa	kilopascal
L	liter
M	molarity (mmol/L)
m	meter
mEq	milliequivalent
mg	milligram
min	minute
ml	milliliter
mm	millimeter
mmol	millimole
mOsm	milliosmole
mV	millivolt
N	normality
ng	nanogram
p	partial pressure
s	second
U	Unit
ug	microgram

Chemical Compounds:

1,3 DPG	1,3-diphosphoglycerate
13-HODE	13-hydroxy-9,11-octadecadienoic acid
ADP	adenosine diphosphate
AMP	adenosine monophosphate
ATP	adenosine triphosphate
Ca^{2+}	calcium
cAMP	cyclic adenosine 3',5'-monophosphate
cGMP	cyclic guanosine 3',5'-monophosphate
CK	creatine kinase
CK-MB	MB fraction of creatine kinase
Cl^{-}	chloride
CO_2	carbon dioxide
CoA	coenzyme A
CP	creatine phosphate
CPD	citrate-phosphate-dextrose
Cu^{2+}	copper
DHAP	dihydroxyacetone phosphate
ECGF	endothelial cell growth factor
EDRF	endothelium derived relaxing factor (nitric oxide)
EDTA	ethylenediamine-tetraacetic acid
F-6-P	fructose-6-phosphate
FAD	flavin adenine dinucleotide (oxidized form)
FADH	dihydroflavin adenine dinucleotide (reduced form)
FC-43	Fluosol-43 emulsion
FDP	fructose-1,6-diphosphate
Fe^{2+}	iron
G-1-P	glucose-1-phosphate
G-6-P	glucose-6-phosphate

G-6-PDH	glucose-6-phosphate dehydrogenase
GIK	glucose-insulin-potassium
Gl-3-P	glyceraldehyde-3-phosphate
Gl-3-PDH	glyceraldehyde-3-phosphate dehydrogenase
H^+	hydrogen
H_2CO_3	carbonic acid
H_2O	water
H_2O_2	hydrogen peroxide
Hb	haemoglobin
HCl	hydrochloric acid
HCO_3	bicarbonate
Hg	mercury
HK	hexokinase
K^+	potassium
LDH	lactate dehydrogenase
Mg^{2+}	magnesium
N_2	nitrogen
Na^+	sodium
NAD	nicotinamide adenine dinucleotide (oxidized form)
NADH	dihydronicotinamide adenine dinucleotide (reduced form)
NADP	nicotinamide adenine dinucleotide phosphate (oxidized form)
NADPH	dihydronicotinamide adenine dinucleotide phosphate (reduced form)
NaOH	sodium hydroxide
NH_2	ammonia
α-KGl	α-ketoglutarate
O_2	oxygen
OAA	oxaloacetic acid
PDH	pyruvate dehydrogenase
PEP	phosphoenolpyruvate
PFK	phosphofructokinase
PGI_2	prostacyclin
PK	pyruvate kinase
PO_4^{3-}	phosphate
SO_4^{2-}	sulfate
SOD	superoxide dismutase
THAM	tromethamine
tPA	tissue plasminogen activator
TRIS	tris (hydroxymethyl) aminomethane hydrochloride
UW-CSS	University of Wisconsin cold storage solution

Other Abbreviations:

AO	aortic flow
BSA	body surface area
CABG	coronary artery bypass graft
CI	cardiac index
CO	cardiac output
DPTI	diastolic pressure time index
e^-	electron
ECG	electrocardiogram
HR	heart rate
IABP	intra aortic balloon pump
LA	let atrium
LAP	left atrial pressure
LV	left ventricle
LV dp/dt	first derivative of LV developed pressure
LVEDP	left ventricular end diastolic pressure
MAP	mean arterial pressure
PB	Plasmalyte B electrolyte solution
PVR	pulmonary vascular resistance
SV	stroke volume
SVI	stroke volume index
SVR	systemic vascular resistance
SWI	stroke work index
TTI	tension time index

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Chapter 1

INTRODUCTION

The first successful cardiac operation was performed by Professor Ludwig Rehn in September 1896 in Frankfurt; repair of a penetrating wound of the right ventricle (R14). However, it was not until September 1952 that the first elective "open-heart" surgical procedure was performed; closure of an atrial septal defect by Dr F John Lewis using moderate systemic hypothermia (28^{o}C) and inflow occlusion (L33). The period that the vena cavae can be safely occluded without sustaining neurological damage is though limited to 8 - 10 min. Further progress in cardiac surgery was thus primarily hindered by the inability to independently maintain cerebral circulation. In addition, the technique of inflow occlusion has a significant risk of systemic air embolism (L33), and there is also no adequate means to rewarm the cold non-beating heart (W5). Nevertheless, surface-induced deep hypothermia and total circulatory arrest without cardiopulmonary bypass was used to correct congenital heart defects till the late 1960's, but with a mortality as high as 58 % (M51). Although hypothermic inflow occlusion is no longer used routinely today, inflow caval occlusion can still be a valuable adjuvant technique during modern cardiac surgery (J11,O4).

The evolution of cardiac surgery required a means of temporarily replacing the function of the heart and lungs, and thereby supporting the systemic circulation. Thus research at this time concentrated on this problem, and an extracorporeal heart-lung apparatus was first used clinically in May 1953 by Dr J Gibbon to close an atrial septal defect (G19). However, further attempts to repair intracardiac defects utilizing these pump-oxygenators failed (L37). The only alternative means of temporarily supporting the patient's circulation was pioneered by Dr C W Lillehei; controlled cross-circulation from a donor parent to the patient (W5). Cross-circulation was used clinically for the first time in March 1954, and the mortality in 45 subsequent corrections of congenital heart

defects was 37 % (L37). Although this high mortality is not good by today's standards it was acceptable in 1955, and better than that associated with other methods used at that time (M51).

Nevertheless, the design and function of extracorporeal pumps and bubble oxygenators progressively improved and cardiopulmonary bypass became routine in July 1955 (K21,W5). Thereafter, the scope of cardiac surgery expanded rapidly, and cardiac surgery was now only limited by the amount of damage the myocardium could sustain during the surgical procedure. Consequently, a great deal of research was therefore directed towards ways of protecting and preventing injury to the myocardium whilst the surgeon operated upon the heart. Nonetheless, there is still no consensus on the ideal technique of perioperative myocardial protection. Moreover, certain cardiac operations require specific intraoperative techniques because of other constraints. In addition, cardiac surgeons should also be aware of the multitude of factors that can alter myocardial function during the perioperative period. These factors and diverse techniques of protecting the myocardium are therefore reviewed.

1.1 MECHANISMS OF MYOCARDIAL INJURY

The myocardium can be injured by mechanical, ischaemic and pharmacological means (W12), and postoperative morbidity and mortality increase if injury results in myocardial necrosis (K20).

Mechanical Injury of the Heart

Mechanical injury of the heart occurs frequently during open-heart surgical procedures (Table 1.1).

Table 1.1

MECHANICAL INJURY OF THE HEART
Surgical Incisions (E2)
Surgical Handling
Myocardial contusion
Myocardial lacerations
Topical Hypothermic Injury
Epicardial Frostbite (H16,S66)
Ventricular Distension (H49)

Any surgical incision of the myocardium (eg. right ventriculotomy for repair of Fallot's tetralogy) will produce local myocardial necrosis. In addition, the incision and its subsequent closure can have more profound effects if coronary arteries are inadvertently divided, if myocardial geometry is altered thus decreasing the efficiency of global contraction (E2). Furthermore, the heart is easily contused and lacerated by "rough" surgical handling, and can be injured by "protective mechanisms" such as extreme hypothermia (H16,R27,S66). Ventricular distention is also harmful as it causes

subendocardial ischaemia, and severe distention can result in an irreversible mechanical "stretch injury" of the myocardium (H49). Cross-linkages between actin and myosin filaments are disrupted and the myocyte is thereafter incapable of contracting.

Therefore, prerequisite myocardial incisions should be limited as far as possible, the heart should be handled with care and ventricular distention avoided.

Ischaemic Injury of the Heart

The energy requirements of the heart can be subdivided into the energy required for electromechanical work (70 % - 88 % of energy requirements depending upon the degree of work (B76,H50)), as well as basal cellular metabolic requirements. These energy requirements are met by aerobic metabolism in the perfused heart, primarily from catabolism of either carbohydrates (38 mmol ATP per mmol extracellular glucose) or fatty acids (129 moles of ATP per mole of palmitate) (O16). However, if the heart is deprived of coronary blood flow and thus oxygen, aerobic metabolism ceases and only a limited amount of energy can be supplied by anaerobic metabolism (2 mmol ATP per mmol extracellular glucose). Ongoing energy requirements cannot therefore be met and myocardial ischaemia occurs.

Myocardial ischaemia exists whenever the energy demand of the myocardium is greater than the energy supply, and is associated with lack of substrates for energy production and insufficient removal of potentially toxic end products of metabolism. The effects of myocardial ischaemia are multiple, progressive and vary according to the degree of ischaemia (H16). In summary, oxidative mitochondrial metabolism diminishes rapidly, fatty acyl CoA accumulates, anaerobic glycolysis is initially stimulated but then inhibited by increasing levels of lactate, NADH, and hydrogen ions (N8,R53), and high energy phosphates (ATP, CP) are depleted (B63,H18,N7,R42). Energy dependant systems can thus no longer function effectively; electromechanical coupling and contractility

declines, cessation of membrane pumps results in changes to intracellular ionic concentrations (F13), and damage occurs to the sarcoplasmic reticulum and mitochondria (G21,M41,O18). Finally, lipases and proteases are activated and cellular necrosis occurs (B53,M37,S27).

Ischaemia thus causes major metabolic, ionic and electrophysiological changes to the myocardium and the pathophysiology of these changes interrelate in a complex fashion. ATP deficiency and altered proton and calcium homeostasis (P14) appears though to be the major damaging factors in the ischaemic process. Initially, ischaemic induced cellular changes are potentially reversible, but cell death eventually occurs if allowed to progress (B53). Nevertheless, myocardial tolerance to ischaemia can be significantly increased by favorably manipulating the ischaemic process; decreasing metabolic demands, stabilizing membranes, and maintaining intracellular homeostasis.

Pharmacologic Injury of the Heart

Pharmacological drugs can be both beneficial and injurious. Excessive doses of so called "protective agents" can result in myocardial damage, as exemplified by the Melrose cardioplegic solution (H16,M23,M29). Excessive doses of blood cardioplegic reperfusion solutions have also been shown experimentally to be potentially deleterious (K25). In addition, pharmacologic agents may be beneficial only during specific time periods, and toxic at others (eg. beneficial effect of preoperative glucose, insulin and potassium which increases myocardial glycogen stores (O11,V23), but possible detrimental effect of glucose and insulin when present during ischaemia (H27,N5,W34)). Finally, inappropriate use of drugs (eg. immediate postischaemic use of inotropic drugs) can also increase myocardial ischaemic damage (K1,L19).

There are thus a number of different mechanisms whereby the heart can be injured, all of which can detrimentally affect postoperative myocardial function. Although ischaemia is probably the major cause of intraoperative myocardial damage, all of the above potentially harmful factors must be taken into account during open-heart surgery.

1.2 MYOCARDIAL PROTECTION DURING OPEN-HEART SURGERY

The majority of cardiac operations performed today involve cross-clamping the ascending aorta, although many cardiac operations can be performed with the heart continuously perfused and beating on cardiopulmonary bypass. Cross-clamping the aorta facilitates the surgical procedure technically by producing a still, bloodless operative field, but also causes myocardial ischaemia. Cardiac surgery is thus dependant upon a compromise between the needs of the surgeon; surgical exposure and sufficient time to complete the necessary procedure, and the needs of the myocardium for a continuous supply of oxygen and substrate (H16).

The intraoperative objective during cardiac surgery should therefore be twofold; correction of the pathological entity necessitating the operation, but without causing additional myocardial damage. Ideally, after the surgical procedure there should be no discernable reduction in myocardial function even if temporary. Although the previously discussed mechanisms of myocardial injury appear simplistic, a multitude of factors must be taken into account if myocardial damage during open-heart surgery is to be prevented; preoperative, intraoperative, anaesthetic and pharmacological factors (M10), effects of cardiopulmonary bypass, surgical incisions and handling of the heart, adjuvant techniques used to reduce the effects of myocardial ischaemia, and postoperative care. Thus "myocardial protection" is the responsibility of not only the cardiac surgeon but also the anaesthetist and intensivist. Furthermore, a fundamental principle during open-heart surgery is that myocardial damage is cumulative, and occurs whenever and wherever there is an imbalance between myocardial energy supply and demand (B73). Adequate myocardial protection can only be achieved if a number of principles are followed throughout the entire perioperative period, and conceptually this can be broken down into various phases of the surgical procedure (B70,B73,B74,H16,W12); preoperative period, during cardiopulmonary bypass, whilst

the aorta is cross-clamped, the postischaemic period following the release of the aortic cross clamp, and the postoperative period.

No single factor provides "myocardial protection" during open-heart surgery.

Furthermore, despite an enormous amount of basic research already done on myocardial protection (M24), only general principles can be tabulated. The "ideal" techniques for protecting the myocardium during open-heart surgery are still being evolved (B60,B61,B69,B74,H14,J24,K20,S77). Intraoperatively, there are a number of different ways of both protecting the myocardium and of correcting cardiac defects. However, of utmost importance is the necessity that the surgeon has a sound knowledge of the principles and specific details of any one technique. Techniques used successfully at one institution may fail at another, because of lack of attention to some "minor" detail (W12).

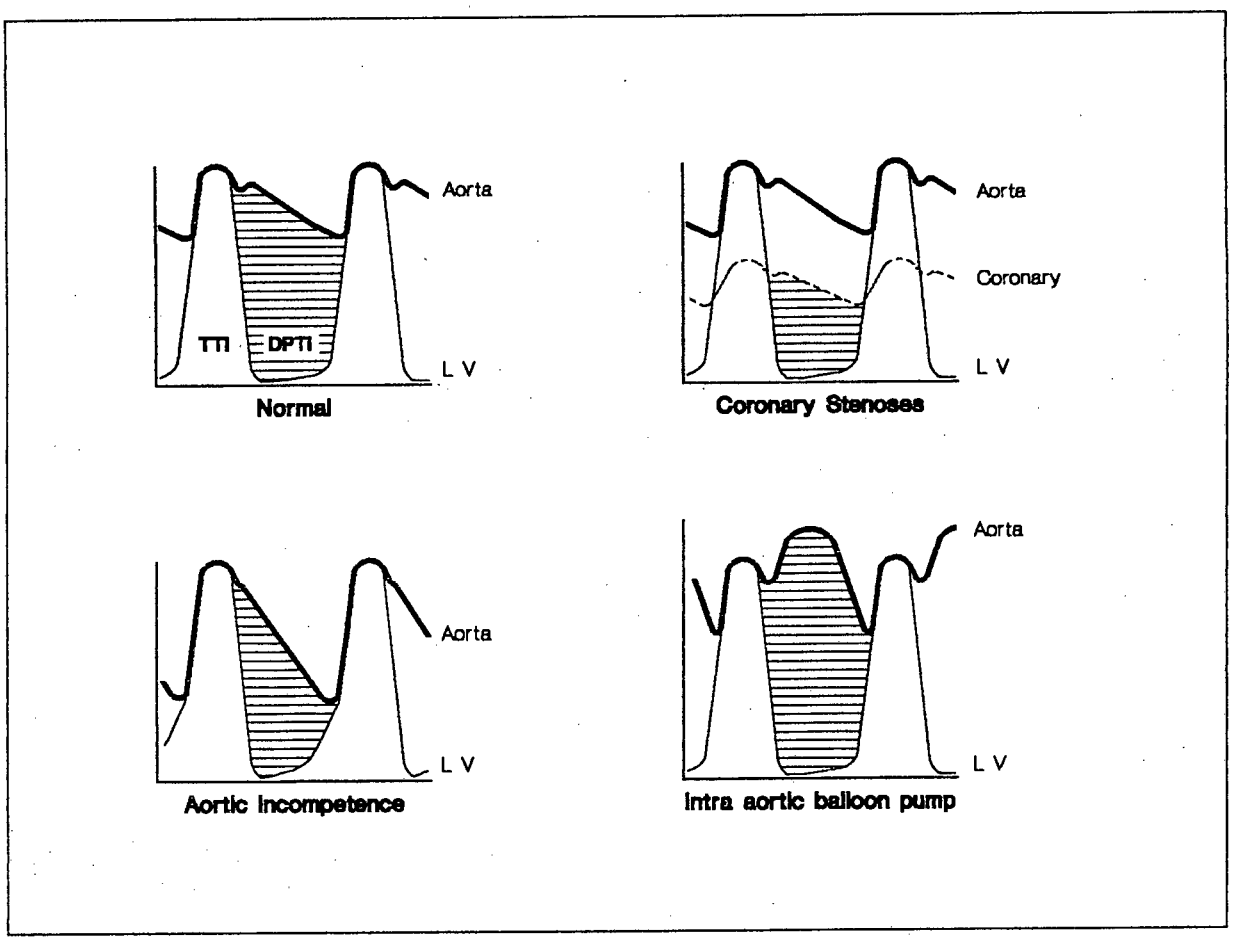
1.3 MYOCARDIAL PROTECTION IN THE PREOPERATIVE PERIOD

The majority of patients undergoing open-heart surgical procedures have already sustained some myocardial damage as a result of the pathological condition necessitating cardiac surgery.

Myocardial ischaemia is usually a common denominator in the preoperative period. Various pathological conditions can result in myocardial ischaemia and conceptually ischaemia occurs if the ratio of the area beneath the left ventricular systolic pressure curve (tension time index or energy demand) and the area between the aortic and left ventricular pressure curves in diastole (diastolic pressure time index or energy supply), is less than 0.8 (B73). Examples of this concept are shown in Figure 1.1; the decreased energy supply in the myocardium distal to coronary artery stenoses or in aortic incompetence, and the increased energy supply provided by intraaortic balloon pump counterpulsation.

An understanding of the pathophysiology of the ischaemic process in these various pathological conditions will assist in the preoperative medical management of these patients. The patient's pathology will dictate the importance of each possible preoperative intervention. For example, in valvular heart disease bradycardia must be prevented in the presence of aortic incompetence, but encouraged if there is significant valvular stenosis. In contrast, in ischaemic heart disease coronary vasodilator therapy and adequate preoperative beta blockade is essential (S54).

Figure 1.1



Legend:

Aortic and left ventricular (LV) pressure curves illustrating the ratio of the energy supply (DPTI - diastolic pressure time index) and energy demand (TTI - tension time index) of the normal heart, the heart with aortic incompetence, in the myocardium distal to coronary artery stenoses, or during intraaortic balloon pump counterpulsation are shown. Modified from Cardiovasc Clin 1981; 12(3):9-30 (B73) with permission G D Buckberg and Cardiovascular Clinics.

Adequate preoperative beta blockade decreases perioperative ischaemia by approximately 50 % (H7,S52), whereas calcium antagonists do not decrease the incidence of ischaemic events during anaesthesia. Furthermore, administration of beta blockers (propranolol 0.05 mg/kg) even a few minutes before the aorta is cross-clamped reduces perioperative infarct size in both ischaemic and valvular heart disease patients (R6).

Furthermore, a number of additional beneficial interventions that diminish perioperative morbidity and mortality can also be commenced preoperatively (B62) (Table 1.2).

Table 1.2

POSSIBLE PREOPERATIVE INTERVENTIONS
Increase myocardial oxygen supply (K7)
Oxygen
Optimize coronary blood flow
Vasodilator therapy
Intra aortic balloon pump
Decrease myocardial oxygen consumption (K7)
Optimize heart rate
Optimize preload & afterload
Vasodilators
Beta-blockers (H7,R6,V15)
Intra aortic balloon pump
Decrease catecholamines (H14)
Beta-blockers (R6,S52,S54)
Adequate premedication (B60,P22)
Anxiolytics
Metabolic interventions
Increase intracellular glycogen (O11)
Glucose-insulin-potassium (M41)
Inhibit free fatty acid metabolism
Nicotinic acid (O23)
Prevent free radical generation (M37)
Allopurinol (B8,C12,S75,W12)
Discontinue certain drugs
Digitalis (C44,K31)
Amiodarone (K42,L34,V2)
The "super fit" athlete

Preoperative metabolic interventions

Preischaemic metabolic enhancement is generally not used routinely, but might well be essential in high risk class IV patients, who have an increased probability of

perioperative myocardial necrosis because of depleted myocardial glycogen reserves (K19).

Glucose-insulin-potassium (GIK) infusions were first reported to be beneficial for patients with ischaemic heart disease by Sodi-Pallares in 1963 (S58). All three components of GIK were necessary, neither single components nor any two components were as effective in decreasing the electrocardiographic signs of acute myocardial infarction. GIK increases intracellular potassium, glycolysis, gluconeogenesis, improves sarcoplasmic reticulum calcium uptake, inhibits lipid metabolism, diminishes potentially harmful accumulation of fatty acyl CoA, increases prostaglandins, and possibly scavenges oxygen free radicals (H35,M41,O19,O22). All of these effects would be beneficial during or prior to ischaemia. Although routine preoperative substrate enhancement is controversial (L28), the majority of patients taking a regular diet will have low preoperative levels of glycogen (L28). Preoperative overnight infusions of GIK (1 L 20% glucose, 45 mmol KCl, insulin 10 U, heparin 2000 U) will increase myocardial glycogen levels (O11), and high preischaemic myocyte glycogen levels are associated with improved tolerance to ischaemia and fewer complications after open-heart surgery (L46,O11). Moreover, even a short period of metabolic enhancement, from induction of anaesthesia till the aorta is cross-clamped (glucose 0.5 - 1 gm, insulin 1 - 1.5 U, KCl 0.25 mmol/kg body weight/h), is beneficial (H4,V23). The trauma of surgery and cardiopulmonary bypass also inhibits insulin secretion because of increased stress hormone release and hypothermia (K40,K41). Therefore, metabolic enhancement in the postischaemic period with GIK is also beneficial (G28,S88,S89,V23). Nevertheless, close monitoring of serum potassium and glucose concentrations are essential both during infusions of GIK, and in addition for 6 - 12 h postinfusion as rebound hyperkalemia is a noted complication (B38).

Chronic use of diuretics reduces intracellular potassium and magnesium (D26,G27), and decreases sodium-potassium pump function (D22). Thus, low serum levels of these electrolytes should be corrected preoperatively.

Long chain fatty acid metabolites - acyl CoA - accumulate in ischaemic myocardium and impair mitochondrial function (O16). Hence, inhibiting fatty acid uptake and metabolism prior to ischaemia, by using GIK or nicotinic acid may be beneficial (O23). High doses of oral nicotinic acid (3.6 gm/24 h) have been used to decrease serum levels of free fatty acids after myocardial infarctions, but these doses are associated with significant side effects (R56). Nevertheless, smaller intravenous doses may be beneficial prior to cardioplegic arrest, by specifically inhibiting myocardial lipolysis without altering serum free fatty acid levels (O23).

Oxygen derived free radicals contribute to perioperative tissue injury and are generated by a number of mechanisms including the catalysis of hypoxanthine (see section 1.6). Therefore, free radical scavengers can be infused into the ischaemic tissue to "mop" up free radicals, or alternatively the generation of free radicals can be prevented (M37,W12). Although the exact role and timing of the administration of free radical inhibitors and scavengers is still to be defined, especially as free radicals are predominantly generated on reperfusion (see section 1.6), preoperative administration of free radical scavengers can be beneficial (B8,S75). Allopurinol has been shown to reduce free radical generation if given preoperatively (50 mg/kg for 72 h) (B8,S75), although pretreatment at a lower dose (20 mg/kg) only improved recovery experimentally after normothermic ischaemia and not after hypothermic ischaemia (C12). Nevertheless, clinical trials suggest that preoperative allopurinol (300 mg twice daily for 2 days and 600 mg on the morning of operation) decreases complications following cardiac surgery (J10,R7,T2). Moreover, preoperative allopurinol also allows build up of the metabolites oxypurinol / alloxanthine, which also inhibit xanthine oxidase and scavenge hydroxyl radicals (T2,V18).

Drugs that should be discontinued prior to cardiac surgery

Certain therapeutic agents should be discontinued preoperatively, because of their potential detrimental effects during subsequent ischaemia.

Digitalis should be discontinued preoperatively if possible, as it possibly increases cytosolic calcium during ischaemia which impairs postischaemic myocardial function (C44,K31).

Amiodarone is an antiarrhythmic drug, and its preoperative use has been associated with increased operative mortality and morbidity; hepatic, pulmonary, and cardiac dysfunction are increased after open-heart surgery (K42,L34,V2). However, if preoperative withdrawal of amiodarone is not possible because of malignant dysrhythmias, then the surgical procedure should be performed without cardioplegia due to potentially dangerous interactions of amiodarone and cardioplegic solutions (Cox J; personal communication). Furthermore, because of its long half life (measurable serum levels recorded as long as 9 months after discontinuation of amiodarone (L34)), it should be stopped for at least 6 months prior to surgery.

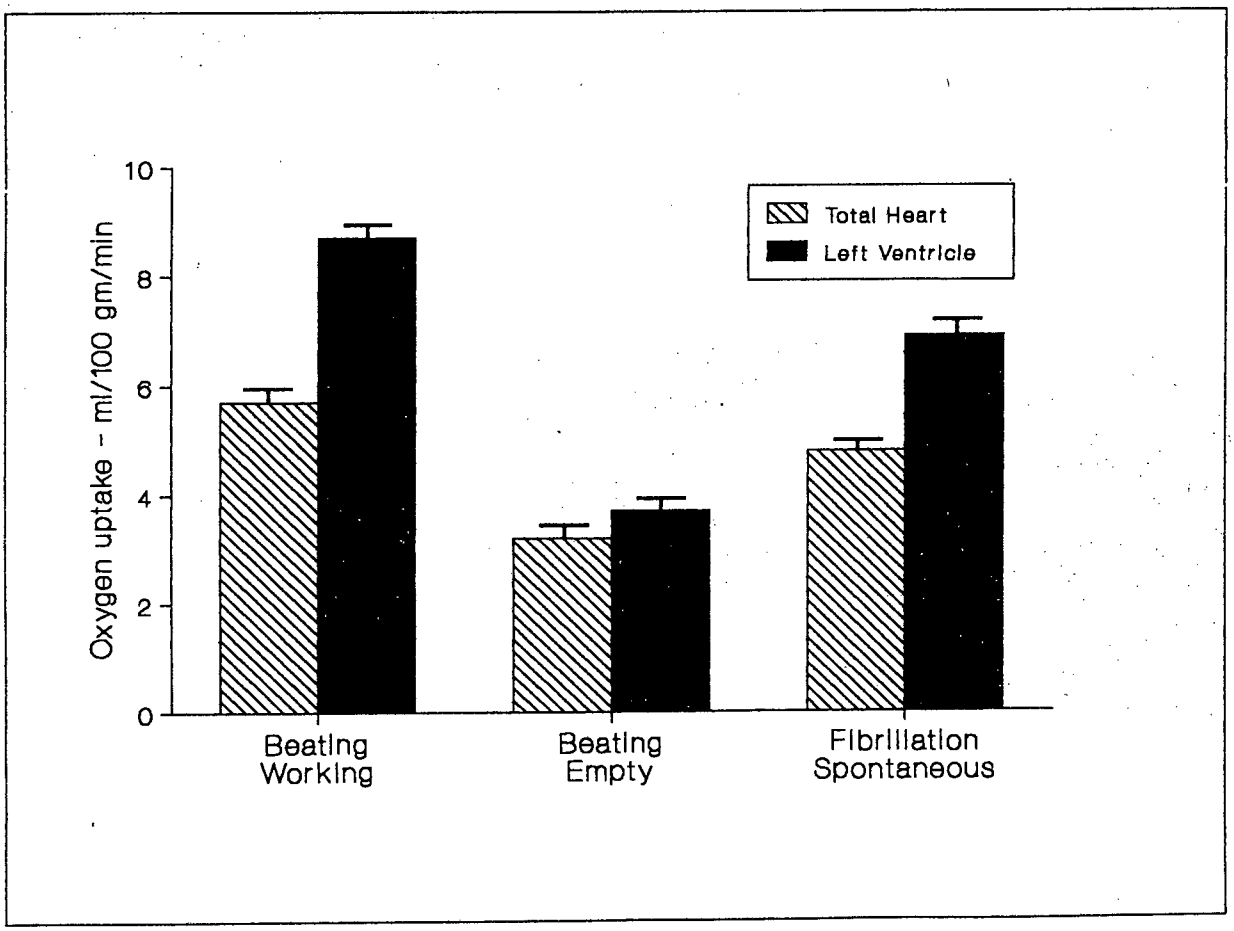
The myocardium of **"super-fit" olympic athletes** undergoes metabolic alterations, and has been associated with irreversible myocardial dysfunction following uncomplicated short cardioplegic protected ischaemic periods during arrhythmia surgery (Cox J; personal communication). Arrhythmia surgery is possibly the only branch of cardiac surgery where the patient can be extremely fit and yet require open-heart surgery. Thus, "detraining" of super-fit athletes is now advised, prior to cardiac surgery if cardioplegia is necessary.

The preoperative management of patients undergoing cardiac surgery can thus significantly alter postoperative myocardial function, morbidity and mortality.

1.4 MYOCARDIAL PROTECTION ONCE ON CARDIOPULMONARY BYPASS

The majority of cardiac operations are performed with the aid of an extracorporeal circulation. The systemic venous blood is drained into an extracorporeal oxygenator where it is oxygenated and then pumped back into the patient's arterial system by a cardiopulmonary bypass machine. The empty heart continues to beat but no longer ejects once total cardiopulmonary bypass is commenced, and this beating "non-working" state decreases myocardial oxygen consumption by more than 40% (Fig 1.2) (H50).

Figure 1.2



Legend:

Oxygen consumption of the total heart and left ventricle during beating working, beating empty or spontaneous ventricular fibrillation conditions. Modified from Hottenrott et al, J Thorac Cardiovasc Surg 1974; 68:615-625 (H50) with permission G D Buckberg and Mosby Year Book Inc.

Thus extracorporeal support of the systemic circulation decreases myocardial energy demands and can assist in reversing myocardial ischaemia. However, although cardiopulmonary bypass decreases myocardial energy demands, extracorporeal circulation also causes cumulative myocardial damage. Four hours of continuous coronary perfusion with no ischaemic stress decreases left ventricular function, compared to four hours of cardioplegic protected hypothermic arrest (R25).

Cardiopulmonary bypass induced myocardial damage

Cardiopulmonary bypass damages all blood components (erythrocytes (R16), leukocytes (S67), platelets (V7), plasma proteins (V6,V16)) by (K21):-

Exposure of blood to unphysiological surfaces,

Sheer stresses,

Incorporation of abnormal substances (pyrogens, plastics, micro-emboli etc).

Furthermore, micro and macro emboli, released vasoactive substances, catecholamines, activated complement, sequestrated leukocytes, free fatty acids and denatured proteins, which can all cause damage to various organs (K18,L28,M2,M55,N17,V3). Thus, cardiopulmonary bypass itself can result in neurologic (P1,S38,T20), pulmonary, renal and myocardial damage (N17). Hence, the duration of cardiopulmonary bypass should be limited whenever possible.

Primary factors causing myocardial damage during cardiopulmonary bypass are listed in Table 1.3.

Table 1.3

DAMAGING EFFECTS OF CARDIOPULMONARY BYPASS
Extracorporeal circulation itself (D21,K18)
Pumping mechanism;
Roller pump > Centrifugal?
Oxygenator;
Bubble > Membrane (D21,G36,H39,P1,V6)
Flow;
Non-pulsatile > pulsatile? (R15,T20)
Duration of cardiopulmonary bypass (D21)
Cardiotomy suckers (turbulence) (V6)
Micro-emboli (P1)
Low perfusion pressures (M20)
Ventricular fibrillation
Further aggravated if:
Continuous electrical fibrillation (H50)
Myocardial Hypertrophy (H51)
Ventricular distention (H49)
Hypothermia (B63,G37,S40)

Myocardial perfusion pressure

The majority of factors listed in Table 1.3 decrease myocardial perfusion directly or indirectly. Myocardial blood flow may be diminished directly by decreased perfusion gradients or indirectly by changes in coronary vascular resistance and regional blood flow. Systemic perfusion pressure is easily controlled, and should be maintained at adequate levels throughout cardiopulmonary bypass (50 - 70 mm Hg (L28)). At normothermia (37^{o}C) the myocardium can compensate for reduced perfusion gradients by autoregulation and compensatory vasodilation (M59), provided there are no coronary artery occlusions. However, coronary autoregulation is inadequate at hypothermia (28^{o}C) (C24,M20), despite adequate perfusion pressures there is decreased collateral blood flow to areas of myocardial ischaemia during cardiopulmonary bypass (M49).

Effect of Ventricular Fibrillation

Ventricular fibrillation increases myocardial wall tension and thus oxygen consumption (Fig 1.2) (H50). In addition, ventricular fibrillation decreases perfusion to the subendocardium because of myocardial compressive forces, thus causing subendocardial ischaemia (H51). Moreover, if perfusion pressures are low or unequally distributed between right and left coronary arteries (B56,N1), if fibrillation is electrically maintained (H50), or if there is ventricular hypertrophy (H52), then myocardial injury is accentuated. Although, myocardial oxygen uptake during hypothermic (28^{o}C) ventricular fibrillation is 52 % less than if at normothermia (Fig 1.3), subendocardial blood flow is still reduced (B56). Hence, hypothermia does not protect the ventricle from subendocardial ischaemia caused by ventricular fibrillation. Furthermore, ventricular fibrillation can result in ventricular dilatation, which would itself produce further cumulative myocardial damage (H49). However, a short period (3 min) of ventricular fibrillation at 28^{o}C prior to cardioplegic arrest is possibly not harmful in nonvented hearts (S9), but even a shorter period of continuous electrical fibrillation (30 s) is harmful (S8).

Ventricular fibrillation causes subendocardial ischaemia and should therefore be avoided, both prior to aortic cross-clamping and during the reperfusion period (L42). Nevertheless, if a period of ventricular fibrillation cannot be avoided then a ventricular vent should be inserted to prevent ventricular distention, an adequate perfusion pressure must be maintained, myocardial temperature should be decreased to approximately 28^{o}C, and the period of ventricular fibrillation should be kept as short as possible.

Effect of Ventricular Distention

Ventricular distention also increases myocardial wall tension (Laplace's law: wall tension is proportional to the distending pressure and principal radii of curvature (G2)), which increases myocardial oxygen consumption. In addition, the increased intracavitory ventricular pressure decreases subendocardial perfusion thus resulting in subendocardial ischaemia (H49). Ventricular distension does not have to be severe for it to be harmful, although should massive distention occur the resultant overstretch of muscle fibres can produce irreversible mechanical damage (see section 1.1). Insertion of a left ventricular vent can prevent ventricular distention. However, complete ventricular collapse may also be damaging in perfused beating hearts because of changes in ventricular geometry and compressive forces (L28), but this can be prevented by manipulating the vent.

The following factors are associated with a higher risk of ventricular distension occurring during cardiopulmonary bypass :-

- Aortic incompetence,
- Inadequate systemic venous drainage (B22),
- Increased bronchial blood flow,
- Ventricular fibrillation.

Ventricular distention can also occur whilst the aorta is cross-clamped, during cardioplegic arrest. However, moderate ventricular distention (LVEDP less than 15 mm Hg) during hypothermic cardioplegic arrest is not harmful (L22), and may be beneficial if the distending solution is cold. Therefore, routine insertion of a LV vent is not necessary, although warm systemic blood allowed to accumulate in the left ventricle will increase rewarming of the hypothermic myocardium which could in turn be detrimental (see section 1.5). Nevertheless, if an LV vent is not inserted prior to the aortic cross-clamp period, then left ventricular distention (LVEDP greater than 20 mm Hg) must be

prevented if it occurs in the early reperfusion period, as LV distention during reperfusion depresses postischaemic myocardial function (L50).

Thus, the left ventricle need not be routinely vented during open-heart surgical procedures, but only provided appropriate steps are taken should distention be seen to occur. Venous drainage should also be ideal and noncoronary flow limited in the absence of left ventricular venting, in order to prevent an accumulation of blood on the left side of the heart (L22). Alternatively, in certain circumstances venting the pulmonary artery as opposed to the left ventricle may be equally effective in decompressing the left ventricle (G4,M48).

Systemic Hypothermia

Systemic hypothermia, that is lowering the temperature of the entire body, decreases oxygen demand of all organs, increases tissue energy states (S90), and thus produces specific advantages for the cardiac surgeon (Table 1.4). The first open-heart surgical procedure in 1952 was performed without cardiopulmonary bypass, and its success was due entirely to the beneficial effects of systemic hypothermia (L33). However, despite routine use of cardiopulmonary bypass from 1955 onwards it was not until 1958 that cardiopulmonary bypass was combined with systemic hypothermia (surface cooling) (S32). Today, systemic hypothermia is induced by a heat exchanger incorporated in the cardiopulmonary bypass circuit, and initial surface cooling is only occasionally used (R21).

Table 1.4

ADVANTAGES OF SYSTEMIC HYPOTHERMIA
Increases the safe cerebral circulatory arrest time (G29,M57,O3,R9,R21)
3 - 5 min at 37°C
10 - 15 min at 28°C
45 - 60 min at 18°C
Cardiopulmonary bypass flow rates can be decreased
Decreases noncoronary collateral flow (Y1)
Decreases pump-oxygenator induced damage (S32)
Helps maintain profound myocardial hypothermia (B48,C34)
Diminishes conducted heat from adjacent organs
Diminishes heat gain from systemic perfusate

Early pump oxygenator systems were extremely destructive to blood elements, especially at high flow rates. Hence, lower flow rates in conjunction with mild systemic hypothermia (30°C) were advantageous (S31,S32). Total body oxygen consumption at 28°C is reduced by 50% - 60%, and at 18°C by 85% - 90% (G29). Thus, normothermic flow rates for adults (2.2 - 2.5 L/min/m^2) can be reduced to 1.6 - 2.2 L/min/m^2 if systemic temperature is decreased to less than 28°C (C34,K21). Furthermore, malfunctions of early cardiopulmonary bypass systems occasionally occurred, and hypothermia provided a measure of safety if the extracorporeal circulation stopped inappropriately.

There are also a number of potential disadvantages to using systemic hypothermia (R21,S91,W19), which can effect the myocardium directly or indirectly (Table 1.5).

Table 1.5

DISADVANTAGES OF SYSTEMIC HYPOTHERMIA

Leftward shift of oxyhaemoglobin dissociation curve
- Less oxygen released at lower temperatures (G2),
  but there is increased plasma dissolved oxygen.
* Optimize pO_2: 250 mm Hg (30 - 35 kPa)

Increased blood viscosity (F33)
* Haemodilute at hypothermia (U1),
  haematocrit at 20°C - 25°C: 0.2 - 0.25

Vasoconstriction
- Increased peripheral and pulmonary vascular resistance
  partially mediated by alpha-receptors (R21)
- Coronary vasoconstriction (B63)

Decreased coronary autoregulatory vasodilation
* Maintain an adequate perfusion pressure (C24,M20)

Positive inotropic effect (R12,S40)
- Increased intracellular calcium (K43)

Change in pH of neutrality (W19)
* Alpha-stat pH management (B19,S91,W30)

Inhibition of insulin release (K40,K41,R21)

Time taken to rewarm the patient intraoperatively,
and "after-drop" following adequate rewarming (N10)
* Pharmacologic vasodilation during rewarming

Increased oxygen consumption by shivering in the postoperative period (Z10)
* Neuromuscular blockade

Legend:
* Measures that should be taken to prevent the deleterious effects of hypothermia.

However, many of these potentially harmful effects of hypothermia can be appropriately counteracted. Some of these possible deleterious effects of hypothermia such as increased viscosity of blood, phase changes in lipid components of cell membranes and increased activation energy of ATPases increase markedly below 25°C

(F33,M26). Thus, possibly 25°C should be the lower limit for routine cardiopulmonary bypass procedures.

Rapid myocardial cooling can also cause coronary vasoconstriction and increased contractility as a result of complex hypothermic induced cytosolic changes of calcium flux (B63,K43,S40). Thus, rapid prearrest cooling of the beating heart can decrease postischaemic myocardial recovery (R11) as a result of increased cytosolic calcium (G37). Electromechanical arrest induced initially at normothermia prior to rapid cooling of the myocardium and systemic circulation has therefore been advocated, in addition to other advantages of normothermic arrest (see section 1.5) (H48,R40,R44,T10,W31). Furthermore, the temperature difference between the blood going into the patient and the patient's actual temperature should not be greater than 10°C - 14°C during systemic cooling (D10), in order to prevent bubbles coming out of solution; when the well oxygenated cold blood is rapidly rewarmed (becomes supersaturated) on contacting the warmer body.

In clinical practise today, moderate hypothermia (25°C - 28°C) with low flow rates is still used for routine cardiac surgery. Low cardiopulmonary bypass flow rates minimize noncoronary collateral flow from obscuring the operative field (K21) and help prevent rewarming of the hypothermic myocardium (see section 1.5) (C20,C34,Y1). In addition, deep systemic hypothermia is used if circulatory arrest during congenital or aortic arch operations is necessary (C41,K33,M57,O3,R21). If circulatory arrest is required, then systemic temperature is lowered to 18°C - 20°C in order to obtain electrocerebral silence and adequate cerebral protection (C38,G29,T20).
Furthermore, a distinction must also be made between the reasons and benefits of systemic hypothermia as apposed to profound myocardial hypothermia (see section 1.5).

1.5 MYOCARDIAL PROTECTION WHILST THE AORTA IS CROSS-CLAMPED

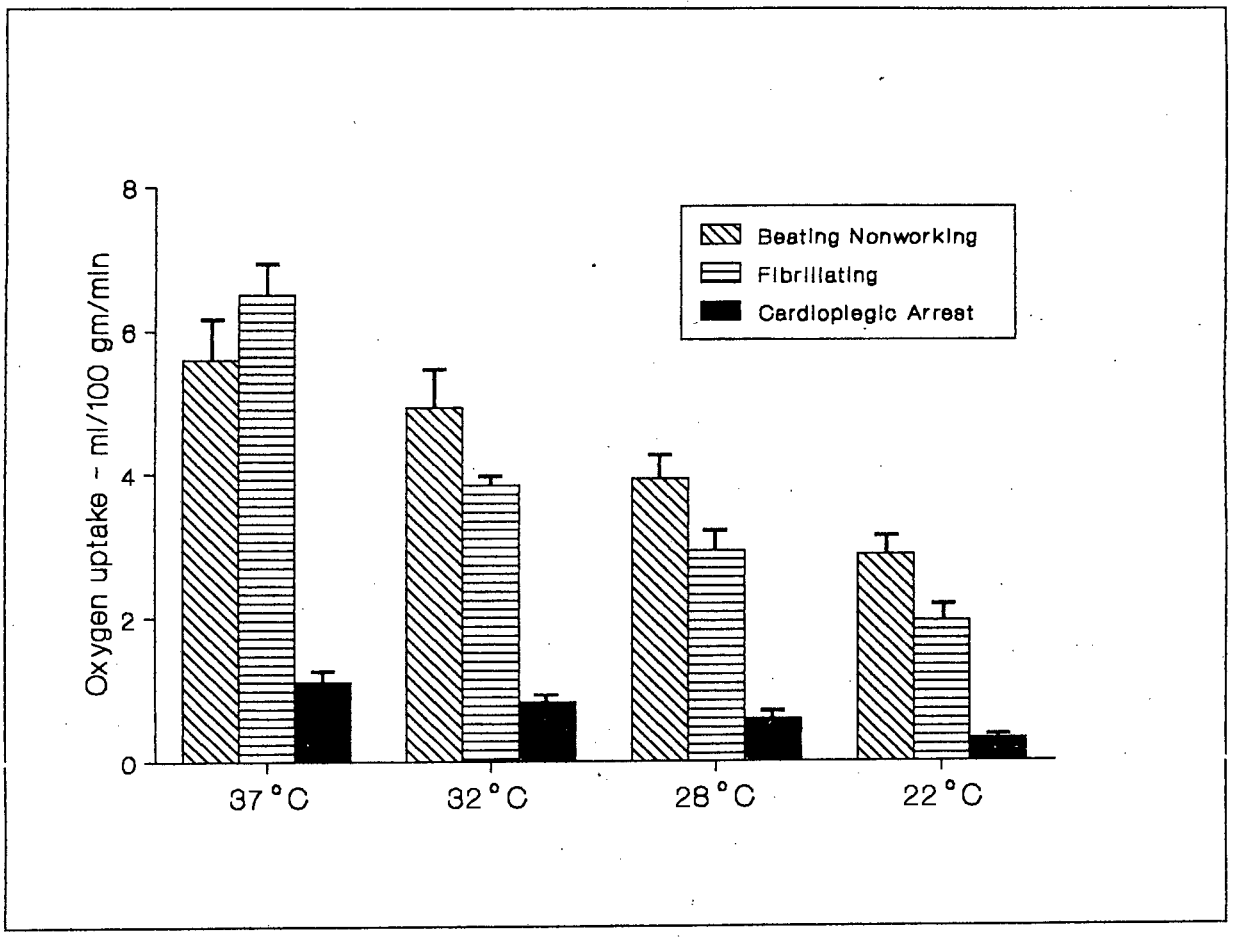
The objective the surgeon wishes to achieve by cross-clamping the aorta is a still, bloodless operative field (L3,W35). The advantages being:-

a) The heart is soft and can be retracted more easily,
b) Microvascular anastomoses can be more accurately constructed,
c) Cerebral air embolism can be prevented (L3),
d) Certain cardiac abnormalities can only be corrected with prolonged cross-clamping of the aorta.

Nevertheless, simple cross-clamping of the aorta produces a still heart as a result of anoxic cardiac arrest due to depletion of high energy phosphates, and therefore tolerance to ischaemia is limited. Ischaemic tolerance can though be increased by maximally decreasing myocardial oxygen demand (basal metabolic and electromechanical requirements) during the ischaemic period, and thus decreasing the decay of high energy phosphates (B62). This can be achieved by both pharmacologic electromechanical arrest (induced by infusing a properly formulated cardioplegic solution), and profound myocardial hypothermia (Fig 1.3).

Cardioplegic arrest of the canine heart at normothermia decreases left ventricular myocardial oxygen demand from 5.6 ml O_2/100 gm/min (beating non-working heart) to 1.1 ml O_2/100 gm/min; the difference would thus approximate myocardial electromechanical energy requirements (B76). If myocardial temperature is then decreased to 22°C, oxygen demand diminishes further to 0.3 ml O_2/100 gm/min; the basal myocardial metabolic requirements at hypothermia (Fig 1.3). Profound cardiac hypothermia reduces metabolic rate, the rate of decline of high energy phosphates (D14,S90), mitochondrial calcium transport (F37), and rapidity of onset of acidosis (H16,T5).

Figure 1.3



Legend: Left ventricular myocardial oxygen uptake of the empty beating nonworking, fibrillating, and cardioplegic arrested hearts at different myocardial temperatures. Reprinted with permission Buckberg et al, J Thorac Cardiovasc Surg 1977; 73:87-94 (B76) and Mosby Year Book Inc.

Non-cardioplegic methods of myocardial protection

During the past years a variety of different methods of protecting the myocardium during cardiac surgery have evolved (H16,K20). Although non-cardioplegic methods are used more infrequently today, they are still occasionally applicable.

1) Continuous cardiac perfusion

The myocardium is continuously perfused with oxygenated blood via the aortic root or during aortic valve surgery by direct cannulation of the coronary arteries. This may be performed at either normothermia or moderate hypothermia (25°C - 32°C) and with or without electrical ventricular fibrillation (to obtain a still operative field).

Continuous cardiac perfusion does not produce ideal operative conditions; the heart is neither still nor bloodless, and muscular tone hampers manipulation and retraction of the heart. Certain surgical complications such as posterior left ventricular rupture following mitral valve replacement are also increased with this method (G33). Furthermore, continuous cardiopulmonary bypass damages all blood elements and produces cumulative myocardial damage and does not prevent myocardial ischaemia from occurring (see section 1.4). Although an empty beating heart has lower myocardial oxygen demands (Fig 1.2) (H50), altered compressive forces and ventricular geometry impede the distribution of intramyocardial blood flow resulting in subendocardial ischaemia (M49). Flow in collateral vessels supplying ischaemic myocardium is also diminished whilst on cardiopulmonary bypass, despite adequate perfusion pressures (M49). Hence, hypothermic cardioplegic arrest with either blood (F26,R25) or crystalloid cardioplegia (A11) is better than an equivalent period of continuous perfusion of the beating heart with blood. Furthermore, the potentially harmful effects of ventricular fibrillation (used to obtain a still operative field) have already been discussed (see section 1.4). Cannulation and perfusion of the coronary ostia during aortic valve surgery is also associated with vascular injury, inadvertent maldistribution of perfusate and consequent myocardial necrosis (N1), and ostial stenosis that can develop within a relatively short postoperative period (incidence of 1 % - 5 % within 6 months) (M44).

Nevertheless, surgery on the continuously perfused beating heart is still essential in certain open-heart procedures. It is occasionally necessary to operate on the beating

normothermic heart in arrhythmia surgery; to enable monitoring of atrioventricular nodal function whilst operating in close proximity to it, whilst mapping arrhythmias, or to ensure successful ablation of ischaemic ventricular tachycardias (C40). Thus if prolonged continuous perfusion of the beating heart is necessary, the most atraumatic cardiopulmonary bypass oxygenators and pumps should be used, adequate perfusion pressures must be maintained, and possibly pulsatile flow should also be used.

In addition, acceptable experimental (G31) and clinical results for both elective and emergency coronary revascularization have been reported using continuous perfusion without cross-clamping the aorta (A14,A15,A16,A17). However, cardioplegic arrest would be used by these cardiac units if there was significant aortic regurgitation on cardiopulmonary bypass (A14). Technical points to ensure safe continuous myocardial perfusion include systemic hypothermia (28^{o}C) with mannitol (25 gm) added to the systemic perfusate, local pericardial cooling with 4^{o}C Ringer's lactate solution, maintaining mean perfusion pressure at 80 - 100 mm Hg, elective ventricular fibrillation, routine left ventricular venting, proximal vein-to-aortic anastomoses done first, local vessel occlusion for distal vein-to-coronary anastomoses and avoiding aortic cross-clamping. Although hospital survival is acceptable, a significant incidence of central nervous system injuries (4 %) (L31), and postoperative low cardiac output requiring an IABP (2.7 - 3.2 % of elective patients) is reported (A16,A17).

2) Intermittent cardiac ischaemia

The aorta is intermittently cross-clamped for short periods (less than 15 min) with subsequent 5 - 15 min periods of reperfusion of the beating heart. Normothermia (M56,V4) or moderate cardiac hypothermia (25^{o}C - 32^{o}C) is usually used, with or without electrical fibrillation in order to obtain a still operative field (B46).

Surgical exposure is not optimum as myocardial tone is maintained. In addition, the risks of both systemic and coronary embolization (air (K20) and particulate matter

(O12)) are possibly increased because of multiple manipulations of the aorta, and it is possible that cumulative repetitive reperfusion injuries are induced by this method (K20). Nevertheless, low morbidity and mortality figures have also been reported using this method for coronary revascularization procedures (B46). Technical points include moderate systemic hypothermia (30°C; lower systemic temperatures may not be necessary (V4)), left ventricular venting, intermittent aortic cross-clamping (usually less than 8 min per distal anastomosis) and ventricular fibrillation, with defibrillation during each reperfusion period. However, cardioplegic arrest is again necessary if long (greater than 15 min) continuous periods of ischaemia are anticipated, with all redo coronary revascularizations or if the aorta is heavily calcified (B44,B46).

Clinical studies comparing intermittent aortic cross-clamping and hypothermic cardioplegic arrest have shown equivalent outcome (B16,M56). Moreover, an advantage of intermittent global or local ischaemia over cardioplegic arrest is easier sizing of the length of bypass grafts when the heart is distended with blood under beating conditions (F20,M56). However, high energy phosphates and cellular ultrastructure are better preserved in the cardioplegia group, although the use of cardioplegic solutions is also associated with an increased incidence of temporary postoperative rhythm disturbances (F20). Furthermore, a large number of experimental studies have shown cumulative damage as a result of intermittent aortic cross-clamping when compared to hypothermic cardioplegic arrest (F26,H16,L2,L32,W35). Hypothermic cardioplegic arrest also facilitates the construction of microvascular anastomoses in a still, relaxed, bloodless surgical field, and requires minimal manipulation of a calcified aorta (O12).

3) Prolonged ischaemic cardiac arrest

The aorta is simply cross-clamped for prolonged periods (15 - 60 min) at profound myocardial hypothermia (22°C). After 20 - 30 min the myocardium becomes electromechanically silent and still, because of exhaustion of intramyocardial energy supplies (H16,K20).

Normothermic ischaemic arrest can produce irreversible ischaemic contracture of the heart; the stone heart (C36). A critical reduction of myocardial ATP content (J12) in the presence of calcium results in hypercontracture of the myocardium and accelerated ATP hydrolysis (H18). However, the time till onset of ischaemic contracture is variable (22 - 110 minutes (C36)), and has been related to the degree of LV hypertrophy and rate of ATP utilization (B30). Furthermore, short periods of normothermic ischaemia (15 min) cause significant postischaemic myocardial dysfunction (R25), hence the need for additional myocardial hypothermia.

Profound hypothermic ischaemic arrest was pioneered successfully by Norman E Shumway (G33,H57,S44), but is now no longer used routinely. A myocardial temperature of 15°C - 20°C was induced by irrigating the pericardium with 4°C saline at 100 - 150 ml/min, in combination with systemic hypothermia (32°C) and diminished cardiopulmonary bypass flow rates (1.5 L/min/m^2 body surface area) (G33,H57,S44). Aortic cross-clamp periods of 16 - 96 min were tolerated with acceptable clinical results, but prolonged cardiovascular support was necessary after this period of anoxic arrest (G33,H57). Nevertheless, a randomized clinical study of aortic valve replacements showed hypothermic ischaemic arrest to be superior to continuous coronary perfusion at 32°C (S16). Furthermore, Griepp et al reported a significant decrease in the incidence of posterior left ventricular rupture complicating mitral valve replacement using aortic cross-clamping combined with profound topical hypothermia (no cardioplegia), in contrast to continuous perfusion without cross clamping the aorta (G33). Thus, although both aortic and mitral valve replacements can be performed on the continuously perfused heart, aortic cross-clamping combined with hypothermia decreases morbidity and mortality.

Hypothermic ischaemic arrest may still have a role today in neonatal cardiac surgery when combined with deep hypothermic circulatory arrest, as neonatal hearts react differently to cardioplegic solutions compared to adult hearts (B3,B50,B51,B77,M7,W10).

Protagonists for the continued use of non-cardioplegic methods of myocardial protection, unless specifically indicated such as in arrhythmic operations, use these methods primarily for primary coronary revascularization procedures. The criticisms levelled against hypothermic cardioplegic arrest include:-

Multitude of cardioplegic solutions being used,

Uneven cooling and distribution of cardioplegic solutions,

Noncoronary collateral flow washes out the cardioplegia,

Increased conduction disturbances with cardioplegia (F20).

These criticisms are valid, but to a large extent have been solved today and will be discussed in the following pages. Moreover, these same surgeons using non-cardioplegic methods do not hesitate to use cardioplegia if indicated.

Cardioplegic arrest

Most modern cardiac surgeons agree that if it is necessary to clamp the aorta continuously for longer than 15 minutes, then pharmacologic cardioplegic arrest should be used to protect the myocardium. Cardioplegic solutions stop all electromechanical activity of the heart and thus decrease myocardial energy demands. Interestingly though, one of the primary objectives of Melrose et al for formulating the first cardioplegic solution in 1955, was to produce elective cardiac arrest in order to decrease the incidence of air embolism and not to provide "myocardial protection" as such (M30).

Melrose et al produced elective diastolic cardiac arrest by injecting 2 ml of a 25 % stock solution of potassium citrate diluted to 20 ml with blood, into the aortic root after occluding the aorta (M30). This dose of potassium citrate (greater than 200 mEq) far exceeded the minimum effective concentration of 1 mg/ml and resulted in a distinctive type of myocardial necrosis (M23,M29), and consequently this method fell into

disrepute. Initially a stock solution of potassium citrate had been used, and the actual concentration of potassium citrate was inadvertently lowered by precipitation which had resulted in less damage than when the solution was later made up fresh (H16). Concurrently, other solutions were also being tried; acetylcholine 10 mg/kg body weight (L3), potassium citrate combined with magnesium sulfate and neostigmine (S32). Nevertheless, cardiac surgeons stopped using cardioplegic solutions for more than 15 years in the English speaking part of the world, because of their potential toxicity (G8,H16). However, more controlled research on the formulation of cardioplegic solutions continued in the 1960's in Germany.

Modern principles of elective cardioplegic arrest

The basic concepts of protecting the ischaemic heart, once the aorta is cross-clamped, are centered on avoiding ischaemic damage by matching myocardial energy demands with available energy production, and are summarized in Table 1.6 (B60,B61,B63,B69,B73,H14,H16,J24).

The majority of myocardial energy expenditure is due to electromechanical activity (Fig 1.2 & 1.3), hence the benefit of diastolic arrest (B76,H50). However, asystole must be produced quickly as a significant amount of ATP can be consumed in the brief period between cross-clamping the aorta and obtaining electromechanical arrest (W35), as myocardial oxygen reserves are rapidly exhausted. The initial ATP losses can be minimized by either precooling the heart by systemic hypothermia, or by an initial infusion of cold oxygenated blood, glucose and 0.2% procaine (B63) (to prevent hypothermic induced increased muscular tone) prior to infusion of crystalloid cardioplegic solutions. Alternatively, cold oxygenated crystalloid or blood cardioplegia may also limit ATP losses prior to initiating diastolic arrest (B69).

Table 1.6

PROTECTION OF THE ISCHAEMIC HEART

Maximize Energy Conservation

- Stop electromechanical activity
  - Chemical arrest of the heart in diastole
    - Produce immediate arrest
    - Must be easily maintained and reversed
    - Must not produce myocardial damage
- Reduce basal metabolic rate
  - Hypothermia
  - Maintain intracellular homeostasis without energy consuming membrane pumps

Prevent Unfavorable Ischaemic Induced Changes

- Modify extracellular ionic environment
  - Specifically calcium
- Maintain energy production
  - Supply substrates
    - Oxygen
    - Glucose?
    - Amino acids?
    - High energy phosphates?
  - Remove end products of metabolism
- Counteract acidosis
  - Modify pH
  - Provide buffers
- Prevent oedema
  - Hyperosmolarity
  - Colloid oncotic pressure

Diastolic cardiac arrest can be produced pharmacologically by a number of mechanisms, but only five primary mechanisms are used in present cardioplegic solutions either singly or in combination (B60,B61,B62,B69,H14).

Namely infusion of;

High concentrations of Potassium or Magnesium

Depletion of extracellular Calcium or Sodium

Local anaesthetic agents:- Lignocaine, Procaine.

Profound myocardial hypothermia used in conjunction with cardioplegic arrest further reduces basal metabolism, assists in maintaining electromechanical arrest and provides additional myocardial protection (Fig 1.3) (B76,C20,E9,H16,H24,H25). Lowering the myocardial temperature of the cardioplegic arrested normothermic heart to 22°C decreases oxygen consumption a further 72 % (B76). Furthermore, adequate hypothermia appears to be essential during cardioplegic arrest to ensure effective protection of both the atrioventricular conduction tissue (C15,C16,S55), and right ventricle (B39,C26,G7,G24). Hypothermic cardioplegic arrest thus provides superior postischaemic recovery and better myocardial protection compared to hypothermic ischaemic arrest provided a properly formulated cardioplegic solution is used (C34). However, one of the clinical objectives when using cardioplegic solutions and myocardial hypothermia is to provide and maintain *uniform* myocardial cooling (Table 1.7).

The rationale for systemic hypothermia has already been discussed (see section 1.4). The importance of systemic hypothermia as a "cofactor" for myocardial protection is illustrated by the inability of many centers to reproduce the early results of Shumway et al (G33), when using hypothermic ischaemic arrest, because systemic flow rates were not decreased concurrently with systemic hypothermia (32°C) (H16). A systemic temperature of less than 25°C is advised by Borst et al, because of the pronounced effect that systemic temperature has on myocardial rewarming (B48). However, temperatures below 25°C, unless specifically indicated for neurological protection during circulatory arrest, may not be appropriate (see section 1.4). Thus most surgeons would compromise and use moderate systemic hypothermia (25°C - 28°C) during periods of cardioplegic arrest.

Table 1.7

MAINTAINING UNIFORM MYOCARDIAL HYPOTHERMIA

Systemic hypothermia
- Lower cardiopulmonary bypass flow rates can be used:
  - Diminishes noncoronary collateral flow
- Decreases conducted heat from adjacent organs

Infusion of cold (4°C) cardioplegic solution
- Single or multidose infusions
- Antegrade via the aortic root
- Retrograde via the coronary sinus

Topical myocardial hypothermia
- Intermittent irrigation with cold saline
- Continuous pericardial lavage (L21,S44)
- Epicardial cooling jackets (B47,D3)
- Inferior pericardial insulation pads (B48)

Left ventricular vent (B48)
- Prevents accumulation of warm systemic blood in the heart

Myocardial rewarming

Infusion of cold cardioplegic solutions produces quicker and better cooling of the more vulnerable subendocardium, than does topical hypothermia (D5). However, a single infusion of cardioplegic solution will not itself be adequate *to maintain* the induced cardiac hypothermia throughout the surgical procedure. Excessive myocardial rewarming to 18°C - 22°C can occur within 20 minutes (B48,D3). The inferior surface of the heart rewarms the most rapidly by heat conducted through the diaphragm from the liver, despite topical hypothermia (B48). The myocardial septum also rewarms as a result of systemic blood collecting in the heart; systemic and bronchial venous return as well as noncoronary collateral blood flow (Table 1.8).

Table 1.8

MYOCARDIAL REWARMING	
Mechanism	Prevention
Theater environment (R39)	
Ambient temperature	Low Theater temperatures ($19^{0}C$ - $22^{0}C$)
Surgical operating lights	Cold light sources
Surgical handling the heart (R39)	Minimize handling Use cold surgical swabs
Adjacent organs (D3)	
Descending thoracic aorta	Pericardial insulation pads
Liver	
Systemic blood flow (R39)	
Noncoronary collateral flow	Decrease systemic flow rates
Bronchial flow	Vent the left ventricle
Systemic venous blood in the right atrium	Total cardiopulmonary bypass

Legend:
Topical hypothermia and multidose reinfusions of cold cardioplegic solutions are additional universally applicable measures that will reverse rewarming of the hypothermic myocardium.

To counteract myocardial rewarming additional intermittent multidose reinfusions of cold cardioplegic solution, and / or topical hypothermia are recommended (D3,D5). However, with certain cardioplegic solutions multidose reinfusions are not recommended (G11,P28,S68), and an alternative method of maintaining hypothermia is then essential.

Topical hypothermia

The two primary reasons to still use topical hypothermia in addition to cold cardioplegia are:-

a) To prevent continued rewarming of the myocardium by radiated, convected and conducted heat (Table 1.8) (D3,R39). The anterior situated right ventricle and inferior myocardial surface being more at risk (B48,C26,G7,G24,V14).

b) To improve myocardial protection when there is heterogeneous delivery of cardioplegic solution in the presence of coronary artery disease (L9,L21).

Therefore, topical hypothermia is still used as an adjunctive method to cardioplegia (Table 1.7), but topical hypothermia itself can also be potentially harmful (Table 1.9).

Table 1.9

DANGERS OF TOPICAL MYOCARDIAL HYPOTHERMIA
Epicardial frostbite injury (H16,S66) Osmotic considerations (R27)
Phrenic nerve injury (C45,R26,R36,R49)
Vagus nerve injury (E16)
Increased postoperative respiratory complications (A22,B21,C45)

Legend:
The majority of these harmful effects can be prevented by using only cold (4°C) saline and not iced slush (C45,R26,R49).

Postoperative phrenic nerve injury (which is usually left sided) has been related to a number of factors (M14), but topical iced slush is probably the most likely incriminating factor even if the internal mammary artery is harvested (C45). Although pericardial insulating pads can protect the phrenic nerve from cryo-injury (E16), topical iced slush as apposed to 4°C cold saline should not be used (R26,R49).

Continuous pericardial lavage with 4°C saline is possibly inconvenient, inconsistent, noisy (because of the necessity to use sump suction) and can waste blood (B47), but does maintain cardiac hypothermia (L9,L21). A possibly superior alternative is the use of myocardial cooling jackets (B47,D3,D4), however, these may also be bulky, inconvenient and have been associated with cryo-injury if incorrectly used (R49), and furthermore cannot be used for all surgical procedures. Thus intermittent application of cold saline

($4^{o}C$) whenever cardioplegia is reinfused, is a frequently used but possibly poorer compromise.

What is the "ideal" myocardial temperature during hypothermic cardioplegic arrest?

The rate of decline of high energy phosphate compounds is directly related to temperature; the lower the temperature the slower the loss (D14). The protective effects of myocardial hypothermia are only really apparent below $28^{o}C$ - $25^{o}C$ (F37,H14,H24), and even better at $18^{o}C$ (G21). However, the lowest possible myocardial temperatures will not necessarily produce superior postischaemic recovery (B6,K12).

Keon et al showed that human atrial tissue immersed in a non-cardioplegic solution is best preserved at $12^{o}C$, a temperature of $4^{o}C$ was associated with depressed postischaemic contraction (K12). At very low temperatures there is impaired sodium / potassium ATPase activity, which results in changes to intracellular electrolyte homeostasis (M15) and decreased fluidity of membrane lipids and cell swelling (H16,L24,T28). Moreover, atrial function is better maintained by hypothermia than ventricular function (N11). Other investigators have also shown that the cardioplegic arrested heart should be kept at a temperature of $12^{o}C$ - $14^{o}C$, and that any further benefit obtained by decreasing the temperature to $4^{o}C$ is of only marginal benefit (B6,R1,R38). The optimal myocardial temperature for hypothermic cardioplegic arrest also differs according to whether the heart is in vivo (clinical open-heart surgery), or isolated ex vivo during organ storage for transplantation.

During clinical open-heart surgery it is extremely difficult to achieve and maintain myocardial temperatures below $10^{o}C$ (B6), and temperatures fluctuate because of rewarming of the myocardium (Table 1.8). Furthermore, the ideal hypothermic myocardial temperature is also influenced by the composition of the cardioplegic solution infused. A higher temperature may be more optimal with blood cardioplegia,

which may be more effective if infused at a temperature of 20°C (mean myocardial temperature 21.7°C), than 10°C (mean myocardial temperature 16.9°C) (M5). Nevertheless, the infusion temperature for cold blood cardioplegia recommended by G D Buckberg is 4°C - 8°C (B70). However, in our experience temperatures below 8°C are rarely obtained at infusion rates used clinically, with the presently available blood cardioplegic delivery systems (unpublished data). With crystalloid cardioplegic solutions the "ideal" myocardial temperature is 10°C - 15°C (R38,T28), which can be achieved by infusing cold cardioplegic solution (4°C - 10°C) into the heart (M5,P27,T28). In addition, rewarming of the myocardium above 18°C should be prevented (H16). Magovern et al showed equivalent recovery with both 20°C blood and 10°C crystalloid cardioplegia (M5). Thus, with blood cardioplegia additional topical hypothermia with its increased pulmonary morbidity (to ensure as cold temperatures as possible), may not be necessary (A22).

In contrast, temperatures as low as 0°C can be easily and inadvertently achieved during isolated organ preservation (H34), and temperatures below 4°C may be harmful (R38,T28). Intracellular sodium-potassium homeostasis must be maintained despite hypothermia, and the Na^+/K^+ pump is possibly inactivated below 4°C - 10°C in the heart (M15,P33). In an isolated rat heart study using St Thomas' cardioplegia the optimal storage temperature was shown to be 7.5°C, lower temperatures being harmful (T8). Thus a temperature of 7.5°C - 10°C is probably the "ideal" temperature for isolated organ storage (T7). Nevertheless, other solutions with different compositions may be associated with better preservation at 0°C (E15,F27,P23,Y6).

Cardioplegic solutions

A multitude of different cardioplegic solutions have been formulated, and these will be discussed more extensively in chapter 3. The composition of the "gold standard" for each of the three major categories of cardioplegic solutions is shown in Table 1.10. We should though also distinguish between cardioplegic solutions used in clinical open-heart

surgery and solutions used for organ preservation. Although in the clinical situation these are often the same solution, they need not be the same solution as the constraints surrounding preservation of an in situ heart (continuous myocardial rewarming, noncoronary blood flow etc) need not apply to an isolated ex vivo organ.

Table 1.10

CARDIOPLEGIC SOLUTIONS

	Crystalloid		Blood
(mmol/L)	Intracellular Bretschneider	Extracellular St Thomas'	4:1 Buckberg
Na^+	15	120	116 - 120
K^+	10	16	18 - 20
HCO_3^-	--	10	20 - 22
Ca^{2+}	--	1.2	0.5 - 0.6
Mg^{2+}	4	16	1.0 - 1.2
Cl^-	51	160.4	110 - 114
Glucose	--	--	42 - 44
THAM	--	--	14 - 15
Mannitol	30		
Histidine	198		
Tryptophan	2		
KH-2-oxygluterate	1		
Osmolality (mOsm/kg H_2O)	298	290	340 - 360
pH	7.1	7.8	7.7 - 7.8

Legend:

Concentrations of components contained in the indicated cardioplegic solutions. The delivered electrolyte concentrations for blood cardioplegia were calculated from the concentrate used for standard cold induction with blood cardioplegia by G D Buckberg (see Table 3.5). With multidose reinfusions of blood cardioplegia the delivered potassium concentration is decreased to 8 - 10 mmol/L.

Despite hypothermia and cardioplegic arrest, basal myocardial metabolic energy requirements remain. Energy requirements can only be further decreased if intracellular homeostasis can be maintained without the use of energy consuming

membrane pumps. This in part is the reason for using intracellular electrolyte equivalent formulations for the preservation of isolated organs. Alternatively, if extracellular electrolyte equivalent formulations are used, the membrane pumps must be maintained by anaerobic glycolysis. However, anaerobic glycolysis is inefficient and therefore the duration of tolerable myocardial ischaemia is limited (B61). Blood cardioplegia though allows limited but more efficient aerobic metabolism, and thus might be the superior extracellular electrolyte formulation. Nevertheless, cardioplegic solutions must also be delivered to the myocardium correctly, whatever the composition.

Delivery of cardioplegic solutions

In order to obtain optimal myocardial protection during the aortic cross-clamp period, not only is the composition of the cardioplegic solution important but also of equal importance is ensuring uniform delivery of the solution throughout the myocardium and maintenance of cardioplegic arrest during the entire ischaemic period (B74). Coronary artery stenoses impede the delivery of cardioplegic solutions beyond critical stenoses (A31,B18,S46). However, delivery can also be heterogeneous even in the absence of coronary artery disease (A18). Hence, the importance of ensuring optimal delivery of cardioplegic solutions (Table 1.11).

The importance of the correct temperature of the cardioplegic solution in order to achieve uniform myocardial hypothermia has already been discussed. The next most important factor is to ensure optimal coronary perfusion pressures during infusion of the cardioplegic solution, which is the principal determinant of regional flow even in the arrested heart (A18). However, the correct infusion pressure also depends upon whether the cardioplegic solution is infused antegrade via the aortic root, or retrograde via the coronary sinus.

Table 1.11

OPTIMAL DELIVERY OF CARDIOPLEGIC SOLUTIONS

A) Temperature

Crystalloid cardioplegia

Temperature of solution ..... : 4°C - 6°C

Myocardial temperature : 12°C - 15°C

Blood cardioplegia

Temperature of solution ..... : 8°C - 10°C?

Myocardial temperature : 15°C - 18°C?

B) Volume of solution

Extracellular electrolyte equivalent cardioplegic solution

Induction:

2 - 3 ml/gm LV mass (M17,T9)

10 - 25 ml/kg body weight; ± 1000 ml in adult patients?

Multidoses:

5 - 10 ml/kg body weight; ± 200 - 300 ml in adult patients?

Intracellular electrolyte equivalent cardioplegic solution

Induction:

7 - 10 ml/gm heart weight; ± 3 - 4 L in adult patients

C) Antegrade via the aortic root

Requires a competent aortic valve

Infusion pressure (A31,B74,J9,T5):-

Induction: 80 - 130 mm Hg

Reinfusions: 50 mm Hg

Affected by coronary vascular resistance

Stenoses (W14)

Vasoconstriction

Cold (B63)

Potassium (C21,S7)

Possible additional vasodilators?

Nitroglycerine (S46)

Adenosine (B41)

Magnesium (C21)

D) Perfusion through completed grafts (B18,B74)

E) Retrograde via the coronary sinus (B70)

Infusion pressure: less than 40 mm Hg (M35,M38)

Legend:

The recommended volume to be infused is for extracellular electrolyte equivalent crystalloid and blood cardioplegic solutions.

Larger volumes are required for intracellular electrolyte equivalent cardioplegic solutions.

Antegrade cardioplegia

The recommended antegrade pressure for infusing cardioplegic solutions is 80 - 100 mm Hg (A31,B70,J9). Excessive perfusion pressures (greater than 150 mm Hg) will injure the endothelium (J9,M53). However, one must take into cognizance that pressure gradients of 30 - 60 mm Hg may exist between the aorta and distal coronary arteries in the presence of coronary artery stenoses (R37). In addition, studies in normal sheep hearts showed that pressures of 130 mm Hg as opposed to 90 mm Hg delayed the onset of myocardial acidosis (T5). Thus, in the presence of coronary artery disease slightly higher infusion pressures (up to 130 mm Hg) on induction of antegrade cardioplegic arrest may be more optimal. However, a lower pressure of 50 mm Hg is recommended during maintenance reinfusions of cardioplegia in the arrested heart, in order to prevent oedema formation (B74,P27).

Pharmacologic vasodilators such as papavarine (S53) and nitroglycerine (1 mg bolus injection during infusion of cardioplegia) (M25,S46) have also been added to the cardioplegic solution in order to improve antegrade cardioplegic solution distribution, by optimally dilating the coronary arteries. However, although these vasodilators decrease coronary vascular resistance without altering systemic vascular resistance (M25), in a clinical trial these vasodilators did not alter the postoperative surgical results (S53). Moreover, it is possible that a "steal" syndrome could be produced by dilating responsive coronary arteries and thus decreasing the effective perfusion pressure in arteries with fixed stenoses supplying more ischaemic areas. Thus these additional vasodilators are probably not indicated in a properly formulated cardioplegic solution.

Volume of cardioplegia

The volume of cardioplegic solution infused clinically is usually 10 - 15 ml/kg body weight, *if extracellular electrolyte equivalent formulations are used.* However, the dose should ideally be rather determined by ventricular mass as opposed to body weight (M17,T9). The optimal dose for the St Thomas' cardioplegic solution is 2.0 ml/gm wet heart weight experimentally (T9), and in a clinical study of patients with left ventricular

hypertrophy 2.5 ml/gm LV mass (LV mass was determined angiographically) (M17). This dose might have been as much as 25 ml/kg body weight, depending upon the patients body weight. Other studies have also confirmed the safety and benefit of giving high volumes of crystalloid cardioplegia (E14). Nevertheless, Preusse et al have shown that temperature equilibration occurs within approximately two minutes of infusion of cold cardioplegia, but that myocardial oxygen consumption equilibration only occurs after a longer period of infusion (P27,P29). Buckberg et al using blood cardioplegia also contend that it is the duration of infusion that is more important than volume, as oxygen is taken up over time and not by dose, and recommend that cardioplegia is infused for 3 - 5 min at 250 - 350 ml/min (B74).

Therefore, when infusing cardioplegic solutions the minimum dose, perfusion pressure and duration of infusion should all be taken into account.

Volume infused = Infusion rate X Time

Infusion rate = Pressure / Resistance.

If infusion rate is kept constant, blood cardioplegia produces higher aortic infusion pressures than crystalloid cardioplegic solutions because of its increased viscosity, and consequently has an improved distribution (R24). However, if infusion pressure is kept constant with crystalloid cardioplegic solutions (which will necessitate higher flow rates in order to produce appropriate perfusion pressures), regional myocardial perfusion is then better with crystalloid as apposed to blood cardioplegia (E17).

Finally, *if intracellular electrolyte equivalent cardioplegic solutions are used*, a prolonged infusion time of 8 - 10 min is required in order to obtain complete equilibration of electrolytes in the interstitial space (B62,P27). Therefore, the recommended dose for the Bretschneider cardioplegic solution is an initial volume of 7 - 10 ml/gm heart weight infused over 8 - 10 min, which would be approximately 3 - 4 L in an average adult patient (P27,P28).

Noncoronary collateral flow

Following the induction of cardioplegic arrest, *diastolic arrest must be maintained throughout the ischaemic cross-clamp period.* However, two primary factors can unpredictably diminish the efficacy of myocardial protection in the clinical situation:-

i) Noncoronary collateral blood flow (B57,H36)

ii) Constant rewarming of the myocardium (Table 1.8)

Blood flow derived from the bronchial arteries (which can be removed by adequately venting the left side of the heart) can be substantial (B42,L1). In addition, although true noncoronary blood flow via posterior pericardial communicating vessels and pericardial adhesions may be minimal, there is a marked variability of this flow in both normal hearts and those with coronary artery obstructions. Noncoronary collateral flow can be substantial in hearts with left ventricular hypertrophy (15 % - 20 % (B57)), or coronary artery disease (6 % (H36)). If significant noncoronary flow is observed (filling of coronary arteries with blood during revascularization, backbleeding from the coronary ostia, recurrence of electrical activity), then this can be diminished by decreasing systemic flow rates and perfusion pressure (B74). In addition, because of the variability of noncoronary flow, as well as the known constant rewarming of the myocardium and the presence of small-amplitude electrical activity (F7,L6) occurring prior to visible mechanical activity (which is detrimental to myocardial protection) (L7), multidose reinfusions of cardioplegic solution are recommended (B54,B74).

Multidose cardioplegia

The strategy of administering multidose cardioplegia is recommended clinically (B74), as a result of experimental (C35,E9,S18,T4) and clinical studies (B54). Myocardial rewarming (D3,D5) and washout of cardioplegia by noncoronary flow (B57,H36) is counteracted, and in addition metabolic end products are removed and substrates resupplied to the myocyte (B74). Intermittent reinfusions of cardioplegic solutions correct metabolic acidosis, wash out lactate and allow reactivation of glycolysis (K38).

In the isolated rat heart model we demonstrated an independent beneficial effect of multidose cardioplegia, as opposed to single dose cardioplegia on postischaemic mechanical recovery (appendix A-2). However, multidose reinfusions also increased postischaemic washout of lactate dehydrogenase in our model. Thus this non-colloid containing solution might have increased protein flux from the interstitial tissue, or alternatively reinfusions of cardioplegia increased cellular membrane damage despite improved mechanical recovery. In our studies the cardioplegic solutions had been aerated with 95 % O_2 5 % CO_2 during preparation, resulting in partial pressures of oxygen of approximately 150 mm Hg. It is possible that intermittent reinfusions of oxygen containing solutions after periods of ischaemia generated oxygen free radicals (B8,S75), especially if partial pressures of oxygen are high (G5). Free radicals in turn can cause damage to cellular membranes (S27), by lipid peroxidation and denaturation of proteins (G5,M37,P32) (See section 1.6).

Nevertheless, multidose reinfusions of cardioplegia have been shown to be superior to single-dose cardioplegia (E9,S18), and should be reinfused approximately every 20 minutes throughout the cross-clamp period (B74). The volume of each multidose reinfusion should be approximately half the induction dose, but infused at a lower pressure (40 - 50 mm Hg).

Taken one step further, is the proposal for continuous cold blood cardioplegia (K15). However, this technique does not produce a "dry" operative field and does not therefore facilitate the surgical procedure.

Various other surgical strategies used during coronary artery revascularization procedures can also improve myocardial protection, by improving the distribution of the cardioplegic solution and preventing inflow limitation upon reperfusion (C23). These include:-

- Perfusing cardioplegia through the completed distal anastomoses during the cross-clamp period. (B74,K9,S46,S48),

- Perfusing grafts after removal of the cross-clamp, while proximal anastomoses are performed with a side-clamp (K9),
- Constructing all anastomoses during one single prolonged cross-clamp period (B18,C23,W14),
- Constructing proximal grafts before aortic cross-clamping.

Retrograde Cardioplegia

Retrograde delivery of cardioplegic solutions via the right atrium (F1) or coronary sinus is another effective alternative to ensure uniform distribution of cardioplegia, in the presence of coronary artery stenoses (S62). An additional experimental method is antegrade cardioplegic infusion with simultaneous pressure-controlled intermittent or continuous coronary sinus occlusion (L20,S81,S82). Although there is a great variation in the venous drainage of the myocardium, the majority of blood drains into the coronary sinus. The remainder of the myocardial venous blood drains via anterior cardiac veins that drain the anterior surface of the right ventricle (L17), and Thebesian veins that drain blood directly into the chambers of the heart. Therefore, retrograde cardioplegia may provide inadequate protection to the right ventricle (P6,S42,S78), and possibly parts of the septum (S42,S78). Nevertheless, there is an extensive valveless network of venovenous, arteriovenous and venoluminal anastomoses, that allows retrograde coronary sinus perfusates to permeate throughout all major areas of the myocardium (M35).

Experimental and clinical studies have confirmed that retrograde cardioplegia is superior to antegrade delivery in perfusing areas distal to occluded coronary arteries (G41,H1,M16,S39), and maintains preferential subendocardial flow (P6). Cannulation of the coronary sinus initially had to be performed under direct vision and was associated with lacerations and rupture of the coronary sinus (M39). However, newer auto-inflating catheters can be inserted "blindly" into the coronary sinus (D23,G43) and venous injury is prevented if infusion pressures never exceed 50 mm Hg (B70). Alternatively, retrograde cardioplegia can be infused via the right atrium if both cavae

are snared and the pulmonary artery occluded (D16,F1). Although right atrial cardioplegia is claimed to be safer (F1) with improved right ventricular protection (N2), it is cumbersome to work with since distention of the RV makes exposure of the posterior myocardium difficult. Moreover, other studies have shown that right atrial retrograde cardioplegia does not provide better right ventricular protection than coronary sinus cardioplegia (D17,S13).

Retrograde cardioplegia is a safe effective clinical method of delivering cardioplegic solutions (D23,F14,G43,M39,S21) and may be the method of choice in redo-revascularization procedures, by averting prograde embolization of atheromatous material from grafts (F17,L17,S57). However, induction of cardioplegic arrest with retrograde infusion of cardioplegia is associated with a prolonged time before arrest occurs (M39). Retrograde Bretschneider cardioplegia, although providing overall good protection, was also noted to cause mild extracellular oedema and microvascular damage (S21).

Nevertheless, promptness of arrest and inadequate right ventricular protection can be overcome by combining antegrade and sequential retrograde cardioplegia (B70,K2,P5), and in a clinical study this strategy improved ventricular recovery following coronary bypass surgery (B28). Combined antegrade / retrograde cardioplegia is probably the modern myocardial protection strategy of choice for patients with coronary artery disease, aortic stenosis, and for redo-revascularization procedures.

Normothermic cardioplegic arrest

Normothermic cardioplegic arrest prevents the increased cytosolic calcium and increased myocardial tone associated with rapid cooling of the myocardium (K43,T10,W31). In addition, enzymatic functions are more optimal at normothermia and hence provided there is adequate distribution of the cardioplegic solution, then

initial normothermic cardioplegic arrest can be used to provide "preischaemic" metabolic enhancement of the myocardium.

Preischaemic Metabolic Enhancement

Hearse et al showed that the time till onset of normothermic ischaemic contracture could be increased by either reducing myocardial energy demands or increasing energy supply (H18). Thus if hearts are depleted of high energy phosphates prior to the ischaemic period, tolerance to ischaemia is diminished. In the clinical situation many patients arrive in the cardiac theater with ongoing ischaemia, dependant on intra aortic balloon pumps, with recent myocardial infarcts, and the emergent nature may have precluded preoperative metabolic enhancement (see section 1.2). Initial repletion of energy stores prior to a prolonged ischaemic period would thus increase tolerance to the ischaemic cross-clamp period in these "energy depleted hearts". Intravenous GIK instituted at the onset of anaesthesia will assist in replenishing energy stores (H4). However, tolerance to ischaemia can also be increased by preischaemic normothermic arrest and continuous perfusion with an oxygenated substrate containing perfusate (P17). Normothermic induction of cardioplegic arrest and perfusion with substrate enriched blood cardioplegic solutions (Table 1.12), prior to hypothermic cardioplegic arrest, has been found to be beneficial both experimentally and clinically (B49,H48,R40,R44,T19,W31).

Furthermore, adequate distribution of this initial normothermic "resuscitating reperfusion cardioplegic solution" in the presence of critical coronary stenoses can now be overcome by using combined antegrade / retrograde cardioplegic infusions (B70). Normothermic induction is though probably not necessary in the standard low risk patient (R23).

Table 1.12

CONCENTRATE FOR 4:1 BLOOD CARDIOPLEGIA

WARM INDUCTION

Additive	Volume (ml)	Delivered concentration	
THAM (300 mmol/L)	225	pH	: ± 7.6
CPD	225	Ca^{++}	: ± 0.15 mmol/L
50% Dextrose H_2O	40	Osm	: ± 400 mOsm/kg H_2O
5% Dextrose H_2O	220	Glucose	: ± 70 mmol/L
KCl (2 mmol/ml)	40	K^+	: 20 - 25 mmol/L
Glutamate (7.32%)	125	Glutamate	: 13 mmol/L
Aspartate	125	Aspartate	: 13 mmol/L

Legend:
Infused at 37°C, at a rate of 250 - 350 ml/min till arrest, then at 150 ml/min for a total of 5 min. Thereafter, 250 - 350 ml/min standard cold (4°C - 8°C) cardioplegia is given for approximately 3 min. CPD: Citrate phosphate dextrose. Modified from Buckberg G D, J Cardiac Surg 1989; 4:216-238 (B70).

Warm heart surgery with continuous warm cardioplegia

An alternative approach to hypothermic cardioplegic arrest is the concept of continuously perfusing the arrested heart at normothermia (P3), either antegrade (L36) or retrograde (S11) with blood cardioplegia. Continuous warm blood cardioplegia is though more cumbersome and does not allow optimal visualization of microvascular anastomoses (S10), although it reportedly decreases the incidence of postoperative conduction disturbances (F38), avoids possible disadvantages of hypothermia, and may be better than continuous cold cardioplegia in patients who have limited ischaemic tolerance (L35). Furthermore, as myocardial energy demands are higher the "safety element" is possibly lower, especially as adequate perfusion to all regions of the myocardium cannot always be ensured (E8). Thus, continuous warm blood cardioplegia must be given as a continuous retrograde technique and not antegrade in the presence of coronary artery occlusions, in order to ensure adequate perfusion of all the myocardium distal to coronary stenoses (M66). Furthermore, metabolic monitoring is possibly necessary in order to ensure that the retrograde flow (50 - 350 ml/min) adequately meets the metabolic requirements of the warm heart. In addition, "overdosing" with

cardioplegia (K25) or its components (eg. K^+, THAM) is possible, and therefore a different crystalloid concentrate is used for continuous warm cardioplegia as opposed to that used for intermittent cold cardioplegia. Continuous warm retrograde cardioplegia also does not result in superior myocardial protection when compared to intermittent cold combined antegrade and retrograde blood cardioplegia (M67).

Conduction defects following cardioplegic arrest

Conduction defects, either episodes of transient or permanent atrioventricular block or postoperative supraventricular tachyarrhythmias, have been observed following cardioplegic arrest. Postoperative new atrioventricular conduction block occurs in 18 % - 45 % of coronary artery bypass graft patients (B1,O1,W17), but the majority resolve by hospital discharge (B1,O1). These conduction defects have been related to the number of vessels bypassed, the duration of both cardiopulmonary bypass and aortic cross-clamping (B32), preoperative longstanding hypertension, left main coronary artery disease, use of digitalis (W17), use of cardioplegia as opposed to hypothermic fibrillation (O1), with blood more than crystalloid cardioplegia (G42), and with intermittent cold blood cardioplegia as opposed to continuous warm blood cardioplegia (F38). Furthermore, endogenous release of adenosine (B20), inadequate cooling of the right atrium (M3), air in the atrioventricular nodal artery, and infusions of high concentrations of potassium (E5) are factors that have also been implicated in increasing conduction defects. Nevertheless, these conduction defects are probably due to ischaemia and incomplete protection of the specialized conduction system (M60), although conduction tissue has higher concentrations of glycogen, glycolytic capacity (B62) and is possibly more resistant to hypoxia than normal myocardium (W34). Moreover, low-amplitude electrical activity that is not detectable on routine ECG monitoring can occur during cardioplegic arrest (F8), and this has been shown to arise from the conduction tissue (F7). Both sinoatrial and atrioventricular nodal tissue has intrinsic electrical activity, as a result of calcium-mediated slow channel activation. Although potassium cardioplegia

prevents activation of myocytes by partial depolarization (-40 mV), this is the potential at which slow channel calcium conduction is increased. Thus standard intermittent hypothermic potassium cardioplegia provides incomplete protection of the conduction system (M3), and continuous warm retrograde blood cardioplegia may more adequately prevent ischaemia to the conduction tissue (F38). Nevertheless, the incidence of atrioventricular dissociation arrhythmias following intermittent hypothermic cardioplegia can be decreased by using lower concentrations of potassium in all multidose reinfusions of cardioplegia (D15,E5). Alternatively, conduction system activity can also be prevented by using a calcium channel blocker - nifedipine (F7), or intracavitory right atrial cooling (M3).

In addition, neither hypothermic cardioplegic arrest with intermittent cold cardioplegia nor continuous warm retrograde cardioplegia adequately protects the right atrium (C18,P15,F38), and poor postischaemic atrial contraction can diminish cardiac output by as much as 20% (E5). Furthermore, persistent atrial activity during cardioplegia and ischaemic injury may cause an increased incidence of postoperative supraventricular tachyarrhythmias (M63,T13). Methods of improving atrial hypothermia by either internal or external cooling improves atrial preservation and diminishes conduction abnormalities (C15,C16,H38). The incidence of postoperative supraventricular tachycardias can also be decreased by prophylactic magnesium supplementation (F4).

Hence, additional methods of preventing ischaemia during the aortic cross-clamp period are important, such as ensuring adequate hypothermia if hypothermic cardioplegic arrest is used, especially for the right atrium, right ventricle and specialized conduction tissue.

1.6 REPERFUSION OF THE POSTISCHAEMIC HEART

Myocardial ischaemia causes progressive cellular injury proportional to the duration of ischaemia and rapidity of energy depletion, and if severe enough will result in myocyte necrosis (B75). However, mild to moderate ischaemia can result in temporary postischaemic myocardial dysfunction, despite the absence of irreversible damage or structural changes; *myocardial stunning* (B40,B55). Furthermore, reperfusion of previously ischaemic tissue can also accelerate or induce myocyte damage and necrosis, that was undetectable prior to the onset of reperfusion; *the reperfusion injury* (B75,G3,H15,V19).

Myocardial "stunning"

The mechanism of myocardial stunning is multifactorial, and has been postulated to be due to oxygen free radicals, excitation-contraction uncoupling and impaired calcium homeostasis (B40,P14), and may require several days of reperfusion prior to complete reversal (B55,B78). Postischaemic injured hearts, even if they were protected with cardioplegic solutions, are unable to fully utilize available oxygen in the immediate reperfusion period (B17,F30,K35,K36), because of diminished mitochondrial function (K3). Furthermore, both glucose and free fatty acid uptake are inhibited in the postischaemic period partly as a result of stress hormone induced insulin suppression (S89), and thus amino acids are the only exogenous substrate taken up by the heart during initial reperfusion (S87). Loss of Krebs' cycle and purine metabolites during the ischaemic period will also limit postischaemic myocardial function and recovery (H3,P7). In addition, a progressive increase in coronary vascular resistance has been noted to occur during the reperfusion period clinically (D18), and is possibly a manifestation of the no-reflow phenomenon (G6).

Reperfusion injury

When oxygen is reintroduced into the myocardium after a period of anoxia or hypoxia, structural cellular changes occur within two minutes of reoxygenation (G3). This results in release of intracellular enzymes and rupture of the sarcolemma (G3), and is associated with mitochondrial calcium uptake (H19). The pathogenesis of this reperfusion injury has been related to a number of factors such as oxygen free radical production (B12), resynthesis of ATP in the presence of intracellular calcium overload causing hypercontracture and tearing of cell membranes (P20), and loss of essential metabolites and substrates. The reperfusion injury is an exacerbation of cellular damage upon reperfusion and can be attenuated by either supplying adequate substrates, myocardial oxygenation, maintaining better cellular high energy phosphate levels during ischaemia (K6), preventing sudden energy-dependent mitochondrial calcium accumulation with respiratory inhibitors (G3), or by supplying calcium chelators (P14) during reoxygenation. Hence, the concept of reperfusing the postischaemic myocardium with a specific reperfusion solution designed to prevent a reperfusion injury.

Reperfusion solutions

Modifying the initial perfusate on reperfusion of ischaemic myocardium can alleviate both myocardial stunning and the more severe reperfusion injury. In principal this has been based on; maintaining the heart arrested (high potassium) to channel all available oxygen to cellular repair, lowering the ionic calcium (0.5 mmol/L) to limit calcium influx while membrane permeability is increased (F24), an alkalotic pH (pH 7.8; approximates the "ideal pH" at 28°C for optimal enzymatic function (S91,W19)), improved buffering (THAM) to counteract acidosis (F25), increased osmolarity (360 mOsm/L) to counteract cellular oedema (F24,S19), and substrate enhancement with glucose (S4) and amino acids (L-glutamate (H3,L18,M32), L-aspartate (B70,E13,R41)) to replace intermediates lost during the ischaemic period and for myocardial energy production.

The composition of the present reperfusion blood cardioplegic solution recommended by Buckberg is tabulated in Table 1.13 (B70). Additional substrates that might also be beneficial during reperfusion are purine precursors; adenosine and ribose (H3,P7).

Reperfusion of the postischaemic myocardium should also be at a controlled lower pressure (less than 50 mm Hg) which prevents oedema formation (S94), diminishes endothelial cell damage (S17) and improves recovery (A6), and should in addition possibly be pulsatile (M58).

Table 1.13

CONCENTRATE FOR 4:1 BLOOD CARDIOPLEGIA

	REPERFUSION SOLUTION		
Additive	Volume (ml)	Delivered concentration	
THAM (300 mmol/L)	225	pH	: 7.5 - 7.6
CPD	225	Ca^{++}	: 0.15 - 0.25 mmol/L
50% Dextrose H_2O	40	Osm	: ± 400 mOsm/kg H_2O
5% Dextrose H_2O	200	Glucose	: ± 70 mmol/L
KCl (2 mmol/ml)	15	K^+	: 8 - 10 mmol/L
Glutamate (7.32%)	125	Glutamate	: 13 mmol/L
Aspartate	125	Aspartate	: 13 mmol/L

Legend:
Infused at 37°C, at a rate of 150 ml/min antegrade 1.5 min, retrograde 1.5 min. Systemic rewarming commenced 3 - 4 min before delivery. CPD: Citrate phosphate dextrose. Modified from Buckberg G D, J Cardiac Surg 1989; 4:216-238 (B70).

Although Rosenfeldt et al suggested a low pressure was not necessary, there protocol included an initial 2 min period when the reperfusion pressure was gradually increased from zero to the test pressure (R37). Thus it is possible that this low pressure is only important for the first 2 min and not 10 min as used by Swanson et al (S94). Although Buckberg and co-workers initially reperfused the postischaemic heart at 28°C (F24,F25), Swanson et al showed no difference between reperfusion at 28°C or 37°C (S94), and reperfusion is now usually done at normothermia (B70,T4). However, lower initial reperfusion temperatures may be necessary if temperatures during isolated organ

storage were very low, or if calcium-free storage solutions (K28) or asanguineous reperfusion solutions are used (M31,M32). The dose of the reperfusion solution should also be limited, to prevent possible myocardial depression related to excessive administration of cardioplegic solution (K25).

In studies on regional ischaemia, which potentially imposes greater reperfusion damage than global ischaemia, an even lower calcium concentration (0.15 - 0.25 mmol/L) (A19), greater hyperosmolarity (greater than 400 mOsm/L), and hyperglycemia (greater than 400 mg%) (O9) is beneficial. However, this might also be a manifestation of a diluting effect from collateral flow into the regionally reperfused ischaemic myocardium. Nevertheless, the importance of decompressing the heart with a left ventricular vent (A20,A23,V20), and initial low pressure reperfusion (O8) with a blood reperfusate was again demonstrated in this model, if optimal functional recovery is to be obtained (A21).

Asanguineous reperfusion solutions can also improve postischaemic recovery. Both a standard cardioplegic solution (R13) and an asanguineous solution with similar constituents to that proposed by Buckberg and co-workers have been shown to be beneficial, but at 28°C (M31,M32). Thus, the provision of oxygen in a reperfusion solution is not the major component that is beneficial (A7), but rather other factors such as calcium content. In an asanguineous reperfusate the optimal calcium is also 0.3 - 0.5 mmol/L (H5). In addition, washout of accumulated metabolic end products and removal or suppression of oxygen free radicals is possibly of equal importance (J13,M31). However, in contrast to blood reperfusates that contain natural free radical scavengers, additional free radical scavengers may be essential in asanguineous reperfusates (A8,J13).

Oxygen free radicals

A free radical is a molecule with an odd number of electrons, and is therefore unstable and chemically reactive (M37). Free radicals occur in vivo and contribute to pathologic

processes such as carcinogenesis (P32) and complement mediated pulmonary damage (T18). Free radical scavengers such as superoxide dismutase (SOD) are found in all aerobic organisms (F31), and deficiencies of these endogenous defense mechanisms are associated with defects in oxygen tolerance (A30,F31). Furthermore, oxygen free radicals are probably formed during ischaemia (L49,M37) but in addition are generated to a much greater extent during reperfusion after an ischaemic period, as their production is dependant upon oxygen tension (generation of free radicals is increased if the pO_2 is greater than 150 mm Hg) (G5). Free radicals are thus primarily generated when oxygen is reintroduced after a period of ischaemia (Fig 1.4), and cause myocardial injury (S27) by lipid peroxidation and denaturation of proteins (G5,M37,P32,S27). The hydroxyl radical is considered to be the most cytotoxic of the known oxygen free radicals (C19,M37), although the superoxide anion possibly causes the most impairment to endothelium-dependant functions (L16,V11).

There are a number of different mechanisms whereby free radicals are generated (P32). However, probably the three primary sites of free radical generation in the reperfused ischaemic myocardium are:-

Intra-mitochondrial,

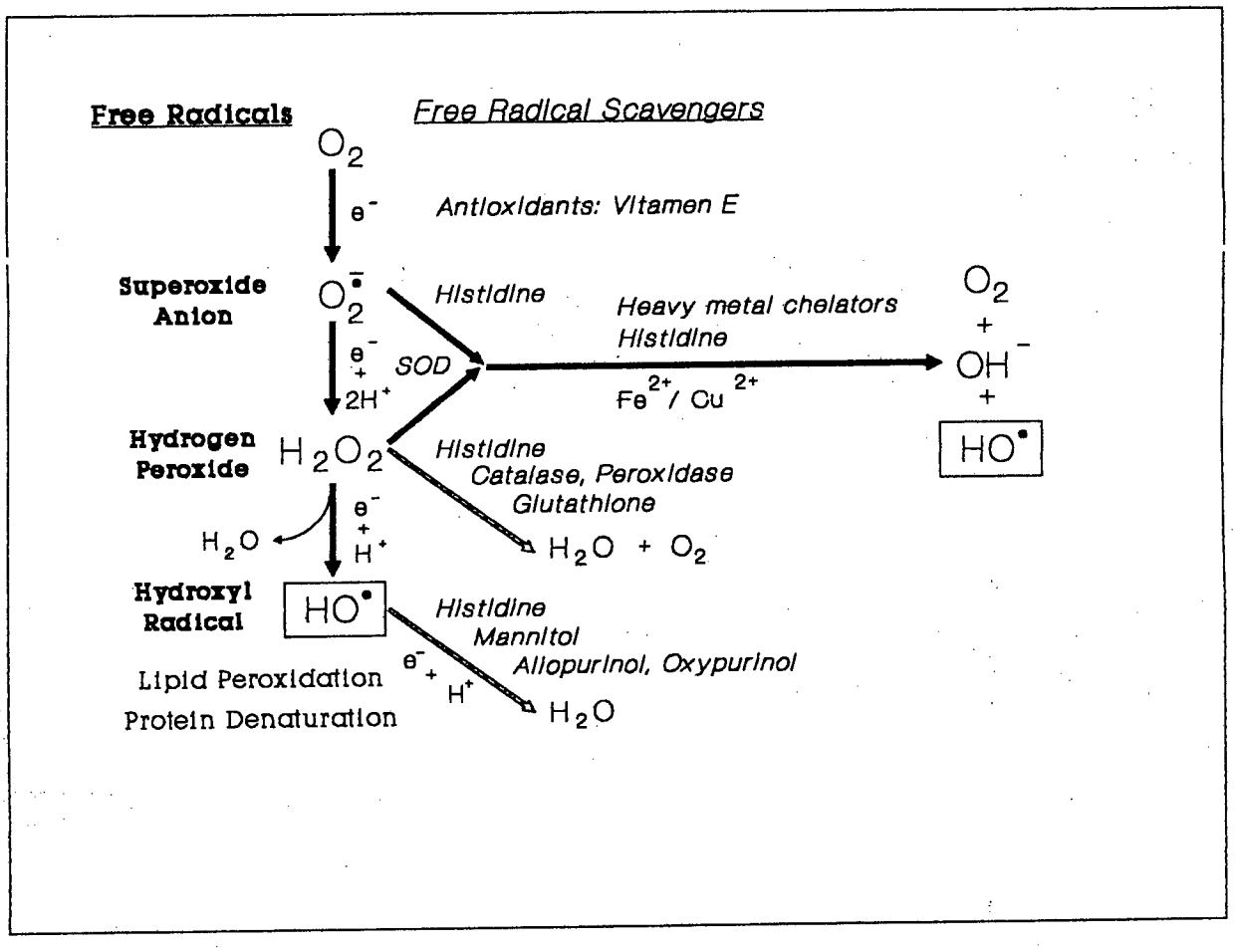
Endothelial; xanthine oxidase mediated,

Polymorphonuclear leukocyte mediated.

Mitochondrial cytochrome oxidase transforms 95 % of oxygen in a tetravalent reduction into water, in the electron transport system. However, 5 % of available oxygen is diverted toward a univalent reduction pathway in which electrons are gained in a sequential fashion with the formation of oxygen free radicals (Fig 1.4) (F31). Nevertheless, intracellularly formed free radicals are normally inactivated by intracellular defence mechanisms (superoxide dismutase, glutathione) (F31,M33,M37).

Figure 1.4

GENERATION OF OXYGEN FREE RADICALS



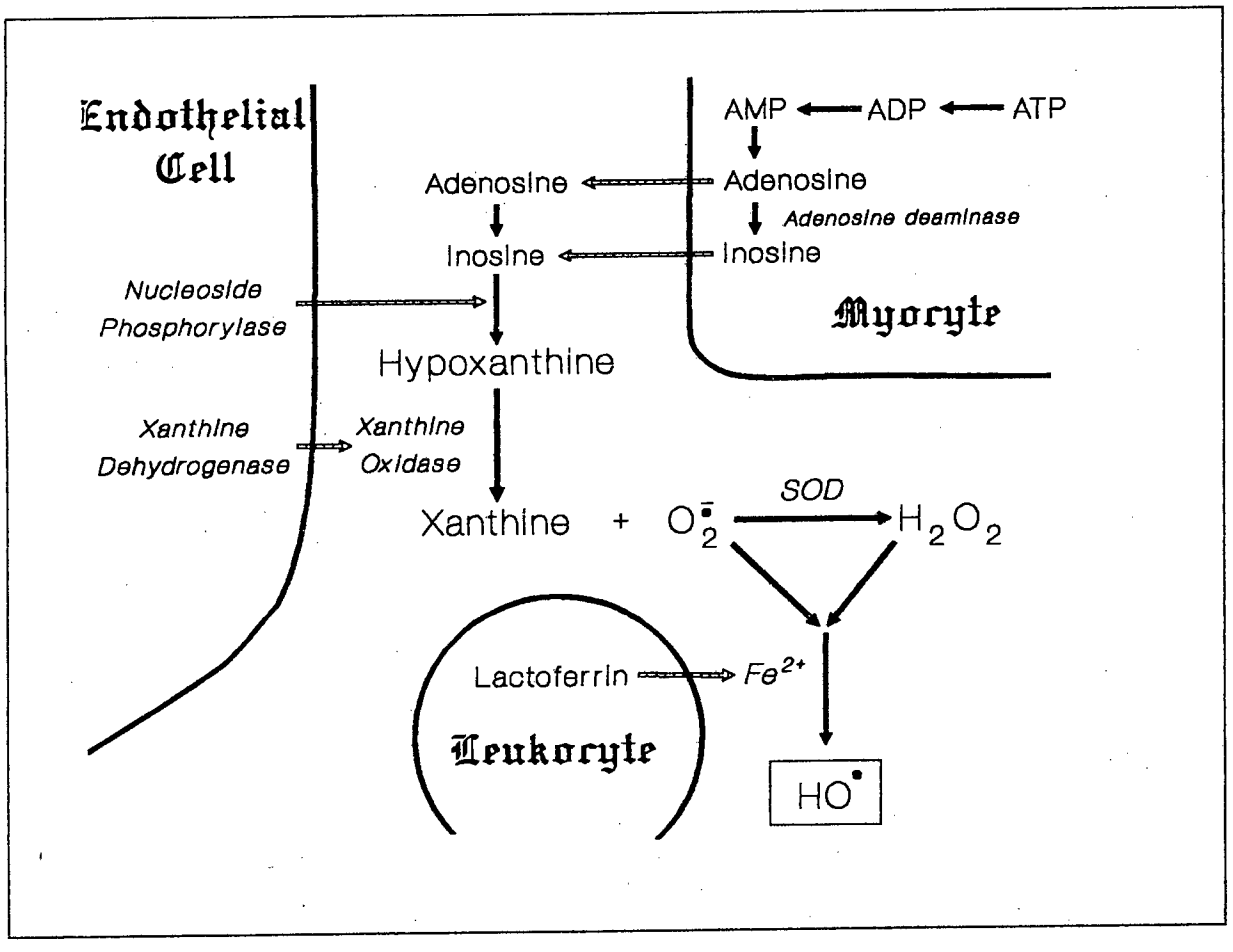
Legend:

The highly toxic hydroxyl radical can be generated by either the univalent reduction pathway of oxygen, or from the combination of superoxide anion and hydrogen peroxide by the Haber-Weiss reaction using a transition metal catalyst. The sites of action of free radical scavengers (italics) are also shown.

Myocardial ischaemia results in metabolic changes that predispose to oxygen free radical generation. Ischaemic induced free radicals are probably produced by the latter two mechanisms of free radical production (xanthine oxidase and polymorhonuclear leukocyte mediated (Fig 1.5)) (M37,S27). Adenine-nucleotides are broken down in the myocyte only to the stage of inosine during ischaemia (G13,S27). Although the enzyme required for further degradation (xanthine oxidase) may be present in human myocardium in only trace amounts compared to the rat and dog (E1), xanthine dehydrogenase is found in endothelial cells of capillaries (J3,J4,S27). Xanthine

dehydrogenase can then be converted to xanthine oxidase by cytosolic proteases (M22). In addition, ischaemia damages the endothelium (F12,H55) and thus increases the release of xanthine dehydrogenase which is rapidly converted to xanthine oxidase in the interstitium (J3,R58). Myocyte released inosine can therefore be degraded to hypoxanthine, that in turn is oxidized by endothelial cell released xanthine oxidase, which generates the superoxide anion in the presence of oxygen (P32).

Figure 1.5
FREE RADICAL GENERATION AND CELLULAR INTERACTIONS



Legend:
Free radicals are generated during reperfusion from inosine released from ischaemic myocytes, reacting with xanthine oxidase released from endothelial cells, and iron released from polymorphonuclear leukocytes.

In addition, injured endothelial cells attract and activate polmorphonuclear leukocytes (K24,M28,R46), which release proteolytic enzymes and generate oxygen free radicals (D20,K24). Moreover, leukocytes by releasing lactoferrin make "free" iron available that catalyzes the Haber-Weiss generation of the hydroxyl radical (Fig 1.5) (C19,M37). Cardiopulmonary bypass itself also activates complement, neutrophils (F5), and generates free radicals that have been implicated in cardiopulmonary induced pulmonary damage (K13,R54,T18). Free radicals in turn will result in further endothelial injury (T18,T24). Thus the primary sites of action of free radical scavengers during postischaemic reperfusion are probably the vascular endothelium and leukocytes, and not the myocyte (I1).

Free radical scavengers

Free radicals are generated during ischaemia and during reperfusion with the reintroduction of oxygen (B8,L49,S75). The metabolic changes that would otherwise lead to oxygen free radical generation upon postischaemic reoxygenation could be prevented by providing adequate myocardial oxygenation during the ischaemic period (B9,C6). However, this is probably rarely achievable and thus the importance of additional free radical scavengers. A number of compounds can reduce free radical mediated cellular injury; low molecular weight antioxidants (Vitamin E), heavy metal chelators (deferoxamine) (C19) and enzymatic scavengers (superoxide dismutase, catalase, reduced glutathione, peroxidase) (M37). Moreover, some of these substances such as histidine can act at a number of sites and unlike mannitol (G5,M4) in low concentrations (C19) (Fig 1.4). However, superoxide dismutase scavenges the superoxide anion (F31) by catalyzing its conversion to hydrogen peroxide. Thus superoxide dismutase can also enhance biological damage by increasing the formation of hydrogen peroxide which is the substrate for the generation of the hydroxyl radical, unless the hydrogen peroxide formed is also reduced (C19). Free radical scavengers are thus beneficial in both cardioplegic and reperfusion solutions, but must be present

before the onset of reperfusion (B9). Moreover, one of the salutary effects of blood cardioplegic and reperfusion solutions are the natural oxygen free radical scavengers contained in erythrocytes (J13,V1).

Measures can also be taken in the preoperative period to decrease the postischaemic generation of free radicals. Preoperative administration of allopurinol as well as increasing concentrations of its metabolite oxypurinol prevent the formation of free radicals by inhibiting xanthine oxidase (Fig 1.5) (S27), scavenge the hydroxyl radical (T2), and decrease perioperative complications after cardiac surgery (see section 1.3). The addition of allopurinol to both blood cardioplegia (1 mmol/L (V18)) and crystalloid cardioplegia (St Thomas'; 0.15 mmol/L (C12)) has also been shown to improve postischaemic functional recovery, and diminish free radical induced chromosomal aberrations (0.75 mmol/L) (E6). However, Myers et al showed that catalase (1.7 ug/ml) was more efficacious than allopurinol (1 mmol/L) in the asanguineous St Thomas' solution (M65). Superoxide dismutase by itself is ineffective (M33,M65) as it results in increased hydrogen peroxide (C19). However, the combination of superoxide dismutase and catalase in crystalloid cardioplegic solutions (B9,G17,G18,G32,J15,S76) and blood reperfusates (O24) have been shown to improve myocardial preservation. The best protection against free radicals is possibly provided by both inhibiting the production of the superoxide anion (allopurinol) and enhancing the degradation of hydrogen peroxide (M65) (catalase, peroxidase, reduced glutathione (10 mmol/L (C11)).

Leukocyte induced free radicals can also be inhibited by administering systemic heavy metal chelators such as deferoxamine (10 - 30 mg/kg), which remove the catalyst required for the Haber-Weiss reaction (Fig 1.4 & 1.5) (C33,M36). Deferoxamine has also been added to crystalloid cardioplegic solutions (0.04 - 1.5 mmol/L) both experimentally (M34) and clinically (F10,M36) and this improved myocardial preservation. Metal chelators may well be even more salutatory in the presence of blood and therefore leukocytes (M65); i.e. blood cardioplegic (I2) or reperfusion solutions, than crystalloid solutions that do not contain leukocytes. Alternatively, either

preventing leukocyte activation with phosphodiesterase inhibitors (antileukocyte action mediated by increased cAMP (C13)) or leukocyte depletion via polyester filters can ameliorate both free radical mediated lung injury (B7) and myocardial reperfusion injury (B58,C13,P19). However, leukocyte depletion is not as efficacious as a substrate-enriched blood reperfusion solution (K26).

There is thus strong evidence for both the production of oxygen free radicals and the benefit of using free radical scavengers in both cardioplegic, reperfusion and organ preservation solutions.

Clinical management of the postischaemic myocardium

In non-emergency open-heart surgical procedures reperfusion solutions are not used routinely by probably the majority of cardiac surgeons. Nevertheless, correct management of the postischaemic myocardium can also significantly improve myocardial recovery.

In the early postischaemic period haemodynamic stresses should be avoided (F30). Ventricular fibrillation should be prevented by either components added to the cardioplegic solution such as lignocaine (F15), procaine or calcium channel blockers, or by a systemic bolus of lignocaine (2 mg/kg) infused immediately prior to the release of the aortic cross-clamp (F3). Furthermore, allowing the heart to remain asystolic in the immediate reperfusion period is beneficial by preventing energy from being "wasted" on electromechanical contraction, provided ventricular distention does not occur (A12,L30). Thereafter, while remaining on cardiopulmonary bypass myocardial energy demands should be maintained as low as possible, in order to allow available energy to be channeled to reparative processes. Thus the heart should be allowed to beat but in a

non working state, that is *not ejecting*, which decreases O_2 demand by more than 40% (Fig 1.2) (B73), and perfusion pressure and oxygenation should be optimized.

Persistent postoperative insulin resistance due to catecholamine induced trauma metabolism can also be overcome with postoperative glucose-insulin-potassium infusions (S88,S89). Furthermore, instead of using inotropic agents which increase myocardial oxygen demand and may aggravate trauma metabolism, heart rate should be optimized with external pacemakers, and preload and afterload optimized by the appropriate use of volume expanders and vasodilators. However, excessive volume or pressure loading of the ventricle will also increase myocardial oxygen demands (V17), which should be kept as low as possible in the postoperative period (F30).

There are a number of causes of postoperative low cardiac output (Table 1.14). Although, inadequate intraoperative myocardial protection and myocardial stunning are major causes of postischaemic low cardiac output, other possible causes must first be actively excluded.

Table 1.14

ETIOLOGY OF POSTOPERATIVE LOW CARDIAC OUTPUT

Haemodynamic:

- Inappropriate preload,
  - Hypovolaemia,
  - Hypervolaemia; LA pressure > 20 mm Hg.
- Inappropriate afterload,
  - Vasoconstriction; SVR > 1500 dynes·s·cm^{-5},
  - Excessive vasodilation; SVR < 600 dynes·s·cm^{-5}.
- Arrhythmias
  - Bradycardia, or tachycardia,
  - Loss of synchronized atrial contraction.
- High Pulmonary vascular resistance
  - PVR > 250 dyne·s·cm^{-5}
- Cardiac Tamponade

Metabolic Causes:

Hypoxia, hypercarbia, acidosis, adrenal insufficiency.

Coronary Air or Particulate Matter Embolism:

A small amount of air remaining in the left side of the heart can easily enter the coronary arteries (L33).

Surgical Causes;

- Myocardial ischaemia
  - Acute myocardial infarction
  - Stenosis, kinking, or spasm of coronary artery bypass grafts,
  - Incomplete myocardial revascularization,
- Mitral valve replacement induced loss of LV function.
- Residual uncorrected cardiac defect

Inadequate Myocardial Protection:

- Composition of cardioplegic solution,
- Inadequate distribution of cardioplegic solution,
- Rapid rewarming of the myocardium;
  - Temperature of cardioplegia,
  - Noncoronary collateral flow,
  - High systemic flow rates.

Other causes:

- Pre-existing ventricular dysfunction
- Allergic reactions
- Urinary retention.

Legend:

Post operative low cardiac output is defined to be present if the cardiac index is less than 2.0 L/min/m^2 in the presence of an adequate preload.

A period of at least 15 - 30 min of optimal reperfusion following the release of the aortic cross-clamp should be allowed prior to introducing inotropic agents, as premature postischaemic use of calcium (A2) and inotropic agents accentuates myocardial damage and impairs functional recovery (K1,L19). Furthermore, an additional therapeutic intervention that could be considered for postoperative low cardiac output is triiodothyronine supplementation (C29,D27,H45,N13). If myocardial failure persists, despite above mentioned resuscitative measures, an intra aortic balloon pump (K5) should be inserted. An IABP both increases myocardial energy supply (Fig 1.1) and decreases myocardial energy demands (S15). Thereafter, if a low cardiac output persists then a left ventricular assist device can be inserted, if indicated (A9).

Chapter 2

MODELS USED TO FORMULATE SOLUTIONS

Cardioplegic solutions need to be developed and evaluated in animal models prior to human use. Thereafter, multivariate clinical trials of experimentally investigated solutions are valuable and necessary. However, the multitude of uncontrolled variables, as a result of the disease processes necessitating cardiac surgery, makes evaluation of cardioplegic solutions difficult in the clinical situation.

In animal studies, it is important to undertake evaluations in more than one species because of interspecies differences. Hearse D J, Jynge P and co-workers evaluated the St Thomas' Hospital No 2 cardioplegic solution in both rat and canine models (J23). However, the ideal species for evaluating solutions intended for human use is possibly the primate, as it is one of the closest species to man. In this thesis three models were used to assess the efficacy of cardioplegic solutions used in South Africa, and experimental modifications of the St Thomas' Hospital cardioplegic solution.

All experimental animals used in our laboratory received care according to the "Principles of laboratory animal care" of the National Society for Medical Research and the "Guide for the care and use of laboratory animals" prepared by the National Academy of Sciences (NIH Publication No 80 - 23, revised 1978). In addition, experimental protocols were reviewed and authorized by the Animal Research Review Committee of the University of Cape Town.

2.1 ISOLATED RAT HEART

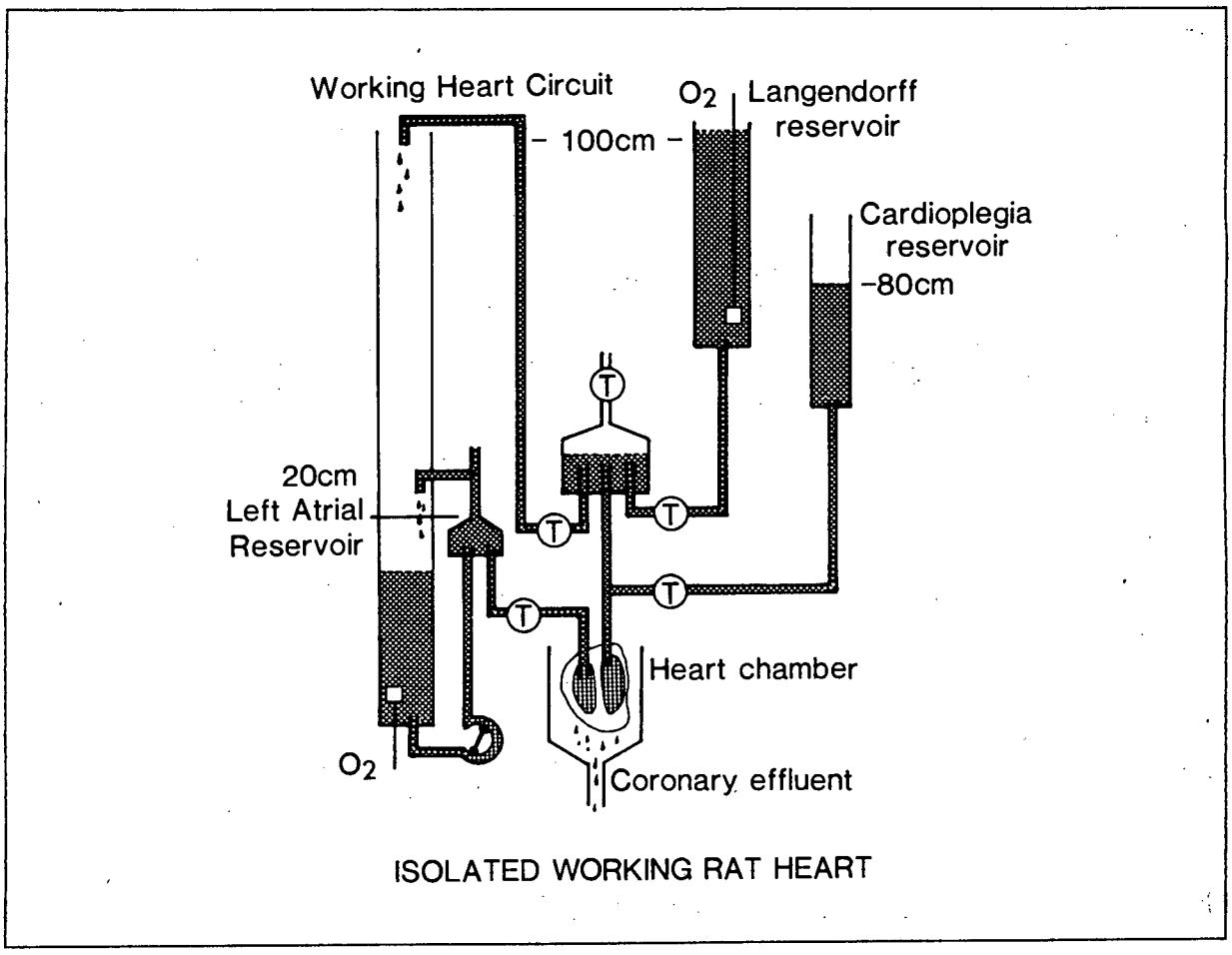
The isolated perfused rat heart was used as the experimental model for the majority of studies in this thesis. This model has two components; the Langendorff preparation and the working heart model.

Langendorff preparation

The Langendorff model was originally described in 1895 (L10), and since then has been used extensively to study cardiac metabolism in a number of different animals (H16). Male rats of the Long Evans strain and maintained on a standard diet were used for our experiments.

The rat was anaesthetised with diethyl ether, heparinized with 200 U heparin injected into the exposed femoral vein, and the heart was then carefully but rapidly excised and placed into cold (4°C - 10°C) Krebs-Henseleit buffer (Table 2.1). The cold temperature arrests the heart and thus provides some protection to the myocardium during this initial ischaemic period. The aorta was then cannulated and the heart mounted on the perfusion apparatus (Fig 2.1), within one minute of excision. Normothermic (37°C) Krebs-Henseleit buffer oxygenated with 95 % O_2 5 % CO_2 (pO_2 greater than 600 mm Hg) was filtered through a cellulose filter (0.8 um size pores; Millipore corporation, Bedford, Massachusetts, USA) and then allowed to flow retrograde into the aorta at a constant pressure of 100 cm H_2O (75 mm Hg), thus establishing coronary perfusion. Normal sinus rhythm recommenced immediately. The pulmonary artery was then incised to ensure adequate drainage of coronary effluent, and the heart was maintained at 37°C in a water jacketed chamber. This model thus allows the perfused heart to continue beating in a nonworking mode, and was used to stabilize the experimental hearts prior to conversion to the working model. In addition, this mode of perfusion was also used for the initial reperfusion of the postischaemic heart.

Figure 2.1



Legend:
Three way taps (T) allow easy conversion to either Langendorff mode, the working heart circuit or infusion of cardioplegic solutions. Reprinted from J Thorac Cardiothorac Surg 1991; 102:405-412 (appendix A-2) with permission Mosby Year Book Inc.

Working heart model

In order to allow the heart to perform external work so that mechanical performance could be monitored, the Langendorff model was then converted to the working model originally described by Neely and colleagues (N6). The left atrium was cannulated through the pulmonary veins, and filled with oxygenated Krebs-Henseleit buffer at a constant pressure of 20 cm H_2O (15 mm Hg). The left ventricle then spontaneously ejects the perfusate against a hydrostatic pressure of 100 cm H_2O (75 mm Hg), and the coronary flow was collected and recirculated. Aortic pressure, aortic flow, coronary

flow, and heart rate were then recorded in the control working period, prior to the ischaemic period, and also in the postischaemic recovery period.

Cardioplegic solutions were infused into the aorta at a pressure of 80 cm H_2O (60 mm Hg) prior to the ischaemic period, by simultaneously closing the aortic and atrial cannulae (Fig 2.1), and infusing the cardioplegic solution through a side arm of the aortic cannula. Constant hypothermia (10°C) was maintained throughout the ischaemic period by another separate cold (10°C) water-jacketed chamber.

Table 2.1

KREBS-HENSELEIT BUFFER

Compound	mmol/L	gm/L	Electrolyte	mmol/L
NaCl	118	6.9	Na^+	143
$NaHCO_3$	25	2.1	K^+	6
KCl	4.8	0.36	Ca^{2+}	1.25
KH_2PO_4	1.2	0.16	Mg^{2+}	1.2
$CaCl_2 \cdot 2H_2O$	1.25	0.18	Cl^-	125.3
$MgSO_4 \cdot 7H_2O$	1.2	0.3	PO_4^{3-}	1.2
D-Glucose·H_2O	11.1	2.2	Glucose	11.1

Experimental protocols

All hearts were stabilized for 10 minutes in the Langendorff mode, prior to changing to the working model. Preischaemic control haemodynamic parameters were measured at 2, 5 and 10 minutes during this working period. Any heart that was unstable during this period was rejected. The 10-minute values were then recorded as the preischaemic controls for future comparisons.

Cardioplegic arrest was induced by infusing 10 ml of cold (10°C) cardioplegic solution into the aorta. In the experiments in which multidose reinfusions of cardioplegia were

provided, 6 ml of cardioplegia was reinfused every 30 min throughout the 3-hour ischaemic period.

At the end of the ischaemic period each heart was reperfused in the Langendorff mode with standard oxygenated Krebs-Henseleit buffer (37^{o}C) for 10 - 20 min at a pressure of 80 - 100 cm H_2O (60 - 75 mm Hg) according to individual experimental protocols. The hearts were then converted to the working model for a further 10 minutes, at which time the postischaemic haemodynamic parameters were recorded. The coronary sinus effluent was collected during the ischaemic period when multidose cardioplegia was administered, as well as during the postischaemic Langendorff reperfusion period. At the end of the experiments the hearts were freeze-clamped with Wollenburg tongs to determine their high-energy phosphate content.

Exclusion Criteria:

In the preischaemic control period the following values were used to discard hearts:-

Aortic output < 30 ml/min

Coronary flow > 22 ml/min

Heart rate < 200 beats/min, or irregular rhythm

In the postischaemic period any heart whose coronary flow increased significantly by more than 50 % - suggesting a left atrial leak - was excluded.

Parameters of recovery.

To evaluate the efficacy of myocardial protection both mechanical, metabolic and morphological parameters may be measured (H16). However, ultimately the most important parameter is mechanical recovery, which was measured under controlled conditions (both preload and afterload), in this isolated rat heart model.

Haemodynamic parameters

Cardiac output (CO) in this preparation is the sum of aortic flow (AO) and coronary flow. Stroke volume (SV) was then calculated using the following formula:

$$SV = CO / HR \ (ml/beat)$$

Values obtained during the postischaemic working period were expressed as a percentage of their individual preischaemic control values. Results were then presented as percentage means and standard errors of percentage means.

Metabolic parameters

Myocardial enzyme leakage (creatine kinase, CK; lactate dehydrogenase, LDH) although not cardiospecific, are thought to indicate damage to cellular membranes. High levels are thought to indicate severe cellular damage although there is a degree of enzyme leakage in nonischaemic healthy tissue (H16). CK-MB levels of greater than 133 U/L within 15-hours of operation will identify 96 % of perioperative infarcts in clinical studies (V5). The enzyme LDH (mU/gm) was used in the studies in this thesis, and was measured enzymatically by the method of Bergmeyer and Bernt (B24). End products of glycolytic metabolism were also measured in some experiments: lactate (umol/gm) was measured by the method of Gutmann and Wahlefeld (G44), and oxygen uptake by subtracting venous from arterial oxygen content (B63).

The weight of the rat hearts was calculated from their body weights using a formula derived from previous laboratory measurements of both heart and body weight of rats of this strain (unpublished data).

$$\text{Heart weight} = (\text{Rat weight} + 75.18) / 359.71$$

Loss of myocardial high energy phosphate stores is an integral component of ischaemia, and Bretschneider and co-workers predicted poor recovery if ATP levels fell below 4 umol/gm wet weight (B63). ATP levels have been used in clinical studies and correlate with postoperative outcome (H6). However, other studies have demonstrated that postischaemic levels of ATP of less than 2 umol/gm wet weight neither predict recovery of contractile function nor irreversible damage (R42,V13). Thus, although newer "on

line" nuclear magnetic resonance techniques of measuring ATP may provide valuable information, one cannot predict functional recovery from these values (R42,S5). High energy phosphates were measured in the studies in this thesis by freeze-clamping the hearts with Wollenberg tongs, freeze drying the samples and levels were then determined by the method of Lamprecht and Trautschold, expressed as umol/gm wet weight (L4). Preischaemic control values for ATP and CP were derived from hearts subjected to the same initial experimental protocol i.e. 10 min Langendorff and 10 min working heart modes, but thereafter immediately freeze clamped. Tissue ATP and CP levels were then also measured at the end of the ischaemic period in some of the studies, by freeze clamping a second set of hearts prior to reperfusion. In addition, high energy phosphate levels were also measured in most of the studies after reperfusion, at the end of the experimental protocol.

Statistics

Extreme observations or "outliers" were tested for by Dixon's criteria (S56), in order to exclude major experimental errors, and if found those hearts were then excluded from further analyses.

The statistical tests used to compare differences between groups were the Anova one-way, two-way or three-way analyses of variance. However, the Anova depends upon an assumption of common variance amongst observations within each treatment group. Therefore, if variance was not consistent necessary adjustments for the violation of common variance were made. If statistical significance was obtained with ANOVA then pairwise comparisons of means were performed using either t-statistics with a nominal level of significance set at 5 %, or by the f-test. Appropriate tables were then used to determine p-values for comparisons of interest. Statistical significance was assumed when the p-value was less than 0.05.

2.2 IN VIVO PRIMATE MODEL

Juvenile and adult Chacma baboons (Papio ursinus) were used in the primate experiments in this thesis. An in vivo model using cardiopulmonary bypass to simulate the clinical situation as close as possible was used.

Experimental protocol

The animals were premedicated with intramuscular ketamine hydrochloride (10 mg/kg body weight). An endotracheal tube was then inserted, which was stabilized and secured with a cardboard ring and elastoplast. A narcotic analgesic; morphine sulfate (0.25 mg/kg), an antisialogogue; atropine sulfate (0.5 mg) and a neuromuscular blocker; pancuronium bromide (0.1 mg/kg) were administered intravenously. Anaesthesia was maintained with a mixture of nitrous oxide (7 L/min), oxygen (3 L/min), and 0.5 % halothane, administered via a Bird Mark 8 respirator (Bird Corporation, Palm Springs, California, USA) at a respiratory rate of 10 - 20 breaths/min. Tidal volume was adjusted according to animal size, ventilator pressures kept at 15 - 20 cm H_2O, and blood gases were monitored throughout the experiment and ventilation adjusted accordingly. The animals were also monitored for signs of insufficient anaesthesia (hypertension, bradycardia, tachycardia, pupillary dilatation, lacrimation, salivation and muscular tension), and the level of anaesthesia appropriately adjusted. Intra-arterial and central venous lines were inserted into the femoral vessels.

Immediately prior to performing the median sternotomy a second dose of morphine sulfate was given. After full heparinisation (500 U/kg) the heart was cannulated in preparation for cardiopulmonary bypass. Venous blood was drained via a single venous cannula inserted into the right atrium, oxygenated by a bubble oxygenator (Polystan Venotherm Oxygenator, low prime adult / paediatric Cat 012500, or infant Cat 011200;

Polystan A/S, Vaerlose, Denmark), temperature controlled with a heat exchanger incorporated in the oxygenator, and the blood was then returned by the cardiopulmonary bypass machine (Polystan A/S; Vaerlose, Denmark), via a cannula inserted into the ascending aorta. A Swan-Ganz thermodilution catheter (American Edwards 7F; American Edwards Laboratory, American Hospital Supply corporation, Santa Ana, California, USA) was inserted through the superior vena cava and positioned in the pulmonary artery for the measurement of cardiac output. Cannulae was also inserted into the left atrium, left ventricle and a thermistor probe (Microprobe thermometer Model BAT-12; Physitemp, Clifton, New Jersey, USA) positioned in the ventricular septum.

Systemic temperature was lowered and maintained at 26^{o}C after commencing cardiopulmonary bypass. The aorta was then cross-clamped and cardioplegic solution (15 ml/kg) infused via a separate cannula inserted into the ascending aorta, at a constant pressure of 80 mm Hg. In addition, the pericardial cavity was irrigated with iced saline during each infusion of cardioplegic solution. Multidose maintenance cardioplegia (100 ml/dose) was reinfused every 30 minutes throughout the 180-minute cross-clamp period. Systemic rewarming was commenced 30 minutes before the release of the aortic cross-clamp, and continued throughout the 15-minute reperfusion period until normothermia was obtained. The left ventricle was vented during the cross-clamp period and initial reperfusion period to ensure left ventricular decompression, by a cannula inserted via the right superior pulmonary vein. The animal was then weaned from cardiopulmonary bypass without the use of any inotropes.

The animals were euthanased while still under full anaesthesia by the intravenous infusion of a strong solution of potassium chloride, thereby effecting immediate cardiac arrest at the completion of each experiment.

Parameters of recovery

Myocardial temperature and haemodynamic parameters - heart rate, arterial blood pressure, right and left atrial pressure, first derivative of left ventricular developed pressure (LV dp/dt), and cardiac output (by thermodilution) were recorded with a Honeywell AR-6 Simultrace Recorder (Electronics for Medicine, Honeywell Inc, Pleasantville, New York, USA). Preischaemic haemodynamic parameters were recorded after the positioning of all cannulae prior to the commencement of cardiopulmonary bypass. A left ventricular function curve was plotted by recording these parameters at increasing left atrial filling pressures (5, 10, 15, 20 mm Hg), obtained by infusing cardiopulmonary bypass prime into the animal. In addition, a left ventricular transmural needle biopsy was taken from the apex of the left ventricle for determination of high-energy phosphates and transmission electron microscopic assessment.

Postischaemic haemodynamic and biochemical parameters were again taken at 5 and 30 minutes after termination of cardiopulmonary bypass, (a postischaemic LV function curve was only obtained at the 30 minute postischaemic period).

Analysis of data

Values were expressed as an index, to exclude the influence of animal size. The following formulae were used to derive cardiac index (CI), stroke volume index (SVI), and left ventricular stroke work index (SWI).

$$BSA\ (cm^2) = Weight^{0.425}\ (kg)\ X\ Height^{0,725}\ (cm)\ X\ 71.84\ (D25).$$

$$CI\ (L/min/m^2) = CO\ (L/min)\ /\ (BSA\ X\ 10^{-4}\ m^2/cm^2)$$

$$SVI\ (ml/beat/m^2) = (CI\ X\ 1000\ ml/L)\ /\ HR\ (beats/min)$$

$$SWI\ (gm{\cdot}m/beat/m^2) = SVI\ X\ (LV\ systolic\ pressure\ (mm\ Hg) - LVEDP\ (mm\ Hg))\ X\ 0.014348\ (S6)$$

Postischaemic values were then expressed as a percentage of each individual preischaemic control, and the same statistical tests as previously described in section 2.1 were used.

For statistical comparisons of SWI left ventricular function curves, the SWI at each left atrial filling pressure was expressed as a percentage of the stroke work achieved in the preischaemic control curve for each individual animal. Mean percentages and standard errors of mean percentages were then compared by ANOVA. However, for display purposes an average preischaemic control left ventricular function curve was derived by pooling the data from all control function curves. Postischaemic function curves for each group were then normalized to the average preischaemic curve; by multiplying the mean percentage recoveries and their standard errors for each group, at each left atrial filling pressure from 0 - 20 cm H_2O, by the average control SWI at the corresponding pressure.

The volume of cardioplegia administered to each animal (15 ml/kg) was related to heart weight by using the following formula derived from previous multiple measurements of both indices, in primates of this species in our laboratory (unpublished data).

Heart weight = (body weight X 8.33) - 37.03.

2.3 LIMITATIONS OF ANIMAL STUDIES

There are several limitations of the isolated rat heart model. The most important being that a non-blood perfusate and small mammalian "normal" hearts are used.

Species

Small mammalian hearts, especially the rat, guinea pig and rabbit have been used extensively to study the effect of cardioplegic solutions, because of low cost, ease of handling, and possibility of studying large numbers (H16). However, physiological differences clearly exist between species (B31,G1,H20,H21), and tolerance to normothermic ischaemia varies between species (G1). Furthermore, cardioplegic protection may be beneficial in one species (rat), but provide no protection in others (ferret and guinea pig) (G1), and susceptibility to reperfusion damage also differs (H20).

Major physiological differences also exist between these smaller mammals and larger animals (pig, canine, primate); eg. smaller animals have high resting heart rates (rat - 355 beats/min, guinea pig - 273 beats/min), and the oxidative capacity of the myocardium of smaller animals is significantly higher, although the glycolytic potential of mammalian myocardium is possibly fairly uniform (B31). Smaller animals also have higher levels of basal metabolism, oxygen consumption (H53), and may have considerably different electrophysiological properties (B60).

Amongst larger animals there are also differences; the pig has extremely limited collateral flow between different regions of the myocardium and develops cardiac oedema rapidly. The calf heart exhibits considerably higher resistance to ischaemia than the dog heart, which is somewhat less than a healthy human heart (B60). The canine heart is less tolerant to cardiopulmonary bypass and develops myocardial oedema more predictably and frequently than the human (R35).

In addition, differences in susceptibility to ischaemic injury also depend upon the maturity of the animal (P30), and cardioplegic solutions used in adult hearts may not have equivalent efficacy in neonatal hearts (B3,B4).

The primate though, has a close evolutionary relationship to man, and is therefore probably the closest available experimental model to man for evaluating the efficacy of cardioplegic solutions. Nevertheless, direct extrapolation of animal experiments to the human clinical situation requires caution.

Ex Vivo Models

Experimental conditions can be rigidly controlled in ex vivo models (H16). However, the "unphysiological" crystalloid perfusates used in the majority of these models allows only limited recovery after ischaemic contracture, and also increases compliance and causes slow deterioration of the heart with time (G1,N6). Furthermore, bubble oxygenation of the crystalloid perfusate denatures added proteins and may cause microemboli. Trace amounts of heavy metals in the reagents of the perfusate can also affect the stability of the model (N6), and Neely et al add Ca-EDTA 0.5 mmol/L to the Krebs-Henseleit perfusate to improve the stability of their model. Thus, alternative paracorporeal and in vivo models using blood perfusates are more physiological, although more complex and expensive (W7).

In vivo models in contrast to ex vivo models, maintain the involvement of metabolic, nervous, hormonal and other factors such as noncoronary collateral flow. However, metabolic, hormonal, biochemical alterations such as ionized calcium concentration (D24) and haematological parameters (haematocrit) in these paracorporeal models can also effect myocardial function and therefore outcome. Cardiac output can also be altered by heart rate, preload and afterload in the in vivo model. Although the former

two variables can be kept constant, afterload can be difficult to keep stable and thus can affect the results. Therefore, isovolumic measurements of contractility may be more relevant in this model, and a more sensitive indicator of ischaemic damage may be postischaemic oxygen utilization (K37).

Nevertheless, the clinical situation of cardiopulmonary bypass, with its associated damaging effects, can be closely simulated with in vivo models.

Experimental Protocol

The experimental protocol used to evaluate the efficacy of cardioplegic solutions is extremely important. Cardioplegic solutions should be evaluated in models that simulate the clinical situation, and two fundamental aspects are the temperature during the ischaemic period and the energy state of the experimental heart.

Hypothermia alters the physiological environment; "fluidity" of proteins and lipids, activity of enzymes (P33), calcium homeostasis (K12,S40), coronary autoregulation (C24,M20), and the neutral pH of water (W19). Therefore, one should critically question the validity of normothermic evaluations of cardioplegic solutions, destined to be used in hypothermic conditions (J21,K11). Nevertheless, it is important to also be aware of the efficacy of cardioplegic solutions at different temperatures (J21), as temperatures may vary during open-heart procedures. In all studies in this thesis, the cardioplegic solutions were evaluated at hypothermia ($10^{o}C$).

The majority of experimental animals have "normal hearts", whereas in the clinical situation operations are performed on diseased hearts. Thus models simulating coronary stenoses to study cardioplegic distribution (G41,L20), or models using energy depleted hearts (by adding an initial ischaemic insult) (R44), hypertrophied hearts (B30), and

cyanotic hearts (F32), are extremely relevant and important (B71). However, when studying the effects of cardioplegic solution composition on functional recovery, an initial ischaemic insult may impose an additional reperfusion injury which itself induces additional physiological changes. Nevertheless, because of the excellent performance of better formulated cardioplegic solutions, an initial normothermic ischaemic insult may be necessary. An ischaemic insult was added to the experimental protocol in a later study in this thesis, in order to produce energy deficient hearts.

Other experimental variables

The preparation of the cardioplegic solution itself can introduce experimental variables. For example, particle induced coronary vasoconstriction must be prevented (H17,R30), and therefore all experimental cardioplegic solutions should be filtered through an 0.8 um sized cellulose filter (Millipore corporation; Bedford, Massachusetts, USA).

Heavy metal contamination

Contaminated salts of the various components of the cardioplegic solution to be evaluated can alter its efficacy. We noted significant myocardial depression if the standard St Thomas' cardioplegic solution was prepared with magnesium chloride obtained from one company ($MgCl_2 \cdot 6H_2O$ - AnalaR, Product 10149; BDH Chemicals Ltd: Poole, England), in contrast to another ($MgCl_2 \cdot 6H_2O$ - Product 5833; Merck Ltd: Darmstadt, Germany). We presumed that this was due to the higher quantities of heavy metal contaminants allowed with the former product. Heavy metals can be used as cofactors in the generation of oxygen free radicals (C19,M37), and other investigators have also noted the detrimental effect of heavy metal contaminants (Wicomb W: personal communication). Thus, calcium or sodium EDTA (0.5 mmol/L) has been added to perfusates (N6) and cardioplegic solutions (W27), in order to chelate residual traces of heavy metals.

Oxygen content of cardioplegic solutions

The majority of solutions prepared in the laboratory are routinely gassed with 95 % O_2 5 % CO_2 during their preparation, in order to prevent precipitations of calcium salts. Thereafter, depending upon how these solutions are stored and the length of elapsed time prior to use, the oxygen content can vary significantly. Thus "non-oxygenated" cardioplegic solutions may contain significant quantities of dissolved oxygen if equilibration to atmospheric pressure has not yet occurred (appendix A-7). Furthermore, some commercial cardioplegic solutions (Bretschneider HTK4) are stored in vacuum in glass bottles, and are therefore anoxic if infused immediately upon opening the container. Hence, the amount of oxygen dissolved in "non-oxygenated" solutions can result in experimental variations.

Processing of samples for electron microscopy

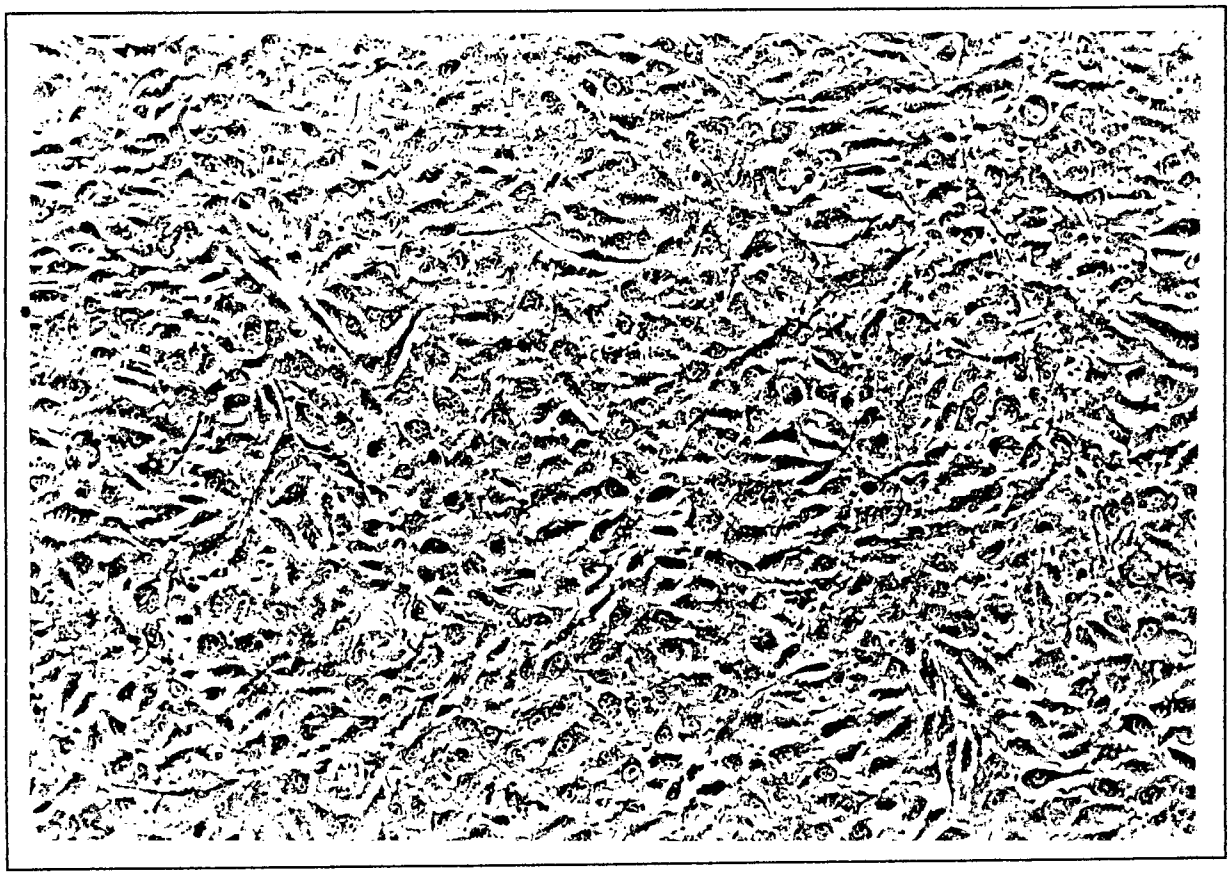
Morphological studies have consistently been in broad agreement with both enzymatic and functional assessments (H16). Characteristic mitochondrial changes correlate well with functional recovery and adequacy of myocardial preservation (S51). However, processing of tissue for transmission electron microscopy is critical, as fixation techniques may alter structural findings (S51,V12). Fixation of tissue samples in these studies was with 5 % buffered glutaraldehyde and processing was by routine techniques employed in our laboratory (osmic acid and dehydration in graded alcohols and embedded in araldite) (R34).

2.4 MONOLAYER ENDOTHELIAL CELL CULTURES

The vascular endothelium is an extremely important component of all organs (see section 6.1), and is the first structure that infused cardioplegic solutions contact. Various isolated cellular and subcellular preparations have been used to evaluate the effect of cardioplegic solutions, but very few evaluations have been made of the effect of cardioplegic solutions on the endothelium (H16). We developed a model of cultured human endothelial cells (Fig 2.2) in order to assess the direct effect of cardioplegic solutions on the endothelium.

Figure 2.2

MONOLAYER CULTURE OF ENDOTHELIAL CELLS



Legend:

Confluent monolayer of human venous endothelial cells. A typical "cobblestoned" surface with no intercellular gaps is observed. Light microscopy X 100. Reprinted from Ann Thorac Surg 1990; 50:902-910 (appendix A-6) with permission The Society of Thoracic Surgeons.

Technique of endothelial cell cultures

Human adult saphenous vein remnants (2 - 3 cm) normally discarded after coronary bypass graft surgery, or saphenous veins obtained from organ transplantation donors were used. The segment of vein was cannulated, flushed with incomplete culture medium (Medium 199 - Earle's salts (Flow Laboratories, Irvine, Scotland)), and then filled with 0.1 % collagenase (CLS II; Cooper Biomedical, Malvern, Pennsylvania) and incubated at 37°C for 15 min (Z3,Z4). The collagenase-cell suspension was then inactivated by flushing with culture medium containing 30% foetal calf serum (Delta Bioproducts, Johannesburg, South Africa), collected and centrifuged for 10 min at 120 *g*. The pellet was then resuspended in 2 ml complete culture medium (Medium 199 plus 20% foetal calf serum and endothelial cell growth factor (ECGF) - (Collaborative Research Incorporated, Lexington, Massachusetts)), and plated into a 9.6 cm^2 well of a six-well culture plate precoated with human fibronectin (Inotech, Zurich, Switzerland, 18 ug/ml). All cultures were incubated at 37°C in a 5 % CO_2 atmosphere.

To remove erythrocytes and cell debris the primary cultures were rinsed after 24 hr, with Dulbecco's phosphate buffered saline (Flow Laboratories, Irvine, Scotland). Thereafter, complete culture medium (2 ml) was added, and 50% of the supernatant was replaced twice weekly with fresh complete culture medium. Endothelial cells were passaged at 80% partial cell coverage, designated by a microgrid technique (Z3,Z4). This partial cell coverage was usually obtained 10 days after endothelial cell harvest. In order to reduce the cell damaging effect of multiple passages (W11), primary cultures were passaged at a ratio of 1:22, using trypsin EDTA (Flow Laboratories, Irvine, Scotland). These first passage endothelial cells were either seeded into a 175 cm^2 flask precoated for 12 hours with gelatin (Difco Laboratories, Detroit, Michigan, 10 ng/ml in 0.1 M $NaHCO_3$ buffer), or onto 9 mm glass cover-slips for immunohistochemical staining of von Willebrand factor (Factor VIII related Antigen) (Z3), which is a typical cell marker for endothelial

cells [J2]. Cultures were managed as before, and confluence again obtained within 10 days. The cells were passaged once more, but in a 1:1 ratio into 12 well culture plates (3.5 cm^2 per well), precoated with gelatin. Confluence of second passage monolayers was obtained after 2 - 3 days. Individual wells were assessed microscopically for completeness and homogeneity of cell coverage, and unevenly covered wells were excluded.

Experimental Protocol

A baseline in situ grid count of each well was performed [Z3,Z4], using an inverted phase contrast microscope (ID-02-MT, Zeiss, West Germany). The cell count in "20 grid squares" was the total number of cells seen in each four corner squares of a marker grid and counted in five different areas of each well. The culture medium was then removed and the preservation solution applied initially as a rinse, to ensure removal of all medium, and then a one ml aliquot reapplied.

The cells were exposed to the preservation solutions for predetermined periods, and the morphology of the cells was then examined by light microscopy. Thereafter, the solution was removed and the cells rinsed with incomplete culture medium, and one ml complete medium but not containing ECGF was reapplied. All cell cultures were again placed in a 37°C, 5 % CO_2 incubator, to assess cell survival and allow for any delayed expression of cell damage. A post exposure cell count was performed after rinsing the cells with incomplete culture medium, to remove debris and dead cells, at 24 h and again at 36 h. The percentage of surviving cells in each well, at the 24 h and 36 h post exposure time periods, was derived from the ratio of each individual post exposure count to the corresponding initial pre exposure count for that well.

In order to ensure reproducibility each solution was tested in different culture plates, and on endothelial cells obtained from separate human donors.

Limitations of this model

This model uses morphology and cell survival to assess the effects of cardioplegic solutions on endothelial cells, although endothelial functions such as the release of vasoactive substances are not assessed. Indirect measurements of endothelial function, such as vasoresponsiveness to infused substances (C21,S7), require both endothelial and vascular smooth muscle function (B14). Whereas in our model the direct effect of cardioplegic solutions on the endothelium alone, were evaluated. Furthermore, venous as opposed to arterial endothelial cells were used in our experiments and differences do exist between different vascular beds (D9,L51,W2). However, extrapolation to the arterial system is probably justified as similarities also exist between vascular beds (J1,P8), and basic morphological changes were evaluated in our model. Nevertheless, extrapolation of these isolated cellular studies to the human clinical situation should be done with caution.

Chapter 3

COMPOSITION OF CARDIOPLEGIC SOLUTIONS

Cardioplegic solutions were initially formulated in the mid 1950's in order to produce elective cardiac arrest (L3,M30,S32), but these solutions also caused myocardial damage (M23,M29), and clinical use was therefore discontinued (G8,H16). However, experimental work continued and the basic physiological and biochemical effects of cardioplegic arrest were more extensively determined (B63,G9,H16). Thus, cardioplegic solutions were reintroduced in clinical open-heart surgery in the 1970's (B54,B63,G8,K22).

Cardioplegic solutions were initially used at normothermia by both Kirsh et al (K22) and Gay et al (Table 3.1) (G9). However, the effectiveness of a high magnesium solution similar to that of Kirsh, diminishes markedly after 30 min normothermia (W4). Nevertheless, acceptable clinical results (S20) were also obtained by the technique of Bleese and Rodewald et al in Hamburg, who used the Kirsh solution to initially arrest the heart at normothermia, but then cooled the heart with a cold oxygenated perfusate (B32). Hypothermia used in conjunction with cardioplegia was then soon reintroduced routinely in the clinical situation (B32,B63,C34,G8,H16).

Table 3.1

CARDIOPLEGIC SOLUTIONS USED AT NORMOTHERMIA

Kirsh (B32,J22,K22)

	gm %	mmol/L
Magnesium-l-Aspartate	2.5	160.9
Procaine-HCl	0.3	11
Sorbitol	4.5	247
Osmolality:	463 mOsm/kg H_2O	

Legend:
Volume infused: 1 - 2 ml/kg body weight (30 - 140 ml)

Gay (G9)

	gm %	mmol/L
NaCl	0.4	68
$NaHCO_3$	0.1	12
KCl	0.25	34
Glucose	0.2	10
Osmolarity:	± 238 mOsm/L	

Legend:
Volume infused: 100 ml in combination with blood in an approximate ratio of 1:1 (canine hearts).

A plethora of different cardioplegic formulations have since been used clinically. Cardioplegic solutions are now subdivided into either pure crystalloid (intracellular or extracellular electrolyte equivalent formulations), or blood cardioplegic solutions.

3.1 CRYSTALLOID CARDIOPLEGIC SOLUTIONS

Crystalloid cardioplegic solutions were initially the predominant category of cardioplegic solution used. However, each cardiac center developed their own "cardioplegic solution" (Table 3.2, 3.3, 3.4). In addition to these *"named cardioplegic solutions"*, many surgeons used cardioplegic solutions made up by merely adding potassium with or without bicarbonate to commercially available standard intravenous electrolyte solutions (R29,Appendix A-1).

Table 3.2

CARDIOPLEGIC SOLUTIONS USED AT HYPOTHERMIA

mmol/L	Guy & Ebert (G8)	Roe's (R33)	Tyers (T27)	Kirklin (UAB) (K20)
Na^+	48.5	27	138	110
K^+	40	20	25	30
HCO_3^-	10	-	20	27
Ca^{2+}	-	-	0.5	0.5
Mg^{2+}	-	1.5	1.5	-
Cl^-	78.5	47	98	85
Glucose	278	278	-	27.7
Mannitol	-	-	-	54.39
TRIS	-	2	-	-
Acetate	-	-	27	-
Gluconate	-	-	23	-
Osmolality (mOsm/kg H_2O)	320	347	275	330
pH	7.7	7.6	7.8	7.5

Comparisons between some of these *"named cardioplegic solutions"* have been done, however, often with various modifications of these solutions or with inappropriate techniques, making detailed comparative analysis difficult. For example, the St Thomas' Hospital No 2 cardioplegic solution (Table 3.3) provides better myocardial protection

than Tyers' (R29) or Kirsh solutions (with or without additional hypothermia), both clinically (S20) and experimentally (J22). A comparison of solutions containing low sodium concentrations showed that Roe's solution was inferior to Bretschneider HTK (Table 3.4), and resulted in progressive acidosis and depletion of ATP in a hypothermic (27°C) canine model (W33).

Although comparisons of all known solutions have not been performed, there are primarily two "gold standard" crystalloid cardioplegic solutions which have been extensively investigated and characterized; Bretschneider HTK4 and St Thomas' Hospital No 2 cardioplegic solutions. These two cardioplegic solutions should therefore be used as the baseline for all future comparisons of "new" crystalloid cardioplegic formulations.

St Thomas' Hospital Cardioplegic Solutions

Extracellular electrolyte equivalent cardioplegic solutions rely primarily on high concentrations of potassium (10 - 40 mmol/L) to partially depolarize the cellular membrane, thereby inactivating sodium channels and preventing the genesis of an action potential (B69,H16). However, it is not only the potassium concentration that is important, but also the concentrations of all components of the cardioplegic solution (H14,H24). Poorly formulated solutions provide incomplete myocardial protection (B5,K30).

Jynge P, Hearse D J and colleagues performed dose response curves to determine the "ideal" concentration of each component of the St Thomas' cardioplegic solution, in the isolated rat heart model (H28,J17,J23). Thus, the St Thomas' Hospital No 2 cardioplegic solution, which is better than the St Thomas' No 1 cardioplegic solution (L26), can be considered to be the best characterized extracellular electrolyte equivalent cardioplegic solution and hence the "gold standard" for this group of cardioplegic solutions.

Table 3.3

ST THOMAS' HOSPITAL CARDIOPLEGIC SOLUTIONS

(mmol/L)	St Thomas' No 2 (J23)	St Thomas' No 1 (L26)	Ringer's Solution	MacCarthy Ampoule
Na^+	120	144	144	-
K^+	16	20	4	16
HCO_3^-	10	-	-	-
Ca^{2+}	1.2	2.4	2.4	-
Mg^{2+}	16	16	-	16
Cl^-	160.4	201.8	152.8	49
Procaine-HCl	-	1	-	1
Osmolality (mOsm/kg H_2O)	282	300	-	-
pH	7.8	5.5 - 7.0	-	-

A cardioplegic solution is infused in order to manipulate the extracellular ionic concentrations which in turn:

i) Causes electromechanical arrest,

ii) Prevents an increase of intracellular calcium during the ischaemic period.

The interactions between the different ions contained in cardioplegic solutions are extremely complex, and changes in the concentration of one ion can markedly effect the efficacy of another. Hence the importance of both understanding these relationships and establishing dose response curves for each individual electrolyte.

Calcium content

Calcium is probably the single most important ion in terms of protecting the ischaemic myocardium, both during ischaemia (H16) and during reperfusion (H5,O20). An increase in intracellular calcium is detrimental because it activates ATPase, proteases,

lipases, accumulates in the mitochondria (H16), and consumes energy independent of excitation-contraction when taken up by the sarcoplasmic reticulum (R19). Intracellular calcium concentrations can be altered via a number of mechanisms; the slow calcium channel, sarcolemmal sodium-calcium exchange, sarcolemmal calcium pumps, and by uptake by the sarcoplasmic reticulum and mitochondria (O21). Calcium fluxes are also intimately related to both pH and the other cations; low extracellular sodium, high pH or high potassium concentrations promote calcium influx (H16,L11,L12,O17), whilst magnesium inhibits sarcoplasmic reticulum release of calcium (K8).

Intracellular calcium increases during both ischaemia and reperfusion (P31), and thus a primary function of a cardioplegic solution is to prevent this increase in calcium. However, calcium is also essential for maintaining the integrity of the cell membrane (H16), and removal of all extracellular calcium predisposes the heart to the *calcium paradox.* The *calcium paradox* is the term used to describe the irreversible cellular damage that occurs when a heart is reperfused with a calcium-containing solution after a short period of calcium-free perfusion (H19,J20,R55). The likelihood of the calcium paradox occurring is increased by either normothermia (R10), ischaemia (J18), high potassium concentrations, alkalosis (H31), prolonged myocardial washout of calcium (K32), or presence of calcium-binding anions (citrate, phosphate, gluconate) (C27,J16,J18). The calcium paradox also occurs in all species (H16), and in organs other than the heart (N18). The original Melrose cardioplegic solution (M23,M29,M30) contained exceptionally high concentrations of the calcium-binding anion citrate, and thus the calcium paradox was the probable cause of the myocardial damage induced by this solution. The calcium paradox can be prevented from occurring by maintaining a minimal calcium concentration of 0.025 mmol/L in the extracellular fluid (H32). Calcium-free cardioplegic solutions have been used successfully clinically (B37), however, some of these cardioplegic solutions may not have been truly calcium-free as even "deionized distilled water" contains trace amounts of calcium (H16,Appendix A-6). The highly variable noncoronary collateral flow also probably prevented complete calcium depletion in the clinical situation (R10). Nevertheless, calcium-free cardioplegic

solutions are potentially dangerous, and the St Thomas' Hospital No 2 cardioplegic solution contains 1.2 mmol/L of calcium.

The optimal concentration of calcium in the St Thomas' cardioplegic solution was originally shown to be 1.2 mmol/L (Y2,Z8) in a normothermic model (R28). However, in hypothermic models the optimal concentration is 0.3 - 0.6 mmol/L (B2,R31,R32). Furthermore, the known complex hypothermic induced changes to cellular calcium fluxes (K43,S40), suggest that the latter results are more appropriate to the clinical situation.

Sodium content

Sodium tends to accumulate intracellularly during ischaemia (P31), as a result of opening of sodium channels (O17), and as the ATP dependant sarcolemmal sodium-potassium pumps fail. This intracellular sodium accumulation can be prevented by using a sodium-poor cardioplegic solution. However, this imposes other constraints on the composition of the cardioplegic solution, such as the necessity for it to be calcium-free (H14,L12) (see next section; Bretschneider cardioplegic solutions).

Hearse et al elected to formulate a calcium containing cardioplegic solution that avoided extremes in ionic concentrations and thus used a sodium concentration approximating that of extracellular fluid, for the St Thomas' cardioplegic solution (J23). The optimal sodium concentration of the St Thomas' cardioplegic solution was found to be 100 - 120 mmol/L, in a 30 min normothermic ischaemic model (J17). A sodium concentration of 60 mmol/L or less increased creatine kinase release, thus indicating cellular damage probably due to an induced increased calcium influx (L12).

Potassium content

High extracellular potassium concentrations (30 - 40 mmol/L) reduce the resting membrane potential from approximately -85 mV (O17) to -40 to -30 mV (H16). Although this membrane potential inactivates the sodium channel and thus prevents an action potential, this membrane potential also activates the calcium channel (O17). Therefore, high extracellular potassium concentrations promote calcium influx which is harmful during ischaemia. In addition, these high potassium concentrations damage the endothelium (F23,J7,M11,S7), cause coronary vasoconstriction (C21,H25), increase postoperative conduction arrhythmias (D15,E5), and accelerate atherogenesis (O13). However, a potassium concentrations of 20 mmol/L reduces the membrane potential to approximately -50 mV which still produces electromechanical arrest, but without the unfavorable calcium effects (H16).

Another disadvantage of potassium induced partial depolarization is that sodium channels open at -70 mV to -60 mV (O17), and therefore partial depolarization still allows sodium influx (H16), which in turn also increases intracellular calcium (O17). However, sodium influx can be partly inhibited by Class I antiarrhythmic agents such as lignocaine (H22,O17). Kyo et al showed that a normokalaemic lignocaine (400 mg/L) cardioplegic solution resulted in higher end-ischaemic adenosine triphosphate content with good functional recovery, than hyperkalaemic cardioplegic solutions (K45). Potassium induced partial depolarization is thus theoretically inferior to polarized arrest, which decreases metabolic demands more than depolarized arrest (S73).

Nevertheless, the minimum concentration of potassium needed to obtain arrest at normothermia with an asanguineous solution is 15 mmol/L (R51), and with blood cardioplegia 20 - 25 mmol/L (B74). However, myocardial hypothermia potentiates potassium arrest as hypothermia ($4^{o}C$ - $10^{o}C$) induces membrane depolarization of approximately 10 mV (K43). Therefore, the potassium concentration in cardioplegic

solutions can be decreased to 8 - 10 mmol/L with subsequent multidose reinfusions, once the myocardium is hypothermic (B74).

Magnesium content

Magnesium is a major intracellular cation, a cofactor for a number of enzymatic reactions, and the majority of intracellular magnesium is complexed with adenine nucleotides (P24). High extracellular magnesium concentrations inhibit sarcotubular calcium release and calcium influx (K8), decrease potassium efflux during ischaemia, decrease intracellular sodium and limit intracellular magnesium loss (S41). These effects of magnesium are advantageous during ischaemia and magnesium is therefore an important protective ion in cardioplegic solutions (B68,H28). The addition of magnesium to a cardioplegic solution also suppresses mitochondrial nonphosphorylating oxygen consumption (S83), prevents calcium-induced increased myocardial tension (G12) and mechanical activity (T22) during initial infusion of the cardioplegic solution.

The optimal concentration of magnesium in an extracellular cardioplegic solution used at either normothermia or hypothermia is 16 mmol/L (B68,H28,R18), which approximates the reported intracellular magnesium concentration of 16 - 17 mmol/kg cell H_2O (P24). Although magnesium may not to be necessary in low calcium containing cardioplegic solutions (K16), other studies have shown that magnesium is beneficial in the presence of both high and low calcium concentrations (B68).

The St Thomas' Hospital No 2 cardioplegic solution contains a magnesium concentration of 16 mmol/L, and therefore a bolus of 16 - 32 mmol of magnesium (1 - 2 L cardioplegia) is infused systemically if the cardioplegic solution is allowed to drain into the systemic venous blood during clinical open-heart surgery. This results in a 70 - 130% increase in serum magnesium levels from 0.7 - 0.95 mmol/L to 1.34 - 1.86 mmol/L, which return to normal within 24 h (M12). Although high magnesium concentrations may depress myocardial contraction, cause bradyarrhythmias and heart block,

cardiovascular toxicity only occurs at higher levels (2.5 mmol/L) (E4). Moreover, this magnesium load might be beneficial by correcting preexisting or cardiopulmonary bypass associated hypomagnesemia, by decreasing systemic vascular resistance and catecholamine response in the postischaemic period (C10), and by suppressing postoperative supraventricular tachyarrhythmias (F4).

Local anaesthetic agents

Lignocaine can be added to the St Thomas' cardioplegic solution in a concentration of 0.05 mmol/L (14.4 mg/L; 0.72 ml 2 % Lignocaine - HCl) (H22). Equivalent postischaemic recovery is also obtained with procaine 0.05 mmol/L. These local anaesthetic agents "stabilize membranes" and may act as cardioplegic agents (prevent depolarization by inhibiting the fast inward sodium channel), as well as inhibiting potassium conductance. Procaine also prevents both hypothermic induced prearrest myocardial contraction which is harmful (R12) and coronary vasoconstriction (B63,S79), and has also been shown to be beneficial in kidney preservation (C32). However, procaine has also been shown to be injurious to the endothelium (S60), and hinders the permeation of H^+ and lactate ions thus reducing the effectiveness of potential buffers (B62). Nevertheless, residual postischaemic amounts of procaine or lignocaine can also help prevent arrhythmias during reperfusion (H24).

The St Thomas' cardioplegic solutions No 1 & No 2

The St Thomas' Hospital No 1 cardioplegic solution (MacCarthy solution; Table 3.3) is available commercially as a concentrated ampoule that is added to standard nonlactated Ringer's solution (Cardioplegic solution 20 ml: John Bell & Croyden; London, United Kingdom). Whereas, the superior St Thomas' No 2 cardioplegic solution is also available commercially (Plegisol: Abbott Laboratories; North Chicago, Illinois, USA) and in South Africa (St Thomas' Hospital Cardioplegic Solution: Sabax Ltd; Aeroton, Johannesburg). Both of these commercial St Thomas' No 2 cardioplegic solutions require 10 ml 8.5 %

sodium bicarbonate (10 mmol) to be added to each liter prior to use. The sodium bicarbonate is not included in the initial formulation because of stability and shelf storage reasons.

The efficacy of the St Thomas' cardioplegic solution is primarily due to its concentrations of potassium and magnesium (J19) and the effectiveness of the St Thomas' cardioplegic solution is independent of temperature and infusion volume (C20,J21), although enhanced by hypothermia. Furthermore, as extremes in electrolyte concentrations are avoided (J23), equilibration is simpler (H14) and its effectiveness is not diminished as much by noncoronary collateral flow compared to other cardioplegic solutions (W7). Therefore, it is an ideal crystalloid cardioplegic solution for clinical open-heart surgical procedures. Furthermore, the St Thomas' No 2 cardioplegic solution has been shown to provide both superior myocardial protection (L26), and be less cytotoxic than the St Thomas' No 1 cardioplegic solution (C4).

Bretschneider cardioplegic solution

The Bretschneider cardioplegic solutions can be classified as intracellular electrolyte equivalent solutions (H16), and Bretschneider HTK4 should be considered the "gold standard" of sodium-poor cardioplegic solutions (Table 3.4). The composition of the Bretschneider cardioplegic solutions has been progressively altered on the basis of experimental data. However, the Bretschneider cardioplegic solution was originally designed to arrest the heart by markedly diminishing the extracellular sodium concentration (Bretschneider No 3) (B63). A low extracellular sodium concentration that approximates that of the intracellular compartment prevents the genesis of an action potential (B63). Procaine was initially included in these solutions, to inhibit the hypothermic induced contraction that occurs when suddenly infusing a cold solution into the heart (B63), however, procaine hinders the permeation of H^+ and therefore reduces the effectiveness of potential buffers (B62). Histidine which is a powerful physiologic

buffer was then added to the solution in order to artificially double the buffering capacity of the myocardium (G10), and thereby delay acidosis and ATP breakdown by allowing ongoing anaerobic metabolism. The buffering capacity of homogenized ventricular myocardium is 40 mmol HCl/L/δ pH (K34), and the buffer capacity of Bretschneider HTK4 cardioplegia is more than double this value; 100 mmol/L/δ pH (appendix B-5).

Table 3.4

BRETSCHNEIDER CARDIOPLEGIC SOLUTIONS

(mmol/L)	Bretschneider No 3 (J22,S63)	Bretschneider -HTK (1980) (G11)	Bretschneider -HTK4 (1984) (G11)
Na^+	12	15	15
K^+	10	10	10
Mg^{2+}	2	8	4
Cl^-	26	46	51
Procaine-HCl	7.4	-	-
Mannitol	239	20	30
Histidine	-	195	198
Tryptophan	-	2	2
KH-2-oxygluterate	-	-	1
Osmolality (mOsm/kg H_2O)	320	300	298
pH (25°C)	5.5 - 7.0	7.2	7.1

The Bretschneider cardioplegic solution contains a low concentration of sodium and therefore must be calcium-free (extracellular calcium less than 0.01 mmol/L (G10)) in order to be effective (H14), as intracellular calcium influx is promoted by a low sodium concentration (L12). The addition of as little as 0.024 mmol/L of calcium reduces the protective effect of Bretschneider cardioplegia (G10,J19). The presence of calcium in a sodium-free solution was the probable cause of the intramitochondrial occlusions observed by Kyosola et al in Helsinki, with their cardioplegic solution (K46). However,

a calcium-free solution in turn predisposes the heart to the *calcium paradox* (discussed above).

Bretschneider HTK or St Thomas'?

The Bretschneider cardioplegic solution should only be used under well controlled constant hypothermic conditions (J22), as temperatures below 27°C prevent the calcium paradox from occurring (R10). Experimental comparisons to determine the superiority between Bretschneider and St Thomas' cardioplegic solutions under normothermic conditions are therefore invalid (J21,K11). In hypothermic canine models, Bretschneider HTK provides better myocardial protection than the St Thomas' cardioplegic solution (S25,W7). However, in the isolated rat heart model St Thomas' cardioplegia is associated with superior hypothermic preservation compared to the Bretschneider No 3 solution (admittedly less efficacious than the latest Bretschneider HTK4 solution) (J19,J22).

We compared Bretschneider HTK4 and a modified St Thomas' plus glucose (10 mmol/L) cardioplegic solution in the isolated rat heart model (appendix B-5). Postischaemic mechanical recovery was similar in both groups, but Bretschneider HTK4 was associated with lower ATP content at the end of the ischaemic period and greater sarcolemmal damage. The recommended high volume induction dose for Bretschneider HTK4 cardioplegia (P28) was used in this study, and although the recommended dose for St Thomas' cardioplegia is lower than that used in this study, high volumes of St Thomas' cardioplegic solution are not detrimental (T9). Furthermore, as a single dose cardioplegic infusion was used in this model (appendix B-5), the inclusion of glucose in the St Thomas' cardioplegic solution might have decreased recovery in the St Thomas' group (see section 5.1). Other studies using isolated rat hearts have also shown Bretschneider HTK to be inferior to extracellular electrolyte equivalent cardioplegic solutions (H32,J20,J22). Nevertheless, the suitability of using calcium-free infusates in

the isolated rat heart model has been questioned (W7), as canine studies have shown excellent protection with Bretschneider HTK (S25,W7).

Schnabel and co-workers demonstrated that Bretschneider HTK4 retards ATP decay twice as much as a modified St Thomas' cardioplegic solution (B62,S25). However, in our study Bretschneider HTK4 was associated with increased ATP decay. Differences in susceptibility to ischaemic injury and responsiveness to myocardial protection exist between species, and it is possible that this could account for some of the discrepancies between these studies (G1). Nevertheless, although smaller animals have a higher oxidative capacity (B31), it is not yet known which species is most representative of man.

Bretschneider HTK cardioplegia produces acceptable clinical results equivalent to the St Thomas' cardioplegic solution (S20), especially if combined with topical hypothermia (S68). However, the myocardium should not be exposed to Bretschneider HTK at or near normothermia because of the potential for the calcium paradox. Bretschneider cardioplegia also requires a more complex and prolonged method of administration; precooling of the heart with a mixture of blood and glucose (B63,S63), thereafter infusion of cold (5°C - 10°C) oxygenated Bretschneider for 8 - 10 min, initially at 80 - 100 mm Hg, then 40 - 50 mm Hg to ensure full equilibration (P27), with an initial total infused volume of 3 - 4 liters (P28) (7 - 10 ml/gm heart weight (P27)). Furthermore, during clinical open-heart surgery conditions are often "uncontrolled", because of constant and occasionally rapid rewarming of the myocardium from the surgical environment and noncoronary collateral flow. Warnecke et al showed that noncoronary collateral flow decreases the efficacy of the Bretschneider solution, but improves postischaemic recovery with the St Thomas' Hospital No 1 cardioplegic solution (W8). Multidose reinfusions of Bretschneider cardioplegia also diminish its efficacy, although this has possibly been corrected by lowering the magnesium concentration in Bretschneider HTK4 (G10,G11). Nevertheless, in controlled isolated hypothermic conditions Bretschneider HTK4 cardioplegia is associated with superior preservation of

adenosine triphosphate, thus it may be the more "ideal" solution for preservation of the isolated heart for transplantation, as opposed to routine clinical open-heart surgery.

3.2 BLOOD CARDIOPLEGIA

There are certain theoretical advantages for blood as the vehicle for delivery of cardioplegic solutions (B70,I3,W12):-

- The heart is kept oxygenated while it is being arrested,
- Oxygen and other substrates are resupplied with each reinfusion thus allowing intermittent aerobic metabolism,
- Improved buffering capacity (W9),
- Improved oncotic properties,
- Free radical scavenging properties (V1),
- Rheological influences; in the absence of particulate matter (red blood cells), flow occurs primarily in arteriovenous capillaries and there is restricted flow in true capillaries (Z7).

A number of experimental and clinical studies have shown blood cardioplegia to be superior to crystalloid cardioplegic solutions (B73,C8,C17,C30,E12,F28,I4,S50). Unfortunately, the majority of these studies compared blood cardioplegia to inferior crystalloid cardioplegic solutions by today's standards (C8,C17,E12,F28,I4,S50). Moreover, few comparisons have been done between blood cardioplegia and *oxygenated* crystalloid cardioplegic solutions. Both blood (haematocrit; 10% - 20%) and oxygenated crystalloid cardioplegic solutions may be equally effective (R48), and crystalloid possibly superior at low temperatures (M5). Nevertheless, the salutary effects of blood cardioplegia are not solely reliant on its oxygen carrying capacity (B71), but also other biochemical properties of the red cell membrane (B29,I3,S79). Blood also has a much higher buffering capacity than the majority of crystalloid cardioplegic solutions (W9), and erythrocytes can decrease free radical mediated damage as they contain significant quantities of free radical scavengers (V1) and can also take up and remove hypoxanthine (B26). Furthermore, although Weisel et al showed incomplete preservation of ATP with blood cardioplegia clinically (W15), other studies have shown almost complete

preservation of myocardial ATP (C8,E12) and possibly better cellular protection compared to the St Thomas' cardioplegic solution (C30).

The "gold standard" for blood cardioplegia should probably be the solution developed by G D Buckberg and colleagues (Table 3.5) (B70,F26), even though there have been very few comparisons between different blood cardioplegic compositions.

Table 3.5

CONCENTRATE FOR 4:1 BLOOD CARDIOPLEGIA

COLD INDUCTION

Additive	Volume (ml)	Delivered concentration		
THAM (300 mmol/L)	200	pH	:	7.7 - 7.8
CPD	50	Ca^{++}	:	0.5 - 0.6 mmol/L
5% Dextrose in		Glucose	: ±	43 mmol/L
1/4 N Saline	550	Osm	: ±	350 mOsm/kg H_2O
KCl (2 mmol/ml)	30	K^+	:	18 - 20 mmol/L

Legend:
Infused at 4°C - 8°C, at a rate of 300 - 350 ml/min antegrade for 2 min, then 200 - 250 ml/min retrograde for 2 min. CPD: Citrate phosphate dextrose. Modified from Buckberg G D, J Cardiac Surg 1989; 4:216-238 (B70).

MULTIDOSE CARDIOPLEGIA

Additive	Volume (ml)	Delivered concentration		
THAM (300 mmol/L)	200	pH	:	7.7 - 7.8
CPD	50	Ca^{++}	:	0.5 - 0.6 mmol/L
5% Dextrose in		Glucose	: ±	43 mmol/L
1/4 N Saline	550	Osm	: ±	350 mOsm/kg H_2O
KCl (2 mmol/ml)	10	K^+	:	8 - 10 mmol/L

Legend:
Infused at 4°C - 8°C, at a rate of 200 ml/min antegrade for 1 min, retrograde for 1 min, at approximately 20 min intervals. CPD: Citrate phosphate dextrose. Modified from Buckberg G D, J Cardiac Surg 1989; 4:216-238 (B70).

Blood cardioplegia should be dilute in order to prevent increased viscosity with hypothermia, which could be harmful (see section 1.4). Therefore, blood cardioplegia is

usually delivered in either a 2:1 or 4:1 ratio (blood:crystalloid concentrate). In addition, haemodilution of the systemic perfusate is usually the norm during hypothermic cardiopulmonary bypass (see section 1.4). The cardioplegic delivery system for clinical use of blood cardioplegia, although more costly than that required for crystalloid, has also now been simplified (B70).

Blood cardioplegia provides excellent left ventricular protection, but right ventricular protection is reported to be superior with crystalloid cardioplegia (M62). The anterior situated right ventricle is much more dependant on adequate hypothermia (G24), and the colder delivery temperatures of crystalloid cardioplegia produce consistently colder right ventricular myocardial temperatures (M62). Blood cardioplegia is also associated with more postoperative conduction disturbances (G42), and is probably contraindicated if cold-reacting autoantibodies are present (H46). However, modified blood cardioplegic solutions can be used for normothermic "resuscitation" of energy depleted hearts (Table 1.12) (R43,R44), and as the basic composition for reperfusion solutions (Table 1.13) (see section 1.6) (B70).

Nonetheless, blood cardioplegia is the least temperature dependant solution should myocardial rewarming occur during clinical open-heart surgery (W12). A properly formulated blood cardioplegic solution probably provides better overall myocardial protection than crystalloid solutions today, especially if ischaemic periods are prolonged (H16).

3.3 PRESERVATION OF THE HEART FOR TRANSPLANTATION

The principals for protecting the myocardium during clinical open-heart surgery, also apply to preservation of the heart for transplantation. However, the surrounding environment can be much better controlled when preserving the isolated heart:-

- Low ($4^{o}C$) temperatures are easily maintained,
- There is no washout by noncoronary collateral blood flow,
- Hearts for transplantation are usually "healthy normal" hearts, free of coronary artery stenoses.

Donor hearts

Donor hearts are usually free of organic disease. However, donor hearts can incur damage as a result of endogenous catecholamine release at the time of brain death, which can cause myocardial calcium uptake and myocyte necrosis (N15,N16). Brain ischaemia and death deplete myocardial ATP and glycogen stores, and decreases myocardial function (W22). These metabolic changes are possibly related to reductions in circulating insulin, cortisol, triiodothyronine and thyroxine levels (N14). Hence, donor hearts may not be entirely "normal", and may benefit from normothermic cardioplegic arrest with a substrate-enriched blood cardioplegic solution prior to hypothermic storage (T19).

Heart storage for transplantation

The majority of clinical transplantation programs transport and store the donor heart by simple hypothermic storage. Furthermore, the preservation solution for storage of the heart is usually the standard cardioplegic solution used at that institution. However, this policy should probably be reevaluated, because of the differences between isolated organ storage and clinical open-heart surgery. In clinical practise, hypothermic storage

of the donor heart is usually limited to less than 4 - 6 hours, because the heart must be able to support the entire circulation at the end of the surgical procedure. However, experimental hypothermic heart storage possibly has an upper limit of approximately 18 hours, in terms of energy stores (T11). In contrast, continuous hypothermic perfusion storage methods can maintain myocardial energy stores for more prolonged periods, but involve more complex systems and can also be damaging (T11). Alternative methods such as supercooling, freezing, and hyperbaric oxygenation have been tried experimentally, but are not used clinically (C37).

Isolated hypothermic storage

During isolated hypothermic organ storage, there is no continued supply of substrates or washout of metabolic end-products in order to allow ongoing energy production. Therefore, ionic pumps eventually cease to function and passive diffusion of electrolytes will occur. Furthermore, severe hypothermia (less than $4^{o}C$ - $10^{o}C$) suppresses the activity of the Na^{+} / K^{+} pump (M15,M26), and this may also alter intracellular homeostasis.

Intracellular electrolyte equivalent solutions, by attempting to abolish all ionic gradients, further reduce metabolic requirements and maintain intracellular ionic homeostasis (R17). These intracellular solutions were originally designed and used to store kidneys for up to 30 hours (C32). Nevertheless, Reitz et al were able to obtain survival of orthotopic transplanted hearts following 18 - 26 hour storage of donor canine hearts with both the Collins and Sacks solutions (Table 3.6) (R17). These results could be reproduced by other investigators using modifications of these solutions (S92,T15), and hypocalcaemic reperfusion solutions improved survival further (D8,S92).

The primary factors that have improved the efficacy of renal preservation solutions are thought to be high osmolality (400 mOsm/kg H_2O), impermeate solutes but preferably not glucose (A24), and replacement of chloride by impermeate anions (G30). This was

partly the basis for the composition of the solution formulated for pancreatic, hepatic and renal preservation by Belzer and co-workers: UW-CSS; University of Wisconsin Cold Storage Solution (P23,W3). Adenosine and free radical scavengers were also considered essential (S65).

Table 3.6

INTRACELLULAR STORAGE SOLUTIONS

(mmol/L)	Collins - C_2 (C32)	Sacks (S1)	Stanford Univ. Sol. (S95)	Belzer UW-CSS (W3)
Na^+	10	14	20	30
K^+	115	126	27	125
Mg^{2+}	30	8	-	5
HCO_3^-	10	20	20	-
Cl^-	15	16	27	-
SO_4^{2-}	30	-	-	-
PO_4^{3-}	57.5	60	-	25
Glucose	139	-	255	-
Mannitol	-	206	63	-
Lactobionate	-	-	-	100
Raffinose	-	-	-	30
Adenosine	-	-	-	5
Glutathione	-	-	-	3
Allopurinol	-	-	-	1
Pentastarch (gm/L)	-	-	-	50
Dexamethasone (mg/L)	-	-	-	8
Insulin (U/L)	-	-	-	100
Bactrim (ml/L)	-	-	-	0.5
Osmolality (mOsm/kg H_2O)	300	430	> 400	320
pH	7.0 (25°C)	7.0 (2°C)	- -	7.4 (25°C)

Preservation of the heart has also been improved by hyperosmolarity (D8) and addition of colloid oncotic pressure to the preservation solution (D8,T21). In addition,

preservation might be more optimal if the myocardium is first arrested with an extracellular electrolyte equivalent cardioplegic solution, and then flushed and stored in an intracellular electrolyte equivalent solution (K27). The "intracellular" Bretschneider HTK cardioplegic solution maintains pH, and delays ATP decay more than the "extracellular" St Thomas' Hospital No 1 cardioplegic solution (E15). However, glucose containing but better buffered "extracellular" cardioplegic solutions might provide even better protection at 0°C (E15). The UW-CSS solution provides superior experimental storage of hearts when compared to Bretschneider (O10), St Thomas' (W24,Y3), Stanford (S95,W24) or modified Collins (S95) solutions, and this might be due to its reported superior preservation of the endothelium (C1).

Nevertheless, the majority of clinical transplant centers presently use either the Stanford University solution or other extracellular based formulations (G26).

Continuous organ perfusion

Continuous perfusion of the isolated heart is technically more difficult, than isolated hypothermic storage. In addition, although it is associated with superior preservation of high energy phosphates, the perfusate can cause myocardial damage (T11). Continuous hypothermic perfusion with oxygenated Bretschneider HTK (F19), and other perfusates has allowed successful experimental transplantation after 24 hour (K17,M40,W21,W22) and 48 hour storage (W25,W26), and has also been used for clinical heart transplantation (W23). Low perfusion pressures (8 - 12 cm H_2O) must be used to prevent myocardial oedema, the maximum pressure being 20 cm H_2O, and colloid is an essential component of the perfusate if this method is used (K17). Continuous perfusion of the beating heart at or near normothermia with modified blood perfusates, has also allowed experimental storage of hearts for 12 - 24 hours (C22,S61).

Nevertheless, continuous perfusion systems for storage of hearts are not widely accepted clinically, primarily because of a concern that any inadvertent technical mishap could

result in unnoticed loss of protection. The transplanted heart must be capable of full function immediately after insertion into the recipient (C37), and therefore a method of assessing viability such as quantitative birefringence (D6) or intramyocardial pH (K14,T6) would probably be essential if these more complicated systems are to be used.

The ideal technique for storage of the donor transplant heart has not yet been determined, despite a large number of experiments. Simple hypothermic storage with an extracellular electrolyte equivalent cardioplegic solution remains the most widely used clinically method, at present. However, attention should also be directed to the preharvesting management of potential donor hearts, the initial solution used to arrest the heart while at normothermia, hypothermic storage and transport solution (H33), storage temperature (7.5°C - 10°C) (H34,R38,T7,T8,T28), the reinfusion of cardioplegic solution during implantation (S93), and the use of a reperfusion solution (B72,D8,M46,S92).

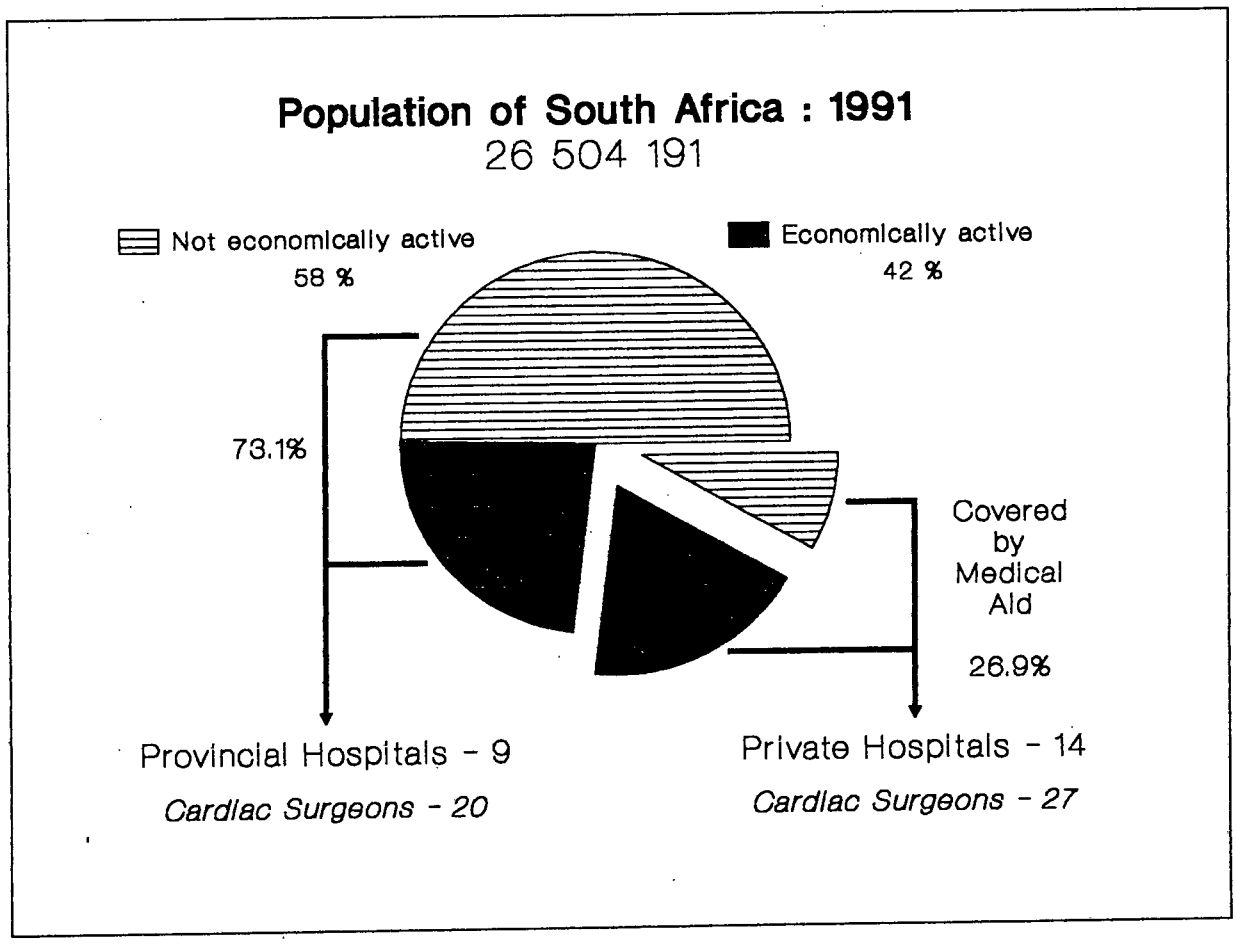
Chapter 4

MYOCARDIAL PROTECTION IN SOUTH AFRICA

Cardiac Surgery in South Africa

The population of South Africa in 1991 was 26.5 million people, made up of 10 separate ethnic groups (S69). However, only 7.1 million of the population are covered by medical aid health schemes, and hence can afford private medical care (Fig 4.1) (A27).

Figure 4.1



Legend:
The percentage of the population treated in either provincial or private hospitals, and the total number of cardiac surgeons in South Africa are shown. The 1986 statistics for the economically active population - 42 % (S64) were extrapolated onto the provisional 1991 population census figures (S69). Furthermore, the assumption was that 50 % of dependants on medical aid schemes (A27) were also economically active.

The health care of the remaining 73.1 % of the population is provided by provincial government funded hospitals, consisting of both academic hospitals with attached University administered medical schools and non-academic hospitals.

Cardiac surgery is performed in 9 provincial and 14 private hospitals in South Africa in 1991, and an estimated 7 000 open-heart surgical procedures are performed annually. Twenty percent of the total deaths in South Africa in 1988 ($\pm$ 7.9 deaths / 1000 population) were due to diseases of the circulatory system, a third of which were classified as either ischaemic or rheumatic heart disease (S64). However, ischaemic heart disease was the cause of 14.8 % of the deaths in the more affluent population groups, as apposed to 0.8 % in the remainder of the population. Hence, ischaemic heart disease surgery is the predominant type of cardiac surgery performed in the private sector. Whereas, in the provincial hospitals more rheumatic valvular, congenital and traumatic induced cardiac surgical procedures are performed, in addition to all postgraduate training. Applied research is also only undertaken at the government supported provincial hospitals attached to universities.

Cardioplegic solutions used in South Africa: 1988

A survey of the type of cardioplegic solutions used in South Africa was obtained in 1988, and revealed that approximately 70 % of hospitals performing cardiac surgery in South Africa were using crystalloid cardioplegic solutions, and the remainder various 2:1 blood cardioplegic formulations (see Appendix A-1). Neither retrograde cardioplegia, normothermic induction, nor reperfusion cardioplegic solutions were used. At Groote Schuur Hospital we had recently changed to the St Thomas' Hospital No 2 cardioplegic solution (Table 3.3), and were the only unit using this cardioplegic solution at that time. Published objective evaluations, either experimentally or clinically, of the crystalloid

cardioplegic solutions being used in the remainder of the country have never been reported (Table 4.1).

Table 4.1
CRYSTALLOID CARDIOPLEGIC SOLUTIONS: SOUTH AFRICA - 1987

mmol/L	SABAX	GSH	PB-1	PB-2
Na^+	141	15	130	140
K^+	24 / 12	20	34	34
HCO_3^-	38	15	27	37
Ca^{2+}	0.9	1.0	-	-
Mg^{2+}	1.5	12	1.5	1.5
Cl^-	119	46	140	140
Glucose	51	240	-	56
Mannitol	68	14	-	-
Osmolality (mOsm/kg H_2O)	416	350	302	357
pH	7.7	7.6	7.7	7.8

Legend:
SABAX; commercial cardioplegic solution available from Sabax Ltd. A high potassium solution is used for induction of cardioplegic arrest and a second low potassium solution for all subsequent multidoses. GSH; cardioplegic solution previously used at Groote Schuur Hospital. PB-; cardioplegic solutions made up by adding potassium and glucose to a liter of Plasmalyte B intravenous electrolyte solution.

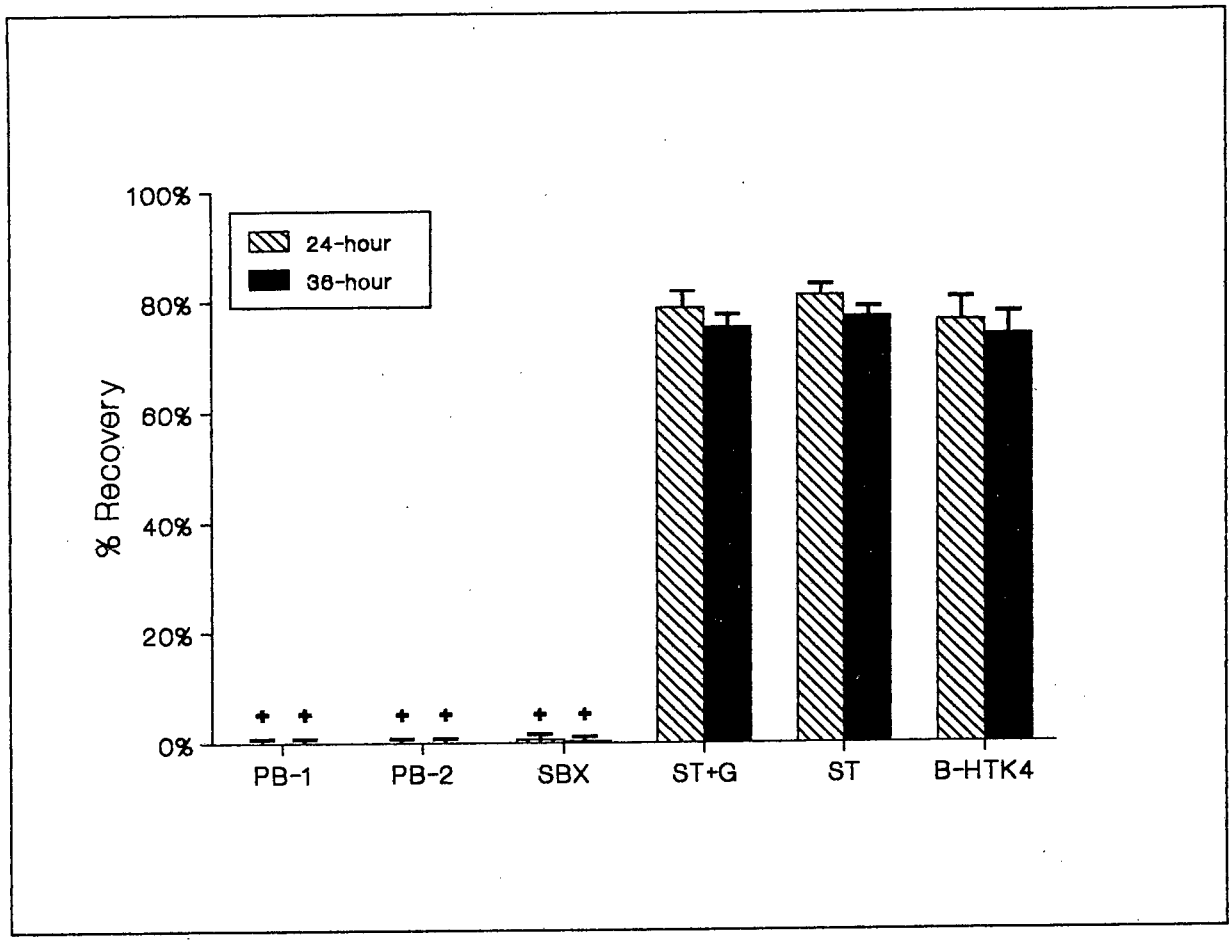
We evaluated both the SABAX and GSH cardioplegic solutions in the in vivo primate model, and compared them to the St Thomas' Hospital No 2 cardioplegic solution (see Appendix A-1). Postischaemic haemodynamic recovery was superior with the St Thomas' Hospital No 2 cardioplegic solution, although no differences in high energy phosphate content could be demonstrated between the different cardioplegic solutions.

In a separate study these cardioplegic solutions (SABAX, St Thomas', PB-1, PB-2) were exposed to monolayer cultures of human venous endothelial cells at room temperature (22^oC) for 12 hours, as described in Chapter 2 (see Appendix A-5). This temperature was selected for this study, as intraoperatively the temperature of the myocardium

usually varies from 15°C to 20°C (D3) and the major beneficial effect of hypothermia occurs below 28°C (F18,H24). Adequate post-exposure endothelial cell survival was only obtained with the St Thomas' No 2 and Bretschneider HTK4 cardioplegic solutions (Fig 4.2). All other crystalloid cardioplegic solutions used in South Africa resulted in loss of adherence, disruption, and cell death of the endothelial cells.

Figure 4.2

ENDOTHELIAL CELL SURVIVAL



Legend:

Endothelial cell survival 24 hours and 36 hours after a 12-hour exposure to cardioplegic solutions at 22°C. ST, St Thomas' Hospital No 2 cardioplegic solution with or without glucose (G; 10 mmol/L); B-HTK4, Bretschneider HTK4 cardioplegic solution; SBX, Sabax cardioplegic solution; PB-, cardioplegic solutions formulated by adding glucose and potassium chloride to a balanced intravenous electrolyte solution Plasmalyte B. Means and standard errors of means are presented.

+ - $p < 0.001$ compared to the respective ST or B-HTK4 time period.

Plasmalyte B cardioplegic solutions

Plasmalyte B cardioplegic formulations were cytotoxic in the endothelial cell model. This cytotoxicity was shown to be due to these solutions being calcium-free and having excessively high potassium concentrations (appendix A-5). One cardiothoracic unit in South Africa had modified the PB-1 solution by adding 200 ml pump blood; that is dilute blood cardioplegia as proposed by Daggett et al (D2,H31), and this unit had subjectively observed improved recovery (personal communication). However, this was probably as a result of the addition of trace amounts of calcium to a calcium-free solution (H29), although red blood cells do provide other beneficial factors (B29,I3,S79,Z7). The dangers and limitations of a calcium-free solution, specifically with regard to the calcium paradox, have already been discussed in Section 3.1 (H19,J20,R55). Furthermore, Plasmalyte B cardioplegic formulations with additional calcium have also been shown to be inferior to the St Thomas' Hospital No 2 cardioplegic solution (L41).

In addition, these Plasmalyte B formulations contain excessively high potassium concentrations which shift the resting membrane potential into the range where calcium channels are opened (O17), and this potassium content is well above the optimal potassium concentration for extracellular electrolyte equivalent cardioplegic solutions (see section 3.1) (R51). In addition, the magnesium content of these Plasmalyte B cardioplegic solutions is well below the optimal concentration of this important cation in cardioplegic solutions (see section 3.1) (H28,R18).

We did not evaluate the Plasmalyte B cardioplegic solutions in either the isolated rat heart or in vivo primate models, however, both our endothelial study and literature review support the conclusion that the Plasmalyte B cardioplegic solutions used in South Africa (Table 4.1) are both potentially harmful and less efficacious than the St Thomas' Hospital No 2 cardioplegic solution.

SABAX cardioplegic solution

In the in vivo primate model the St Thomas' Hospital No 2 cardioplegic solution provided better myocardial protection and superior postischaemic haemodynamic recovery than the SABAX cardioplegic solution (appendix A-1). The SABAX cardioplegic solution (Table 4.1) has a high potassium concentration (24 mmol/L) for induction of cardioplegic arrest and then a lower concentration (12 mmol/L) for all multidose reinfusions of cardioplegia, which is theoretically beneficial (B74). Nevertheless, the multidose SABAX cardioplegic solution with the lower concentration of potassium was evaluated in the endothelial cell model, and found to be cytotoxic to the endothelium (appendix A-5). This endothelial cytotoxicity was thought to be due to the low concentration of magnesium, which is well below the optimal concentration recommended for crystalloid cardioplegic solutions (H28,R18). Furthermore, the concentration of both mannitol and glucose contained in this cardioplegic solution has been shown to be harmful in crystalloid solutions (H27,appendix A-2), although we were unable to demonstrate a deleterious effect of high glucose (50 mmol/L) in our endothelial model (appendix A-5).

The SABAX cardioplegic solution is also hyperosmolal, and although hyperosmolality has been shown to be beneficial during reperfusion of the postischaemic myocardium (S19), excessive hyperosmolality is not beneficial in cardioplegic solutions (H27) and should probably never exceed 400 mOsm/kg H_2O (A24,W28). Although we did not add methylprednisolone to the SABAX solution we evaluated as practised by some units in South Africa (personal communication), steroids have not been shown to be beneficial in cardioplegic solutions (K23).

The SABAX cardioplegic solution is therefore both potentially harmful and less efficacious than the St Thomas' Hospital No 2 cardioplegic solution.

GSH cardioplegic solution

In the in vivo primate model, the GSH cardioplegic solution provided "myocardial protection" that was no better than a "non-cardioplegic" solution (standard Krebs-Henseleit buffer) (appendix A-1). The GSH cardioplegic solution was not evaluated in the endothelial cell model.

This cardioplegic solution previously used at Groote Schuur Hospital, is a prime example of the dangers of "adding a bit of this and a bit of that" without understanding the important inter-relationship of the different ionic components. GSH cardioplegic solution was originally based on the early Bretschneider cardioplegic formulations (Table 3.4), but was then progressively altered. Calcium and magnesium where added to the original formulation, presumably because of concern for the calcium paradox subsequent to perfusion with calcium-free solutions (H19,J20), and reported beneficial effect of the cation magnesium (H28). The basic premise that a cardioplegic solution with a low sodium content must be calcium-free (G10) was not taken into account (see section 3.1). This "modified" cardioplegic solution was then used clinically, despite no experimental or clinical evaluations of this specific formulation.

The GSH cardioplegic solution is an extremely poor formulation for a cardioplegic solution, and is no better than a hypothermic buffered physiological saline solution.

Other crystalloid cardioplegic solutions used in South Africa

All questionnaires in the 1988 survey of cardioplegic solutions used in South Africa were not returned. Nevertheless, one other additional cardioplegic solution is being used in South Africa; the St Thomas' Hospital No 1 cardioplegic solution (Table 3.3).

The St Thomas' Hospital No 2 cardioplegic solution provides better myocardial protection than the St Thomas' No 1 cardioplegic solution (L26). Furthermore, I doubt whether in South Africa the imported "MacCarthy ampoule" (Cardioplegic solution 20 ml: John Bell & Croyden; London, United Kingdom) has even been used to formulate

the correct St Thomas' No 1 solution. This ampoule of potassium, magnesium and procaine (Table 3.3) should be added to Ringer's solution (L26); a balanced electrolyte solution which is not freely available in South Africa. The addition of the "MacCarthy ampoule" to other balanced electrolyte solutions (Ringer's lactate, Plasmalyte B) would not result in the correct formulation of the St Thomas' No 1 cardioplegic solution (Table 4.2).

Table 4.2

ST THOMAS' HOSPITAL NO 1 CARDIOPLEGIC SOLUTION

mmol/L	St Thomas' No 1	MacCarthy Ampoule	Ringer's Solution	Ringer's Lactate	Plasmalyte B
Na^+	144	-	144	131	130
K^+	20	16	4	5.4	4
HCO_3^-	-	-	-	-	27
Ca^{2+}	2.4	-	2.4	2.0	-
Mg^{2+}	16	16	-	-	1.5
Cl^-	201.8	49	152.8	111	110
Procaine	1.0	1.0	-	-	-
Lactate	-	-	-	29	-

Legend:
The St Thomas' Hospital No 1 cardioplegic solution is made up by adding the MacCarthy ampoule to Ringer's solution.

Moreover, the MacCarthy ampoule is not registered with the Medical Control Council of South Africa and thus the commercial sale and use of this cardioplegic ampoule is at present illegal in South Africa (personal communication).

The addition of the MacCarthy ampoule to Ringer's lactate, although approximating the St Thomas' No 1 formulation, will thus contain the anion lactate. Lactate may be harmful during global ischaemia, although lactate possibly does not enhance damage during normothermic ischaemia (G14), and may be beneficial in the prime solution of the cardiopulmonary bypass circuit (T-19). Nevertheless, lactate ions can induce morphological mitochondrial changes (A29), and there is thus a potential danger of

including lactate in crystalloid cardioplegic solutions (H26). Lactate containing cardioplegic solutions provide inferior myocardial protection compared to the St Thomas' Hospital No 2 cardioplegic solution (R29).

The addition of the MacCarthy ampoule to the Plasmalyte B electrolyte solution would result in a calcium-free cardioplegic solution, the dangers of which have already been discussed (see section 3.1) (H19,J20,R55).

In conclusion, the best crystalloid cardioplegic solution presently available in South Africa is the St Thomas' Hospital No 2 cardioplegic solution (St Thomas' Hospital Cardioplegic Solution: Sabax Ltd, Johannesburg:- 10 ml 8.5 % sodium bicarbonate (10 mmol) must be added to each vacoliter before use, as sodium bicarbonate is not included in the vacoliter because of stability and shelf storage considerations).

Myocardial protection in South Africa: 1991

Another questionnaire regarding techniques of myocardial protection, was forwarded to all cardiac surgeons in South Africa in 1991; 80 % of questionnaires were returned. The approximate distribution of open-heart surgical procedures presently performed in South Africa is shown in Table 4.3.

Table 4.3

DISTRIBUTION OF OPEN-HEART SURGICAL PROCEDURES

Provincial Hospitals ± 270 / month	Procedures	Private Hospitals ± 400 / month
55%	Valvular heart surgery	23%
26%	CABG	63%
16%	Congenital heart surgery	14%
< 2%	Transplantation	--
< 1%	Arrhythmia heart surgery	--

Legend:
The estimated number and type of open-heart surgical procedures presently being performed in South Africa is shown. CABG - coronary artery bypass grafting.

Cardioplegic solutions: 1991

The majority of cardiac surgeons in the provincial hospitals now use the St Thomas' Hospital No 2 cardioplegic solution (Table 4.4).

Table 4.4

CARDIOPLEGIC SOLUTIONS USED IN SOUTH AFRICA: 1991

Provincial Surgeons (N = 20)	Cardioplegic Solution	Private Surgeons (N = 27)
16	St Thomas' No 2 cardioplegic solution	4
3	Blood Cardioplegic solutions	6
1	Other Crystalloid cardioplegic solutions	17

Clinical use of the St Thomas' Hospital No 2 cardioplegic solution

The subjective clinical experience of cardiac surgeons using the St Thomas' Hospital No 2 cardioplegic solution has been variable. Most surgeons reported good results with this

cardioplegic solution. However, criticisms about the St Thomas' Hospital No 2 cardioplegic solution put forward are:

i) A high incidence of the occurrence of ventricular fibrillation and therefore the need for defibrillation following the release of the aortic cross-clamp.

ii) Resumption of electrical activity within 15 - 20 min of cardioplegic arrest.

This was in contrast to the experience of surgeons using the St Thomas' Hospital No 1 cardioplegic solution, who reportedly rarely need to use defibrillation.

The St Thomas' No 1 cardioplegic solution contains the local anaesthetic and antiarrhythmic agent procaine-HCl (1 mmol/L), which additionally has a prolonged duration of action, and its use is associated with prolonged asystole during reperfusion of the postischaemic myocardium. Nevertheless, the incidence of spontaneous normal sinus rhythm, following the use of the St Thomas' No 2 cardioplegic solution and release of the aortic cross-clamp, can be increased by the addition of lignocaine or procaine to this solution as well. The recommended dose of lignocaine or procaine that can be added to the St Thomas' No 2 cardioplegic solution is 0.05 mmol/L (H22) (0.72 ml/L of 2% lignocaine-HCl for intravenous use; 14.4 mg/L: see section 3.1). This amount of lignocaine or procaine was recommended as a result of a dose response curve for postischaemic myocardial recovery performed on isolated rat hearts. However, it is possible that a greater amount of lignocaine or procaine (similar to that used in the St Thomas' No 1 cardioplegic solution) may be necessary, for the prevention of ventricular fibrillation during the reperfusion of the postischaemic human myocardium. A dose of lignocaine in the St Thomas' No 2 cardioplegic solution of 0.7 mmol/L (10 ml 2% lignocaine-HCl; 200 mg/L) is being used by two cardiac surgeons, who report that the majority of their patients return spontaneously to normal sinus rhythm after a period of asystole following the release of the aortic cross-clamp. However, caution should be taken as to the total dose of cardioplegic solution administered if this amount of lignocaine is used in order to prevent possible lignocaine toxicity. Therefore, we will be shortly commencing a clinical trial of the addition of different doses of lignocaine to the St Thomas' No 2 cardioplegic solution.

Two cardiac surgeons reported early resumption of electrical activity with the use of the St Thomas' No 2 cardioplegic solution. However, this is not the experience of the majority of surgeons using this solution. In addition to the composition of the cardioplegic solution being used, there are a multitude of additional factors that also must be attended to if adequate myocardial protection is to be obtained (see chapter 1).

Blood cardioplegia used in South Africa

The blood cardioplegic solutions being used in South Africa are delivered to the patient in a 1:1 ratio of "pump blood" to crystalloid concentrate. Two different crystalloid concentrates are being used (Table 4.5). The calculated delivered concentrations of the indicated variables would differ slightly from those indicated in Table 4.5 in the clinical situation because of systemic haemodilution while on cardiopulmonary bypass (see section 1.4)

Table 4.5

CONCENTRATE FOR 1:1 BLOOD CARDIOPLEGIA

CONCENTRATE 1

Component	Quantity	Delivered concentration
Plasmalyte B	1 L	Hb : 7 g% Ca^{2+} : ± 1.2 mmol/L
15% KCl	13.5 ml	K^+ : 17.8 mmol/L Osm : ± 300 mOsm/kg H_2O
Occasional additional additives		
50% $MgSO_4$	6 ml	Mg^{2+} : 6.6 mmol/L
50 % Dextrose	10 - 20 ml	Glucose : 17 - 31 mmol/L Osm : 310 - 340 mOsm/kg H_2O

Legend:

Calculated delivered concentrations, assuming no haemodilution and normal serum levels; i.e. Hb 14 g %, Ca^{2+} 2.4 mmol/L, K^+ 4.6 mmol/L, Mg^{2+} 0.9 mmol/L, Glucose 6 mmol/L. (NB: Plasmalyte B contains K^+ 4 mmol/L, Mg^{2+} 1.5 mmol/L)

CONCENTRATE 2

Component	Quantity	Delivered concentration
Ringer's Lactate	1 L	Hb : 7 g%
		Ca^{2+}: ± 2.2 mmol/L
15% KCl	20 ml	K^{+} : 25 mmol/L
50% $MgSO_4$	8 ml	Mg^{2+}: 8.7 mmol/L
8.5% $NaHCO_3$	50 ml	
25% Mannitol	10 ml	Osm : ± 340 mOsm/kg H_2O
20% Albumin	50 ml	
Lignocaine	200 mg	

Legend:
Calculated delivered concentrations, assuming no haemodilution and normal serum levels; i.e. Hb 14 g%, Ca^{2+} 2.4 mmol/L, K^{+} 4.6 mmol/L, Mg^{2+} 0.9 mmol/L, Glucose 6 mmol/L. (NB: Ringer's Lactate contains K^{+} 5.4 mmol/L, Ca^{2+} 2.0 mmol/L). The fraction of delivered ionized Ca^{2+} might be less than that estimated because of calcium binding to albumin.

Other crystalloid cardioplegic solutions in South Africa

Various other crystalloid cardioplegic solutions are still being used in South Africa. These include the previously discussed SABAX cardioplegic solution (at least 4 surgeons), the St Thomas' Hospital No 1 cardioplegic solution (at least 3 surgeons), and another formulation made up from Plasmalyte B but having a final electrolyte composition similar to the St Thomas' No 2 cardioplegic solution (5 surgeons). The following is added to a liter of Plasmalyte B:- 10 or 6 ml 15% KCl (20 or 12 mmol), 7.1 ml 50% $MgSO_4$ (14.5 mmol) and 1.76 ml 10% $CaCl_2$ (1.2 mmol), to make a final composition of Na^{+} - 130 mmol/L, K^{+} - 24 or 16 mmol/L, Ca^{2+} - 1.2 mmol/L, Mg^{2+} - 16 mmol/L & HCO_3^{-} - 27 mmol/L (A composition similar to the St Thomas No 2 cardioplegic solution, except for a higher bicarbonate content). The higher potassium concentration is used in the initial solution for induction of cardioplegic arrest and thereafter the lower potassium concentration for subsequent multidose reinfusions.

Strategy of myocardial protection

Approximately 11 % of cardiac surgeons institute a preoperative adjuvant therapy in selected patients in order to improve myocardial protection. This includes preoperative GIK (3 surgeons), and / or preoperative allopurinol (3 surgeons) in selected patients.

Possibly the most significant advance in the field of myocardial protection during the past few years has been the introduction of retrograde administration of cardioplegic solutions via the coronary sinus (see section 1.5). A Strategy of combined antegrade / retrograde infusion of cardioplegic solutions in selected patients, specifically CABG and aortic valve patients, is now being used by approximately 47 % of surgeons in South Africa. Modified reperfusion solutions are also being used by ± 8 % of surgeons, although a further 19 % infuse a bolus of either lignocaine or potassium into the aortic root prior to the release of the aortic cross-clamp in an attempt to modify the initial reperfusion period and to decrease the incidence of reperfusion ventricular fibrillation.

Chapter 5

MODIFICATIONS OF THE ST THOMAS' HOSPITAL CARDIOPLEGIC SOLUTION

The electrolyte content of the St Thomas' Hospital No 2 cardioplegic solution (Table 3.3) is based on careful dose response curves for each individual cation, and is a well formulated cardioplegic solution (see section 3.1). However, the St Thomas' No 2 cardioplegic solution does not contain a substrate for ongoing energy production, has a poor buffering capacity, is isotonic and lacks colloid oncotic pressure. We therefore evaluated various modifications of the St Thomas' cardioplegic solution, in order to assess whether these and other perceived deficiencies in its composition could be corrected.

5.1 SUBSTRATE IN THE ST THOMAS' CARDIOPLEGIC SOLUTION

The principal source of energy for the heart is free fatty acids (N3). However, the well oxygenated heart is not a "fussy eater" and can also utilize both glucose and lactate (M41,O16). Nevertheless, during anoxia and ischaemia some fuels can no longer be used because of major alterations in energy metabolism (R53).

Glucose and Fatty acid metabolism

Glucose metabolism in aerobic conditions

The cell membrane of the myocyte is not freely permeable to glucose (M41) and glucose uptake requires a sarcolemmal glucose carrier which though does not utilize energy (O14). Insulin, epinephrine and hypoxia all increase glucose uptake (M41,O16). Intracellular glucose is then converted to glucose-6-phosphate by utilizing ATP, and from here further metabolism can proceed in three directions (Fig 5.1) (L27,M41,N3,O14):

Glycogenesis,

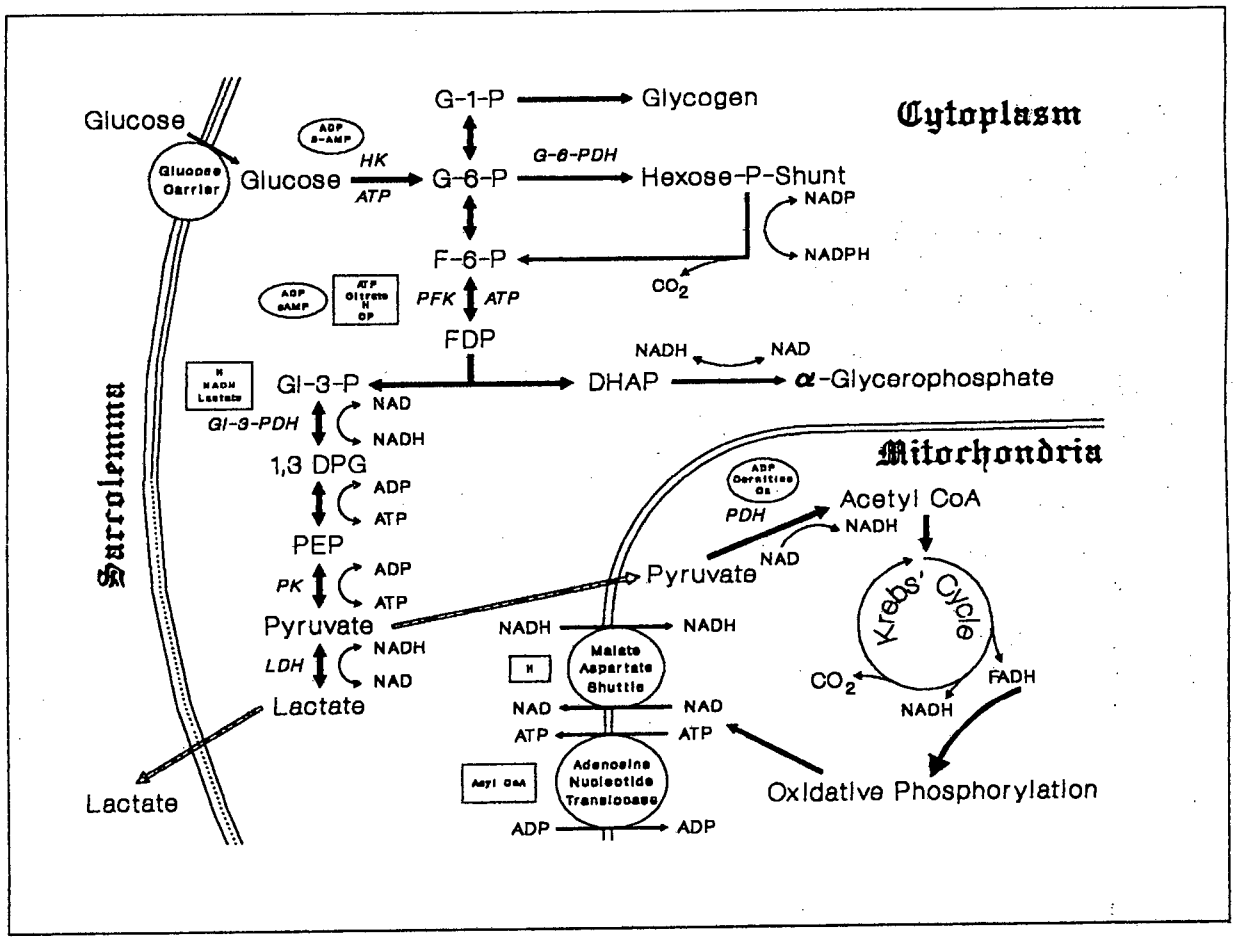
Glycolysis, Krebs' cycle, Oxidative phosphorylation,

Hexose-mono-phosphate shunt.

Glucose-6-phosphate is metabolized in the cytoplasm by glycolysis to pyruvate. Pyruvate is converted to acetyl CoA in the mitochondria which is then oxidized in the tricarboxylic acid cycle (Krebs' cycle) to CO_2, H_2O and NADH, and finally ATP is produced by oxidative phosphorylation in the electron transport chain. NADH formed in the cytoplasm during glycolysis can also be transported into the mitochondria by the aspartate-malate shuttle, where it can also undergo oxidative phosphorylation (S3).

Figure 5.1

MYOCARDIAL METABOLISM OF GLUCOSE



Legend:

The primary rate limiting enzymes are PFK, phosphofructokinase; Gl-3-PDH, glyceraldehyde-3-phosphate dehydrogenase; and other important enzymes are HK, hexokinase; G-6-PDH, glucose-6-phosphate dehydrogenase; PK, pyruvate kinase; PDH, pyruvate dehydrogenase; LDH, lactate dehydrogenase. Factors that inhibit specific enzymes or processes are shown in square boxes, and stimulating factors in oval boxes.

ATP produced in the mitochondria is then transported back into the cytoplasm via a carrier; adenine nucleotide translocase. Thus complete oxidation of a molecule of extracellular glucose produces 38 molecules of ATP (D7,O16). Glycolysis is controlled by the cytoplasmic levels of ATP and citrate produced by oxidative phosphorylation and the levels of ADP and AMP produced by the hydrolysis of ATP (D7), which causes feedback inhibition or stimulation of rate limiting glycolytic enzymes (Fig 5.1). Alternatively, glucose-6-phosphate can be converted to glycogen in the presence of insulin (O14).

Glucose metabolism during anoxia

Oxidative phosphorylation cannot occur in the absence of oxygen. Thus, in anoxia the only source of ATP is from anaerobic glycolysis, but now each molecule of extracellular glucose produces only two molecules of ATP. Glucose uptake and phosphofructokinase activity is stimulated during anoxia, and cytosolic NADH is used to reduce both pyruvate to lactate, and dihydroxyacetone phosphate to α-glycerophosphate (Fig 5.1). Thus both intracellular lactate increases (approximately twofold), as the sarcolemma is not freely permeable to lactate, as well as extracellular lactate (approximately tenfold) (R53). In perfused nonischaemic hypoxic or anoxic hearts the metabolic end products of glycolysis; NADH, protons, and lactate, are removed, and an increased rate of glycolysis is maintained (R53,S22). Thus glucose and insulin are beneficial during anoxia or moderate ischaemia if metabolic end products are removed (A28). Nevertheless, anaerobic glycolysis can produce only 10% - 20% of the normal aerobic cellular ATP requirements (D7,N3), and thus adult hearts cannot maintain normal function by accelerated anaerobic glycolysis (J5,J6,Y5). However, hypoxic induced accelerated glycolysis is greater in neonatal hearts. Nonischaemic hypoxic neonatal hearts can thus maintain normal myocardial ATP levels (J5) and normal mechanical function (J6) with minimal damage to mitochondrial function (Y5), by accelerated anaerobic glycolysis.

Glucose metabolism during ischaemia

If the myocardium is ischaemic as well as hypoxic or anoxic, there is diminished blood flow and thus inadequate washout of metabolic end products. Furthermore, we should also make a distinction between regional ischaemia and total global ischaemia, both of which are associated with slightly different metabolic effects (O15). Following the onset of global ischaemia there is an initial increased glycolytic flux due to rapid glycogenolysis. However, this is followed by feedback inhibition of glycolysis (R53), as a result of accumulation of both extracellular (hundredfold) as well as intracellular (tenfold) lactate (R53), protons (N8), NADH, and α-glycerophosphate (D7). The

glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase is inhibited primarily by increased NADH levels (N3), as well as by increased protons and lactate (M50,R52). Lactate ions may also impair mitochondrial function (A29). Increased protons result in intracellular acidosis which also inhibits the activity of phosphofructokinase (K39,O14), the malate-aspartate shuttle, alters calcium homeostasis and the permeability of plasma membranes (W32). Furthermore, intracellular acidosis is not secondary to only lactic acid accumulation, as protons are also generated by hydrolysis of ATP and from CO_2 retention (G16).

Ischaemia thus markedly inhibits both the capacity of the cell to produce energy from glycolysis and also impairs other cellular functions as a result of accumulation of metabolic end products. Furthermore, diminished postischaemic myocardial recovery has been related to increased lactate, protons and NADH levels rather than to reduced ATP levels (N5).

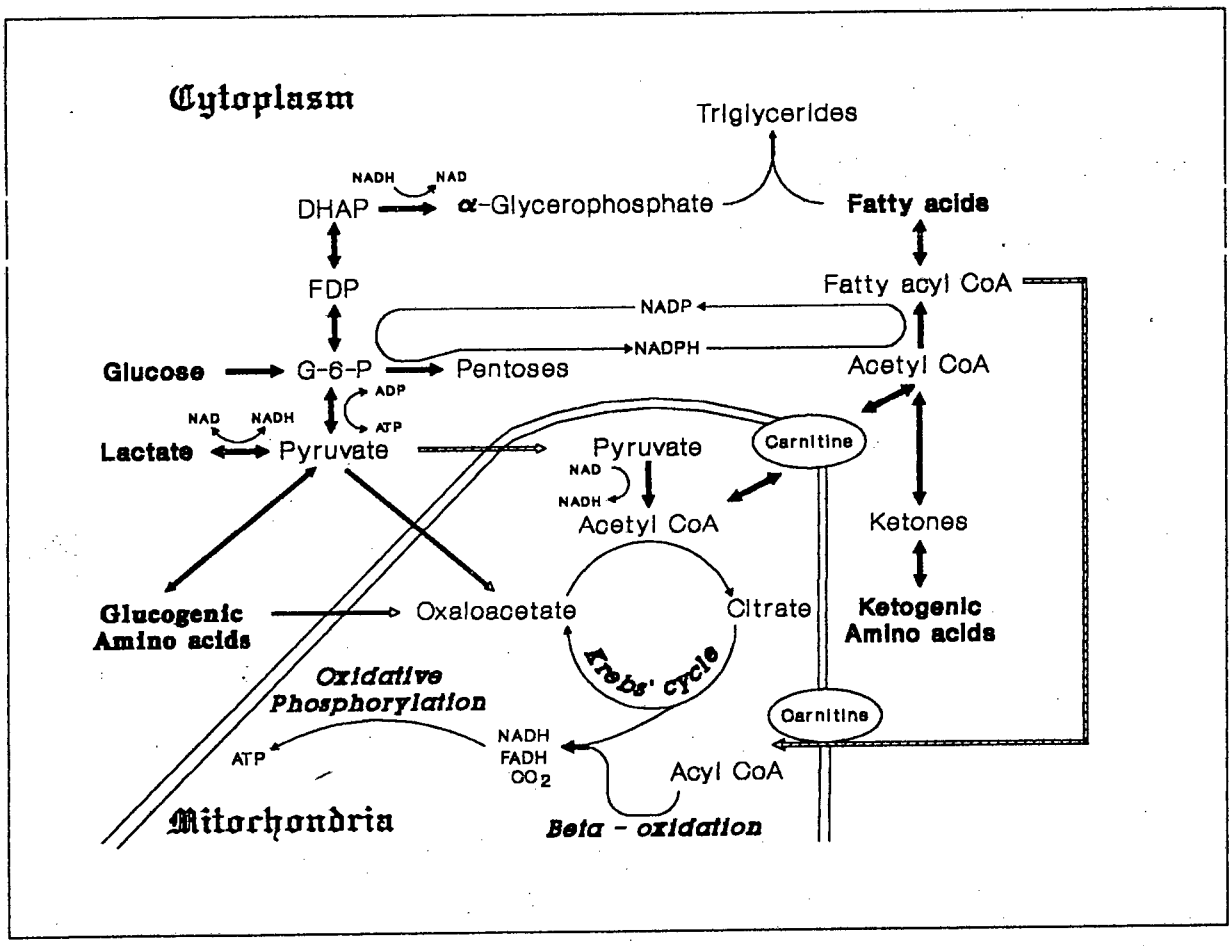
Lipid metabolism in aerobic conditions

Triglycerides cannot pass through the cell membrane and must be first hydrolyzed by lipoprotein lipase located in the capillary endothelium. Free fatty acids then enter the cell by either passive diffusion or a carrier mediated mechanism. Intracellular fatty acids are activated to fatty acyl CoA (ATP dependant reaction), which is transferred into the mitochondria by a carnitine carrier where beta-oxidation produces ATP (Fig 5.2) (M41,O14,O18).

An intimate relationship exists between lipid and carbohydrate metabolism, for example insulin stimulates glycolysis but inhibits fatty acid oxidation. Furthermore, regardless of whether oxidation of either carbohydrate or lipid predominates at any one moment, a certain amount of carbohydrate is always converted to lipid (Fig 5.2) (C3). Myocardial accumulation of lipid also occurs from uptake of exogenous free fatty acids and in hypoxia from glycolytic derived α-glycerophosphate and pyruvate (G22,S23,S30).

However, conversion of lipid to carbohydrate is not possible in animal tissue, because the conversion of pyruvate to acetyl CoA is thought to be an irreversible reaction (L27).

Figure 5.2
INTERRELATIONSHIP OF GLUCOSE, LIPID and AMINO ACID METABOLISM



Legend:
Interrelationship of the primary myocardial fuels; fatty acids, glucose, lactate, and glucogenic or ketogenic amino acids is shown. Beta-oxidation, the Krebs' cycle and oxidative phosphorylation all take place in the mitochondria, and the transfer of acetyl or acyl CoA across the mitochondrial membrane requires the carrier carnitine.

Lipid metabolism during anoxia and ischaemia

The inability to reoxidize NADH in the mitochondria as a result of diminished oxidative phosphorylation during ischaemia, impairs beta-oxidation of fatty acyl CoA which therefore accumulates and increases esterification of fats (O18). Furthermore, pyruvate

is strongly ketogenic in isolated mitochondria when oxaloacetate or other tricarboxylic acid cycle precursors are absent and carbon dioxide assimilation is impossible (C3), which results in increased lipogenesis (G22). The subsequent accumulation of acyl CoA (D7) inhibits adenine nucleotide translocase, which decreases both the transfer of ATP into the cytosol and inhibits the production of ATP as less extra-mitochondrial ADP is transported back into the mitochondria (M41,O18). In addition, acyl carnitine accumulates (D7,O18), which is arrhythmogenic and inhibits the sodium-potassium pump (O14). Furthermore, beta-oxidation remains inhibited during postischaemic reperfusion, as the high concentrations of NADH are not rapidly reversed (D7).

Thus the cellular uptake or production of fatty acids is harmful (B65) and should be prevented during ischaemia (O23).

Carnitine

Carnitine (trimethylbetaine of ß-hydroxy, τ-aminobutyrate) has a specific function intimately related to lipid metabolism and is essential for oxidation of fatty acids. Carnitine levels in the cytosol decrease during experimental ischaemia and acyl CoA and acyl carnitine accumulates in the mitochondria (O18), which as previously discussed is potentially harmful. Thus, supplementing carnitine during total global ischaemia could be harmful, by further increasing intramitochondrial acyl carnitine. Carnitine is therefore not beneficial in cardioplegic solutions, and causes a dose dependent decrease of postischaemic myocardial function (B61,H23). However, Silverman et al showed a beneficial effect of preischaemic administration of carnitine added to the systemic perfusate (100 mg/kg, 30 min normothermic arrest), but this beneficial effect may have been due to the increased carnitine levels present during reperfusion (S47). Nevertheless, additional carnitine may be necessary once aerobic metabolism resumes during reperfusion, as the myocyte cannot synthesize carnitine and is dependant on an extracellular supply (M41).

Thus, carnitine may be beneficial in the preischaemic and reperfusion periods when aerobic lipid metabolism can take place, but carnitine is probably not beneficial in a crystalloid cardioplegic solution during total global ischaemia.

Glucose in cardioplegic solutions

Glucose and increased glycolysis is beneficial during hypoxia provided there is no ischaemia and metabolic end products are constantly removed (J5,J6,R53,Y5). Glucose is also beneficial prior to ischaemia by increasing intracellular glycogen stores (M41,O11), as well as during the postischaemic aerobic reperfusion period (O9). However, it is controversial whether glucose is beneficial in a cardioplegic solution during global ischaemia. We therefore investigated the effect of adding glucose to the St Thomas' cardioplegic solution.

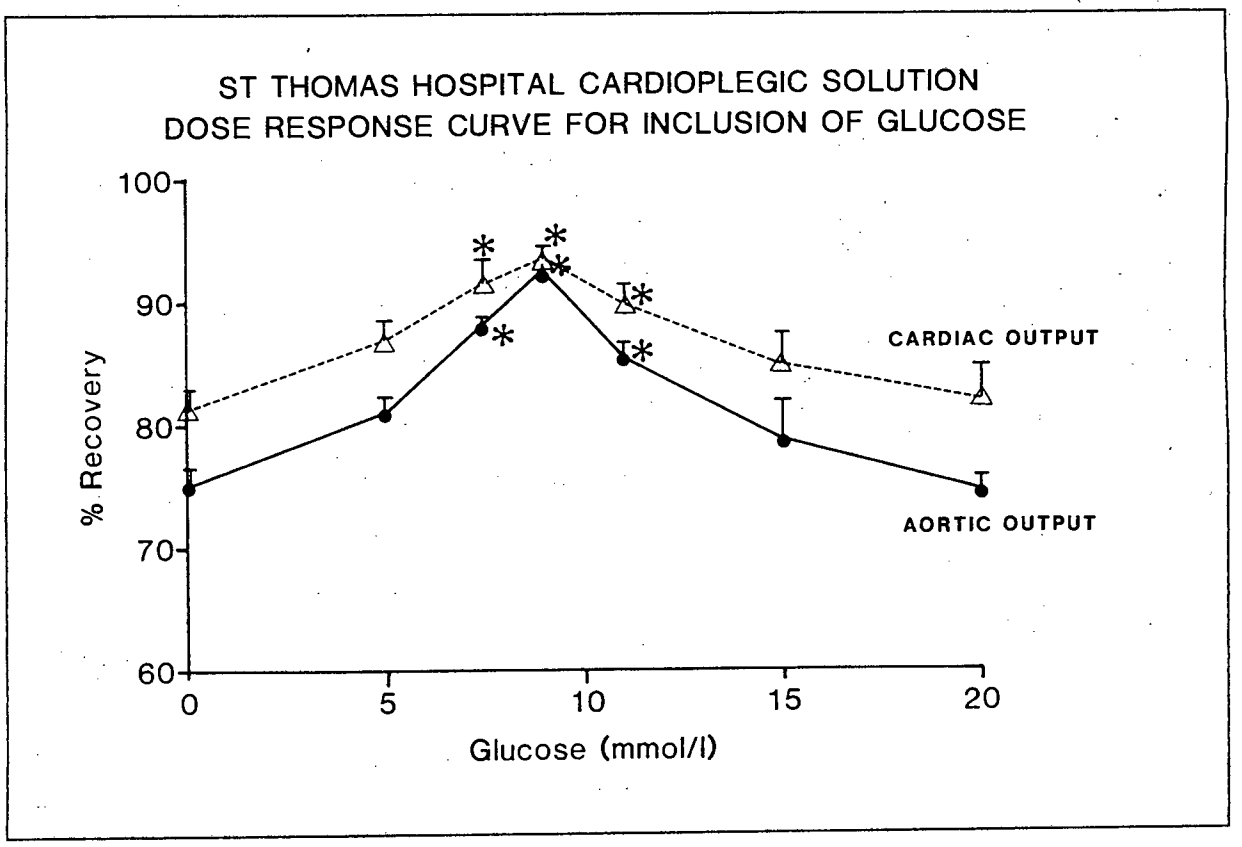
Isolated rat heart model

In the isolated rat heart model, subjected to a 3-hour hypothermic (10^{o}C) cardioplegic protected ischaemic period, we showed that glucose is beneficial when added to the St Thomas' cardioplegic solution (appendix A-2). However, glucose was only beneficial in the concentration range 7 - 11 mmol/L and provided multidose cardioplegia was administered (Fig 5.3).

In contrast, Hearse and co-workers showed a dose dependent decrease in postischaemic function if glucose was added to the St Thomas' cardioplegic solution (H27). However, these experiments where carried out at 28^{o}C with a 70-minute ischaemic period and no reinfusions of cardioplegia. During total global ischaemia increased glycolysis results in progressive accumulation of end products of metabolism (A28,N8,R53), which subsequently inhibits further glycolysis (M50,N3,R52) and causes other potentially harmful effects (discussed previously) (W32). However, a single dose of a similar

cardioplegic solution containing 20 mmol/L glucose was beneficial in an isolated rabbit model (G38).

Figure 5.3



Legend:

Dose response curve for the addition of glucose to the St Thomas' Hospital cardioplegic solution. Multidose reinfusions of cardioplegia were given every 30 min throughout a 3-hour 10°C ischaemic period. Values represent the mean postischaemic recovery of aortic flow (aortic output) and cardiac output, expressed as a percentage of each individual preischaemic value. The vertical bars represent the standard errors of the percentage means. Reprinted from J Thorac Cardiovasc Surg 1991; 102:405-412 (appendix A-2) with permission Mosby Year Book Inc.

* - $p < 0.005$ compared to 0 mmol/L and 20 mmol/L glucose concentration.

If only a single dose of cardioplegia was given prior to the ischaemic period in our model, then the addition of glucose to the St Thomas' cardioplegic solution did not improve postischaemic mechanical recovery and increased postischaemic loss of lactate dehydrogenase (appendix A-2). We did not though observe a decrease in mechanical recovery, as observed by Hearse et al (H27). However, a lower temperature was used during the ischaemic period in our study, which would have decreased the rate of

glycolysis (D14). Nevertheless, we can conclude that glucose is not beneficial during total global ischaemia if end products of metabolism are not removed (A28).

In contrast, if end products of glycolytic metabolism are intermittently removed by frequent reinfusions of cardioplegia as suggested by Apstein et al (A28), then the addition of glucose (7 - 11 mmol/L) to the St Thomas' cardioplegic solution is beneficial (appendix A-2). The addition of glucose resulted in increased glycolysis as indicated by the higher efflux of coronary sinus lactate during each reinfusion of cardioplegia (appendix A-2), and this glycolytic derived ATP could be important because of postulated compartmentation of ATP (B64,B65,W16). However, the beneficial effect of glucose might have not been solely due to increased anaerobic glycolysis. Multidose cardioplegia can also result in intermittent aerobic metabolism, as cardioplegic solutions exposed to the atmosphere will contain oxygen ($pO_2 \pm 150$ mm Hg) (see section 2.3 & 7.1). Intermittent aerobic metabolism not only increases ATP levels but also removes end products of glycolytic metabolism (O7,appendix A-3). In the study by Oguma et al, the protective effects of a cardioplegic solution containing glucose and insulin were completely lost with deoxygenation of the cardioplegic solution (O7). Steinberg et al showed that glucose (28 mmol/L) and insulin was beneficial in both anoxic and oxygenated high volume hypothermic multidose cardioplegic solutions (S72). However, in this study insulin augmented recovery if anoxic cardioplegia was used but did not further improve the oxygenated glucose containing cardioplegic solution. Schwalb et al also showed the salutary effects of glucose (11.1 mmol/L) in an oxygenated St Thomas' cardioplegic solution (S29) in the isolated rat heart model, during a normothermic ischaemic period protected with high volume multidose cardioplegia.

However, a higher concentration of glucose in a cardioplegic solution (111.1 mmol/L) was not beneficial in a canine model despite multidose cardioplegia (A13). We also determined a dose response curve for the concentration of glucose in the St Thomas' cardioplegic solution. Glucose concentrations lower than 7 mmol/L had no significant effect, perhaps because of insufficient glycolysis or an altered threshold for glucose

uptake during hypothermia (appendix A-2). The reported threshold for glucose uptake at normothermia in the absence of insulin is 3.3 mmol/L, (W34). Glucose concentrations above 11 mmol/L also did not improve postischaemic function, possibly because of insufficient washout of increased metabolic end products. The study by Steinberg et al showed the beneficial effect of a higher glucose concentration (28 mmol/L), but used higher volumes and more frequent reinfusions of cardioplegia (S72). Glucose containing cardioplegic solutions have been used clinically, and are associated with better preservation of postischaemic glycogen levels and good clinical results (S12).

Thus glucose is beneficial in a cardioplegic solution, but its beneficial effect is dependent upon the balance between the rate of glycolysis and efficiency of removal of the potentially harmful metabolic end products by either metabolic pathways (intermittent aerobic metabolism) or tissue washout.

Endothelial cell model

Cultured human venous endothelial cells were exposed to cardioplegic solutions for 12 hours at 22°C (see section 2.4). The addition of either 10 mmol/L or 50 mmol/L glucose, to the St Thomas' cardioplegic solution did not alter post exposure endothelial cell morphology or survival, when compared to the standard St Thomas' Hospital No 2 cardioplegic solution (appendix A-5). However, there was a relatively large volume of cardioplegic solution compared to endothelial cell mass in this model, and therefore it is unlikely that there was any significant increase of metabolic end products as would occur in vivo.

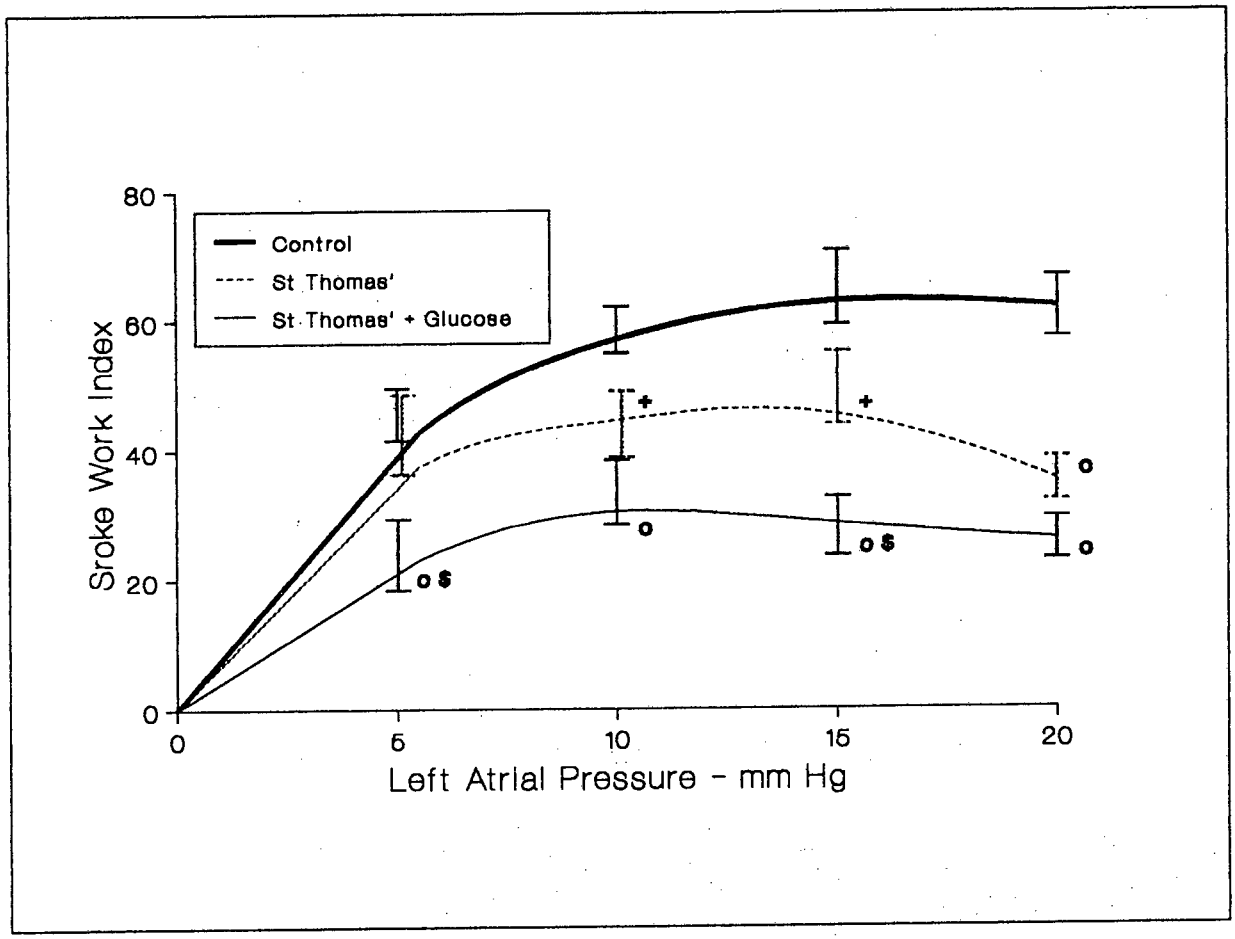
In vivo primate model

The St Thomas' plus glucose (10 mmol/L) cardioplegic solution was also compared to the standard St Thomas' Hospital No 2 cardioplegic solution in the in vivo primate model (appendix B-1). Multidose cardioplegia was reinfused every 30 min throughout a 3-hour hypothermic ischaemic period on cardiopulmonary bypass. The addition of

glucose (10 mmol/L) to the St Thomas' cardioplegic solution caused a decrease in postischaemic myocardial mechanical recovery (Fig 5.4). Furthermore, although the addition of glucose resulted in better preservation of ATP during the ischaemic period, ATP production was suppressed in the postischaemic period (appendix B-1).

Figure 5.4

ST THOMAS' PLUS GLUCOSE - IN VIVO PRIMATE MODEL



Legend:

Left ventricular stroke work index (gm.m/beat/m^2) at increasing left atrial pressures before and after a 3-hour hypothermic ischaemic period protected with multidose St Thomas' cardioplegia with or without glucose (10 mmol/L). A postischaemic left ventricular function curve of stroke work index was obtained 30 min after weaning from cardiopulmonary bypass, and compared to a similar preischaemic curve in each group (N = 6). Mean percentage postischaemic recovery was calculated from each individual postischaemic and preischaemic ratio. An average preischaemic control function curve (Control; N = 12) was derived by pooling the data from both groups, and postischaemic function for each group normalized to this curve for display purposes.

+ - $p < 0.05$ compared to preischaemic control,

o - $p < 0.01$ compared to preischaemic control,

$ - $p < 0.01$ compared to St Thomas' group.

Furthermore, the addition of the powerful buffer histidine to the St Thomas' plus glucose cardioplegic solution also did not ameliorate the harmful effects of the inclusion of glucose in this model (see section 5.4) (appendix B-6).

Thus in the primate model, glucose decreased postischaemic myocardial mechanical and metabolic function when added to the St Thomas' cardioplegic solution despite multidose administration of cardioplegia. Increasing the buffer capacity of the cardioplegic solution also did not prevent the glucose induced postischaemic myocardial depression.

Discussion

We have shown that glucose (7 - 11 mmol/L) is beneficial in the St Thomas' cardioplegic solution provided end products of metabolism are removed in the isolated rat heart model, but not in the in vivo primate model. However, higher concentrations of glucose (greater than 11 mmol/L) were also not beneficial in the isolated rat heart model.

Why do high concentrations of glucose in a cardioplegic solution depress postischaemic recovery?

One postulate is that high concentrations of glucose result in intracellular accumulation of metabolic end products (lactate, protons, NADH). High concentrations of lactate ions can induce mitochondrial changes (A29), and may be harmful in globally ischaemic hearts (N5). However, in an isolated myocyte preparation similar concentrations of lactate do not cause cellular damage (G14). Increased buffering to prevent the harmful effects of possible increased proton accumulation was also of no benefit in the primate model (see section 5.4). Furthermore, we have not been able to show increased intracellular accumulation of either lactate or protons as a result of a high glucose concentration in cardioplegic solutions in the isolated rat heart model in our laboratory (P Owen: unpublished data). Anaerobic glycolysis is inhibited by increased concentrations of NADH, lactate, and protons at two levels; glyceraldehyde-3-phosphate

dehydrogenase, and phosphofructokinase, and thus feedback inhibition possibly prevents excessively high levels of these metabolic end products.

A second postulate is that high glucose concentrations in cardioplegic solutions result in accumulation of fatty acids (acyl CoA and acyl carnitine) during ischaemia, which would be harmful (D7,M41,O14). NADH produced by anaerobic glycolysis is reoxidized by reducing pyruvate to lactate, and by converting dihydroxyacetone phosphate to α-glycerophosphate. In turn, α-glycerophosphate is used in the intracellular biosynthesis of triglycerides from fatty acids (C3,O14,O18). Thus enhanced anaerobic glycolysis, by increasing α-glycerophosphate will encourage esterification of intracellular free fatty acids (B53). Moreover, glucose catabolism by the hexose mono-phosphate shunt produces NADPH which is used in the reductive biosynthesis of fatty acyl CoA from acetyl CoA (C3,L27). Glycolysis also produces pyruvate which is used for lipogenesis during anaerobic conditions (C3,G22). In anoxic conditions excessive acetyl CoA, that cannot be degraded by the Krebs' cycle, is transferred out of the mitochondria (O14,O18), and can thus be reduced to fatty acids in the cytoplasm. Furthermore, the rate of production of lactate correlates with the rate of lipogenesis during anaerobic conditions (G22). Therefore, it is possible that during ischaemia high levels of glucose may increase the synthesis of acyl CoA and acyl carnitine, which is potentially harmful (discussed above). This postulate would also correlate with our findings in the in vivo primate model, in that high ATP levels could be maintained during the ischaemic period when anaerobic metabolism predominates. However, in the aerobic postischaemic period mitochondrial and myocardial function would be depressed as a result of the secondary effects caused by accumulation of acyl CoA and acyl carnitine during the ischaemic period.

Why was St Thomas' plus glucose (10 -11 mmol/L) beneficial in the isolated rat heart model but not in the in vivo primate model?

Glucose (11 mmol/L) was beneficial in the St Thomas' cardioplegic solution in the isolated rat heart model if multidose cardioplegia was provided, but not in the in vivo

primate model. This discrepancy can possibly be explained as a result of interspecies differences in dependence on either lipid or glycolytic metabolism. Guilbeau et al (G38) and Hearse et al (H27) used virtually identical solutions and protocols and yet single dose cardioplegia with glucose (20 mmol/L) was beneficial in the rabbit but detrimental in the rat. Differences exist in the capacity for anaerobic glycolytic flux, and mitochondrial oxidative metabolism between species (B31,H20,H21) and also at different stages of maturity (H44,J5,J6). Foetal and neonatal animals which have a greater dependence and capacity for glycolytic metabolism (H44), have a greater tolerance to ischaemia (B3,B50,B51,Y5). In contrast, the primary fuel for energy in adult animals is lipids, and increased lipolysis is harmful during ischaemia.

In addition to a possible interspecies and maturity difference, the amount of "washout" of end products of metabolism provided by the multidose reinfusions of cardioplegic solution might have been different in the two models. Relatively more "washout" would have been provided in the isolated rat model (reinfusions of cardioplegia (6 ml); 15 - 24 ml/kg body weight, 4.5 - 6.7 ml/gm wet heart weight), than the primate model (reinfusions of cardioplegia (100 ml); 4.2 - 6.7 ml/kg body weight, or 0.6 - 1.1 ml/gm heart weight) (appendix B-1). Thus relatively lower volumes of cardioplegia were reinfused in the primate model, and decreased washout of metabolic end products could have contributed to the poor recovery associated with the glucose containing cardioplegic solution in this model. Other studies using higher volumes and more frequent reinfusions of cardioplegia have also shown beneficial effects of cardioplegic solutions containing higher concentrations of glucose than in our isolated rat heart study (S72). Nevertheless, the volumes used in the primate model simulate the clinical situation more closely than those used in the isolated rat heart model.

In conclusion, until more is known about the mechanism of the harmful effects of glucose during ischaemic arrest protected with or without multidose crystalloid cardioplegic solutions, glucose should not be added to the St Thomas' Hospital No 2

cardioplegic solution. Adequate removal of end products of glycolytic metabolism by either metabolic pathways or by washout appears to be essential if glucose is included in cardioplegic solutions. Multidose reinfusions of cardioplegic solutions also have other beneficial effects. However, simply increasing the buffering capacity of the cardioplegic solution does not alone prevent the potentially harmful effects of the inclusion of glucose in a cardioplegic solution. Nevertheless, preischaemic metabolic enhancement with glucose is beneficial (GIK regime) (H4,H35,L46,O11,V23), as is glucose in the reperfusion period (S4,S88).

Amino acids

Amino acids are used by cells to manufacture tissue proteins, enzymes and hormones as well as specialized products such as purines, pyrimidines, and creatine. The metabolism of amino acids involves oxidative deamination, transdeamination and transamination (C3). The nitrogen contained in amino acids may be used in synthetic pathways or disposed of as ammonia or urea, while the carbon skeleton is used in glucogenic, ketogenic and synthetic pathways (Table 5.1).

Table 5.1

AMINO ACIDS

Glucogenic	Ketogenic
Alanine	Leucine
Arginine	
Aspartate	Isoleucine
Glutamate	Phenylalanine
Histidine	Tyrosine
Oxaloacetate	
Taurine	
α-Ketoglutarate	

Legend:
The ketogenic amino acids, except for leucine, can also be metabolized via glucogenic pathways. Only a few of the more important glucogenic amino acids are shown.

Aspartate and glutamate are the most active amino acids involved in transamination reactions (C3), catalyzing:-

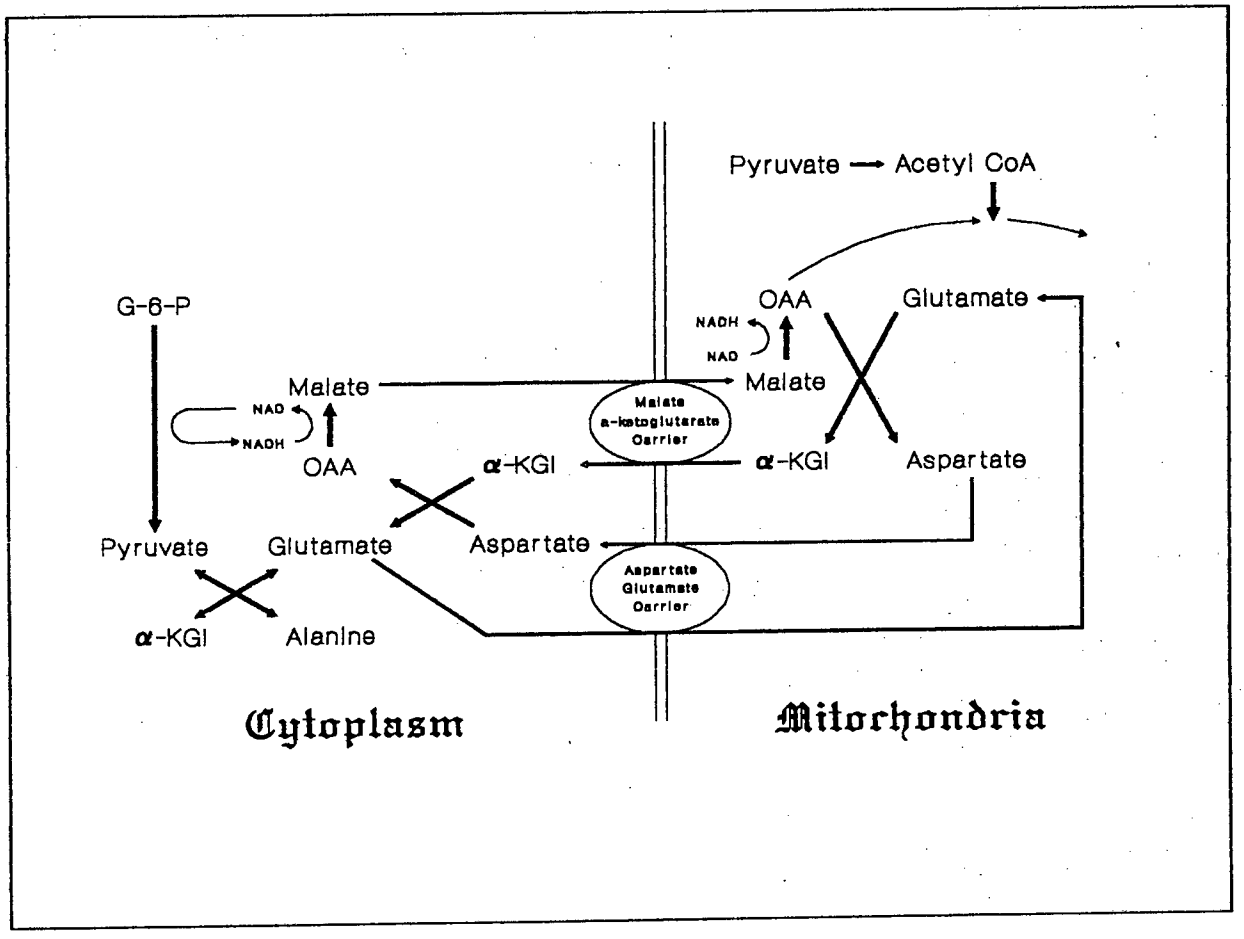
L-Glutamate + Oxaloacetate = α-Ketoglutarate + L-Aspartate,

L-Glutamate + Pyruvate = α-Ketoglutarate + L-Alanine.

Furthermore, both glutamate and aspartate play a major role in the malate aspartate cycle (Fig 5.5), which is used to transfer NADH and NAD between the cytosol and mitochondria and to coordinate cytosolic and mitochondrial energy metabolism (S3).

Figure 5.5

MALATE - ASPARTATE SHUTTLE



Legend:

NAD and NADH are transferred across the mitochondrial membrane by a carrier mechanism, the malate - aspartate shuttle.

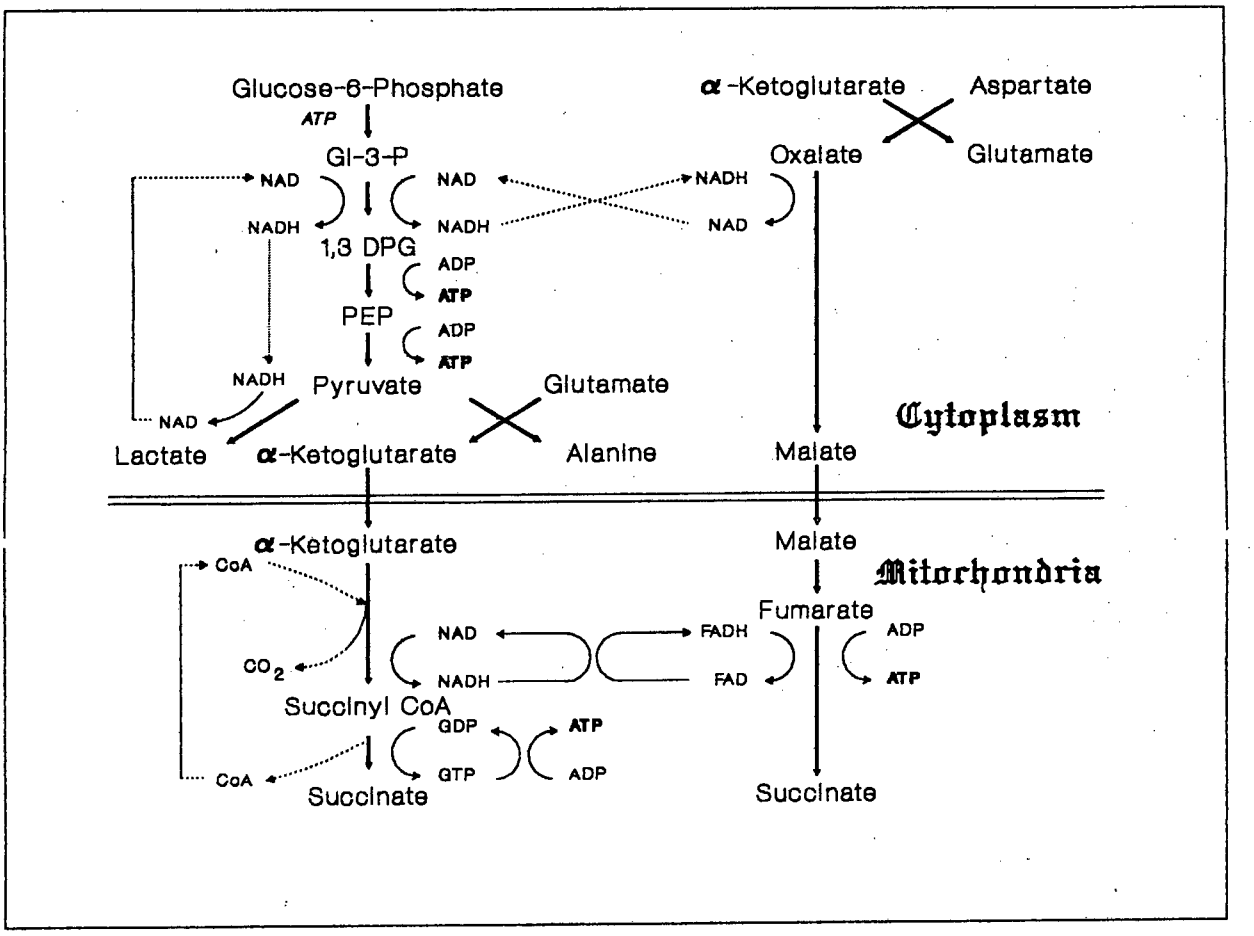
Although the primary metabolism of amino acids requires aerobic conditions, amino acids can also produce high energy phosphates anaerobically independent to glycolysis in the mitochondria (B25,H56,P16,R8,S14). Tissue glutamate and aspartate decrease during ischaemia or hypoxia (P21), and the supply of exogenous glutamate in this situation is beneficial (M18). In patients with ischaemic heart disease glutamate is extracted by the heart with simultaneous loss of alanine (M61,T16).

Perfusion of rabbit interventricular septa before or during anoxia with L-arginine or L-glutamate (1 mmol/L) increases recovery of mechanical performance in the absence of glucose (R8). Furthermore, perfusion before and after global ischaemia with L-aspartate, L-glutamate, L-ornithine or L-arginine enhanced recovery, but this enhancement could be prevented if transamination was metabolically inhibited. Lysine, glycine, methionine and glutamine were ineffective. Thus, only amino acids that accumulate in the cytoplasm, are involved in transamination reactions, and are available for the malate-aspartate cycle are effective. Other investigators have also confirmed that mitochondrial energy production can take place during anaerobic conditions from an extraglycolytic source, usually with concomitant accumulation of succinate (P16,S14,T3). The mechanism of anaerobic ATP production is by conversion of either α-ketoglutarate to succinate or malate to succinate in the mitochondria (Figure 5.6). These reactions are also dependant upon transamination of aspartate and glutamate (B25,H43,T3), and may possibly be enhanced if glucose is also present (S14). If amino acid transamination is prevented during ischaemia, then ischaemic tolerance decreases (J14).

During initial aerobic reperfusion of the postischaemic myocardium, amino acids are the only exogenous substrate taken up by the heart (S87). Amino acids are used to replenish Krebs' cycle intermediates lost during ischaemia (H3), and for myocardial energy production (S87).

Figure 5.6

ANAEROBIC PRODUCTION OF ATP



Legend:
The metabolism of glucose-6-phosphate to lactate produces cytoplasmic ATP (Nett: 3 mmol ATP/mmol G-6-P). Alternatively, the simultaneous metabolism of both glucose-6-phosphate and the amino acid aspartate will produce both cytoplasmic and mitochondrial ATP anaerobically (Nett; 7 mmol ATP/mmol G-6-P) without an accumulation of NADH, but with the production of succinate and CO_2.

Amino acids are thus potentially beneficial both during anoxia or ischaemia and during aerobic reperfusion, but by different mechanisms.

Amino acids in cardioplegic solutions

Anaerobic transamination of amino acids during ischaemia can augment intracellular high energy phosphates. Enrichment of the St Thomas' Hospital cardioplegic solution with L-aspartate (20 mmol/L) decreased high energy phosphate decay during an 8-hour hypothermic ($4^{o}C$) global ischaemic period protected with single dose cardioplegia, and

improved postischaemic mechanical recovery (C25). However, addition of L-glutamate to this enriched solution did not result in further improvement and glutamate alone was ineffective in this model. The amino acid fumarate was shown to be more beneficial than succinate, malate, and glutamate at higher concentrations (100 mmol/L) in another hypothermic crystalloid cardioplegic solution (R47). However, other investigators have shown that L-glutamate (20 - 100 mmol/L) in multidose crystalloid cardioplegic solution decreases high energy phosphate decay (E10), and improves postischaemic recovery (B27). Branch chain amino acids added to an oxygenated modified St Thomas' cardioplegic solution containing glucose (11.1 mmol/L) also improved recovery after a normothermic cardioplegic protected ischaemic period (S29). However, this study did not separate the specific beneficial effects of branched chain amino acids (leucine, isoleucine, valine), from the other amino acids contained in their formulation. Moreover, ketogenic amino acids (leucine, isoleucine) can be metabolized to fatty acids, and fatty acids are harmful during ischaemia (discussed previously) (B65). Nevertheless, from these studies we can conclude that anaerobic amino acid transamination metabolic reactions as discussed above, but specifically glucogenic amino acids, improves preservation during cardioplegic protected hypothermic ischaemia.

The addition of L-glutamate (4 - 25 mmol/L) to oxygenated blood reperfusion solutions also improves postischaemic recovery (H3,H2,L18), and further synergistic enhancement with L-aspartate (13 mmol/L) results in extra recovery (E13,R41). However, aerobic metabolism takes place during reperfusion, in contrast to cardioplegic protected ischaemia. Thus, amino acid enhancement during reperfusion possibly acts by improving oxidative metabolism through both replenishment of Krebs' cycle intermediates and improved transportation of cytoplasmic NADH into the mitochondria via the malate-aspartate shuttle. Metabolic enhancement during reperfusion is also possibly of greater importance than the presence of free radical scavengers (L23).

The amino acids L-glutamate and L-aspartate are present in low concentrations in human serum, less than 0.05 mmol/L [S71], and supplementation of these amino acids during both anoxia and reoxygenation is beneficial [M18].

5.2 OXYGENATION OF CRYSTALLOID CARDIOPLEGIC SOLUTIONS

The cardioplegic arrested heart has a basal metabolic requirement, despite profound hypothermia. This basal oxygen requirement is; 0.3 ml O_2 / 100 gm / min at 22°C (B76), 0.27 ml O_2 / 100 gm / min at 15°C and 0.13 ml O_2 / 100 gm / min at 5°C in the dog heart (B63). However, the more efficient aerobic metabolic pathways only continue to function if oxygen is available, and the oxygen storage capacity of the myocardium is limited (myoglobin; 0.6 volume %) (B60). Once all available oxygen has been used, the basal myocardial energy requirements can only be partially met by ongoing anaerobic metabolism. However, oxygen can also be supplied intermittently throughout the ischaemic period by either multidose blood or oxygenated crystalloid cardioplegic solutions. In an isolated rat heart preparation, approximately 3 ml O_2 / 100 gm was taken up with each reinfusion of a poorly formulated oxygenated crystalloid cardioplegic solution, reinfused for one minute after every 10 minutes of ischaemia (D11). If one extrapolates from the known oxygen demands of the hypothermic cardioplegic arrested canine heart, although the dog has a lower normothermic basal metabolic demand than the rat (H53), the metabolic demands for each 10 minute ischaemic period were nearly met by intermittent accelerated aerobic metabolism.

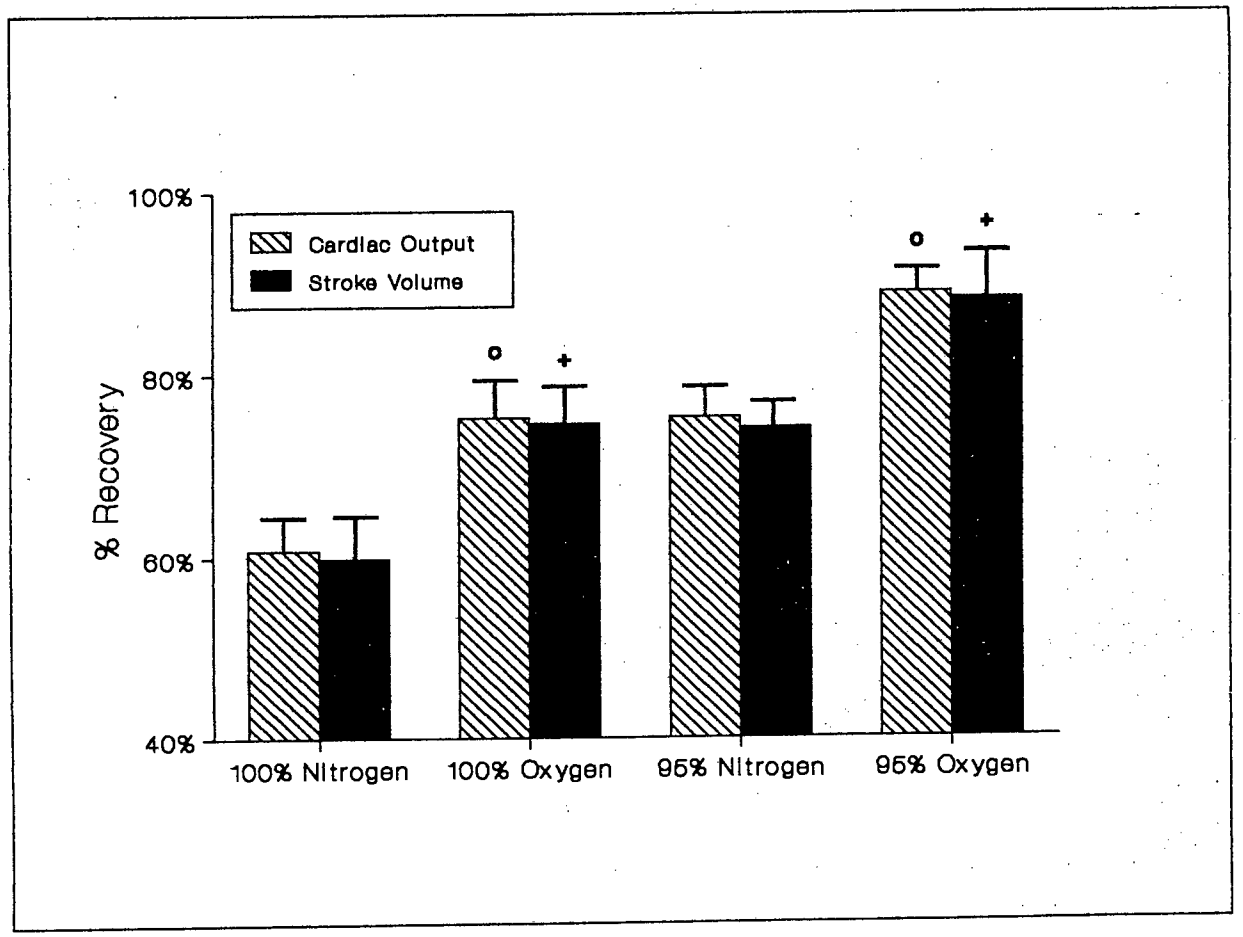
In a crystalloid cardioplegic solution all dissolved oxygen is available to the myocardium (D19), in contrast to blood cardioplegia where less oxygen is available at low temperatures because of the leftward shift of the oxygen-dissociation curve (G2). Furthermore, crystalloid cardioplegic solutions are usually infused at temperatures of 4°C - 10°C (see section 1.5), and at these low temperatures the maximum oxygen content of the cardioplegic solution increases to 3.7 ml O_2 % (at 760 mm Hg) (B13). Thus, at low temperatures oxygenated crystalloid cardioplegic solutions can deliver as much oxygen as blood cardioplegic solutions (D19). Moreover, oxygenation of crystalloid solutions is much simpler, and bubble oxygenation of crystalloid cardioplegic

solutions easily achieves oxygen contents greater than 3.0 ml O_2 % (appendices A-3,A-7).

In the isolated rat heart model, we showed that oxygenation of a crystalloid cardioplegic solution (St Thomas' plus 11.0 mmol/L glucose) improved postischaemic mechanical recovery and decreased washout of lactate dehydrogenase compared to an anoxic solution (Fig 5.7) (appendices A-3,A-4).

Figure 5.7

OXYGENATION OF CRYSTALLOID CARDIOPLEGIC SOLUTIONS



Legend:

Postischaemic mechanical recovery, expressed as mean ratios of individual preischaemic values, of isolated rat hearts after 3-hour 10°C cardioplegic arrest. Hearts were protected with multidose St Thomas' plus glucose (11 mmol/L) cardioplegic solution gassed with either 100% N_2 (N =6), 100% O_2 (N = 8), 95% N_2 5% CO_2 (N = 7) or 95% O_2 5% CO_2 (N = 9). The vertical bars represent standard errors of means. Modified from Ann Thorac Surg 1991; 52:903-907 (appendix A-4) with permission The Society of Thoracic surgeons.

o - $p < 0.01$ compared to the respective N_2 group.

+ - $p < 0.05$ compared to the respective N_2 group.

Other investigators have also shown that oxygenation of both the St Thomas' and other crystalloid cardioplegic solutions retards degradation of myocardial high energy phosphates and enhances postischaemic mechanical recovery (B33,C31,G45,L25).

Clinical studies have shown both no benefit of oxygenating crystalloid cardioplegic solutions (W13), and others significant beneficial effects especially if the aortic cross-clamp period is longer than 28 minutes (G45). These discrepancies may imply that the minimum amount of oxygen required in a cardioplegic solution may yet have to be determined, as non-oxygenated but "aerated solutions" also contain oxygen. In our study we did not compare an oxygenated cardioplegic solution to an aerated solution (appendix A-3). Furthermore, the "aerated" St Thomas' plus glucose cardioplegic solution (appendix A-2) resulted in similar percentage recovery of aortic flow, cardiac output and stroke volume if compared to the oxygenated (95 % O_2 5 % CO_2) St Thomas' plus glucose cardioplegic solution (appendix A-3). However, these studies in appendix A-2 and appendix A-3 were undertaken at different time periods, and the "aerated" solution in the former study had been gassed with 95 % O_2 5 % CO_2 during preparation and although not oxygenated thereafter, oxygen contents (which were not measured) could have remained high (see section 2.3). Thus, this comparison does not necessarily refute the proposal of oxygenating crystalloid cardioplegic solutions.

Oxygenation of crystalloid cardioplegic solutions is beneficial as it allows intermittent aerobic metabolism with each reinfusion of cardioplegia, which is more efficient for energy production. Furthermore, the beneficial effects of oxygen in crystalloid cardioplegic solutions is proportional to their oxygen content (D11), and the protective effects of some crystalloid cardioplegic solutions are primarily due to the oxygen dissolved therein (O7).

Oxygen carriers - Perfluorocarbons

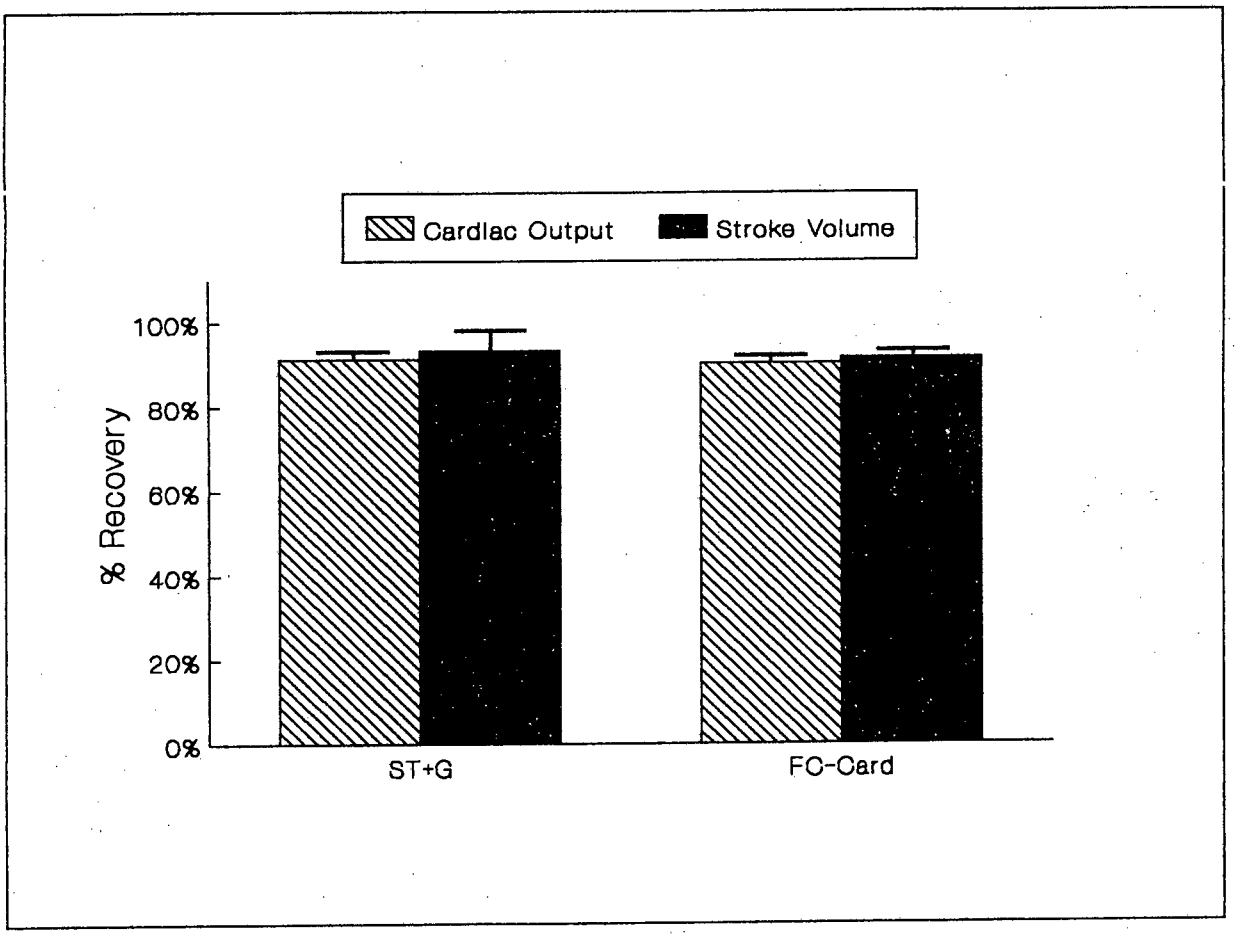
Inert perfluorochemical emulsions have been used experimentally as blood replacements because of their capacity to carry oxygen, as well as for rheological properties (F6). However, although these emulsions are inert, large particles can be potentially toxic and perfluorocarbons also accumulate in reticulo-endothelial cells. Fluosol-43 emulsion (FC-43) is possibly the most suitable perfluorochemical emulsion, although not for human use (F6). The high solubility coefficient for oxygen in FC-43 (0.077 ml O_2 / ml at 10^oC (F6)) increases the oxygen carrying capacity of crystalloid solutions, and at normothermia increases oxygenation of myocardial myoglobin (M8). In addition, all oxygen carried by FC-43 can be released as the oxygen-dissociation curve is linear (F6).

Fluorocarbons have been used experimentally as artificial blood perfusates during cardiopulmonary bypass (C14,E11), and for long term storage of isolated hearts by hypothermic (4^oC) continuous perfusion (B9,G23,K17). Oxygenated perfluorocarbon cardioplegic solutions enhance oxygen utilization during multidose cardioplegic arrest and can provide adequate myocardial protection at 4^oC - 10^oC (M6). Furthermore, perfluorocarbon cardioplegic solutions have been shown to be superior to both blood and crystalloid cardioplegic solutions (H37,K4,N12). However, in these studies the crystalloid cardioplegic solutions were not oxygenated and had different electrolyte compositions to the fluorocarbon cardioplegic solutions. Thus these studies only showed the beneficial effect of supplying oxygen.

In the isolated rat model (appendix A-3), we show that an oxygenated perfluorocarbon cardioplegic solution with an identical electrolyte composition to a good oxygenated crystalloid cardioplegic solution (St Thomas' plus 11 mmol/L glucose), conferred no extra protection despite an increased oxygen-carrying capacity (Fig 5.8).

Figure 5.8

ST THOMAS' CARDIOPLEGIC SOLUTION PLUS PERFLUOROCARBONS



Legend:

Postischaemic mechanical recovery, expressed as mean ratios of individual preischaemic values, of isolated rat hearts after 3-hour 10°C cardioplegic arrest. Hearts were protected with multidoses of either St Thomas' plus glucose (11 mmol/L) cardioplegic solution (ST+G; N = 11) or a perfluorocarbon containing cardioplegic solution (FC-Card; N = 6) having an electrolyte content identical to ST+G. Both solutions were gassed with 95 % O_2 5 % CO_2. The vertical bars represent the standard errors of the means.

Thus, in agreement with other studies we do not recommend the use of perfluorocarbons in hypothermic crystalloid cardioplegic solutions (R50,T1).

Potential harmful effects of oxygenating crystalloid cardioplegic solutions

The beneficial effect of supplying oxygen in cardioplegic solutions is no longer questioned. However, oxygenation of crystalloid cardioplegic solutions can potentially also be harmful:-

Formation of air emboli

If a solution is fully saturated with a gas at a low temperature, rewarming of the solution will alter the temperature dependant dissociation constant for that gas, resulting in the gas coming out of the now supersaturated solution, as bubbles (see section 7.1, 7.2 (appendices A-7,A-8)).

Induced pH changes

The introduction of any gas into a solution containing bicarbonate alters the partial pressure of carbon dioxide in that solution, thereby producing a change in hydrogen ion concentration and thus pH (Fig 5.10). Oxygenation without carbon dioxide will induce an alkalotic pH shift, and this would increase the potential for the calcium paradox if the cardioplegic solution is calcium-free (H31). Bicarbonate containing cardioplegic solutions should be oxygenated with 95 % O_2 5 % CO_2 and not 100 % O_2. (see section 5.3 (appendices A-3,A-4)).

Generation of oxygen free radicals

Oxygen free radicals cause myocardial injury (S27), and are generated when oxygen is reintroduced into previously hypoxic or ischaemic tissue (see section 1.6 (B8,S75)). Free radical generation is also increased by exposure to high partial pressures of oxygen (pO_2 greater than 150 mm Hg (G5)). Therefore, it is possible that free radicals are generated with intermittent reinfusions of multidose oxygenated crystalloid cardioplegic solutions.

5.3 CARDIOPLEGIC SOLUTION pH

"Life is a struggle, not against sin,

but against hydrogen ions".

H L Mencken - *Exuent Omnes* 1919 (B53)

Intracellular metabolic processes continually generate protons, which are removed by oxidative phosphorylation during aerobic metabolism (G16). However, protons accumulate as a result of glycolysis, ATP hydrolysis, and lipid metabolism, during hypoxia and ischaemia, and the resulting cytoplasmic acidosis affects myocardial function (G16). Intracellular acidosis decreases myocardial contractility (P25,S70), decreases energy production both by inhibiting the transfer of reducing equivalents into the mitochondria and by inhibiting glycolysis (W32), alters calcium binding and calcium flux (P18,V22,W32), and finally activates degradative enzyme systems.

The pH of a solution is the negative logarithm of the hydrogen ion concentration, and maintaining a stable pH in body fluids is essential for life (G2). The normal pH of extracellular fluids at normothermia is 7.4, although a pH range from 7.0 to 7.7 is compatible with life. Intracellular pH which is difficult to measure directly, approximates the neutral pH of water; pH 7.0 at normothermia (S91,W19). This is the optimal intracellular pH for the majority of metabolic reactions, which are very sensitive to changes in pH; eg. ATP disappears most rapidly at or below pH 6.3 (B53).

Intramyocardial pH measurements obtained during hypothermic cardioplegic arrest correlate with the degree of ischaemic damage, and thus pH can be used to assess the adequacy of intraoperative myocardial protection (K14). An interstitial pH of less than 7.0 at the end of ischaemia is associated with depressed postischaemic left ventricular functional recovery, following either normothermic or hypothermic global ischaemia without cardioplegia (T6). In an intraoperative study using a bicarbonate containing crystalloid cardioplegic solution, intramyocardial pH increased with infusions of this

alkalotic solution and the magnitude of rise or integrated mean intramyocardial pH correlated with the clinical postoperative assessment of the adequacy of myocardial preservation (K14). However, although intramyocardial pH and the degree of acidosis is an index of the metabolic status of the myocardium, this does not imply that cardioplegic solutions must be alkalotic. Myocardial pH can be maintained at the correct level by using an appropriate buffer (see section 5.4). Furthermore, when assessing the correct pH of a cold cardioplegic solution the effect of temperature on pH must also be taken into account.

pH and Temperature

Biological neutrality is defined to occur when the ratio of hydroxyl and hydrogen ions equals one (S91), and in water and intracellular fluids occurs at pH 7.0 at 37°C. However, the dissociation constant for water decreases as temperature decreases, thus reducing the concentration of hydrogen ions. The neutral intracellular pH at 10°C is therefore pH 7.46 (S91,W19). Similarly, if biological neutrality is to be maintained in blood, normothermic neutral blood pH 7.4 should be pH 7.8 at 10°C.

Although, the "correct" systemic blood pH during hypothermic cardiopulmonary bypass has been a contentious point in the literature, most studies support using the alpha-stat concept during hypothermia (B19,M21,S91,T26,W30). Poikilotherms maintain biological neutrality by allowing pH to rise and pCO_2 to fall when body temperature drops ("alpha-stat" concept of acid base management; (S91)). Maintaining biological neutrality as temperature changes optimizes enzymatic reactions, helps maintain constant cellular volume (W19), and improves cardiac metabolism and function (M21). In contrast, homeotherms including hibernators maintain blood at pH 7.4 *regardless of temperature* ("pH-stat" concept), by increasing the CO_2 and thus hydrogen ion content. This, "acidotic" blood pH of 7.4 at 10°C possibly inhibits metabolism and conserves energy. However, whilst biological neutrality and its advantages might be appropriate for

hypothermic systemic perfusate pH, the aim of cardioplegic induced ischaemic arrest is to diminish metabolic demand maximally. Therefore, the "metabolic inhibitory" effect of a relatively "acidotic" pH might be more appropriate for cardioplegic solutions.

An additional factor that must be taken into account when interpreting pH values, is that all blood gas analyzers measure the partial pressures of gases and pH at 37°C. Therefore, the true pH for the temperature of the sample in question must be calculated from the "meter reading pH" (K10), now done automatically by most modern blood gas analyzers.

pH and Cardioplegic solutions

Although Becker et al using blood cardioplegia showed depression of postischaemic myocardial performance if systemic pH was kept at 7.4, as opposed to an "alpha-stat" alkalotic pH, this study did not separate the effect of cardioplegic solution pH from the effect of systemic pH during reperfusion (B19). In contrast, studies with crystalloid cardioplegic solutions have shown a beneficial effect of a relatively "acidotic" hypothermic cardioplegic solution; pH 7.0 - 7.1 (B27,N19). However, an "acidotic" cardioplegic solution is essential for a calcium-free cardioplegic solution as used by Nugent et al (N19), in order to limit the potential for the calcium paradox (H31,J16).

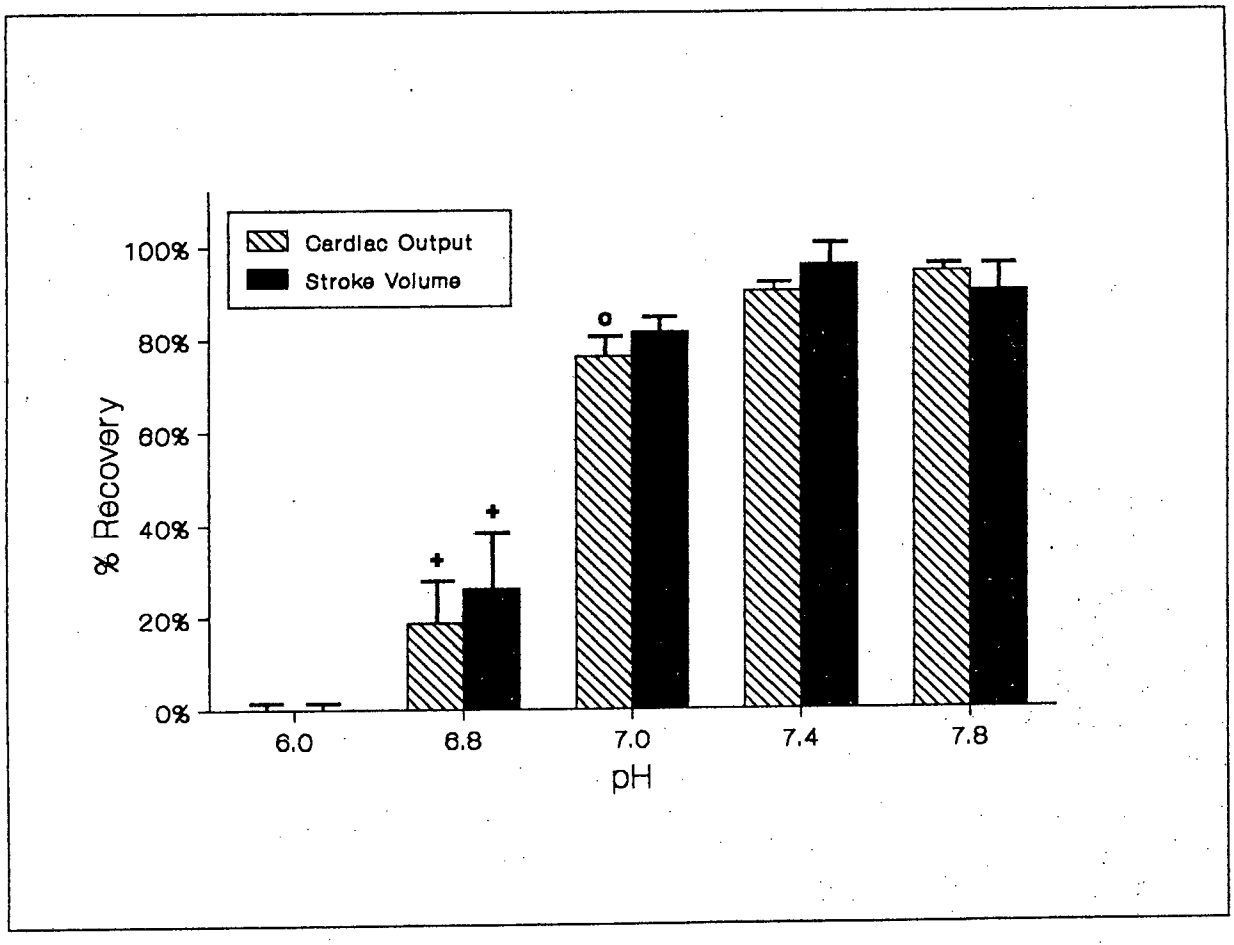
Nevertheless, a reduction of intracellular pH markedly decreases sarcolemmal calcium transport by Na^+ / Ca^{2+} exchange (P18), and decreases K^+ efflux (P25). Furthermore, Ca^{2+} binding to the phospholipid of sarcolemmal sites is pH dependent; At a 37°C pH of 5.5 there is 0% calcium binding, 50% at pH 7.0, and 100% binding at pH 8.5 (L11). In contrast, extracellular alkalosis could promote intracellular calcium accumulation by Na^+ / H^+ and Ca^{2+} / Na^+ exchange (D13). These ionic effects would support the use of a slightly acidotic cardioplegic solution.

"Metabolic acidosis" - induced pH shift

In the isolated rat heart model we examined the effect of altering the pH of a modified St Thomas' plus glucose (11 mmol/L) cardioplegic solution (appendix B-2). Equivalent postischaemic recovery was obtained with pH 7.4 and pH 7.8 (measured at 37°C), and a pH of 7.0 or less was associated with diminished postischaemic mechanical recovery (Fig 5.9) (appendix B-2).

Figure 5.9

EFFECT OF "METABOLIC" INDUCED pH SHIFTS



Legend:

Postischaemic mechanical recovery, expressed as mean ratios of individual preischaemic values, of isolated rat hearts after 3-hour 10°C cardioplegic arrest. Hearts were protected with multidoses of St Thomas' plus glucose (11 mmol/L) cardioplegic solution titrated to the indicated pH's with either 1 N HCl or NaOH. The vertical bars represent the standard errors of the means.

o - $p < 0.05$ compared to pH 7.4

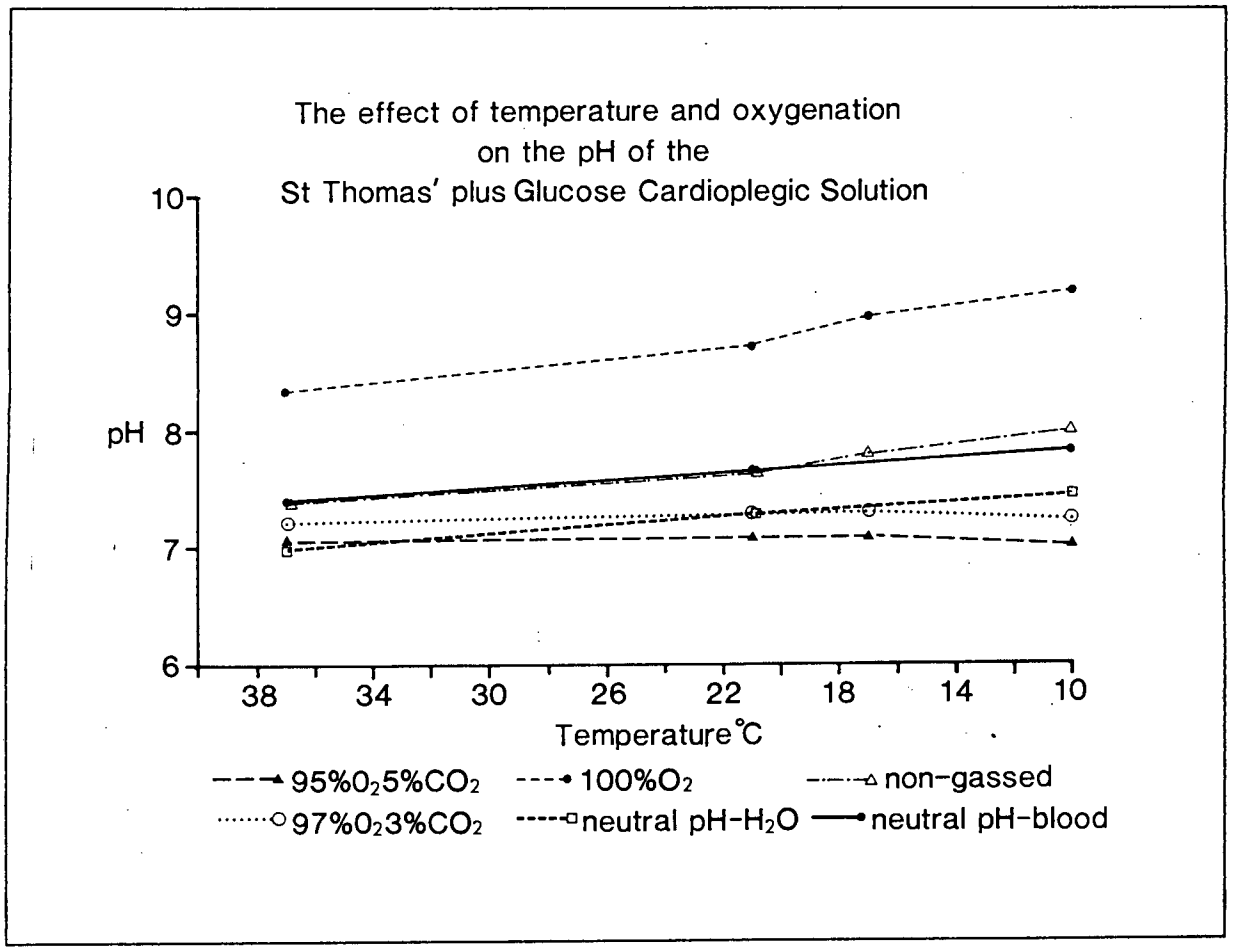
+ - $p < 0.01$ compared to pH 7.4

However, in this model pH was altered by adding HCl or NaOH, and therefore the "acidotic" solutions (pH less than 7.4) would also have had an associated decrease in buffering capacity. The associated diminished buffering capacity could have had a significant effect on postischaemic recovery, as the St Thomas' cardioplegic solution contains a weak buffer; bicarbonate.

"Respiratory acidosis" - induced pH shift

Another study was undertaken in the isolated rat heart model to examine the effect of alterations of pH independent to any alterations of buffer capacity (appendices A-3,A-4). The effect of pH changes were studied in the presence and absence of oxygen without altering either the composition or buffering capacity of the solution, by gassing the cardioplegic solution with or without 5 % CO_2. Oxygenating St Thomas' cardioplegic solution with 100 % O_2 results in an alkalotic pH of 9.2 at 10^{o}C, whereas oxygenation with 95 % O_2 5 % CO_2 results in an equivalent pO_2 but a pH of 7.0 at 10^{o}C (Fig 5.10).

Figure 5.10

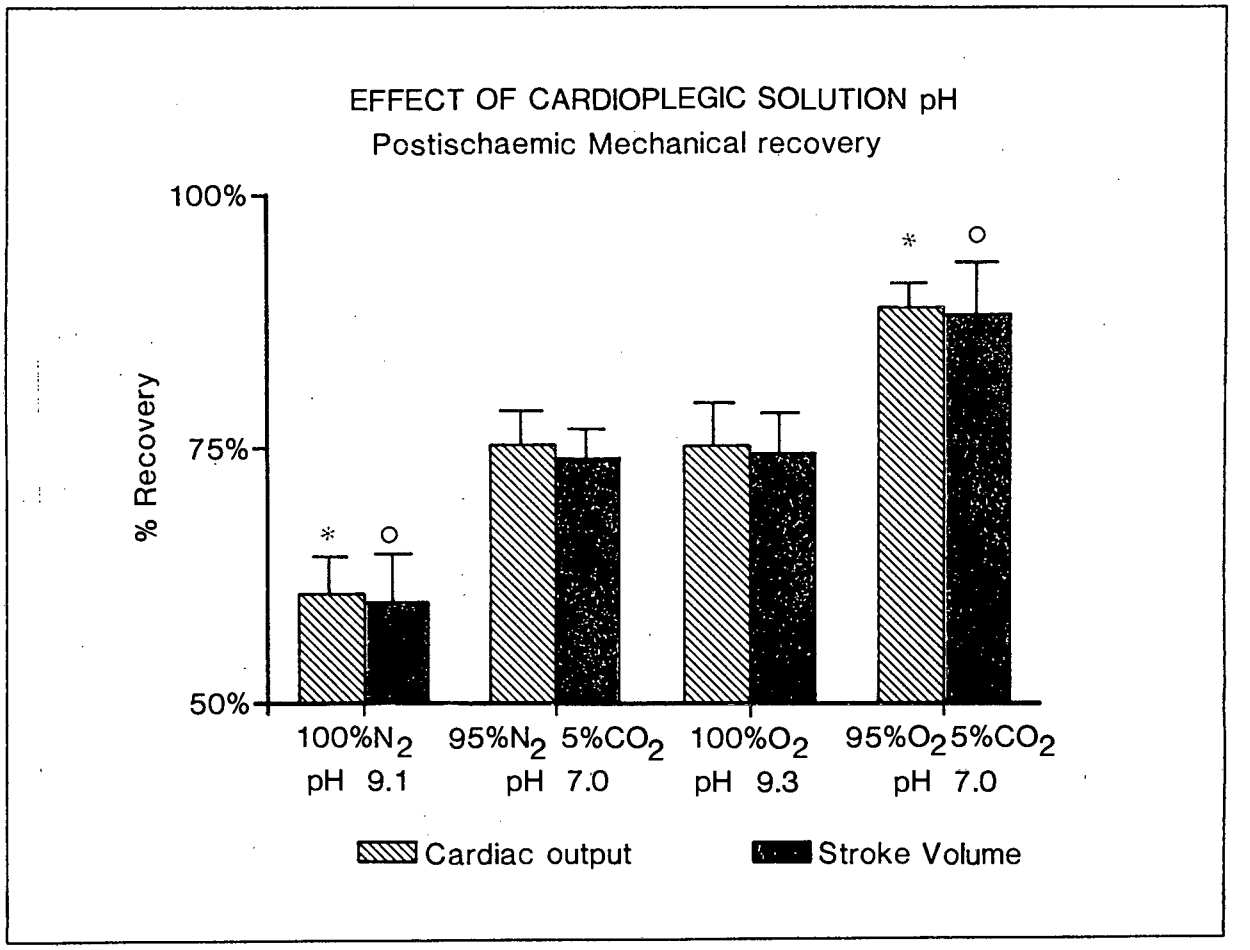


Legend:

The St Thomas' plus glucose (11 mmol/L) cardioplegic solution was gassed with either 100% O_2, 97% O_2 3% CO_2 or 95% O_2 5% CO_2, and the pH measured at different temperatures. The pH of intracellular neutrality (neutral pH of H_2O) and neutral pH of blood is also indicated. Reprinted from J Thorac Cardiovasc Surg 1991; 102:396-404 (appendix A-3) with permission Mosby Year Book Inc.

A cardioplegic solution pH of 7.0 at 10°C in the presence of oxygen (induced by oxygenating with 95 % O_2 5 % CO_2), was associated with increased oxygen uptake from the cardioplegic solution, better maintenance of membrane integrity, and improved postischaemic mechanical recovery (Figure 5.11; (appendices A-3,A-4)). If anoxic cardioplegic solutions were used, postischaemic mechanical recovery also improved at pH 7.0. However, increased postischaemic washout of lactate dehydrogenase suggested increased sarcolemmal damage at pH 7.0 during anoxia, which was possibly due to inhibition of anaerobic glycolysis at this pH. A pH of 6.8 at 37°C (pH < 7.25 corrected to 10°C) inhibits hypoxic accelerated glycolysis (R52).

Figure 5.11



Legend:

Postischaemic mechanical recovery, expressed as mean ratios of individual preischaemic values, of isolated rat hearts after 3-hour 10°C cardioplegic arrest. Hearts were protected with multidose St Thomas' plus glucose (11 mmol/L) cardioplegic solution gassed with either 100 % N_2 (N =6), 95 % N_2 5 % CO_2 (N = 7), 100 % O_2 (N = 8) or 95 % O_2 5 % CO_2 (N = 9), which induced the indicated pH shifts. The vertical bars represent the standard errors of the means. Reprinted from Ann Thorac Surg 1991; 52:903-907 (appendix A-4) with permission The Society of Thoracic Surgeons.

* - $p < 0.01$ compared to 95 % N_2 5 % CO_2 and 100 % O_2.

o - $p < 0.05$ compared to 95 % N_2 5 % CO_2 and 100 % O_2.

The observed beneficial effects of oxygenating with 95 % O_2 5 % CO_2 (pH 7.0 at 10°C) were as a result of the increased pCO_2 and induced pH shifts. Acid-base alterations primarily effect myocardial contraction by altering intracellular pH (C28), which can be rapidly modulated by the free sarcolemmal passage of CO_2 (P25). Therefore, a so-called "respiratory acidosis" (increased CO_2) will have a more pronounced effect on intracellular pH than a "metabolic" acidosis (C28,P25).

Changes in extracellular pH by altering sodium bicarbonate (metabolic acidosis) and maintaining pCO_2 constant do not alter myocardial contractility significantly, in contrast to changes in pCO_2 even if pH is maintained constant (C28). Thus the previously observed detrimental effect of pH 7.0 (Fig 5.13) induced by adding HCl to the cardioplegic solution, was probably due to the associated decrease in buffer capacity (see section 5.4) than to a change in intracellular pH. Lochner et al also demonstrated deleterious effects of oxygenating crystalloid cardioplegic solutions with 100% O_2, and suggested that this was due to complex ionic interactions (L41).

A relatively "acidotic" cardioplegic solution pH of 7.0 at 10°C in the St Thomas' plus glucose cardioplegic solution was better than an alkalotic pH, provided the buffering capacity was maintained constant. The optimal pH for calcium-free cardioplegic solutions such as the Bretschneider HTK4 solution is also less than 7.0 (37°C) (B60,P27), but slight acidosis is essential in this solution in order to prevent the calcium paradox from occurring (H31,J16). Nevertheless, Bernard et al also showed that pH 7.0 (20°C) was better than pH 7.4 or 7.7 using a cardioplegic solution containing calcium and glutamate (20 mmol/L) (B27). Furthermore, the same group also showed that in the reperfusion period a glutamate containing reperfusate was more effective at a pH of 7.7 (28°C) (M32). This thus supports the hypothesis that during cardioplegic arrest inhibition of metabolism by slight "acidosis" is the correct strategy, but that during reperfusion biologic neutrality should be maintained, in order to now optimize enzymatic function.

In conclusion, the St Thomas' cardioplegic solution should be oxygenated with 95% O_2 5% CO_2 which induces a relatively acidotic pH of 7.0 at 10°C, and not 100% O_2. In addition, all bicarbonate containing calcium-free cardioplegic solutions should also be oxygenated with 95% O_2 5% CO_2 to prevent an alkalotic induced increased potential for the calcium paradox (H31).

5.4 CARDIOPLEGIC SOLUTION BUFFERS

Buffers in blood

A buffer is a substance that can bind or release hydrogen ions, thus maintaining the pH of a solution relatively constant despite the addition of considerable quantities of acid or base. The major intracellular buffers in muscles are soluble proteins, histidine related compounds and phosphate, which increase the anaerobic potential of tissues (A4,P4). In blood, the major buffers are plasma proteins, haemoglobin (has six times the buffering capacity of plasma proteins, primarily due to its histidine residues), carbonic acid - bicarbonate system (enhanced by the enzyme carbonic anhydrase contained in red blood cells), and the mono and dibasic phosphate system (Table 5.2) (G2).

The buffering capacity of blood is relatively constant between pH 6.4 and 7.8 at 30 mmol HCl/L/δ pH (CO_2 constant, and at 27^{o}C) (K34). Nevertheless, the buffering capacity of any single system is greatest when the amount of free anion is equal to the amount of undissociated acid, and this occurs when the pH of the fluid equals the pK for that buffer system.

Table 5.2

BUFFER SYSTEMS IN BLOOD
Protein Buffers
$R - COOH \leftrightarrow R - COO^- + H^+$
$R - NH_3^+ \leftrightarrow R - NH_2 + H^+$
Haemoglobin
$Hb - imidazole\ H \leftrightarrow Hb - imidazole^- + H^+$
Carbonic acid - Bicarbonate : (pK = 6.3)
$H_2CO_3 \leftrightarrow H^+ + HCO_3^-$
$H_2CO_3 \leftrightarrow CO_2 + H_2O$ (Carbonic anhydrase)
Mono & Dibasic Phosphate : (pK = 7.2)
$H_2PO_4^- \leftrightarrow H^+ + HPO_4^{2-}$

Buffers in cardioplegic solutions

Providing a strong buffer in a cardioplegic solution facilitates removal of hydrogen ions from the myocyte during the ischaemic period, and thus maintains a stable intracellular pH. In addition, the buffer chosen for a cardioplegic solution should have its greatest buffering capacity (pK) close to the optimal pH for that crystalloid cardioplegic solution.

The phosphate buffer system is potentially harmful in a cardioplegic solution, because phosphate binds calcium; precipitation of calcium phosphate may occur, and in calcium-free solutions the potential for the calcium paradox may be increased because of binding of remaining traces of calcium (H16). Therefore, the three buffers primarily used in crystalloid cardioplegic solutions are; bicarbonate, tromethamine (THAM / TRIS) and histidine. However, both bicarbonate (27 mmol/L) which has a maximum buffering capacity of 15 mmol HCl/L/δ pH at pH 6.3, and THAM (27 mmol/L) with a maximum buffering capacity of 15 mmol HCl/L/δ pH but at pH 8.1 (27^{o}C), have a narrow optimal zone of buffering bracketing their pK (K34). Moreover, Tris has a negative inotropic effect that is maintained after removal of the buffer and possibly inhibits anaerobic glycolysis and oxidative metabolism (G20). In contrast, histidine (161 mmol/L) has a buffering capacity greater than 20 mmol HCl/L/δ pH between pH 5.5 and 8.0, and a maximum buffering capacity at pH 6.0 of 60 mmol HCl/L/δ pH (K34).

Crystalloid cardioplegic solutions with high buffering capacities provided by the imidazoles glyoxaline (25 mmol/L; pK 7.0 at 25^{o}C) or histidine (195 mmol/L) delay ATP decay during ischaemia and improve postischaemic functional recovery, in contrast to poorly buffered solutions (D12,T4,V9,W33). Furthermore, multidose reinfusions of poorly buffered cardioplegic solutions also do not prevent acidosis from developing during ischaemia (T4). Thus, histidine is possibly a better buffer for cardioplegic solutions than bicarbonate. Furthermore, histidine can also be transported into the cell by a carrier mediated system and therefore possibly provides intracellular buffering

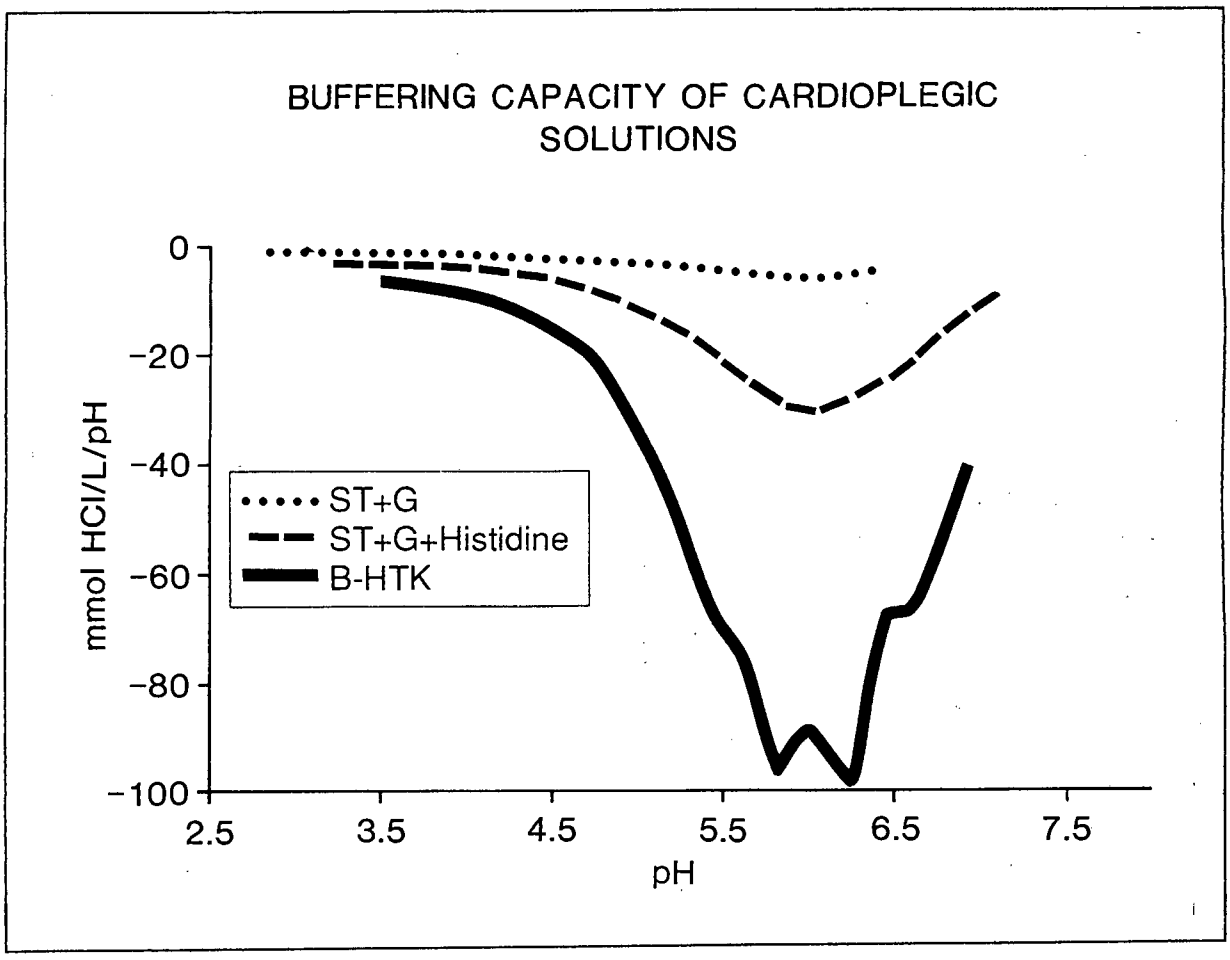
(L29), and has free radical scavenging properties (C19,G5). The major catabolic pathways of histidine are; deamination by histidase to urocanate and thence to glutamate, methylated to 1- or 3-methyl-histidine, and decarboxylated to histamine, and is a naturally occurring substance (C3).

In the isolated rat heart model we investigated whether a modified St Thomas' cardioplegic solution with an increased buffer capacity provided by histidine (50 mmol/L), would be beneficial (appendix B-5). The sodium content of the St Thomas' cardioplegic solution was decreased to 100 mmol/L, in order to make osmotic space available for the addition of histidine.

A pilot dose response curve for the sodium concentration of the St Thomas' cardioplegic solution confirmed the optimal sodium content to be 80 - 120 mmol/L (appendix B-3), similar to the findings of P Jynge (J17). In addition, changes to the osmolality of the St Thomas' cardioplegic solution were also evaluated; if the sodium content was 120 mmol/L then a higher osmolality (350 mOsmol/kg H_2O) was beneficial, if the sodium content was 100 mmol/L a higher osmolality (340 mOsmol/kg H_2O) was also not detrimental (see section 5.5) (appendix B-3). Therefore, the St Thomas' cardioplegic solution was modified by decreasing the sodium content to 100 mmol/L and the osmolality increased to 320 mOsmol/kg H_2O, which allowed the addition of 50 mmol/L of histidine and 10 mmol/L of glucose (see Appendix B-5).

The buffering capacity of this modified St Thomas' cardioplegic solution containing histidine was 30 mmol HCl/L/δ pH at its pK 6.0 - 6.3 (Figure 5.12) (appendix B-5). In contrast, the buffering capacity of the control St Thomas' plus 10 mmol/L glucose cardioplegic solution was significantly lower at 6 mmol HCl/L/δ pH at pK 6.0, and Bretschneider HTK4 which contains 198 mmol/L histidine was greater at 100 mmol HCl/L/δ pH at its pK 5.8 - 6.6.

Figure 5.12

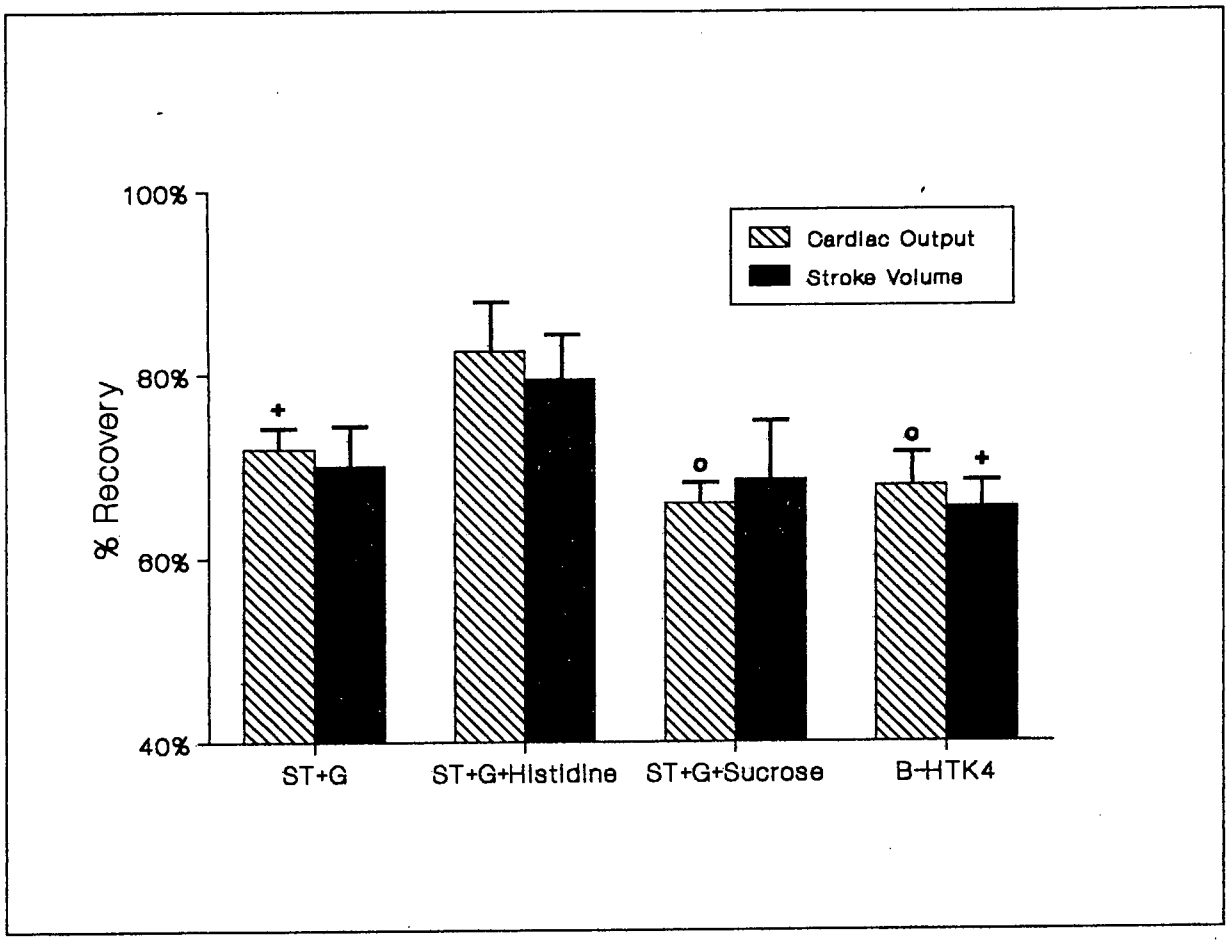


Legend:

The buffering capacity of each cardioplegic solution; St Thomas' plus glucose (ST+G), Bretschneider HTK4 (B-HTK), and ST+G+Histidine, was calculated as the instantaneous three point derivative slope of the titration curve, obtained by titrating each solution with 1 N HCl.

The addition, of histidine (50 mmol/L) to the St Thomas' plus glucose (10 mmol/L) cardioplegic solution, increased the buffering capacity of the St Thomas' plus glucose solution fivefold, delayed ATP decay during a 3-hour ischaemic period and improved postischaemic mechanical recovery (Fig 5.13 (appendix B-5)).

Figure 5.13
ADDITION OF HISTIDINE TO THE ST THOMAS' CARDIOPLEGIC SOLUTION

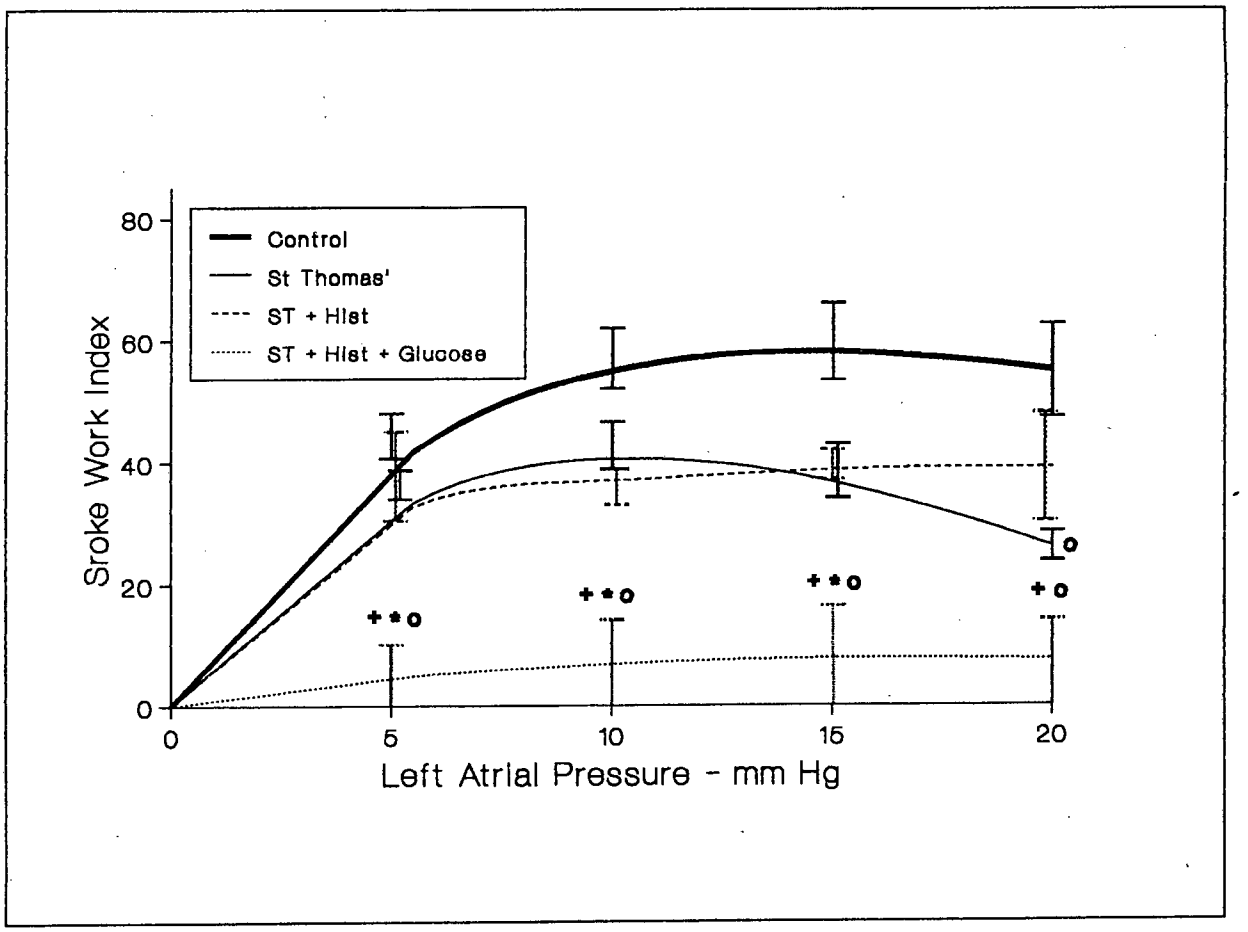


Legend:
Postischaemic mechanical recovery, expressed as mean ratios of individual preischaemic values, of isolated rat hearts after 3-hour 10°C cardioplegic arrest. Hearts were protected with a single dose of either St Thomas' plus 10 mmol/L glucose (ST+G), ST+G plus histidine (50 mmol/L), ST+G plus Sucrose (50 mmol/L) or Bretschneider HTK4 (B-HTK4) cardioplegic solutions. The vertical bars represent the standard errors of the means.
o - $p < 0.01$ compared to ST+G+Histidine,
+ - $p < 0.05$ compared to ST+G+Histidine.

The beneficial effect of including histidine was not due to the associated osmolality or sodium changes, as a similar St Thomas' plus glucose plus sucrose cardioplegic solution was no more effective than St Thomas' plus glucose cardioplegic solution. In addition, Bretschneider HTK4 with a buffering capacity 16 times that of the St Thomas' cardioplegic solution did not improve either ATP preservation or postischaemic recovery, and increased sarcolemmal damage (appendix B-5).

We also compared the efficacy of St Thomas', St Thomas' plus Histidine (50 mmol/L), and St Thomas' plus 10 mmol/L glucose plus histidine (50 mmol/L) cardioplegic solutions, in a pilot study in the in vivo primate model (appendix B-6). Although only a limited number of animals were used (N = 3) in this pilot study, making statistical analysis difficult, the trend in postoperative myocardial function between the groups is notable (Figure 5.14).

Figure 5.14
THE ADDITION OF HISTIDINE TO THE ST THOMAS' CARDIOPLEGIC SOLUTION: PRIMATE STUDY



Legend:

Left ventricular stroke work index (gm.m/beat/m^2) at increasing left atrial pressures before and after 3-hour hypothermic ischaemia protected with either multidose St Thomas' (N = 3), St Thomas' plus histidine (N = 3) or St Thomas' plus histidine (50 mmol/L) plus glucose (10 mmol/L; N = 2) cardioplegic solution. A postischaemic left ventricular function curve of stroke work index was obtained 30 min after weaning from cardiopulmonary bypass, and compared to a similar preischaemic curve. Mean percentage postischaemic recovery was calculated from each individual postischaemic and preischaemic ratio. An average preischaemic control function curve (Control; N = 8) was derived by pooling the data from all groups, and postischaemic function for each group normalized to this curve for display purposes.

* - $p < 0.01$ compared to St Thomas',

+ - $p < 0.05$ compared to St Thomas' plus Histidine,

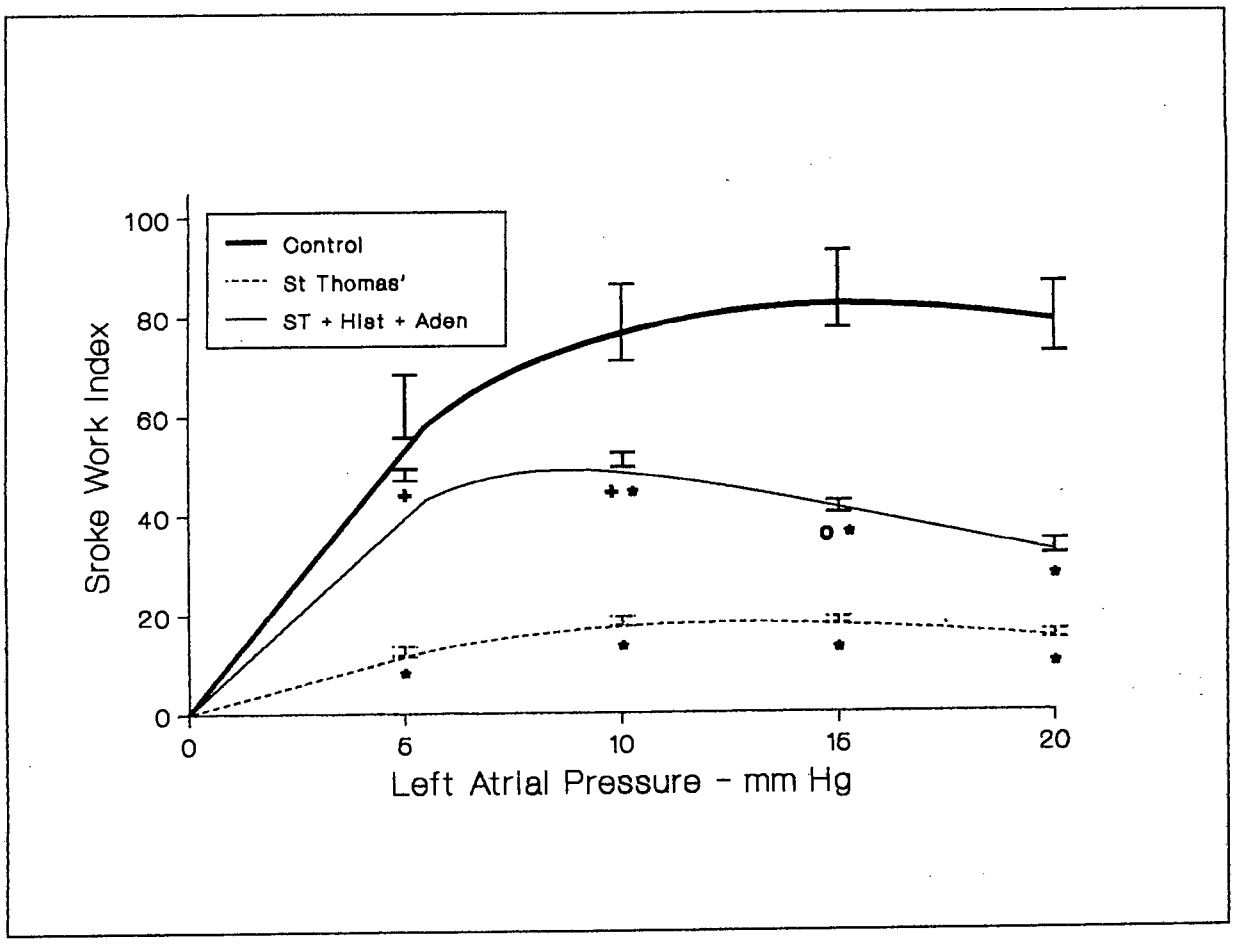
o - $p < 0.05$ compared to Control

The St Thomas' cardioplegic solution already provided good myocardial preservation in this model, thus we felt that further significant improvements would be difficult to obtain. Therefore, we elected not to continue with this model and changed to a model using preischaemic energy deficient hearts (see appendix B-9). Nevertheless, the addition of histidine to the St Thomas' cardioplegic solution tended to improve postischaemic function. However, the addition of glucose (10 mmol/L) to this modified St Thomas' cardioplegic solution containing a greater buffering capacity again decreased postischaemic recovery (appendix B-6) (see section 5.1). The increased buffer capacity provided by histidine did not prevent the depression of myocardial function in the primate model noted previously with the addition of glucose (10 mmol/L) to the St Thomas' cardioplegic solution (appendix B-1). We could though conclude that the addition of histidine to the St Thomas' cardioplegic solution was not deleterious in the primate model.

In a separate in vivo primate study, we examined the efficacy of a modified St Thomas' cardioplegic solution containing both histidine (50 mmol/L) and adenosine (1 mmol/L), and a lower calcium content (0.6 mmol/L) in energy depleted hearts (see Appendix B-9). The hearts were made globally ischaemic and fibrillated for 20 min prior to a 3-hour period of cardioplegic arrest. The optimum concentration of calcium in the St Thomas' Hospital No 2 cardioplegic solution is 0.6 mmol/L and not 1.2 mmol/L (B2,R31,R32) (see section 3.1). Furthermore, the optimum concentration of adenosine in the St Thomas' cardioplegic solution was established in our laboratory to be 1 mmol/L (D Boehm; unpublished results). We therefore felt justified for both financial reasons and because of our preceding results to introduce all three modifications to the St Thomas' cardioplegic solution. This modified cardioplegic solution containing both a higher buffering capacity, a lower concentration of calcium and adenosine was associated with improved postischaemic recovery compared to the standard St Thomas' Hospital No 2 cardioplegic solution (appendix B-9) (Figure 5.15).

Figure 5.15

MODIFIED ST THOMAS' HOSPITAL CARDIOPLEGIC SOLUTION: PRIMATE STUDY



Legend:

Animals were subjected to a 20-min period of ventricular fibrillation with global ischaemia at 32°C prior to 3-hour hypothermic cardioplegic arrest with multidose reinfusions of either the St Thomas' Hospital No.2 cardioplegic solution (N = 8) or a modified St Thomas' cardioplegic solution (St Thomas' + Hist + Aden; N = 6) containing a lower concentration of calcium (0.6 mmol/L), histidine (50 mmol/L) and adenosine (1 mmol/L). A postischaemic left ventricular function curve of stroke work index was obtained 30 min after weaning from cardiopulmonary bypass at increasing left atrial pressures, and compared to a similar preischaemic curve. Mean percentage postischaemic recovery was calculated from each individual postischaemic and preischaemic ratio. An average preischaemic control function curve (Control; N = 14) was derived by pooling the data from both groups, and postischaemic function for each group normalized to this curve for display purposes.

o - $p < 0.05$, + - $p < 0.01$; compared to St Thomas' No 2, * - $p < 0.01$ compared to Control.

In conclusion, the buffering capacity of an extracellular electrolyte equivalent solution such as the St Thomas' cardioplegic solution, can be increased without significantly altering its ionic formulation. An intracellular electrolyte formulation such as Bretschneider HTK4 is not necessary, in order to provide osmotic space for sufficient buffer.

5.5 OSMOLALITY AND COLLOID ONCOTIC PRESSURE IN CARDIOPLEGIC SOLUTIONS

Myocardial oedema can occur secondary to both hypothermia (E7) and ischaemia (F21,L24), because of failure of ATP dependant cellular homeostasis. Ischaemia results in accumulation of end products of anaerobic metabolism and is reported to increase intracellular osmotic pressure by 40 mOsm/L during the first 50 min of ischaemia (T23). Fluid is then shifted from the extracellular to the intracellular space, resulting in cellular oedema. Induced changes in myocardial water content are usually less than 4%. However, this would imply large changes in left ventricular mass; an increase of up to 22% (R35). In the clinical situation, sternal closure is occasionally not possible because of cardiac compression (following difficult prolonged procedures that resulted in severe myocardial oedema (M43,O5)). Hence, there is a need to prevent myocardial oedema.

Tissue fluid is compartmentalized; intravascular fluid, interstitial fluid and intracellular fluid, and each compartment has a different composition (G2). The capillary wall separates the intravascular and interstitial compartments, and allows diffusion and ultrafiltration. At this interface, the majority of fluid is filtered according to Starling's forces; capillary hydrostatic pressure (40 - 15 mm Hg) and colloidal oncotic pressure (25 mm Hg) (S24). Thus the colloid oncotic pressure exerted by intravascular proteins is the primary means of retaining water in the intravascular space and preventing interstitial oedema, as the majority of proteins do not pass through the capillary wall. In the absence of plasma proteins there are no forces drawing fluid back into the capillaries, and fluid can only return into the vascular compartment by lymphatics.

The interstitial fluid is in turn separated from the intracellular compartment by the semi-permeable cell membrane. Ions are distributed across the cell membrane according to both their concentration and electrical gradients, and water then diffuses according to the effective osmotic pressure (G2).

All fluid compartments are in osmotic equilibrium; and plasma isotonicity corresponds to an osmolal concentration of 290 mOsm/kg H_2O. Interstitial and intravascular hypotonicity would cause water to move into the intracellular compartment and thus result in intracellular oedema. Swelling of cells is not only a manifestation of injury but may also contribute to the process leading to cell necrosis (L24). Postischaemic myocardial oedema depresses left ventricular function and increases coronary vascular resistance (F22,P26,W29), and thus should be prevented during cardiac surgery.

Colloid Oncotic Pressure

Colloid; either albumin, hydroxyethyl starch or pentastarch, are used clinically both as the priming fluid for cardiopulmonary bypass circuits and for volume expansion, in order to prevent somatic and pulmonary fluid accumulation (L47,M13,S2). In addition, dextran has been used systemically to prevent the deleterious effects of both systemic and myocardial hypothermia; that is increased viscosity, haemoconcentration, sludging, and cellular oedema both experimentally and clinically (A5,F33,M51).

We therefore evaluated the effect of adding either 2 % or 3 % dextran-40 to the St Thomas' Hospital No 2 cardioplegic solution in the isolated rat heart model (appendix B-7). The oncotic pressure was increased from below 0,2 mm Hg in the standard St Thomas' cardioplegic solution to 15 and 25 mm Hg respectively. In this model (3-hour hypothermic (10^{o}C) ischaemia protected with a single dose of cardioplegia), the addition of either 2 % or 3 % Dextran did not improve postischaemic mechanical recovery. Furthermore, there was no difference in the myocardial water content following reperfusion between the three groups (appendix B-7).

In contrast, Foglia et al prevented potassium cardioplegia induced postischaemic myocardial oedema by adding albumen (oncotic pressure 21 mm Hg) to the cardioplegic

solution (F22). However, the canine is thought to be more susceptible to cardiopulmonary bypass and ischaemic induced myocardial oedema than humans (R35). This is substantiated by the significant amount of oedema induced in their model in a short 10-minute ischaemic period. Moreover, in clinical trials no difference in postischaemic myocardial function or outcome could be demonstrated by including either 3.5 % dextran-40 or albumin (5 gm %) in a crystalloid cardioplegic solution (B34,L39). Our study would support the conclusion that colloid does not appear to be necessary in crystalloid cardioplegic solutions, when administered as either single or multiple doses during clinical open-heart surgery. However, colloid may still be necessary during prolonged hypothermic perfusion of isolated stored hearts, when a continuous hydrostatic pressure would otherwise induce interstitial oedema (see section 3.3) (K17).

Crystalloid osmotic pressure

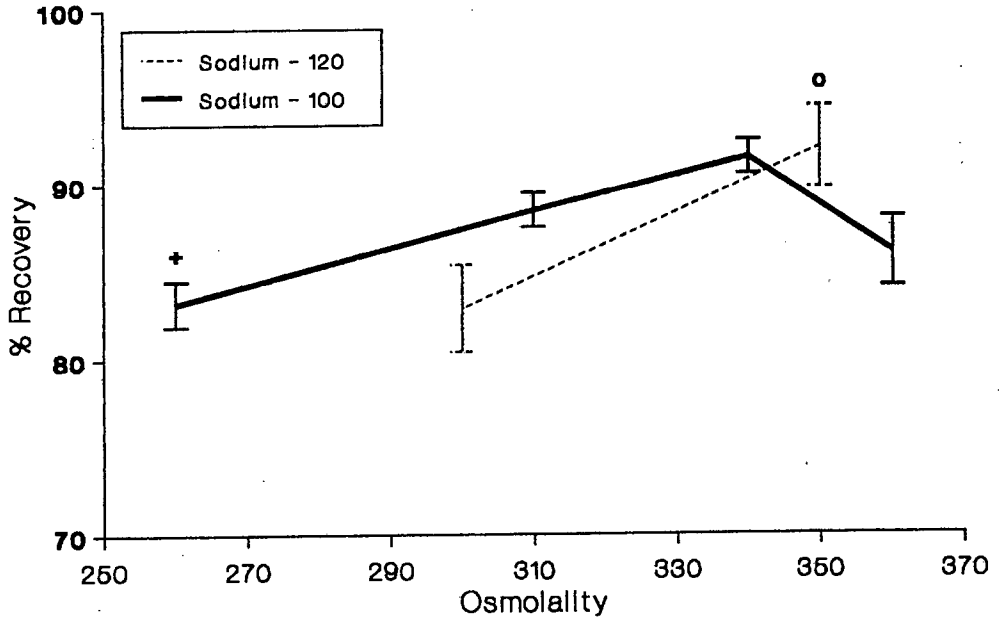
Sixty percent of body weight consists of water, of which 80 % is intracellular (G2). Hence, the osmotic pressure of fluids infused intravascularly are of great importance, as osmotic pressure is a primary determinant of intracellular volume.

In the isolated rat heart model using a 3-hour hypothermic (10^{o}C) ischaemic period protected with multidose of cardioplegia, we evaluated the effect of changes in the osmotic pressure of modified St Thomas' cardioplegic solutions (appendix B-3). The osmolality of the St Thomas' cardioplegic solution was altered by adding sucrose, an inert substance that is not metabolized intravascularly. Although these studies were performed as pilot studies to determine if the available osmotic space could be increased in the St Thomas' cardioplegic solution, significance was obtained despite the low numbers in each group (N = 4). In this model, the osmotic pressure of a modified St Thomas' cardioplegic solution with a sodium concentration of either 100 or 120 mmol/L could be increased from 300 mOsm/kg H_2O to 340 - 350 mOsm/kg H_2O (appendix B-

3). Postischaemic mechanical recovery was either not altered or increased with this moderate hypertonicity (Fig 5.16). Similar to the findings of Foglia et al (F22), cardioplegic solution hypotonicity (osmolality - 260 mOsm/kg H_2O) was harmful.

Figure 5.16

CARDIOPLEGIC SOLUTION OSMOLALITY



Legend:

Postischaemic recovery of cardiac output expressed as a mean ratio of each individual preischaemic value, of isolated rat hearts after 3-hour 10°C cardioplegic arrest. Hearts were protected with multidose cardioplegic infusions of either the St Thomas' Hospital No 2 cardioplegic solution which has a sodium concentration of 120 mmol/L, or a modified St Thomas' solution with a sodium concentration of 100 mmol/L. Osmolality was then altered in each solution by the addition of sucrose.

o - $p < 0.05$ compared to 300 mOsm/kg H_2O, sodium - 120 mmol/L,

+ - $p < 0.05$ compared to 310 mOsm/kg H_2O, sodium - 100 mmol/L.

Hearse et al suggest that the composition of cardioplegic solutions should not differ markedly from normal extracellular values (H14,H16), and showed that an increase of osmolality of $\pm$ 20 mOsm/kg H_2O did not alter mechanical recovery significantly (H27), and the addition of mannitol (50 mmol/L) diminished postischaemic recovery.

Nevertheless, Goto et al enhanced myocardial protection by increasing the osmolarity of the St Thomas' cardioplegic solution to 360 mOsm/L by the addition of mannitol (G25). Moreover, isotonicity of crystalloid cardioplegic solutions is associated with postischaemic myocardial oedema (F22,T23).

In the canine model, which might be more prone to myocardial oedema (R35), cardioplegic osmolality of 370 mOsm/kg H_2O produced $\pm$ 2.3 % dehydration (F22). However, excessive hypertonicity (osmolality greater than 350 mOsm/kg H_2O) in our model tended to diminish postischaemic recovery. Furthermore, in the normothermic working heart moderate hypertonicity (increases of 20 - 40 mOsm/kg H_2O) are beneficial in ischaemic myocardium (P26,W29), but hyperosmolality greater than 400 mOsm/kg H_2O is harmful (W28). Nevertheless, a clinical study failed to show a beneficial effect of increasing cardioplegic solution osmolality to 347 mOsm/kg H_2O with mannitol, although there was possibly more rapid postischaemic metabolic recovery (B43).

We would suggest that cardioplegic solutions should never be hypotonic, but rather isotonic or moderately hypertonic (300 - 350 mOsm/kg H_2O). More marked hyperosmolality may though be beneficial in reperfusion of the postischaemic myocardium (F9,F24,O25,S19). However, the majority of these studies have used mannitol to increase tonicity, and mannitol has additional effects other than preventing swelling of cells (P26), such as free radical scavenging properties (F9,M4,O25).

5.6 ANIONS IN CRYSTALLOID CARDIOPLEGIC SOLUTIONS

The formulation of cardioplegic solutions has been centered predominantly on the cation content of the solution. Nevertheless, the associated anions can influence the efficacy of the solution. The included anions can act in buffer systems (HCO_3^-, PO_4^{2-}), as metabolic substrates (acetate, lactate, aspartate, glutamate), chelators (citrate), or merely as counterions (Cl^-).

Undesirable anions

Phosphate salts are potentially dangerous in calcium containing cardioplegic solutions, because of the possibility of precipitates of calcium phosphate. Lactate is also potentially harmful (H26). Citrate inhibits glycolysis and has a strong affinity for calcium, and will thus change the calcium content of the final cardioplegic solution. Potassium citrate as apposed to potassium chloride is associated with diminished postischaemic recovery, and the citrate ion should therefore also be avoided in crystalloid cardioplegic solutions (H25).

Potentially useful anions

Hearse et al showed that either the chloride or sulfate salt of magnesium could be used in the St Thomas' cardioplegic solution (H28). However, the chloride salt was chosen for the final composition because chloride salts have high solubility coefficients. Although Hearse et al suggested that the aspartate salt did not improve the efficacy of the solution (H24), aspartate and glutamate have since been shown to be potential substrates for both anaerobic and aerobic metabolic pathways (B27,E10,E13,M18,R41). In contrast, the chloride anion has been implicated in the production of cellular oedema, as it is a freely permeable anion. A significant proportion of intracellular anions are nondiffusable, and this hinders the diffusion of cations and favours intracellular

diffusion of chloride ions (Donnan effect) (G2). Ischaemic induced increases in intracellular cations will tend to shift chloride ions into the intracellular space, which will concomitantly draw water intracellularly to maintain isotonicity (R20). The replacement of chloride ions by nonpermeable high molecular weight anions (lactobionate, gluconate) therefore helps regulate cellular oedema (W3).

In the isolated rat heart model using a 3-hour hypothermic (10°C) ischaemic period protected with a single dose of cardioplegic solution, we evaluated whether partially replacing the chloride ion in the St Thomas' cardioplegic solution would be beneficial (appendix B-4). The chloride concentration in the St Thomas' cardioplegic solution was reduced from 160.4 mmol/L to 34.4 mmol/L, by using sodium and potassium gluconate. However, neither postischaemic mechanical recovery nor postreperfusion water content was altered by this modification (appendix B-4).

We also evaluated the effect of this modified St Thomas' cardioplegic solution containing a low chloride content on the endothelium (appendix A-6). In this model, exposure of monolayer cultures of human endothelial cells to the low chloride concentration cardioplegic solution improved endothelial survival at hypothermia (10°C), but not at normothermia. However, gluconate also binds calcium (C27) and therefore alters the free ionized calcium concentration in the solution. Nevertheless, we demonstrated that a change in calcium content was not the mechanism for the improved survival with this low chloride solution (appendix A-6).

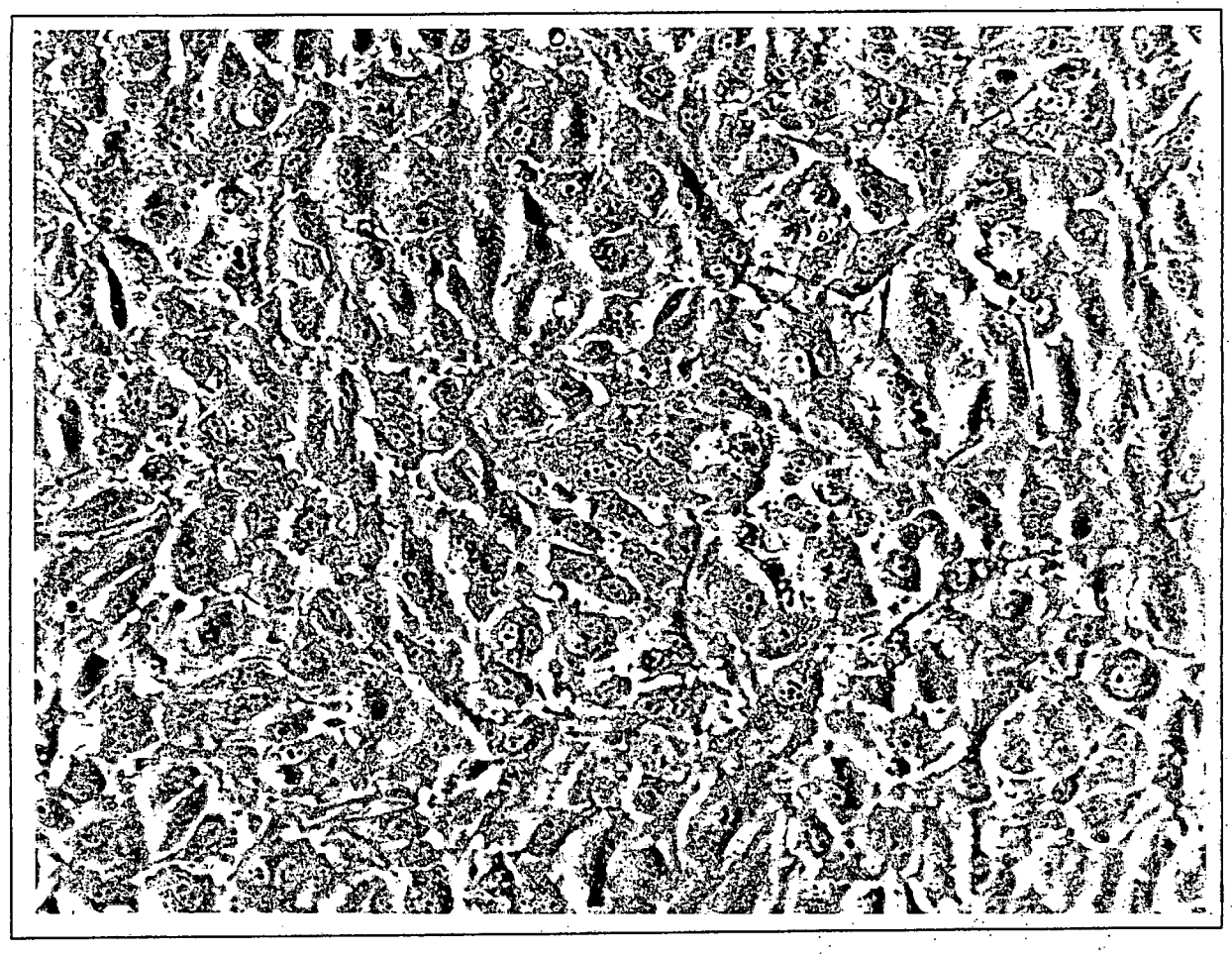
In conclusion, a low concentration of chloride in the St Thomas' cardioplegic solution does not adversely effect postischaemic myocardial function. Furthermore, a low chloride content may well have alternative beneficial effects, and should probably therefore be investigated further.

Chapter 6

THE CARDIAC SURGEON AND THE ENDOTHELIUM

The first barrier between the blood stream and the extravascular space is a monolayer of squamous epithelial cells; the vascular endothelium (Fig 6.1).

Figure 6.1



Legend:
Phase contrast microscopy appearance of a confluent monolayer of human saphenous vein endothelial cells, which demonstrates the typical cobblestone morphology. (Magnification X 100). Reprinted from Applied Cardiovascular Biology 1990, Vol 2, 1991:(in press) with permission S Karger AG, Basel.

In the average adult the endothelium occupies a surface area of approximately 1000 m^2, and performs a multitude of functions without which we could not survive [J1]. In fact,

the endothelium should be considered as an "organ" in its own right, although distributed throughout the entire body. The endothelium also responds to various stimuli not only as a target for injury, but also by altering its function, metabolism and structure and thereby influencing secondary responses to injury (C39,R45). Injury to any organ results in concurrent damage to the vascular endothelium of that organ. Hence, the function, response to injury and preservation of the endothelium should be an important consideration of all cardiovascular surgeons. Furthermore, of specific importance to the cardiac surgeon is the effect on the endothelium of ischaemia and "protective" solutions infused into the coronary circulation, and the preservation of the endothelium of saphenous veins harvested for coronary artery bypass grafts. The patency of prosthetic grafts used to replace portions of the vascular system or bypass small diameter vessels can also be improved by autologous endothelialization of their internal surfaces (Z3,Z4,Z5). Moreover, rapid acute endothelialization of vessels denuded of endothelium as a result of injury or endarterectomy, may also improve vascular surgical results (S26).

6.1 THE FUNCTION OF THE ENDOTHELIUM

The vascular endothelium consists of a haemocompatable monolayer of cells that synthesize subcellular stroma, regulate angiogenesis, modulate capillary permeability, inflammation, the immune response, haemostasis, and vascular smooth muscle tone (C39,F2,P8). The endothelium modulates these biologic systems by a number of mechanisms: converting circulating precursors into vasoactive products, degrading vasoactive substances, secreting vasoactive substances into both the blood stream and subcellular matrix, and also by binding various substances (B14,B59,J1,R57) (Table 6.1). Furthermore, the endothelium interacts continuously with circulating platelets, leukocytes, and vascular smooth muscle cells (G35,H42,M27).

Table 6.1

FUNCTIONS OF THE ENDOTHELIUM

Regulates Haemostasis and Thrombosis

Antithrombotic activities

Secretes / expresses:
- EDRF
- Prostacyclin (PGI_2)
- Thrombomodulin
- Heparan sulfate
- Antithrombin III
- Plasminogen activator (tPA)
- Adenosine
- 13-HODE

Inactivates:
- Thrombin
- ATP, ADP
- Serotonin
- Prostaglandins

Procoagulant activities

Secretes / expresses:
- Collagen
- Fibronectin
- Thromboxane A_2
- Thromboplastin
- Von Willebrand factor
- Factor V
- Inhibitor of tPA
- Platelet activating factor

Binds:
- Thrombin
- Fibrinogen
- Factors V_a, IX_a, X_a

Modulates the Inflammatory Process

Modulates adhesion of leukocytes
- 13-HODE, PGI_2, EDRF

Secretes:
- Interleukin-1
- Platelet-derived growth factor
- Platelet activating factor

Immunoreactivity

Manifests:
- ABO blood groups
- HLA antigens
- Complement binding receptors

Modulates Vascular Tone

Vasodilator properties

Secretes:
- EDRF
- Prostacyclin
- Adenosine

Inactivates vasoactive substances
- Angiotensinase
- Monoamine oxidase
- Kinase II

Vasoconstrictive properties

Secretes:
- Endothelins
- Thromboxane A_2

Activates Angiotensin I:
- Angiotensin converting enzyme

Antithrombotic and haemostatic functions

The normal quiescent endothelium is antithrombogenic, as a result of complex endothelium-mediated mechanisms that either interfere with platelet aggregation, thrombus formation or activate fibrinolysis (Table 6.1). The primary mechanism is probably the inhibition of platelet adhesion and aggregation by both intracellular endothelial 13-hydroxy-9,11-octadecadienoic acid (13-HODE), and a continuous endothelial release of both prostacyclin (PGI_2) and endothelium derived relaxing factor (EDRF) (D20,G35,M1). PGI_2 and EDRF discourage both platelets and leukocytes from adhering to the endothelial lining by increasing intracellular platelet and leukocyte levels of cAMP and cGMP (D20,R2,R3).

Nevertheless, endothelial cells also play a dynamic role in haemostasis; the formation of a platelet plug, fibrin clot and its subsequent intravascular limitation and dissolution, should the vascular endothelium be damaged (Table 6.1). The basal release of PGI_2 and EDRF are lost if the endothelium is denuded or damaged and platelets therefore aggregate and adhere (P8). In addition, the subendothelial extracellular matrix which contains collagen, fibronectin, von Willebrand factor etc, is produced by the endothelium (J2,W1,W6), and activates the clotting cascade and enhances platelet adhesion if exposed to blood. Platelet aggregation in turn potentiates both further platelet aggregation and clot formation by the release of vasoactive substances contained in platelets (ADP, ATP, serotonin, thromboxane A_2, platelet activating factor (G15,P8,V10)). Moreover, the clotting cascade is further enhanced by endothelium binding of various activated clotting factors (P8) and release of the platelet aggregator thromboxane A_2 (R4).

The endothelium is thus not only antithrombogenic but in addition promotes coagulation if it is damaged, and thereafter modulates the clotting process by inactivating platelet released factors, and by activating fibrinolysis through the release of plasminogen activator and PGI_2 (G35,L48) (Table 6.1). Furthermore, endothelial activation by injury

However, the vasodilator activity of the majority of vasoactive agonists is dependent upon the endothelium for their vasodilating effects (B14,F16,F35,G35). In contrast, the simultaneous vasoconstrictive effects of these same vasoactive agonists are due to a direct action on the vascular smooth muscle, although possibly requiring higher concentrations. Thus, vasoactive substances such as acetyl choline and serotonin will cause vasodilation of a vessel in the presence of intact functioning endothelium (endothelium-dependent vasodilation), but vasoconstriction in the absence of the endothelium (endothelium-independent vasoconstriction). In contrast, endothelium-independent vasodilators (papaverine, sodium nitroprusside) cause vasodilation in the presence or absence of the endothelium.

The endothelium also metabolizes and removes many circulating vasoactive substances from the blood stream. Vasoconstrictor responses are therefore enhanced if endothelial function is impaired, and the endothelium is thus an essential structure that modulates vascular tone.

Endothelium-dependent vasodilation

The primary mechanism of endothelium-dependent vasodilation is the release of EDRF and PGI_2 in response to a number of stimulants (B14,P9). Furthermore, it is postulated that the endothelium provides a constant vasodilator tone, which prevents vasospasm by circulating or platelet released vasoconstrictors (G35,S74). Moreover, arteries and veins demonstrate differences in their vasomotor responses, and this difference is due to the variability of EDRF release by different vascular beds (L51,S34,T17).

PGI_2 is produced by both endothelial and smooth muscle cells, has a longer half-life than EDRF, inhibits platelet aggregation, has cytoprotective and fibrinolytic properties and relaxes vessels by increasing platelet and smooth muscle cAMP, in response to various stimuli (G35,S28) (Fig 6.2). Furthermore, the endothelium utilizes prostaglandin precursors released from platelets to form additional PGI_2 (D20). The rate of synthesis of PGI_2 also possibly accounts for differing incidences of atherosclerosis in the

population; increased atherosclerosis in men and transplant recipients from testosterone and cyclosporine inhibition of PGI_2 (G35,S33), and in different vascular beds; the low incidence of atherosclerosis of the internal mammary artery (R45). Thus, PGI_2 is a factor contributing to the internal mammary being the ideal conduit for coronary artery bypass grafts (A1,S80).

Nevertheless, EDRF is thought to be the dominant endothelium-dependent vasodilator, has a half life of less than six seconds in vivo and has now been identified as nitric oxide (P2). EDRF induces smooth muscle relaxation by stimulating guanylate cyclase and thus increasing cGMP which in turn decreases smooth muscle cytosolic calcium (F16,F34), in similarity to the nitrovasodilators (sodium nitroprusside, glyceryl trinitrate). The release of EDRF though requires oxygen, and EDRF induced vasodilation can be inhibited by anoxia, free haemoglobin (binds nitric oxide and inhibits guanylate cyclase) and methylene blue (inhibits guanylate cyclase) (B14,B59). Furthermore, oxygen free radicals and more specifically the superoxide anion destroys EDRF (L16,V11), whereas the free radical scavenger superoxide dismutase increases the stability of EDRF (B14).

Endothelium-dependent vasoconstriction

The endothelium also synthesizes and releases a potent vasoconstrictor; a 21-amino acid peptide - endothelin, which increases cytosolic calcium (N4). Endothelin can thus produce severe coronary vasoconstriction (E18,H41), as well as a positive chronotropic, inotropic and direct arrhythmogenic effect in the heart (N4,Y4). Furthermore, endothelin has a mitogenic effect on vascular smooth muscle and therefore may be a mediator of atherosclerosis, in contrast to EDRF which inhibits mitogenesis (N4,R2,W20). Nevertheless, endothelin also stimulates the release of EDRF and PGI_2, which counteracts its effects to a certain extent (W36). However, endothelin has a slow sustained effect in contrast to EDRF, which has a rapid short duration of action.

Endothelium-dependent relaxation of vascular smooth muscle usually dominates over the endothelium-independent constrictor effects. Moreover, physiochemical factors such as shear stress, pulsatile flow and hypoxia induce endothelium-dependent vasodilation, and endothelial cells can thus act as flow sensors. Peripheral resistance arterioles, where endothelium-dependent vasodilation is more pronounced, can therefore respond to more proximal increases of blood flow (B14). The endothelium is thus an essential structure that normally induces a constant "background tone" of vasodilation. Any imbalance between endothelium released vasoactive substances (endothelin, EDRF, PGI_2), as well as platelet, leukocyte and circulating vasoactive substances would effect vascular tone both locally and globally.

The endothelial response to injury

Removing the endothelium from a vessel is obviously harmful; the barrier effect of the endothelium is lost, local endothelium-dependent vasodilation is lost, blood is exposed to the thrombogenic subcellular matrix, and both platelets and leukocytes aggregate and adhere to the site of injury. However, the endothelium also responds to various stimuli not only as a target for injury, but also by altering its function, metabolism and structure and thereby influencing secondary responses to injury. Thus, sublethal injury of the endothelium without disruption of monolayer integrity can also be harmful (C39). Endothelial injury can then as discussed above, result in the endothelium becoming procoagulant, immunoreactive, adhesive to platelets and leukocytes, as well as increasing vascular permeability and promoting vasoconstriction.

Reendothelialization of denuded arterial endothelium although dependent upon the severity of the initial trauma (L40), occurs in 7 - 10 days (H13,L44). Nevertheless, the function of regenerated endothelium remains abnormal for 4 - 12 weeks, and is characterized by a reduced ability to release EDRF and increased endothelium-dependent vasoconstriction (C42,C5,D20,L38,S43). Furthermore, even in the absence of

initial denudation of the endothelium, ischaemic induced injury endothelial dysfunction can also persist for a prolonged period (F12,P12,P13). Endothelial injury thus produces an immediate as well as a more prolonged predisposition (up to 3 months) for both thrombosis and vasospasm. In addition, endothelial injury in combination with platelet and leukocyte interactions are mechanisms for the pathogenesis of atherosclerosis (B52,D20,R45,W20), and intimal hyperplasia in arterialized saphenous veins (B23,E3).

A multitude of factors can cause endothelial dysfunction and injury (B52), and these include direct mechanical factors such as probes (J8), angiographic catheters, angiographic contrast media (H10), endothelial hypoxia (O6), diabetes, cigarette smoking (Z6), low density lipids (Z9), radiation, endotoxins, immune complexes and haemodynamic stresses. In addition, both the underlying pathology resulting in the need for cardiac surgery (atherosclerosis), and cardiac surgery itself can damage the endothelium.

Atherosclerosis

Atherosclerosis is not only the result of endothelial injury, but atherosclerosis also causes further progressive impairment of endothelial vasoactive functions (Z1). For example, in normal coronary arteries both acetyl choline, sympathetic stimulation and shear stresses all cause vasodilation, but in the presence of atherosclerosis these normal vasodilator responses are lost and vasoconstrictive responses then predominate (V21,Z2). Furthermore, overt atherosclerotic plaques need not be present as endothelial dysfunction also occurs in both "angiographically normal" coronary arteries and "normal" collaterals, in patients with hypercholesterolaemia or evidence of atherosclerosis elsewhere (S36,S37,Z1). Therefore, in patients with atherosclerotic induced angina pectoris and myocardial infarctions, loss of endothelial-mediated vasodilation may contribute to vasospasm (B14,D20,H42).

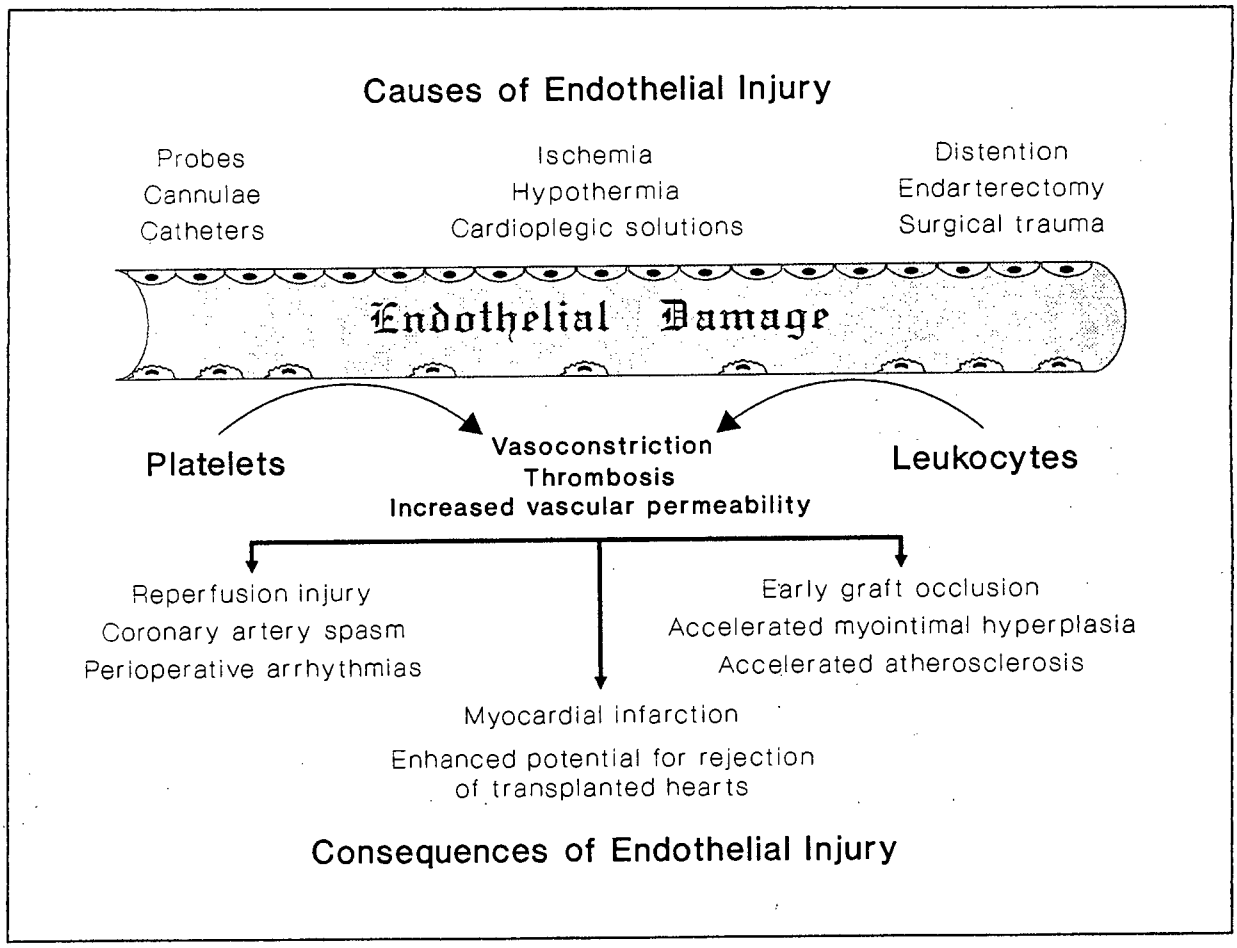
"Elective" cardiac ischaemia and reperfusion

Surgically induced cardiac ischaemia and subsequent reperfusion produces both myocyte and endothelial damage (F12,S85). Ischaemia and hypoxia cause endothelial changes that result in increased endothelial permeability and procoagulant properties (H40,O6), and reperfusion with initial high pressures also increases endothelial cell damage (S17). Furthermore, ischaemic induced myocardial damage occurs primarily in the interstitium (H55) and endothelial interface during the first few minutes of reperfusion (T24,T25), and the endothelium is possibly more vulnerable to ischaemia than the myocyte (S86). The reperfusion injury is produced by oxygen free radicals (see section 1.6) (T24,T25) generated by endothelial and leukocyte interactions (M37), which in addition induce further endothelial damage.

Endothelial injury thus results in increased endothelial permeability, platelet aggregation, leukocyte adhesion and migration (K24,M28,S86), increased production of endothelium-derived contracting factor (P10), impaired endothelium-dependent relaxation in response to aggregating platelets and vasoactive drugs (P11,P12,V8), vasoconstriction and procoagulant properties which in turn causes further secondary myocardial damage. Moreover, dysfunction of endothelium-dependent vasodilation primarily alters postischaemic blood flow to the more vulnerable subendocardium (P13). Plasma levels of the powerful vasoconstrictor endothelin are also increased for 2 - 3 days after surgery (N4). In fact, it is possible that myocardial stunning and the reperfusion injury are merely manifestations of the consequence of endothelial injury.

Hence, there are a multitude of factors that can potentially injure the vascular endothelium during cardiac surgery, and ideally, endothelial injury and its potentially disastrous consequences should be prevented (Figure 6.3). Furthermore, endothelial dysfunction may be present as a result of preexisting pathology; atherosclerosis, and occasionally endothelial injury cannot be prevented; endarterectomies of severely diseased vessels.

Figure 6.3



Legend:
A number of factors may injure the vascular endothelium during cardiac surgery, and the injured endothelium and vessel wall then interacts with both platelets and leukocytes causing the tabulated possible consequences. Endothelial injury induces both early and late myocardial dysfunction.

Therefore, pharmacologic modifications of endothelium, platelet and leukocyte interactions with aspirin, beta-blockers, calcium antagonists, nitrates, thromboxane synthetase inhibitors and thromboxane receptor antagonists, are therefore possibly essential for up to 3 months after any injury to the endothelium (D20). In addition, low dose eicosapentaenoic acid (cod-liver oil) may also be beneficial by facilitating endothelium-dependent vasodilation (G35), inhibiting atherosclerosis and in the long term by decreasing intimal hyperplasia (D20,L5).

6.2 PRESERVATION OF SAPHENOUS VEIN ENDOTHELIUM

Cardiac surgeons possibly first became aware of the importance of preserving the endothelium when harvesting saphenous veins for use as coronary artery bypass grafts, in order to decrease the incidence of early graft occlusion. Rough dissection techniques, excessive distention, low storage temperatures, and the composition of solutions used for irrigation and storage, have all been implicated in damaging the endothelium of saphenous vein grafts.

However, in addition to this surgically induced endothelial dysfunction, vein grafts also undergo endothelial cell loss, smooth muscle hyperplasia (S49), medial fibrosis (B67,C2), lipid uptake (B35) and functional changes (B79,F11,K29,M45) as a result of implantation into the arterial side of the circulation; arterialization of vein grafts (H30). These changes associated with arterialization of vein grafts can though be limited by optimum harvesting techniques (A10). Increased endothelial damage at operation may predispose to both early graft occlusion as a result of endothelial cell dysfunction (C42), endothelial cell loss, platelet and leukocyte adherence (L44), as well as late occlusion due to increased myointimal hyperplasia (O2,R5), lipid uptake (B45) and atherogenesis (B66,O13,S84). Arterialized vein grafts have also though been shown to secrete high concentrations of prostacyclin from the media despite significant endothelial loss, which would help counteract the effects of endothelial damage (B79,H47,H30).

Each aspect of the harvesting of saphenous veins has been evaluated in order to prevent saphenous vein endothelium damage.

Dissection techniques

Standard dissection techniques, consisting of adventitial stripping and uncontrolled manual distention results in decreased release of EDRF and endothelial cell loss (A25). Therefore, atraumatic dissection techniques are essential. In addition, venous side

branches must be ligated away from the vessel wall in order to prevent intraluminal stenoses due to protrusions of the intima and media (G40). Furthermore, despite reversal of saphenous veins, venous valves are associated with thrombosis and late fibrosis and stenosis due to turbulent flow (M47,S84). Therefore, routine incision of valves with a valvutome has been advocated (M47,M52,S84).

Storage temperature

Cold induces venous constriction, which is harmful. Veins stored at 4°C compared to 21°C or 37°C have been shown to have increased morphological changes (L45,S59) and thereafter reduced secretion of both EDRF (L15) and PGI_2 (B80). However, if topical or infused papaverine is used during harvesting to prevent venospasm then storage temperature is possibly not a significant factor (B10), although initial distention with a hypothermic solution may still be damaging (L45). Moreover, Gundry et al reported better preservation of veins stored at 4°C as apposed to 28°C, but no statistical analyses were performed on these limited morphological studies (G39,G40). Nevertheless, the consensus appears to be that veins are best stored at room temperature (B11,L14).

Distention

Uncontrolled distention of saphenous veins to check for leaks can easily produce pressures in excess of 600 mm Hg (A3). Distending venous grafts with pressures of 400 - 500 mm Hg is harmful to both the endothelium (B45,C2,L15,M9), and tunica media (A26,R5), and is possibly the most harmful variable during harvesting of vein grafts (K44). Therefore, the pressure used to distend saphenous veins should be restricted to less than 300 mm Hg, by using a pressure limiting device (B45,L43). Nevertheless, for optimum preservation venous distention should probably be limited to physiological pressures of not more than 100 - 150 mm Hg (A26,G39,G40), although distention to either 125 mm Hg or 350 mm Hg does not alter lipid uptake (B35).

Prevention of venospasm

Venospasm causes intimal tears and endothelial detachment (H12). Despite careful "no-touch" dissection techniques venous contracture and collapse occur often during harvesting of saphenous veins, and this can be prevented by perivenous infiltration with papaverine (Plasmalyte A, papaverine 0.12 mg/ml, heparin sodium 4 U/ml) (H12,L45), and flushing of the vein with the same solution (L43). Alternatively, intermittent irrigation and topical spraying of the vein every 2 - 3 min as soon as it is exposed, with Plasmalyte and papaverine (0.05 - 0.6 mg/ml) during harvesting might be equally effective (B15). This method produced superior early graft patency rates clinically, than atraumatically harvested veins stored in blood (C9). Papaverine (0.12 - 1 mg/ml) though has now also been added to autologous heparinized (10 U/ml) blood with reported good preservation of the endothelium (A10,B10). However, Roberts et al showed that the addition of papaverine to an electrolyte solution used to flush saphenous veins lowered the release of unstimulated prostacyclin release, although no differences in morphology could be found (R22). However, the interpretation of this observation is debatable (C7), as prostacyclin production can be similar in the presence or absence of endothelium (H30), and other studies have shown that the addition of papaverine does not alter the release of EDRF (L15). Nevertheless, the consensus appears to be that the use of papaverine before and during harvesting of saphenous veins is beneficial. Papaverine can be added to either blood or a balanced electrolyte solution, but the solution should be slightly acidic in order to prevent the formation of precipitates (C43).

Irrigation and storage solution

Blood or tissue culture media have been found to be superior to normal saline, regardless of temperature (B80,G39,G40,L45,O2,R5). The use of tissue culture medium though carries an increased risk of contamination (B11). Storage in heparinized blood as apposed to a balanced electrolyte solution (Hartmann's solution), has though also been associated with a time-related reduction in EDRF release (A25). Storage in 10°C blood may also produce more endothelial damage than storage in 10°C Plasmalyte (B15). However, Lawrie et al showed equivalent EDRF release in veins stored in either

heparinized blood or Plasma-Lyte (L15). Nevertheless, although blood may be an adequate preservation medium (A10), it also contains platelets and leukocytes which could become activated and adversely interact with the saphenous vein endothelium (L14). Although there is no consensus as to whether veins should be stored in blood or a balanced electrolyte solution, a balanced electrolyte solution containing papaverine provides excellent endothelial preservation.

Furthermore, following the completion of distal coronary artery to saphenous vein graft anastomoses, many surgeons infuse cardioplegic solutions through the vein grafts to improve myocardial protection (see section 1.5). Hence, it is possible that these reinfusions of cardioplegic solution could also damage the endothelium of the vein grafts (H8,M19,S49), and lead to accelerated atheromatous degeneration (O13).

In conclusion, minimizing both endothelial and medial damage of saphenous veins harvested for use as coronary artery bypass will help improve both short and long term vein graft patency. Atraumatic surgical techniques, prevention of venospasm by the use of papaverine in a balanced electrolyte solution, limitation of venous distention to less than 100 - 150 mm Hg and storage at room temperature are advised. Furthermore, it is possible that aspects of endothelial preservation learned in these saphenous vein studies could also be applicable to the coronary vasculature.

6.3 CARDIOPLEGIC SOLUTIONS AND THE ENDOTHELIUM

Cardioplegic solutions are infused into the heart prior to periods of ischaemia in order to maximally decrease myocardial energy demands, and thereby limit ischaemic induced myocardial damage (see section 1.5). The primary determinant when formulating the composition of cardioplegic solutions has therefore been their ability to induce reversible myocardial electromechanical arrest (see chapter 3). Nevertheless, cardioplegic solutions should also be nontoxic to both the myocyte and endothelial cells.

Hyperkalaemic (K - 30 mmol/L) crystalloid cardioplegic solutions were first shown to cause endothelial damage by Follette et al in 1980 (F23). Intermittent infusions of an unspecified hypothermic (16^{o}C) crystalloid cardioplegic solution, every 20 minutes throughout a 2-hour ischaemic period, caused arterial endothelial disruption and fibrin deposition which could be minimized by the addition of blood to the cardioplegic solution. Harjula A, Mattila S and co-workers also showed that the addition of either blood, albumen or Fluosol DA to a crystalloid cardioplegic solution reduced both coronary artery and saphenous vein graft endothelial damage, experimentally (H9,H11,M19) and clinically (H8). However, the crystalloid cardioplegic solution evaluated in these studies was a sodium-poor calcium-rich solution; a poorly formulated potentially damaging composition for a cardioplegic solution (see section 3.1 & 3.2), and hence we cannot infer that the addition of blood or colloid (which would also have altered the electrolyte composition) is an essential prerequisite for a cardioplegic solution from these studies. Nevertheless, these early studies highlighted the potential for "myocardial protective" cardioplegic solutions to damage the vascular endothelium. Endothelial damage could increase myocardial reperfusion injury, predispose to perioperative coronary artery spasm and thrombosis, increase myocardial ischaemia and hence decrease postischaemic myocardial function (see section 6.1).

The majority of research on the efficacy of cardioplegic solutions has been centered on improving postischaemic myocardial recovery, and few studies have been performed to evaluate the effect of cardioplegic solutions on the endothelium. Furthermore, endothelial changes induced by both hypothermia and the composition of cardioplegic solutions should be assessed separately.

Effect of hypothermia on the endothelium

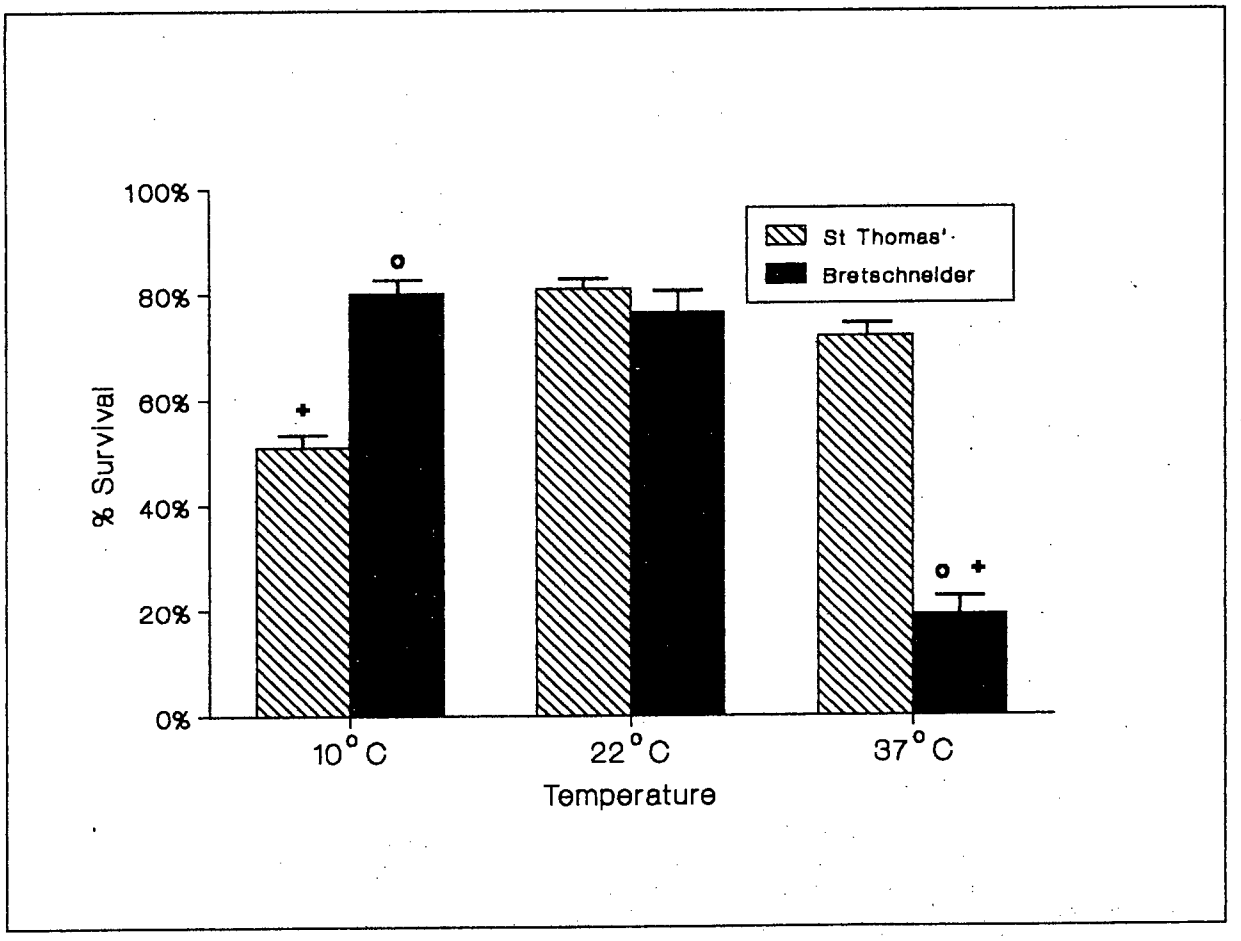
Extreme hypothermia was shown to be detrimental to the endothelium of saphenous veins, but possibly as a result of hypothermic induced venospasm. Nevertheless, hypothermia alters intracellular endothelial homeostasis by inhibiting the function of the sodium-potassium pump and results in increased detachment of cultured endothelial cells (L13). Hypothermic induced alterations of the cytoskeleton are thought to be due to increased cytosolic calcium levels, occurring secondary to intracellular sodium accumulation (L13). Furusho et al demonstrated increased acetyl choline induced coronary vasoconstriction after hypothermic as apposed to normothermic perfusion with either Krebs Henseleit buffer or a calcium-free cardioplegic solution (F36). However, significant endothelial damage had probably already occurred prior to commencing their experimental protocol, as acetyl choline induced vasoconstriction and not endothelium-dependent vasodilation (B14,F16,F35,G35) was present in their pre-exposure controls. Nevertheless, hypothermia possibly accentuated endothelium-independent vasoconstriction.

We evaluated the effect of the St Thomas' Hospital No 2 and Bretschneider HTK4 cardioplegic solutions on cultured human venous endothelial cells at different temperatures (appendices A-5 & A-6). Endothelial cell survival increased following 12-hour hypothermic as apposed to normothermic exposure to the calcium-free Bretschneider HTK4 cardioplegic solution (Fig 6.4). In contrast, the extracellular

electrolyte equivalent St Thomas' cardioplegic solution was associated with superior endothelial cell survival at normothermia.

Figure 6.4

CYTOTOXICITY OF CARDIOPLEGIC SOLUTIONS: EFFECT OF TEMPERATURE



Legend:

Cultured human venous endothelial cell survival 24 hours after 12-hour exposure at the indicated temperatures, to either ST Thomas' Hospital No 2 or Bretschneider HTK4 cardioplegic solutions. Cell survival is expressed as a mean percentage of each individual pre-exposure cell count. Vertical bars represent standard errors of percentage means.

+ - $p < 0.01$ compared with the same solution at 22°C.

o - $p < 0.01$ compared with St Thomas' cardioplegic solution at that temperature.

Furthermore, in contrast to the minimal endothelial cell morphological changes observed after normothermic exposure to the St Thomas' cardioplegic solution, hypothermic exposure to either the St Thomas' or Bretschneider HTK4 cardioplegic solutions caused cellular contraction and intercellular gaps (Fig 6.5). However, as also

shown by Solberg et al (S60) these effects were reversible after reincubation in culture medium (see appendix A-6).

Hypothermia thus has an independent effect on the endothelium and may cause endothelial cell contraction and dysfunction.

Effect of the composition of cardioplegic solutions on the endothelium

The composition of an infused cardioplegic solution can produce cytotoxic effects on the vascular endothelium (C4), although as discussed above, these effects may also be temperature dependent.

Evaluation by cell cultures

Carpentier et al were the first to evaluate the cytotoxic potential of various cardioplegic solutions by tissue culture (C4). The St Thomas' Hospital No 2, Bretschneider No 3 and Roe's cardioplegic solutions were the least cytotoxic to both fibroblasts and endothelial cells, although no statistical analyses were performed. Furthermore, the addition of blood attenuated the more toxic cardioplegic solutions evaluated.

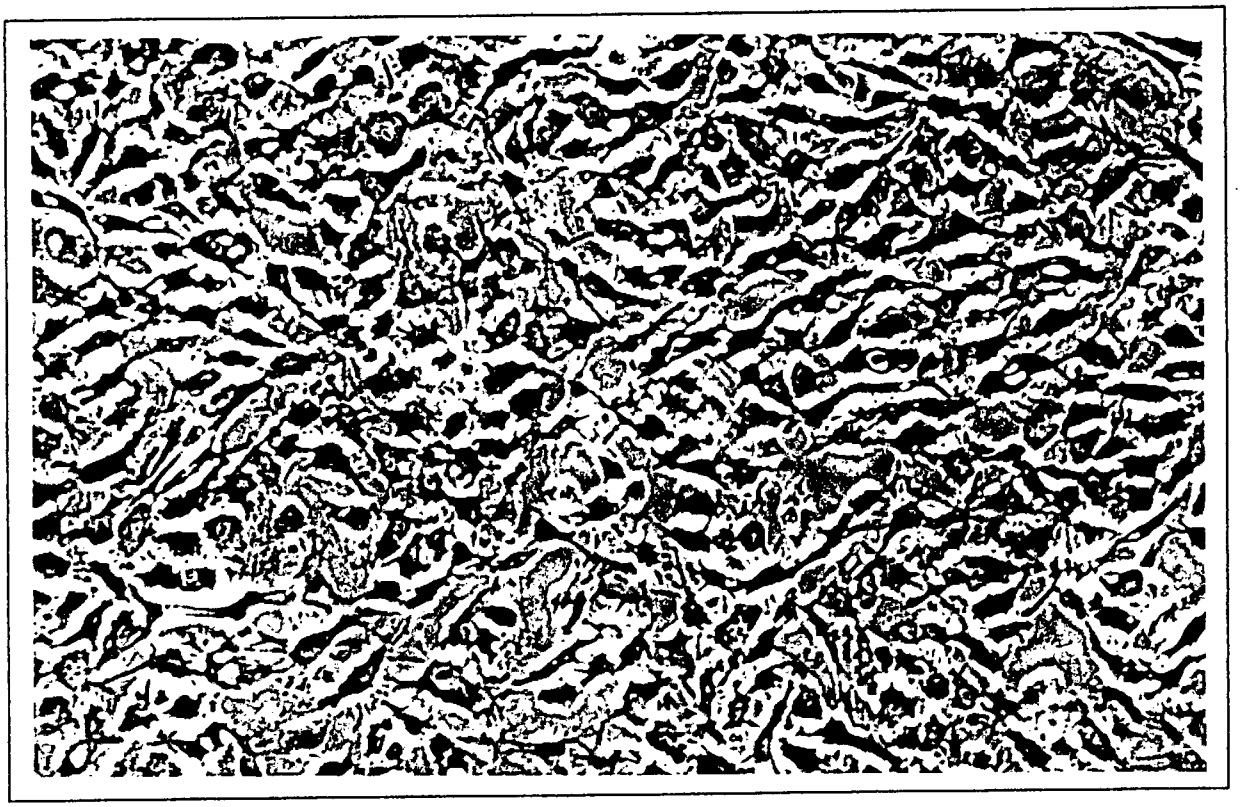
We evaluated the cytotoxicity of various hypothermic cardioplegic and organ preservation solutions on cultured human venous endothelial cells (see appendices A-5 & A-6). When various cardioplegic solutions used in South Africa were evaluated at $22^{o}C$ (appendix A-5), both St Thomas' No 2 and Bretschneider HTK4 cardioplegic solutions produced the best endothelial cell survival following 12-hour exposure to these solutions. However, intracellular electrolyte equivalent formulations were superior to the St Thomas' cardioplegic solution when evaluated at $10^{o}C$, and Bretschneider HTK4 provided the overall best endothelial cell protection at this temperature (appendix A-6). However, the UW-CSS which has extended the storage period of donor livers for

transplantation, was associated with the best morphological preservation of the endothelium (Fig 6.5).

This concurs with the findings of other investigators that the UW-CSS provides superior hypothermic endothelial protection compared to the Euro-Collins and other organ preservation solutions (C1,F29), who postulated that superior endothelial protection might be the reason for the superiority of UW-CSS.

Figure 6.5

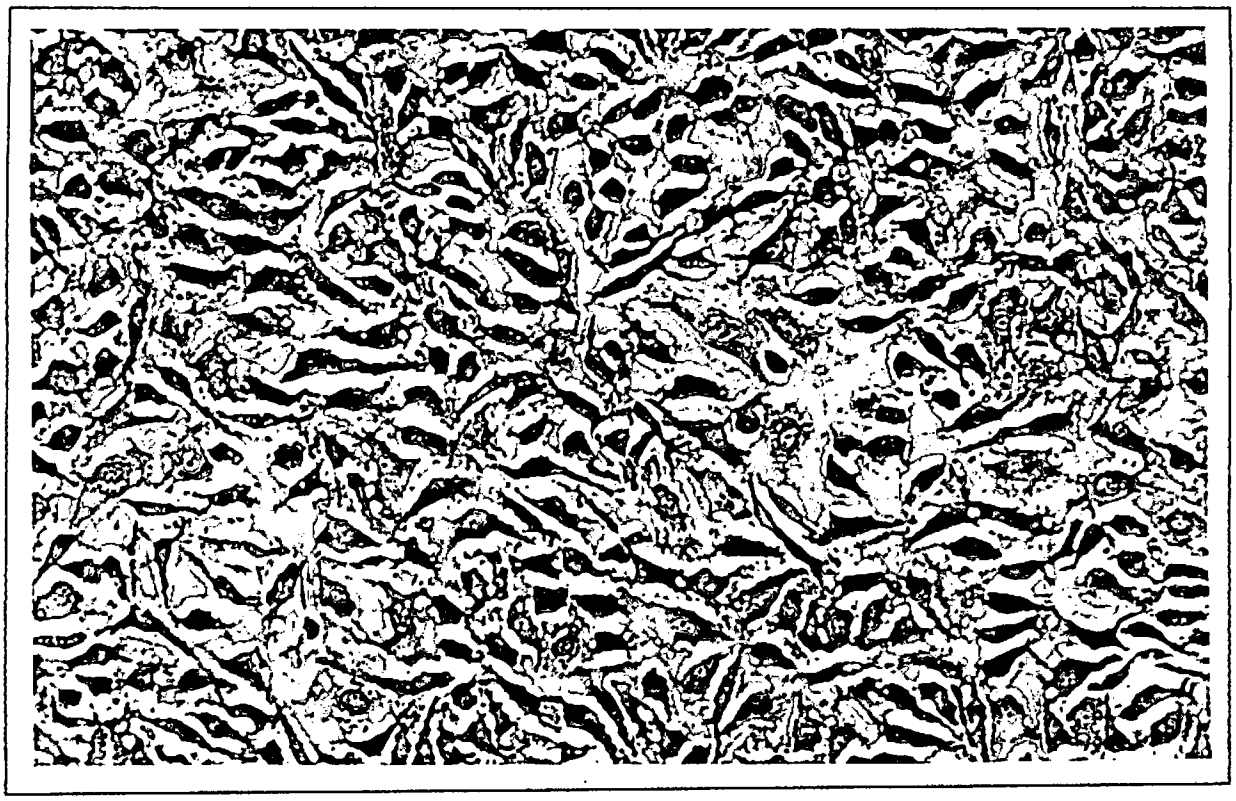
A



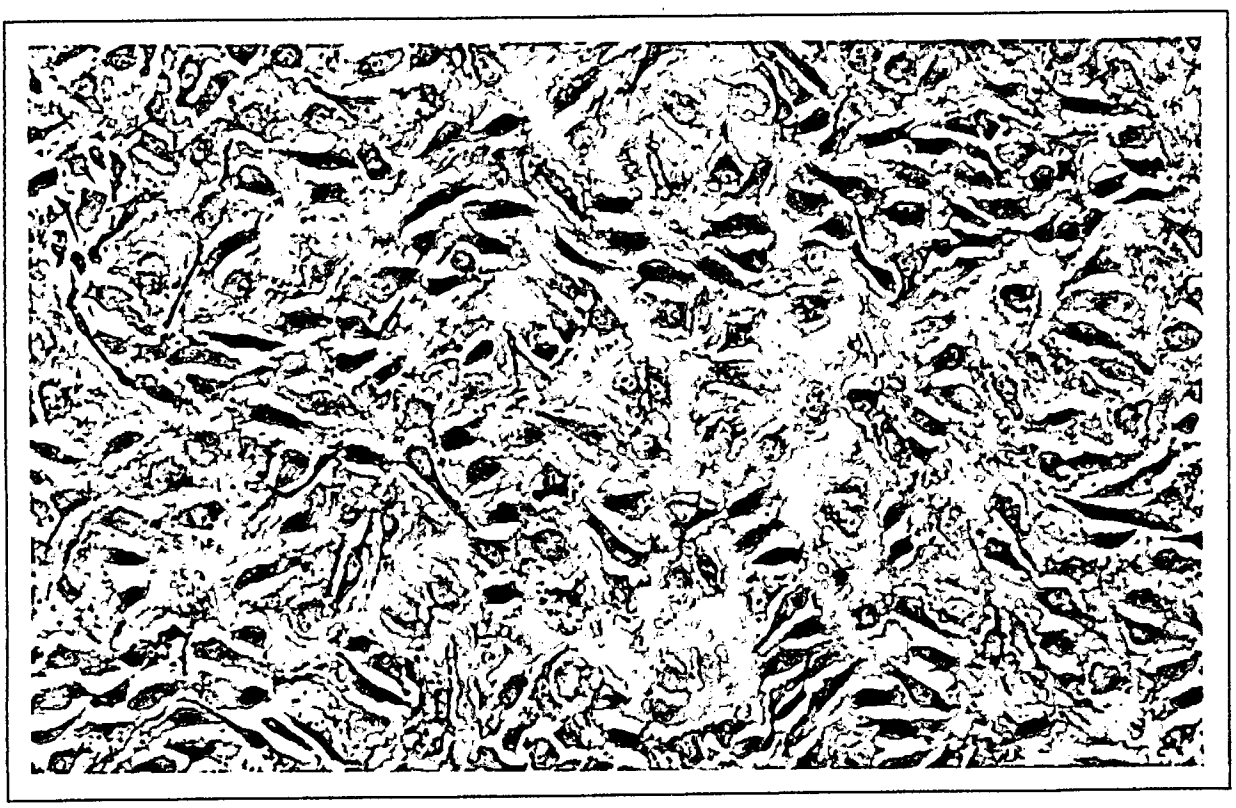
Legend:

Phase contrast microscopy appearance of monolayers of endothelial cells after 12-hour exposure to either St Thomas' Hospital No 2 (A), Bretschneider HTK4 (B) cardioplegic solutions, or University of Wisconsin cold storage solution (C), at 4 - 10°C. Contracted endothelial cells with condensed cytoplasmic granulations, wide intercellular gaps and disruption of monolayer integrity are observed in A & B, whereas in C there is minimal morphological changes the endothelium. (Magnification X 100). Reprinted from Applied Cardiovascular Biology 1990, Vol 2, 1991:(in press) with permission S Karger AG, Basel.

B



C



Furthermore, in our model cytotoxicity could be related to the following factors:-

Chapter 7

PRACTICALITIES OF DELIVERING CARDIOPLEGIC SOLUTIONS

Adequate myocardial protection can only be ensured if the multitude of factors discussed in the preceding chapters are all considered. The composition of the cardioplegic solution to be used being a major consideration. However, the cardioplegic solution must also be delivered to the patient at the correct temperature, pressure and volume (Table 7.1) (see section 1.5).

Table 7.1

OPTIMAL DELIVERY OF CARDIOPLEGIC SOLUTIONS

Temperature of Cardioplegic Solution (B70,M5,P27,T28)
- Crystalloid Cardioplegia: 4°C - 6°C
- Blood Cardioplegia: 8°C - 10°C?

Optimal Myocardial Temperature (M5,R38,T28)
- Crystalloid Cardioplegia: 12°C - 15°C
- Blood Cardioplegia: 15°C - 18°C?

Volume Infused (M17,T9)
- Crystalloid (Constant Pressure)
  - Induction: 10 - 25 ml/kg body weight
  - Multidose: 5 - 10 ml/kg body weight
- Blood (Constant Infusion Rate) (B70)
  - Induction: 300 - 350 ml/min (3 - 5 min)
  - Multidose: 200 ml/min (2 min)

Infusion Pressure of Cardioplegic Solutions
- Antegrade (A31,B70,B74,J-3):
  - Induction: 80 - 130 mm Hg
  - Multidose: 50 mm Hg
- Retrograde (B70,M35,M38):
  - Less than 40 mm Hg

Oxygenated (B33,C31,G45,L25,appendices A-3,A-4)

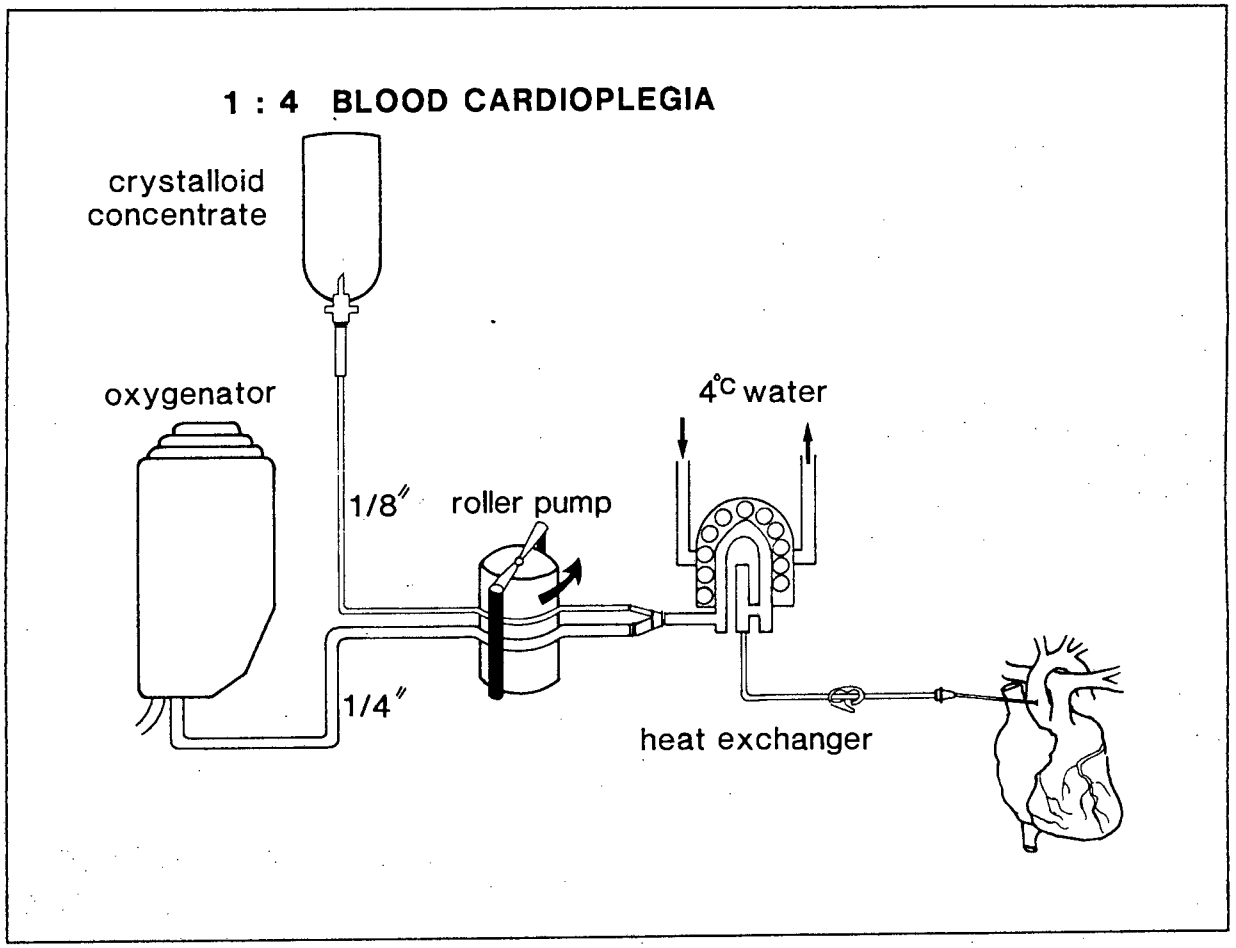
No Particulate Matter or Microemboli (H17,R30)
- Cardioplegic filter: 0.2 um pore size

These secondary variables are easily controlled and monitored in the experimental laboratory. However, in the clinical situation the cardiac surgeon is usually more concerned with the complexities of the operation and may therefore not be able to give as much attention to these variables. Hence, practical means of delivering cardioplegic solutions in the clinical situation are necessary, and if possible should require minimal control from the operating surgeon.

There are two primary categories of cardioplegic solutions; viz. blood and crystalloid cardioplegic solutions, both of which can carry oxygen (D19). Blood cardioplegia, a combination of a crystalloid *concentrate* and oxygenated blood withdrawn from the cardiopulmonary bypass circuit, will supply oxygen bound to haemoglobin. The delivery system for blood cardioplegia has been fairly well standardized, and depends upon the ratio in which the crystalloid *concentrate* is added to the blood component, either as a 1:2 or 1:4 dilution. The composition of the crystalloid concentrate would thus depend upon the intended delivery ratio. The international gold standard is the 1:4 blood cardioplegia developed by G D Buckberg (B70), and delivered by using tubing of differing internal diameters passed through the same "roller-pump head" (Fig 7.1). A heat exchange mechanism, either a coil in ice or an integral heat exchanger is included in the system to ensure that the final mix of blood cardioplegia is delivered to the patient at the correct temperature.

Crystalloid cardioplegic solutions on the other hand, can and are delivered by various methods; roller pump infusion, simple gravity infusion or "pressure bag" infusion. Furthermore, it is more essential with crystalloid cardioplegic solutions as apposed to blood cardioplegia that these solutions are infused into the heart at a cold enough temperature (see section 1.5), correct perfusion pressure (see section 1.5), and the efficacy of crystalloid cardioplegic solutions can be increased by oxygenation (see section 5.2).

Figure 7.1



Legend:

Blood cardioplegic solution delivery system. One revolution of a standard roller-pump using 1:4 delivery, would provide 15 ml of "blood cardioplegia" by combining 3 ml of crystalloid concentrate (1/8" tubing) and 12 ml oxygenated blood (1/4" tubing) withdrawn from the cardiopulmonary bypass oxygenator. Reprinted with permission from Cardiovasc J South Afr 1991; 2:108-114 (appendix A-7).

7.1 OXYGENATING CRYSTALLOID CARDIOPLEGIC SOLUTIONS

Oxygenation of crystalloid cardioplegic solutions improves postischaemic myocardial recovery (B33,C31,G45,L25,appendices A-3,A-4) (see section 5.2).

Commercial crystalloid cardioplegic solutions will be either anoxic if bottled in vacuum in glass (Bretschneider HTK4; Dr F Köhler Chemie GmbH, Alsbach, Germany), or the oxygen content will approximate atmospheric pressures (Table 7.2) if non-vacuum packed in plastic (St Thomas' Hospital cardioplegic solution: Sabax Ltd; Johannesburg, South Africa).

Table 7.2

PARTIAL PRESSURES OF ATMOSPHERIC GASES

	Dry air		Saturated with H_2O
	Percent	Partial Pressure	Partial Pressure
O_2	20.98%	159.4	149.6
CO_2	0.04%	0.3	0.3
N_2	78.06%	593.3	556.6
H_2O	0%	0	47

Legend:
Partial pressures (mm Hg) of atmospheric gases in dry air and air saturated with water at 37°C, at sea level (1 atmosphere; 760 mm Hg) (G2).

The quantity of oxygen dissolved in a solution depends upon both the partial pressure and solubility coefficient for oxygen in that fluid (G2). Furthermore, the solubility coefficient for a gas is inversely proportional to temperature (Table 7.3). Thus, the oxygen content of a solution can be increased by both increasing the partial pressure (oxygenating with 95 % - 100 % O_2) and by decreasing the temperature of the solution (B13). Therefore, crystalloid cardioplegic solutions should be oxygenated at low temperatures (4°C - 6°C), in order to maximally increase the dissolved oxygen content in the solution.

Table 7.2

SOLUBILITY COEFFICIENT FOR OXYGEN					
$4^{o}C$	-	4.256	$24^{o}C$	-	2.768
$10^{o}C$	-	3.689	$26^{o}C$	-	2.670
$12^{o}C$	-	3.524	$28^{o}C$	-	2.578
$14^{o}C$	-	3.373	$30^{o}C$	-	2.495
$16^{o}C$	-	3.216	$32^{o}C$	-	2.428
$18^{o}C$	-	3.107	$34^{o}C$	-	2.361
$20^{o}C$	-	2.989	$36^{o}C$	-	2.300
$22^{o}C$	-	2.875	$37^{o}C$	-	2.273

Legend:
The solubility coefficient for oxygen in a physiological saline solution at different temperatures: milliliters of oxygen dissolved per 100 ml of 0.155 *N* NaCl at 760 mm Hg pressure (B13).

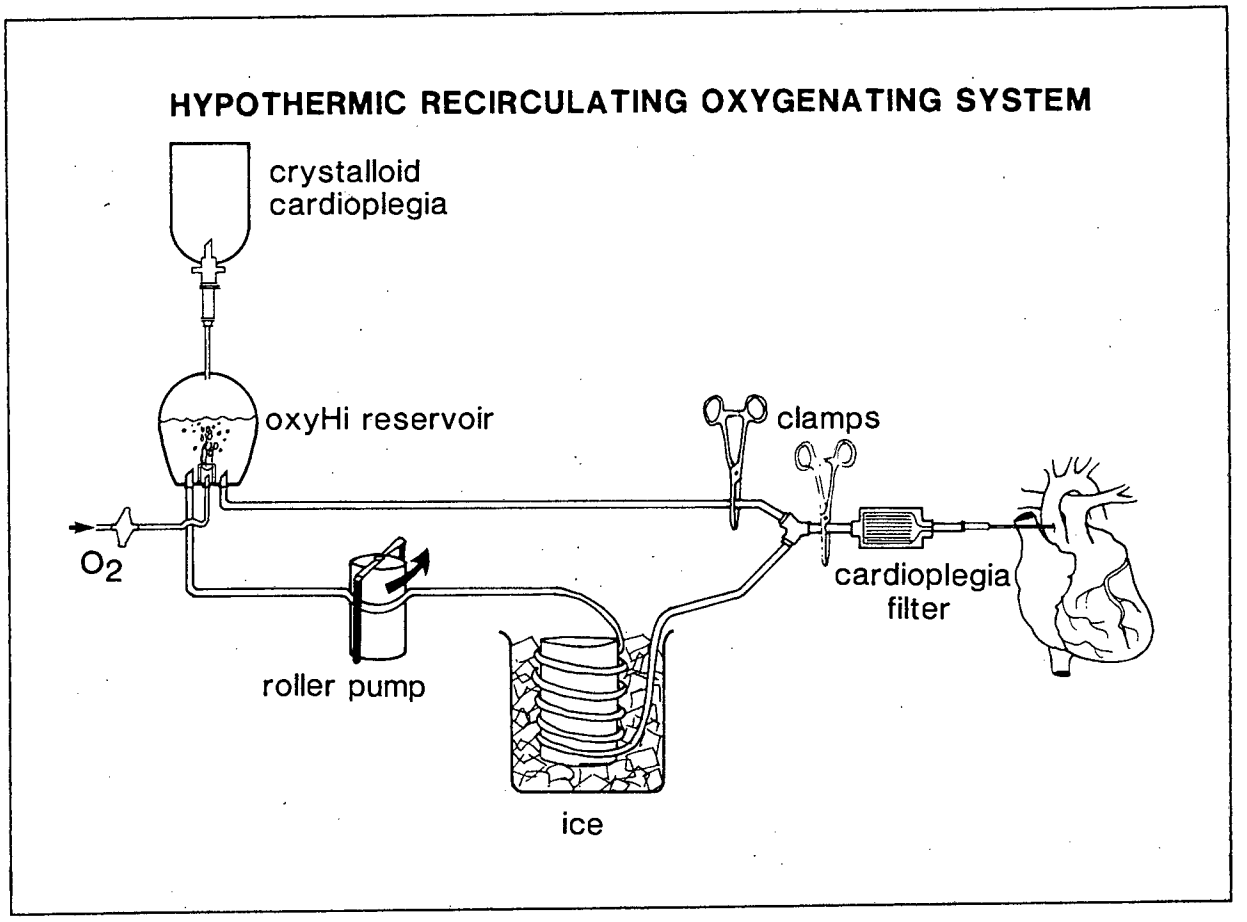
Various methods of oxygenating crystalloid cardioplegic solutions have been proposed, and include:-

1) Bubble oxygenating systems,
2) Hyperbaric bubble oxygenation; 15 cm H_2O positive pressure during bubbling (D1),
3) Hyperbaric pressurization at hypothermia (M42,W18),
4) Membrane oxygenating systems (L8),
5) Hypothermic recirculating oxygenating systems (Fig 7.2).

We compared the oxygen content of "unoxygenated" St Thomas' Hospital No 2 cardioplegic solution (0.45 - 0.84 ml O_2 / 100 ml solution), with the oxygen contents obtained by various oxygenating methods at different gas flow rates (appendix A-7). Oxygenating the St Thomas' cardioplegic solution with 95 % O_2 5 % CO_2 at 1.0 L/min at hypothermia ($4^{o}C$) in a standard 1 liter glass vacolitre kept in ice, produced an oxygen content of 3.48 $\pm$ 0.14 ml O_2 / 100 ml solution within 30 min. This oxygen content was no different to the oxygen content obtained with the more complex and expensive hypothermic recirculating oxygenating method (Fig 7.2) (appendix A-7). Furthermore, after 30 min of oxygenation and provided the cardioplegic solution was kept in ice ($4^{o}C$)

and not rewarmed, the oxygen content in the glass vacolitre did not decrease during a subsequent 2-hour period.

Figure 7.2



Legend:
Delivery system for crystalloid cardioplegia that ensures both cold (4°C) cardioplegia and maximal oxygenation, by continually recirculating the cardioplegic solution through a cooling coil placed in ice (CCAS-OC; American Bentley, Irvine, California, USA). The removal / application of the indicated clamps allows the cardioplegic solution to be continuously recirculated or intermittently delivered to the patient. Reprinted with permission from Cardiovasc J South Afr 1991; 2:108-114 (appendix A-7).

Alternative techniques of oxygenating cardioplegic solutions such as overnight cold pressurization require broaching of the sterility seal of the vacolitre well prior to the procedure (M42,W18), and thus may increase the risks of bacterial contamination. Membrane oxygenating systems for crystalloid solutions are more expensive than simple bubble oxygenation, and have no additional advantage if there are no proteins in the solution (L8).

Potential dangers of maximally oxygenating cardioplegic solutions

If a solution is fully saturated with a gas at a low temperature, rewarming of the solution may result in the gas coming out of the now supersaturated solution as bubbles. In the clinical situation, cardioplegic solution contained in the cardioplegic delivery line leading to the patient rewarms to the prevailing theater temperature ($19^{o}C$ - $24^{o}C$) in the period between each reinfusion of cardioplegia (see section 7.2). If gaseous emboli are then formed in the cardioplegic delivery line they could be harmful if infused with subsequent doses of cardioplegia. Therefore, if crystalloid cardioplegic solutions are maximally oxygenated, then the delivery system should incorporate a means of removing these bubbles prior to subsequent reinfusions. Bubbles can be removed by either a microfilter (CAS-filter; American Bentley, Irvine, California, USA) inserted immediately before the ascending aorta, or alternatively the "warm cardioplegic solution containing gaseous emboli in the line" can be removed through an aortic vent, before administering each subsequent dose of cardioplegic solution.

Alternatively, if the cardioplegic solution is oxygenated at a temperature equal or higher than it is likely to be rewarmed to (i.e. room temperature), the solution would not become supersaturated and bubbles would not come out of solution. However, the solution would then have to be cooled to the correct temperature ($4^{o}C$ - $6^{o}C$) prior to infusion. In addition, this method would imply accepting an oxygen content approximately 40 % lower than if the cardioplegic solution was oxygenated at $4^{o}C$ (appendix A-7). Sub-maximal cardioplegic solution oxygen contents have been associated with a parallel decrease in the effectiveness of the cardioplegic solutions' ability to protect the myocardium (D11). Nevertheless, we showed that only approximately 50 % of the oxygen available in a hypothermic maximally oxygenated cardioplegic solution is taken up by the myocardium (appendices A-3,A-4). Moreover, all oxygen contained in a crystalloid cardioplegic solution is theoretically available to the myocyte as oxygen is released in a linear fashion from crystalloid solutions (D19).

An additional danger of maximally oxygenating crystalloid cardioplegic solutions is that high partial pressures of oxygen may increase the formation of free radicals (G5). Thus the optimal content of oxygen contained in a crystalloid cardioplegic solution may yet need to be determined.

In conclusion, simple oxygenation of cold (4^{o}C) St Thomas' Hospital No 2 cardioplegic solution with 95 % O_2 5 % CO_2 at 1.0 L/min in its original glass vacolitre is as effective as more complex hypothermic recirculating oxygenating delivery systems, in producing a maximal oxygen content. However, if maximally oxygenated cardioplegic solutions are used then measures should be taken to ensure that gaseous emboli, that may form in the cardioplegic delivery line, are not infused into the coronary circulation with subsequent doses of cardioplegia.

7.2 TEMPERATURE OF INFUSED CARDIOPLEGIC SOLUTION

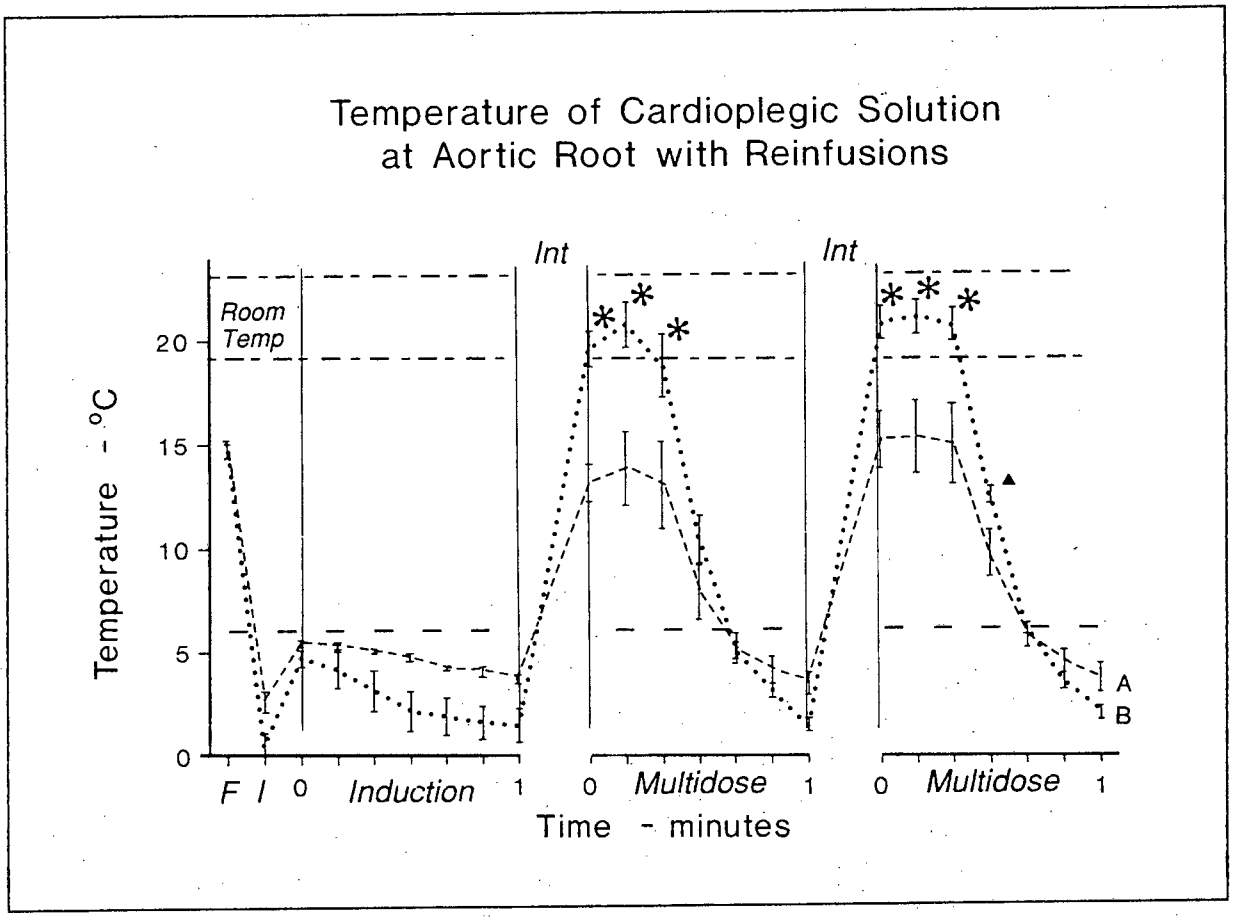
Profound cardiac hypothermia is an essential component of the majority of techniques used to protect the myocardium during the aortic cross-clamp period (see section 1.5). However, myocardial temperatures fluctuate during the ischaemic cross-clamp period because of continued rewarming of the myocardium (R39). Rewarming of the myocardium can though be prevented by either multidose reinfusions of cold cardioplegia or by myocardial protection jackets (see section 1.5). Cold ($4^{o}C$) crystalloid cardioplegic solutions are therefore intermittently reinfused into the heart throughout the aortic cross-clamp period (B54,B74,E9,S18,appendix A-2), in order to maintain myocardial temperatures at $12^{o}C$ - $15^{o}C$ (R38,T28). Multidose reinfusions of cardioplegia also have other beneficial effects such as replenishing substrates and removing metabolic end products (see section 1.5).

We therefore evaluated whether methods of infusing crystalloid cardioplegic solutions deliver the solution to the myocardium at the correct temperature, that is less than $6^{o}C$ (appendix A-8). Crystalloid cardioplegic solutions stored in a standard fridge are often too warm ($11.1 \pm 0.6^{o}C$). Therefore, solutions stored in a fridge should in addition be immersed in ice for at least 30 min before use, to ensure a sufficiently low temperature. Simple infusion of the cold cardioplegic solution, without any accessory cooling coils, will then deliver the cardioplegic solution at the recommended temperature independent of flow rate (100 - 500 ml/min). Alternatively, if cardioplegic solutions are stored or oxygenated at room temperature then a cooling mechanism must be used in the delivery system. However, a cooling coil immersed in ice may not adequately cool the cardioplegic solution at cardioplegic flow rates greater than 200 ml / min (appendix A-8).

Rewarming of solution in delivery lines

Cardioplegic solution contained in the delivery lines leading to the patient rewarms towards ambient room temperature (19°C - 23°C) during the interval (10 min - 30 min) in between each reinfusion of cardioplegia (14.1 ± 0.8°C and 20.1 ± 0.6°C respectively) (Figure 7.3).

Figure 7.3



Legend:

The temperature of the cardioplegic solution at the "aortic end" of the cardioplegic delivery line was measured. Cardioplegic solution removed from the fridge (F) was kept in ice for 30 min (I) to ensure a temperature of less than 4°C. An "induction dose" was infused (250 ml/min for 1 min) immediately after flushing the lines. Thereafter, a second and third multidose of cardioplegia (250 ml/min for 1 min) were infused after either a 10 min (A) or 30 min (B) interval (Int). Means and standard errors of means (vertical bars) for N = 6 observations are presented. The recommended temperature at which crystalloid cardioplegic solutions should be infused (6°C) and the temperature range of operating theaters are indicated. Reprinted with permission from Cardiovasc J South Afr 1992; (in press) (appendix A-8).

* - $p < 0.01$ compared to A at corresponding time.

▲ - $p < 0.05$ compared to A at corresponding time.

Thus with subsequent reinfusions of cardioplegia a significant volume of each dose could consist of "warm cardioplegia" (i.e. cardioplegia with a temperature of greater than 6^{o}C that was contained in the delivery lines) (appendix A-8). Moreover, this volume of "warm cardioplegia" could be as much as 50 % of the total dose of cardioplegic solution reinfused, and can decrease the efficacy of myocardial protection.

One must therefore ensure that cardioplegic solutions are always at a sufficiently low temperature prior to infusion, and in addition "dead space" in delivery lines should be reduced as far as possible. Furthermore, if the "warm cardioplegic solution" contained in the delivery line forms a significant percentage of subsequent multidose reinfusions, this volume should be vented to waste before each reinfusion; by using a special aortic cardioplegic infusion catheter (aortic root cannula with vent - DLP; Grand Rapids, Michigan, USA).

7.3 INFUSION PRESSURE OF CARDIOPLEGIC SOLUTIONS

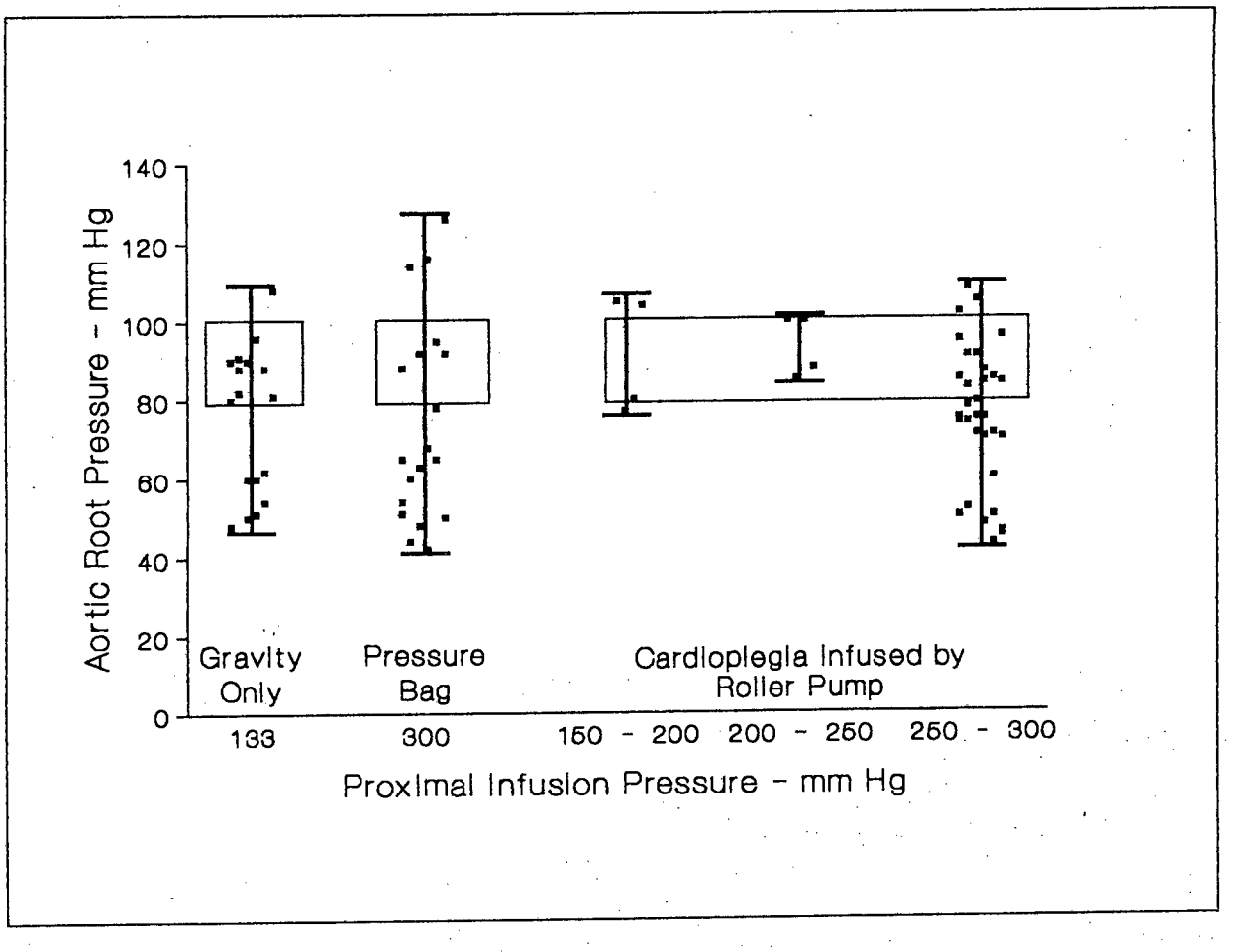
An adequate perfusion pressure to ensure uniform delivery of cardioplegic solutions is essential (A18). Nevertheless, coronary artery stenoses can result in heterogeneous delivery of cardioplegic solutions despite adequate perfusion pressures (A31,B18,S46). However, excessive perfusion pressures (greater than 150 mm Hg) promote myocardial oedema, decrease postischaemic functional recovery (J9), and damage the vascular endothelium (M53). Thus, prograde infusion of cardioplegia into the ascending aorta should be at pressures of 80 - 130 mm Hg (measured in the ascending aorta) on induction of cardioplegic arrest (B69,M54), and at 50 mm Hg with subsequent doses (B74,P27) (see section 1.5). Furthermore, a proximally monitored perfusion pressure may not correlate with the actual ascending aortic root pressure, because of resistances in the cardioplegic delivery line (S45). This was therefore evaluated in the clinical situation.

Crystalloid cardioplegic solutions can be delivered by a variety of means (G34): infusion by gravity (infused from a height of ± 178 cm; 133 mm Hg), external pressurization of the vacolitre of cardioplegia (external pressure bag inflated to 300 mm Hg), or by roller-pump. We compared the delivered ascending aortic pressure during clinical administration of cardioplegia, with proximally monitored cardioplegic line pressures with each of the three delivery systems (appendix B-8).

A wide range of aortic root pressures were measured during infusion of cardioplegia with all three methods (42 - 126 mm Hg) (Fig 7.4) (appendix B-8). Furthermore, there was a poor correlation between the proximal cardioplegic "line pressure" and the actual aortic root pressure, as observed by Sievertsen et al (S45). Thus, cardioplegic infusion pressures should be monitored directly in the ascending aorta as the proximal cardioplegic line pressure is unreliable. Excessive pressures (greater than 150 mm Hg) were not observed with either the gravity or pressure bag method. However, higher pressures would be obtained if larger cardioplegic root cannulas were to be used (M53).

Furthermore, in contrast to the gravity or pressure bag infusion methods, the roller pump delivery system could to a certain extent prevent high and compensate for low aortic root pressures.

Figure 7.4
AORTIC ROOT PRESSURE DURING INFUSION OF CARDIOPLEGIC SOLUTIONS



Legend:
The aortic root pressure was measured during administration of cardioplegia by three different methods and compared to the proximal cardioplegic line pressure. Cardioplegia was infused by gravity (N = 6) from a height of 178 cm H_2O (133 mm Hg) above the right atrium. If a pressure bag was used (N = 6), this was inflated to 300 mm Hg. Alternatively, a roller pump was used (N = 12), in which case the perfusionist attempted to maintain the measured aortic root pressure between 80 - 100 mm Hg.

Thus in agreement with other studies, the roller pump is the superior method of delivering cardioplegic solutions during clinical open-heart surgery (G34). If ascending aortic pressures are not monitored then the cardioplegic solution should be initially infused at a rate of 250 - 300 ml/min with a roller pump (for adult hearts only), and then

the rate increased if the ascending aortic pressure was insufficient by manual palpation. However, when harvesting donor hearts at outlying hospitals, cardioplegia can be infused simply by gravity (height of 173 cm above the right atrium). This method ensures that excessive infusion pressures will not be obtained, without monitoring ascending aortic root pressures. In contrast, pressurization of the cardioplegic solution container can result in excessive infusion pressures, should different perfusion cannulae be used.

7.4 CONTAMINATION OF CARDIOPLEGIC SOLUTIONS

Particulate matter

The majority of commercial intravenous crystalloid solutions contain particulate matter (2 - 20 um in diameter), which may cause coronary vasoconstriction and consequent maldistribution of cardioplegic solution (H17,R30,S35). These particles cause coronary vasoconstriction during infusion of cardioplegic solutions and decrease postischaemic myocardial recovery if large doses of cardioplegia are administered. Particle induced coronary vasoconstriction can be prevented by either a microfilter of at least 0.8 um pore size, or alternatively attenuated by including either nifedipine, ATP, procaine, lignocaine, dipyridamole or steroids in the cardioplegic solution (H17,S35).

Nevertheless, Munsch et al using both a canine model and a clinical study failed to demonstrate particle induced coronary vasoconstriction, and suggested that 0.2 um pore size cardioplegic line filters are not necessary (M64). However, the cardioplegic solutions in the studies by Hearse and Robinson contained significantly higher numbers of 2 - 10 um sized particles (Table 7.4) (H17,R30), and their results suggested that the critical size range for particle induced deleterious effects was 8 - 10 um pore size (H17). Therefore, the study by Munsch et al does not refute the findings of the previous studies, as in their study cardioplegic solutions with significantly lower particulate counts were used.

A particulate matter count was done on the St Thomas' No 2 cardioplegic solution formulated by Sabax Ltd (Aeroton, Johannesburg, South Africa) with a HIAC Model PC 320 "Criterion" particle size analyser (HIAC / Royco Instruments, Pacific Scientific Company, Menlo Park, California), and the results are shown in Table 7.4. The St Thomas' solution formulated by Sabax contains significantly fewer particles than the cardioplegic solutions tested by Hearse, Robinson and co-workers.

Table 7.4

Particle Size (um)	Particle Count / ml			
	Hearse	Robinson	Munch	Sabax
> 2	772	379 - 1505	36	67
> 5	188	81 - 165	7	17
>10	7	2 - 8	2	4
>15	2	2	2	3

Legend:
Particulate counts in cardioplegic solutions reported by Hearse (H17), Robinson (R30), Munch (M64) and in the St Thomas' No 2 cardioplegic solution prepared in South Africa by Sabax Ltd.

Bacteriological contamination

Bacteriological contamination of cardioplegic solutions can result in clinical septicaemia, despite aseptic preparation under laminar flow hoods (H54). Thus, sterility of cardioplegic solutions is essential and the solution should be kept at hypothermia and for no longer than a few hours after additives are added to commercial solutions. In line cardioplegic filters (0.2 um pore size) would also minimize the potential risk, should there have been inadvertent contamination of the cardioplegic solution during preparation.

Microemboli

Oxygenation of crystalloid cardioplegic solutions can result in gaseous micro-emboli which may be harmful (see section 5.7). Rewarming of cardioplegic solution, contained in the delivery line leading to the operating table in between each reinfusion of cardioplegic solution, may cause the formation of bubbles (see section 7.1). Therefore, we suggest that a microfilter (0.2 um CAS-filter; American Bentley, Irvine, USA) should be included in the cardioplegic delivery systems if hypothermic oxygenation of the cardioplegic solution is used.

Heavy Metal Contamination

Heavy metals, specifically iron and copper can act as cofactors for the generation of oxygen free radicals (see section 1.6). Removal of intravascular physiological quantities of these metals reduces free radical generation and the postischaemic reperfusion injury (C33,M36). We noted diminished efficacy of the standard St Thomas' cardioplegic solution when formulated with magnesium chloride containing higher levels of heavy metals (see section 2.3). Hence, contamination of cardioplegic solutions with heavy metals is potentially dangerous. Strict quality control of the production of cardioplegic solutions is therefore essential.

Chapter 8

SUMMARY

The scope of cardiac surgery has increased primarily as a result of two factors; the development in 1953 of a temporary means of replacing the function of the heart and lungs, and improvements during the 1970's and 1980's in techniques of protecting the ischaemic myocardium whilst the aorta is cross-clamped. Nevertheless, poor myocardial protection still contributes to both the mortality and morbidity of cardiac surgery, and is the topic of this thesis.

The myocardium is injured by mechanical, ischaemic and pharmacologic factors during cardiac surgery, and not only myocytes but also endothelial cells need to be protected. A review of techniques used to provide myocardial protection during open-heart surgery highlights the concept that no single factor provides "myocardial protection" during open-heart surgery. Preoperative, intraoperative and postoperative interventions must all be considered in order to obtain optimal myocardial protection. Nevertheless, one of the principle components of modern techniques of intraoperative myocardial protection is the use of cardioplegic solutions, which are infused into the heart to stop electromechanical activity and thereby diminish myocardial energy demands whilst the aorta is cross-clamped and thus the myocardium rendered ischaemic. Some cardiac surgical procedures can be accomplished without cross-clamping the aorta, however, cross-clamping the aorta often facilitates the surgical procedure technically and is thus commonly used.

Crystalloid cardioplegic solutions in clinical use in South Africa were evaluated in an in-vivo primate model that simulated clinical open-heart surgery. Baboon hearts were subjected to 3-hour hypothermic cardioplegic arrest whilst on cardiopulmonary bypass, and thereafter postischaemic haemodynamic recovery was assessed. The best available

crystalloid cardioplegic solution in South Africa was the St Thomas' Hospital No 2 cardioplegic solution. The effect of these various cardioplegic solutions on cultured human venous endothelial cells was also assessed. The St Thomas' Hospital No 2 cardioplegic solution which is an extracellular electrolyte equivalent formulation, was the least cytotoxic solution when exposed to endothelial cells at normothermia. The St Thomas' cardioplegic solution also provided endothelial cytoprotection equivalent to the Bretschneider HTK4 cardioplegic solution (an intracellular electrolyte equivalent formulation) at 22^{o}C, but at 10^{o}C Bretschneider HTK4 was the least cytotoxic cardioplegic solution. The University of Wisconsin cold storage solution though, although not associated with the best postexposure endothelial cell survival at 10^{o}C, caused the least morphologic alterations to the endothelial cells at this temperature.

The best currently available crystalloid cardioplegic solution - the St Thomas' Hospital No 2 cardioplegic solution - was then used as the basic formulation for further experimental modifications. The St Thomas' cardioplegic solution does not contain a substrate for ongoing energy production, has a poor buffering capacity, is isotonic and lacks colloid oncotic pressure. Therefore, experimental modifications of the St Thomas' cardioplegic solution that "corrected" these perceived deficits were evaluated.

Glucose was shown to be a beneficial additive to the St Thomas' cardioplegic solution in the isolated rat heart model, but only at a concentration of 7 - 11 mmol/L and provided multidose reinfusions of cardioplegia were administered. This concentration of glucose also did not alter the previously assessed endothelial cytotoxicity. However, the addition of glucose (10 mmol/L) to the St Thomas' cardioplegic solution depressed postischaemic myocardial recovery in the in vivo primate model, possibly because of either insufficient washout of metabolic end products or interspecies differences. Thus, although both preischaemic and postischaemic metabolic enhancement with glucose is beneficial, we would not advise the addition of glucose to the St Thomas' Hospital No 2 cardioplegic solution in the clinical situation.

An alternative means of increasing myocardial energy supplies during the ischaemic cross-clamp period is to promote aerobic metabolism, by intermittently reinfusing oxygenated crystalloid cardioplegic solution whilst the aorta is cross-clamped. Oxygenating the St Thomas' cardioplegic solution was shown to be beneficial in the isolated rat heart model. However, oxygenation could also induce potentially harmful pH shifts of the cardioplegic solution as a result of altering the pCO_2 of this bicarbonate containing cardioplegic solution. Consequently, the St Thomas' Hospital No 2 cardioplegic solution should be oxygenated with 95 % O_2 5 % CO_2, which optimizes the pH of the solution at pH 7.0. Oxygenating with 100 % O_2 caused the pH of the St Thomas' cardioplegic solution to shift to pH 9.3, which was associated with depressed postischaemic myocardial recovery. The addition of perfluorocarbon oxygen carriers to the well oxygenated St Thomas' cardioplegic solution was also shown to provide no additional myocardial protection.

The efficacy of the St Thomas' Hospital cardioplegic solution was also improved experimentally by increasing the buffering capacity of the St Thomas' solution fivefold, by the addition of histidine. Osmotic space for this additive was obtained by decreasing the sodium concentration of the St Thomas' solution from 120 mmol/L to 100 mmol/L, and increasing the crystalloid osmolality from 295 mOsm/kg H_2O to 320 mOsm/kg H_2O. This moderate hypertonicity was also shown to have an independent salutary effect. The addition of histidine (50 mmol/L) to the St Thomas' cardioplegic solution independently improved the postischaemic recovery of isolated rat hearts subjected to 3-hours hypothermic cardioplegic arrest. Furthermore, a pilot study in the in vivo primate model tended to show improved postischaemic recovery although statistical significance was not achieved in the experimental model used. This experimental cardioplegic solution was further modified by decreasing the calcium content to the optimal value of 0.6 mmol/L and the addition of adenosine 1 mmol/L, and evaluated in the in vivo primate model. Energy depleted baboon hearts were subjected to 3-hour hypothermic multidose cardioplegic arrest, and this modified St Thomas' cardioplegic solution was

associated with superior postischaemic myocardial recovery when compared to the standard St Thomas' No 2 cardioplegic solution

Further modifications evaluated in the isolated rat heart model were the addition of the colloid Dextran-40, and the effect of decreasing the chloride content of the St Thomas' cardioplegic solution. No conclusive advantage of adding 3 % Dextran-40 to the St Thomas' cardioplegic solution and thus increasing the colloid oncotic pressure from 0 mm Hg to 25 mm Hg could be demonstrated. However, this might have been because only single dose and not multidose cardioplegic solution was administered in this experimental model. In addition, decreasing the concentration of the chloride anion in the St Thomas' cardioplegic solution was neither advantageous nor detrimental in the isolated rat heart model. However, a low chloride concentration decreased the observed endothelial cytotoxicity, and should therefore be further evaluated.

Finally, methods of delivering cardioplegic solutions to the patient in the clinical situation were evaluated. Adequate oxygenation of the St Thomas' cardioplegic solution can be obtained by simple bubble oxygenation of the cardioplegic solution in its original packaged container, and complex oxygenating systems are not necessary for crystalloid cardioplegic solutions. However, techniques either preventing or removing bubbles formed in the cardioplegic delivery lines should be employed. Furthermore, the potential harmful effect and rapidity of rewarming of the cardioplegic solution contained in the delivery lines leading to the patient were identified. Thus, in the clinical situation the cardioplegic solution contained in the delivery lines, the volume of which could be as much as 50 % of each subsequent dose of cardioplegia, should be vented to waste prior to each reinfusion of cardioplegia, in order to ensure that the cardioplegic solution is infused at a sufficiently cold temperature.

In summary, we would advise that for clinical open-heart surgery the best presently available crystalloid cardioplegic solution is the St Thomas' Hospital No 2 cardioplegic

solution. Furthermore, the efficacy of the St Thomas' cardioplegic solution can be improved by simple oxygenation with 95 % O_2 5 % CO_2. Blood cardioplegic solutions were not evaluated in the studies in this thesis and might also provide equivalent and possibly superior myocardial protection. Additional potentially beneficial experimental modifications of the St Thomas' cardioplegic solution are the addition of the buffer histidine, increasing the osmolality and possibly decreasing the concentration of chloride ions of the St Thomas' cardioplegic solution, which though require further evaluation. Nevertheless, not only should the foremost cardioplegic solution be used during clinical open-heart surgery, but a number of additional perioperative factors must be considered as well in order to ensure optimal myocardial protection.

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Appendix A-1

Cardioplegic Solutions used in South Africa: An experimental comparison.

Boehm D H,
Human P A,
Von Oppell U,
Reichenspurner H,
Owen P,
Opie L H,
Rose A G,
Reichart B.

Cardiovasc J South Afr 1990; 1:11-16.

The following typographical errors should be corrected in this manuscript:

Page 12

In Table I, the sodium concentration of the St Thomas' Hospital No 2 cardioplegic solutions should read 120 mmol/L, and the glucose concentration of Pl B(3) is 0 mmol/L.

Page 13

In the paragraph titled "Expression of Results", the units of SVI should read $ml \cdot beat^{-1} \cdot m^{-2}$, and the formula for SWI is:-

SWI = SVI X (LV systolic pressure - LVEDP) X 0.014348

Cardiovascular Medicine

Cardioplegic solutions used in South Africa

An experimental comparison

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Abstract

Cardioplegic solutions are routinely used to arrest and protect the myocardium during open-heart surgery. Most of the hospitals performing cardiac surgery in South Africa employ various crystalloid cardioplegic solutions as opposed to blood cardioplegia. In this report we compare the locally manufactured cardioplegic solution (obtainable from SABAX Ltd) and a modified Bretschneider solution previously employed at Groote Schuur Hospital with the current international standard, the St Thomas' Hospital cardioplegic solution No. 2.

Chacma baboon hearts were subjected to a 3-hour *in vivo* ischaemic period during which the myocardium was protected with each of these cardioplegic solutions. Post-ischaemic recovery was assessed in terms of haemodynamic variables (stroke volume index, cardiac index, stroke work index, and left ventricular function curves), levels of adenosine triphosphate and creatine phosphate, and refunction time. In the model used, the post-ischaemic functional recovery of hearts protected with the St Thomas' Hospital cardioplegic solution No. 2 was superior.

Cold chemical cardioplegic solutions are almost universally used to protect the myocardium during bloodless open-heart surgery while the ascending aorta is cross-clamped.[1] Improved cellular protection has enhanced myocardial recovery and thus contributed to a reduction in surgical mortality over the years. In addition, as longer ischaemic periods are now more easily tolerated, the complexity and extent of cardiac surgery has increased. The cardiac surgeon today can cross-clamp the aorta, infuse a cardioplegic solution, and then unhurriedly operate on the protected, cold, non-beating heart. At the end of the procedure the aorta is unclamped and a rapid recovery of myocardial function is expected.

The primary function of a cardioplegic solution is to halt all myocardial electromechanical activity, thereby conserving energy.[1,2] This is achieved by manipulating the extracellular concentrations of the important cations (sodium, potassium, magnesium, calcium). Myocardial hypothermia, obtained by infusing the solution into the coronary arteries at a temperature of 4°C, lowers energy demands even further.[2] Since the use of a hyperkalaemic cardioplegic solution by Melrose *et al.* in 1955[3] an ever-increasing number of formulations are being used experimentally and clinically, thus complicating the objective comparison of solutions and reported beneficial additives. A vast amount of basic research has, however, demonstrated the complex interrelationship of all components in a cardioplegic solution and highlighted the need for careful experimental assessment before any alterations are made to existing solutions.

In 1988 a questionnaire was forwarded to all perfusion technologists registered with the South African Society of Cardiovascular Perfusion Technology regarding the cardioplegic solution used by their unit. Eighty per cent of the questionnaires were returned, and the answers revealed that three hospitals performing cardiac surgery in South Africa were using a form of blood cardioplegia while a further seven employ various formulations of crystalloid cardioplegic solutions. The solution prepared by SABAX Ltd (Samuel Evans Road, Aeroton, Johannesburg) was used by three units, a basic solution of Plasmalyte B plus 30 mmol potassium chloride and various additives was employed by a further three, and only Groote Schuur Hospital was at that time using a modified Bretschneider solution made up by the hospital dispensary

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TABLE I. CRYSTALLOID CARDIOPLEGIC SOLUTIONS USED IN SOUTH AFRICA (mmol/ℓ)

	Na	*K*	*Ca*	*Mg*	HCO_3	*Glucose*	*Mannitol*	*Osmolarity*
ST2	110	16	1,2	16	10	—	—	±300
SABAX	141	24/12*	0,9	1,5	38	51	68	±450/432*
GSH	15	20	1,0	12	15	240	14	±350
Pl B(1)	140	34	—	1,5	37	56	—	±400
Pl B(2)	130	34/14†	±0,4†	1,5	27	±4†	—	±330
Pl B(3)	130	34	—	1,5	27	42	—	±370
KHB	143	5,9	1,25	1,2	25	11	—	±300

ST2 – St Thomas' Hospital cardioplegic solution No. 2.
SABAX – cardioplegic solution available from SABAX Ltd.
GSH – cardioplegic solution previously used at Groote Schuur Hospital.
Pl B – cardioplegic solution made up by the addition of 30 mmol KCl, ± sodium bicarbonate to 1 litre of Plasmalyte B solution.
KHB – Krebs-Henseleit physiological buffer solution used as a non-cardioplegic control solution.
* In the maintenance cardioplegia the potassium concentration is diminished to 12 mmol/l.
† The maintenance cardioplegia has only 10 mmol KCl added to 200 ml pump blood per 800 ml crystalloid solution. This would thus change the solution from an acalcaemic solution to one with an approximate calcium content of 0,4 mmol/l and an approximate glucose concentration of 4 mmol/l.

(GSH). The composition of these solutions is tabulated in Table I. In principle, the St Thomas' Hospital solution No. 2 relies on a high-potassium, high-magnesium system; the GSH solution on a high-potassium, low-sodium system, and the SABAX solution has a high-potassium composition.

The majority of these clinically used cardioplegic solutions have, however, never been adequately or objectively evaluated, neither experimentally nor clinically. This study was therefore undertaken to compare the efficacy of two crystalloid cardioplegic solutions used in this country with an internationally accepted and extensively investigated solution, the St Thomas' Hospital cardioplegic solution No. 2 (ST2).[4-7] In addition, a solution containing normal extracellular concentrations of the major cations, and therefore unable to induce chemical cardiac arrest, was employed as a control in order to highlight the effects of cardioplegic solutions as opposed to the beneficial effects of hypothermia. The physiological saline solution, Krebs-Henseleit buffer (KHB), was selected as the control non-cardioplegic solution (Table I).

The ST2 cardioplegic solution used in this study is the improved St Thomas' Hospital solution No. 2 (Plegisol; Abbott Laboratories, North Chicago, Illinois) and not the original St Thomas' hospital solution No. 1 (MacCarthy).[7] This solution has previously been shown in the rat heart to be superior to other solutions such as Ringer-lactate solution with added potassium and balanced saline solution with glucose and potassium.[6]

In contrast, the SABAX solution, although used clinically in South Africa, has not been reported to have been experimentally evaluated or clinically compared with other attested solutions.

The GSH cardioplegic solution was originally based on the initial Bretschneider solution which was formulated with an 'intracellular' concentration of electrolytes, specifically sodium.[8] Over the years the GSH solution was altered without any reported experimental or clinical trials, resulting in the composition indicated in Table I. The Bretschneider HTK-4 solution in clinical use in Europe today differs markedly from both the GSH solution and the original Bretschneider solution.

Materials and methods

The chacma baboon (*Papio ursinus*) was selected because of its close evolutionary relationship to man. All animals received care according to the *Ethical Considerations in Medical Research*, revised edition, 1987, set out by the South African Medical Research Council.

Animals weighing 13-28 kg were anaesthetised with ketamine hydrochloride (10 mg/kg body weight intramuscularly). Following intubation with an appropriately sized endotracheal tube the animals were ventilated by means of a Bird Mark 8 positive-pressure ventilator. Anaesthesia was maintained with nitrous oxide (70%) and oxygen (30%). Morphine sulphate (0,5 mg/kg intravenously) and pancuronium bromide (0,1 mg/kg intravenously), together with atropine sulphate (0,5 mg intravenously) was administered. Central venous and femoral arterial lines were then inserted. Prior to aortic cannulation blood pressure was controlled by the addition of halothane (0,5-1,0%).

A median sternotomy was performed and after full heparinisation (1 000 U/kg) the heart was cannulated in preparation for cardiopulmonary bypass. Extracorporeal circulation could be commenced by draining venous blood from a single venous cannula inserted into the right atrium, oxygenating it with a Polystan Venotherm low-prime adult/paediatric No. 011520 bubble oxygenator (blood temperature controlled with a heat exchanger), and finally returning the blood to the body via a cannula inserted into the ascending aorta.

A Swan-Ganz thermodilution catheter (American Edwards 7F) was inserted through the superior vena cava and positioned in the pulmonary artery for the measurement of cardiac output. Cannulas were also inserted into the left atrium via the left atrial appendage, into the left ventricle through the apex, into the pulmonary artery and into the ascending aorta for haemodynamic measurements. Finally, a thermistor probe was positioned in the intraventricular septum to monitor myocardial temperature. During the aortic cross-clamp and initial recovery period the left ventricle was vented by means of a cannula inserted via the right superior pulmonary vein. Control haemodynamic measurements were recorded after posi-

tioning of all cannulas, prior to commencement of cardiopulmonary bypass.

Cardiopulmonary bypass was commenced and systemic temperature lowered and maintained at 26°C. The aorta was then cross-clamped and cold (4°C) cardioplegic solution (15 ml/kg) infused through a separate cannula inserted into the ascending aorta. In addition, iced saline (4°C) was used for topical hypothermia. The cardioplegic solution was delivered via a separate roller pump at constant pressure (80 mmHg), measured in the ascending aorta. All topical cold saline was removed after infusion of cardioplegia to simulate the clinical situation. Maintenance cold cardioplegia (100 ml) together with topical cold saline was re-infused every 30 minutes throughout the 180-minute ischaemic cross-clamp period.

Systemic rewarming of the animal was commenced during the ischaemic period, 30 minutes before the release of the aortic cross-clamp, and continued throughout the 15-minute reperfusion period until normothermia was obtained. The animal was then weaned from cardiopulmonary bypass and haemodynamic measurements were taken 5 and 30 minutes after termination of cardiopulmonary bypass. At the end of the experiment, while under full general anaesthesia, the animals were administered a lethal dose of potassium chloride.

Haemodynamic measurements

The following variables were measured with a Honeywell AR-6 six-channel simultrace recorder: heart rate, arterial blood pressure, right atrial pressure, left atrial pressure, the first derivative of the left ventricular developed pressure (LV dP/dt), and cardiac output (CO). All control and post-ischaemic haemodynamic values were taken with the animal volume-loaded to a left atrial pressure of 5 mm/Hg to standardise the preload on the left ventricle. No attempt was made to control the heart rate.

Tissue high-energy phosphates

Left ventricular drill biopsy specimens were taken from the apex of the left ventricle for determination of myocardial high-energy phosphate content. Samples were taken before commencement of cardiopulmonary bypass at the time of control haemodynamic measurements, after the 3-hour ischaemic period prior to release of the cross-clamp, and 5 and 30 minutes after termination of cardiopulmonary bypass. The samples were immediately frozen by immersion in liquid nitrogen and subsequently stored in liquid nitrogen. Determination of adenosine triphosphate (ATP) and creatine phosphate (CP) content was by enzymatic analysis.[9]

Electron microscopy

Drill biopsy specimens from the apical area of the left ventricle were assessed for ultrastructural changes. Samples were taken at the same time for the determination of tissue high-energy phosphate content. Following fixation in 5% buffered glutaraldehyde the samples were processed according to the technique routinely employed in our laboratory.[10]

Crystalloid cardioplegic solutions tested

The composition of the tested solutions is given in Table I. The ST2 cardioplegic solution and the control non-cardioplegic KHB were prepared in the laboratory and filtered through a 0,8 μm filter to remove any bacteria and all particulate matter. The SABAX solution was kindly donated by SABAX Ltd. The GSH solution was the standard solution made by the hospital pharmacy at Groote Schuur Hospital.

Expression of results

Cardiac index (CI) was calculated from the formula:

$$CI = CO/\text{body surface area}$$
$$[\ell.\text{min}^{-1}.\text{m}^{-2}]$$

Stroke volume index (SVI) from the formula:

$$SVI = CI/\text{heart rate} \times 1\,000$$
$$[\text{ml}.\text{min}^{-1}.\text{m}^{-2}.\text{beat}^{-1}]$$

Stroke work index (SWI) from the formula:

$$SWI = SVI/(0{,}01438 \times \text{LV systolic pressure} - \text{LV end-diastolic pressure})$$
$$[\text{g}.\text{m}.\text{m}^{-2}.\text{beat}^{-1}]$$

Haemodynamic values obtained during the post-bypass period are expressed as a percentage of the individual pre-ischaemic control values. Tissue high-energy phosphate content is expressed in absolute values (μmol.g^{-1} wet weight).

Statistical methods

All data were expressed as means ± standard error. Comparison of haemodynamic and biochemical data between the post-ischaemic periods was by two-way analysis of variance. Comparison of data between groups at 30 minutes after cardiopulmonary bypass was by one-way analysis of variance. Individual comparisons were by Student's *t*-test for unpaired data with the Bonferonni correction for multiple comparisons. Statistical significance was assumed with a P value $< 0{,}05$.

Results

The myocardial hypothermia produced by the above experimental protocol was uniform in all animals. Ventricular septal temperature consistently fell to 10°C with each infusion of cardioplegic solution. The myocardium then rewarmed to approximately 20°C in the 30 minutes between each re-infusion.

Haemodynamic recovery

Since there were no differences between haemodynamic recovery at 5 minutes and 30 minutes, as determined by two-way analysis of variance, only the functional recovery at 30 minutes is presented in Table II.

Functional recovery as assessed by SVI in the ST2 group was superior to that in all the other groups. The recovery of CI and SWI of the ST2-treated animals was significantly better than that of animals treated with GSH and KHB. CI and SWI for ST2-treated animals, although higher than that for SABAX-treated animals, were not statistically different.

However, when comparing the functional recovery (SVI, CI, SWI) of hearts treated with SABAX to GSH cardio-

TABLE II. HAEMODYNAMIC RECOVERY (PERCENTAGE OF PRE-ISCHAEMIC CONTROL) OF BABOON HEARTS AT 30 MINUTES AFTER RELEASE OF AORTIC CROSS-CLAMP

	N	*CI*	*SVI*	*SWI*	*LV dP/dt*
ST2	8	94,75 ± 5,81	113,95 ± 8,30	97,14 ± 12,68	87,00 ± 12,39
SBX	7	84,30 ± 5,79	81,35 ± 7,84*	71,67 ± 14,03	83,96 ± 10,22
GSH	7	73,58 ± 3,01*	80,21 ± 6,02*	51,91 ± 2,52*	71,43 ± 6,49
KHB	7	59,86 ± 9,93*	55,61 ± 10,00**	34,98 ± 9,24**	54,72 ± 6,82

The percentage recovery of CI, SVI, LV dP/dt and SWI compared with the pre-ischaemic control, at 30 minutes after termination of cardiopulmonary bypass at a left atrial pressure of 5 mmHg, following a 3-hour ischaemic period. The hearts were protected with the mentioned solutions delivered every 30 minutes at 4°C during this period in addition to topical cold saline.
* $P < 0,05$ v. ST2.
** $P < 0,01$ v. ST2.

plegia or KHB buffer, SABAX cardioplegia was not superior to either the KHB control solution or the GSH solution.

LV function curves were determined at the end of the experimental protocol in some animals. The SWI was measured at different preloads by varying the left atrial pressure between 5 mmHg and 20 mmHg (Fig. 1). The post-ischaemic LV function curve of hearts perfused with ST2 solution approximated the basal pre-ischaemic LV function curve measured in some animals prior to the 3-hour ischaemic period. This finding was in contrast to the depressed post-ischaemic curve for hearts protected with GSH cardioplegia *or* KHB buffer. This technique was not available for the animals treated with SABAX solution due to technical reasons.

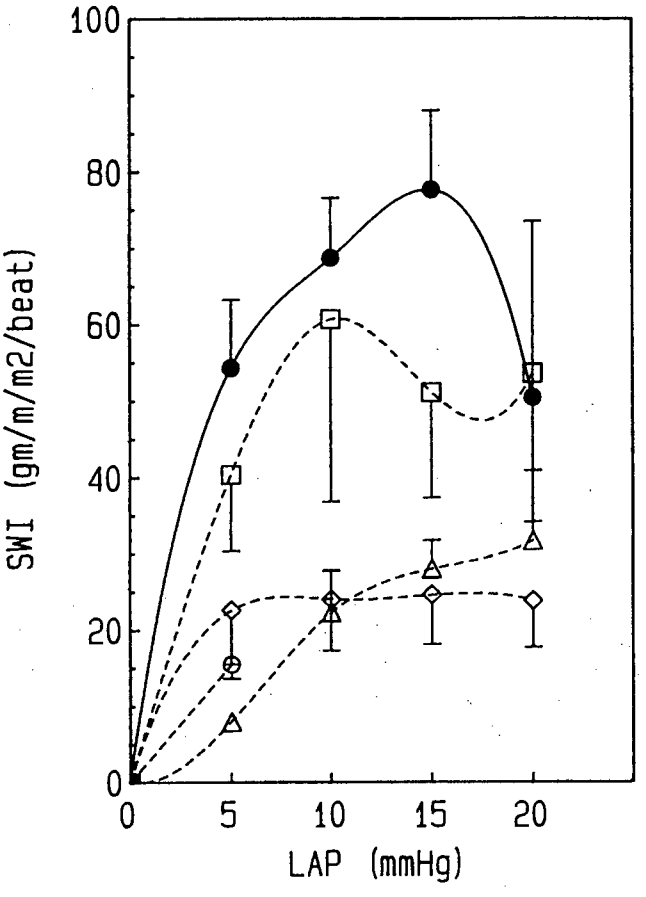

Fig. 1. Post-ischaemic LV function of hearts protected during a 3-hour ischaemic period with St Thomas' Hospital cardioplegic solution No. 2 (-□-), Groote Schuur Hospital cardioplegia (-△-), *or* Krebs-Henseleit non-cardioplegic physiological buffer (-◇-) compared with a pre-ischaemic control LV function curve (-●-). LV function assessed as stroke work index (SWI) was measured at 30 minutes after termination of cardiopulmonary bypass at a varying left atrial pressure (LAP). For hearts treated with SABAX solution (-O-) SWI was only measured at LAP of 5 mmHg. Values are expressed as means (g/min/m²/beat) and standard error of the means.

Recovery of high-energy phosphates

The ATP and CP content of all hearts decreased during the 3-hour ischaemic period despite maintenance doses of cardioplegia (Table III). This loss of ATP was not different between the various groups. However, after release of the aortic cross-clamp there was a marked post-ischaemic recovery of high-energy phosphate levels. The ATP content 5 minutes after cardiopulmonary bypass in hearts treated with ST2 solution was slightly better than that in hearts perfused with SABAX solution, but the differences were not statistically significant. Also, at 30 minutes after bypass, there was no difference in the ATP content of hearts treated with any of the solutions.

The CP levels at 5 minutes and 30 minutes post-bypass were similar in all groups. However, levels in the ST2-treated hearts tended to be slightly superior compared with hearts perfused with SABAX and GSH solution. In addition, at 5 and 30 minutes, the CP content of the non-cardioplegic KHB-treated hearts was not lower than that of hearts perfused with any of the cardioplegic solutions (Table III).

Ultrastructure

Only a limited number of samples could be assessed so far. All groups showed mild to moderate changes after 3 hours of ischaemia (Fig. 2) which were partially reversible after reperfusion (Fig. 3). Further details will be published elsewhere.

Comparison of time to induce cardiac arrest and refunction time

Comparison of the time intervals taken by each solution to induce complete cardiac arrest revealed no statistical differences, and all solutions stopped electromechanical activity within 8-11 seconds (ST2 8,3 ± 1,4 s, SABAX 10,7 ± 2,5 s, and GSH 7,9 ± 1,9 s). The average time to wean the animals off CPB, which is also referred to as 'refunction time', and which is considered to be an index of myocardial protection,[11] was longest for the GSH solution (9,4 ± 3,9 min). Animals in the SABAX group had a mean refunction time

TABLE III. ATP AND CP CONTENT

	Control	*ACC*	*5′*	*30′*
ATP (μmol/g wet wt)				
ST2	4,08 ± 0,49 (5)	2,68 ± 0,23 (5)	3,09 ± 0,45 (5)	2,57 ± 0,33 (3)
SBX	3,78 ± 0,50 (5)	2,98 ± 0,45 (5)	2,58 ± 0,51 (5)	2,40 ± 0,20 (5)
GSH	3,75 ± 0,35 (7)	3,24 ± 0,45 (7)	2,77 ± 0,49 (7)	2,86 ± 0,53 (4)
KHB	3,89 ± 0,28 (7)	1,92 ± 0,21 (5)	2,23 ± 0,34 (7)	2,11 ± 0,54 (7)
CP (μmol/g wet wt)				
ST2	6,59 ± 0,97 (5)	1,98 ± 0,53 (5)	6,78 ± 0,80 (5)	6,10 ± 0,36 (3)
SBX	7,32 ± 0,84 (5)	2,50 ± 0,74 (5)	4,88 ± 0,67 (5)	3,90 ± 0,74 (5)
GSH	7,18 ± 0,65 (7)	1,43 ± 0,44 (7)	4,61 ± 0,96 (7)	5,21 ± 0,96 (4)
KHB	6,81 ± 0,46 (7)	0,49 ± 0,27 (5)	5,69 ± 0,88 (7)	5,76 ± 0,52 (7)

Measurements were taken prior to commencement of cardiopulmonary bypass (control), prior to the release of the aortic cross-clamp (ACC) after a 3-hour ischaemic period, at 5 minutes and at 30 minutes after termination of cardiopulmonary bypass. Number of hearts for each mean is given in parentheses.

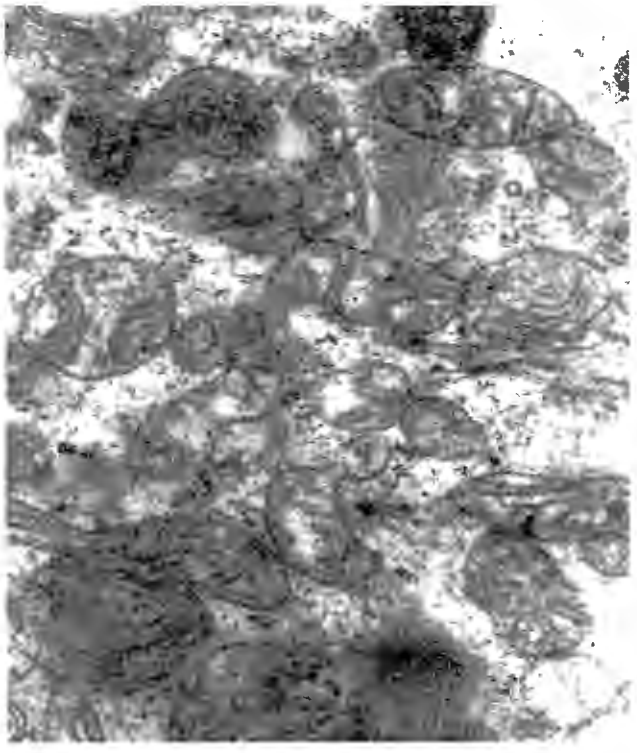

Fig. 2. Ultramicroscopic appearance of a portion of a myocyte of a baboon heart after perfusion with SABAX solution following an ischaemic cross-clamp period of 180 minutes. There is evidence of swelling of the mitochondria with an abnormal crystal arrangement and intracytoplasmic oedema. (Electron photomicrograph x 51 000.)

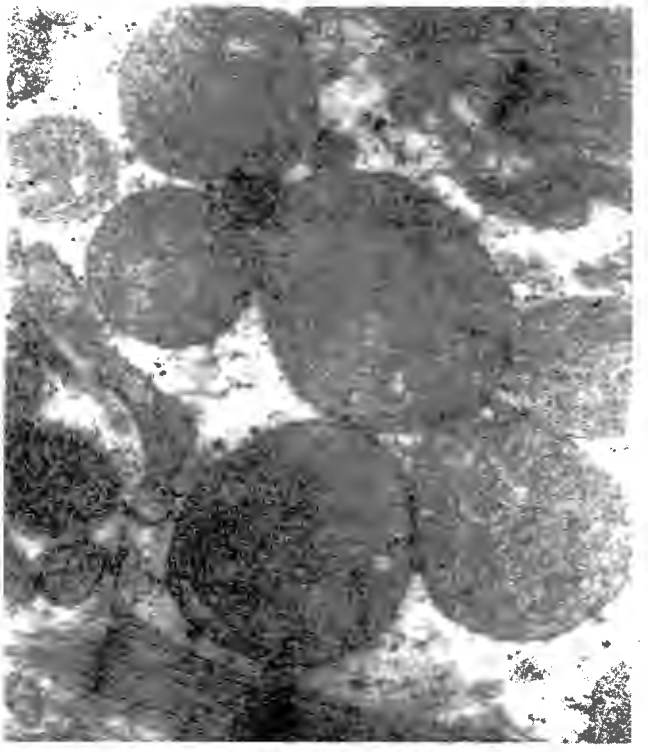

Fig. 3. Relatively normal appearance of mitochondria is evident in this myocyte of a baboon heart which has undergone 3 hours cardiac arrest and perfusion with SABAX solution followed by 1 hour reperfusion with blood. (Electron photomicrograph x 51 000.)

of 6,9 ± 1,8 min compared with 4,6 ± 1,3 min in the ST2 group. In addition, the average energy required to cardiovert the hearts back to sinus rhythm after ischaemia (defibrillation energy) was highest in the SABAX group (85,7 ± 23,8 joules), whereas animals in the GSH and ST2 groups required 34,3 ± 15,9 and 38,8 ± 6,7 joules respectively.

Electrocardiographic criteria

Transient ST-segment elevations in the post-ischaemic ECG recordings were observed in all groups (4/7 cases for the GSH solution, 4/7 cases for the SABAX solution and 4/8 cases for the ST2 solution). New Q waves were not discernible in any of the cardioplegic groups. All animals in the SABAX-treated group recovered in normal sinus rhythm after defibrillation. Of the hearts treated with GSH solution, one remained in atrial fibrillation, whereas in the group treated with ST2 solution, one heart recovered with multiple unifocal ventricular extrasystoles.

Discussion

This study, which closely resembles the clinical situation, shows that the indices of haemodynamic recovery (CI, SVI and SWI) in hearts protected with ST2 was above 95%, in hearts protected with SABAX solution recovery was 70-85%, with the GSH solution it was 52-80%, and with KHB 35-60%. ST2 was superior to the SABAX solution in SVI and to the GSH solution in the majority of measured haemodynamic variables. LV function curves of SWI calculated at left atrial pressures of 5, 10, 15 and 20 mmHg showed that hearts protected with ST2 had good recoveries, in constrast to those treated with GSH solution and KHB. These findings confirm the importance of thorough laboratory testing of any cardioplegic solution. ST2 was extensively investigated in two different animal species and the electrolyte content was formulated according to dose-response curves for each ion.[4,5,8,12]

Although there was no significant difference in the various series in the post-ischaemic recovery of ATP at 5 and 30 minutes, previous studies have shown that ATP levels are not the only factor controlling viability,[13] and that the rate of ATP synthesis may be more important.

Although ATP and CP levels decreased during the ischaemic period, they were quickly restored to acceptable levels during reperfusion in hearts protected with ST2. However, post-ischaemic ATP and CP levels observed in the other groups in this study do not correlate with functional recovery.

The Plasmalyte B with added potassium used in some centres in South Africa has a similar electrolyte content to Ringer's lactate except that Plasmalyte B is *acalcaemic*. Coronary perfusion with a calcium-free solution may lead to increased sarcolemmal permeability to calcium, and when the myocardium is reperfused with a calcium-containing solution a massive influx of calcium can occur, causing severe cellular damage.[14-17] The addition of 200 ml pump blood to the maintenance Plasmalyte B (thus introducing a minimal concentration of calcium), as practised by one unit, could prevent this calcium paradox and consequently be beneficial. In addition, Plasmalyte B contains a high potassium concentration. It has been shown that the optimal potassium concentration for a crystalloid cardioplegic solution is approximately 15 mmol/l and that higher potassium concentrations may be damaging.[18-20] For these reasons this solution was not included in this study.

In the clinical situation, because of the large number of variables such as coronary artery anatomy, LV dysfunction, cross-clamp time, non-collateral flow and the extent of the procedure, it is difficult to objectively evaluate the performance of any one myocardial protection regimen.[12] Variability of non-collateral coronary flow in the diseased heart may influence the rate of 'wash-out' of the cardioplegic solution, so diminishing its advantages; likewise the 'wash-in' of substances (e.g. calcium) may be beneficial when using an acalcaemic cardioplegic solution. It is not yet known whether undetected myocardial damage, resulting from poor myocardial protection, will result in late myocardial fibrosis and decreased left ventricular function. Therefore, we should continue to seek improvements in our techniques of myocardial preservation.

Conclusion

The result of this comparative control study, in conjunction with other reports in the literature, lead us to propose that the optimum crystalloid cardioplegic solution for use in South Africa today should be based on the St Thomas' Hospital cardioplegic solution No. 2. Even minor alterations to this solution should be fully evaluated experimentally before clinical use. The Cardio-Thoracic Department at Groote Schuur Hospital now uses St Thomas' solution No. 2 plus glucose (10 mmol/l) cardioplegic solution. The beneficial effect of adding glucose to the St Thomas' Hospital solution No. 2 at this concentration has been confirmed in our laboratory (unpublished data).

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Appendix A–2

St. Thomas' Hospital Cardioplegic Solution:

Beneficial effect of glucose and multidose reinfusions of cardioplegic solution.

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J THORAC CARDIOVASC SURG 1991;102:405-12

St. Thomas' Hospital cardioplegic solution

Beneficial effect of glucose and multidose reinfusions of cardioplegic solution

The intention of this study was to determine whether glucose is beneficial in a cardioplegic solution when the end products of metabolism produced during the ischemic period are intermittently removed. The experimental model used was the isolated working rat heart, with a 3-hour hypothermic 10° C cardioplegic arrest period. Cardioplegic solutions tested were the St. Thomas' Hospital No. 2 and a modified Krebs-Henseleit cardioplegic solution. Glucose (11 mmol/L) was beneficial when multidose cardioplegia was administered every 30 minutes. Including glucose in Krebs-Henseleit cardioplegic solution improved postischemic recovery of aortic output from 57.0% ± 1.8% to 65.8% ± 2.2%; $p < 0.025$. The addition of glucose to St. Thomas' Hospital No. 2 cardioplegic solution improved aortic output from 74.6% ± 1.9% to 87.4% ± 1.9%; $p < 0.005$. Furthermore, a dose-response curve showed that a glucose concentration of 20 mmol/L gave no better recovery than 0 mmol/L, and glucose in St. Thomas Hospital No. 2 cardioplegic solution was beneficial only in the range of 7 to 11 mmol/L. In addition, we showed that multidose cardioplegia was beneficial independent of glucose. Multidose St. Thomas' Hospital No. 2 cardioplegia, as opposed to single-dose cardioplegia, improved aortic output recovery from 57.4% ± 5.2% to 74.6% ± 1.9%; $p < 0.025$, and with St. Thomas' Hospital No. 2 cardioplegic solution plus glucose (11 mmol/L) aortic output recovery improved from 65.9% ± 2.9% to 87.4% ± 1.9%; $p < 0.005$. Hence, at least in this screening model, the St. Thomas' Hospital cardioplegic solution should contain glucose in the range of 7 mmol/L to 11 mmol/L, provided multidose cardioplegia is given. We cautiously suggest extrapolation to the human heart, on the basis of supporting clinical arguments that appear general enough to apply to both rat and human metabolisms.

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Crystalloid cardioplegic solutions of markedly varying compositions are used both clinically and experimentally to aid in the prevention of intraoperative myocardial damage.[1] This diversity has resulted in difficulties when attempting to attribute improvements to a specific component because of inseparable interactions with other constituents. In particular, controversy exists as to the beneficial effect of glucose.[2-5]

There are two fundamental types of crystalloid cardioplegic solutions: those with an "intracellular" ionic equivalent composition, notably Bretschneider's solution,[6] and those with an "extracellular" form. In the latter group the St. Thomas' Hospital No. 2 (ST) cardioplegic solution has been fully characterized with respect to each individual cation—sodium, potassium, magnesium, and calcium[1, 7, 8]—and is used internationally. Hearse, Stewart, and Braimbridge[2] showed that the addition of glucose to a solution similar to ST was dam-

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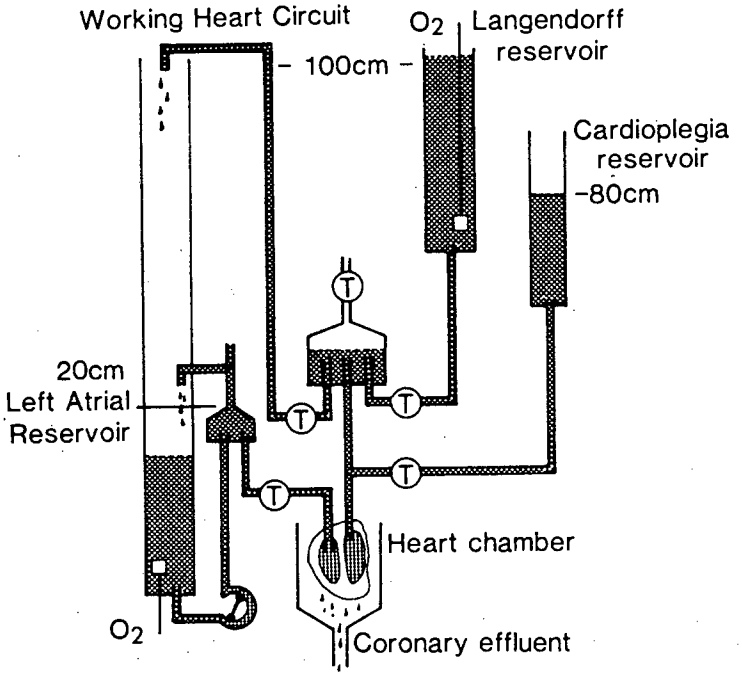

Fig. 1. Isolated working rat heart perfusion apparatus. Three-way taps *(T)* provide easy conversion to either the Langendorff mode or the working heart circuit and allow for infusion of cardioplegic solution. Oxygenation (O_2) of the perfusion fluid (Krebs-Henseleit buffer) is with 95% oxygen:5% carbon dioxide.

Table I. *Cardioplegic solutions*

	Na	*K*	*Mg*	*Ca*	*HCO_3*	*Glucose*
KC cardioplegic solution	143	16	1.2	1.25	25	0 or 11
ST cardioplegic solution	120	16	16	1.2	10	0 or 11

KC cardioplegic solution, modified Krebs-Henseleit solution; ST cardioplegic solution, St Thomas' Hospital No. 2 cardioplegic solution. (The standard solution does not contain glucose.) Values are given in millimoles per liter.

aging in a 70-minute 28° C ischemic model protected with single-dose cardioplegia.[2] However, accumulation of glycolytic end products, as a result of total ischemia, is known to cause detrimental feedback inhibition of metabolism.[9]

The object of this study was to determine the effects of glucose, included in an "extracellular" cardioplegic solution, on postischemic metabolic and mechanical functional recovery after hypothermic cardioplegic arrest. In addition, we examined the necessity for intermittent reinfusions of cardioplegic solution throughout the ischemic period to wash out metabolic end products. The initial experiments were performed with a modified physiologic perfusion solution (Krebs-Henseleit; KC) but with additional potassium (16 mmol/L) to produce diastolic arrest, with or without glucose, 11 mmol/L (KC + G versus KC; Table I). The final experiments followed a similar protocol but used ST, with and without glucose, 11 mmol/L (ST + G versus ST; Table I).

A dose-response curve for glucose in ST was also performed to determine the concentration range in which glucose was beneficial in the ST solution.

Materials and methods

All animals received care according to the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH Publication No. 80-23, revised 1978).

Experimental protocol. The isolated perfused rat heart was used as our experimental model[10] (Fig. 1). Male (Long Evans) rats (250 to 400 gm) were anesthetized with diethyl ether; 200 IU of heparin was injected into the exposed femoral vein and the hearts were rapidly excised and placed into 4° C Krebs-Henseleit buffer ($NaHCO_3$, 25 mmol/L; NaCl, 118 mmol/L; KH_2PO_4, 1.2 mmol/L; KCl, 4.8 mmol/L; $MgSO_4$, 1.2 mmol/L; $CaCl_2$, 1.25 mmol/L; and glucose, 11.1 mmol/L).

The aorta was cannulated within 1 minute, and Langendorff perfusion at a constant pressure of 100 cm of water was com-

menced. The pulmonary veins were then identified, the left atrium cannulated, and the pulmonary artery incised to ensure ejection of coronary effluent. The Krebs-Henseleit buffer was gassed with 95% oxygen:5% carbon dioxide and maintained at 37° C. The hearts were stabilized for 10 minutes before changing to the working mode. The left atrium was then filled at a pressure of 20 cm H_2O, and the perfusion fluid was ejected from the left ventricle against an afterload pressure of 100 cm H_2O. The aortic pressure was measured by a Statham P23dB pressure transducer (Spectramed Inc., Critical Care Division, Oxnard, Calif.) connected to a sidearm of the aortic cannula. After 10 minutes of work (control period) the aortic and coronary flow, peak systolic aortic pressure, and heart rate were recorded. Any heart that was unstable was rejected.

At the end of the control work period the atrial and aortic cannulas were closed off and 10 ml of 10° C cardioplegic solution was infused into the aorta at a pressure of 60 mm Hg. The hearts were maintained at constant hypothermia for 3 hours by means of a 10° C water-jacketed chamber. In the groups receiving multidose cardioplegia, 6 ml of 10° C cardioplegic solution was reinfused every 30 minutes throughout the arrest period. At the end of the ischemic period each heart was reperfused for 10 minutes at 37° C in the Langendorff mode, followed by the working mode for an additional 10 minutes to measure postischemic functional recovery. During the ischemic and postischemic reperfusion periods the coronary effluent was collected, and at the end of the experiment the hearts were freeze clamped with Wollenberg tongs to determine their high-energy phosphate content, expressed as micromoles per gram wet weight.

Preischemic control values for adenosine triphosphate and creatine phosphate were derived from hearts ($n = 6$) subjected to the same initial experimental protocol, that is, 10-minute Langendorff and 10-minute working heart modes, but thereafter immediately freeze-clamped.

Expression of results. Cardiac output (CO) in this preparation is the sum of aortic flow (AO) and coronary flow, while stroke volume (SV) is CO/heart rate. Values obtained during the postischemic working period were expressed as a percentage of their individual preischemic control values. Results are presented as percentage means and standard errors of percentage means.

Exclusion criteria. In the preischemic control period the following values were used to discard hearts: AO <30 ml/min; coronary flow >22 ml/min; heart rate <200 beats/min, or irregular rhythm. In the postischemic period any heart whose coronary flow significantly increased by more than 50% (suggesting a left atrial leak) was excluded. No extreme observations were detected with the use of Dixon's criteria, and therefore no additional hearts were discarded.

The statistical test used to compare differences between the factors, cardioplegic solution (KC or ST), addition of glucose, and provision of multidose cardioplegia, was the three-way analysis of variance (ANOVA). However, the ANOVA depends on an assumption of common variance among observations within each treatment group. In this study the variance within groups was not consistent. Therefore, when focusing on specific comparisons of interest after ANOVA was conducted, necessary adjustments for the violation of common variance were made. Pairwise comparisons of means with no assumption of common variance were performed using t statistics with a nominal level of significance set at 5%.

A consequence of multiple comparisons at a specified nominal level is that some statistics for comparisons may be declared significant when chance alone is operating over essentially equivalent groups. However, we relied on the fact that the number of statistically significant comparisons was a large fraction of the total number of comparisons studied, too large to be explicable in the vagaries of chance and the nominal level.

In the dose-response relationship of AO and CO recovery to glucose concentration, the evidence of some ordinal effect of dose was assessed by the nonparametric "runs-up-and-down" test. Thereafter suitable multiple-comparison t tests were used to establish significance.

Appropriate tables were then used to determine p values for comparisons of interest. Statistical significance was assumed when the p value was <0.05, and those p values reported are conservative.

Table II. *Crystalloid cardioplegic solution*

	Single-dose cardioplegia		
	KC (n = 6)	*KC + G (n = 7)*	
Preischemic control values			
AO (ml/min)	45.3 ± 1.3	39.7 ± 2.7	
CO (ml/min)	62.3 ± 2.4	54.6 ± 3.4	
SV (ml)	0.24 ± 0.01	0.23 ± 0.02	
AP (mm Hg)	107 ± 1	104 ± 2	
Postischemic functional recovery			
AO	50.6% ± 1.5%	51.7% ± 1.7%	NS
CO	56.5% ± 1.8%	60.9% ± 2.7%	NS
SV	60.1% ± 3.5%	60.3% ± 4.4%	NS
AP	92.4% ± 1.4%	89.5% ± 2.4%	NS
	Multidose cardioplegic solution		
	KC (n = 6)	*KC + G (n = 7)*	
Preischemic control values			
AO (ml/min)	38.7 ± 2.3	43.4 ± 3.9	
CO (ml/min)	54.8 ± 4.4	60.0 ± 5.3	
SV (ml)	0.21 ± 0.02	0.22 ± 0.02	
AP (mm Hg)	104 ± 4	108 ± 3	
Postischemic functional recovery			
AO	57.0% ± 1.8%	65.8% ± 2.2%*	$p < 0.025$
CO	70.5% ± 3.0%†	73.3% ± 2.4%‡	NS
SV	71.3% ± 3.9%	76.8% ± 4.4%‡	NS
AP	96.3% ± 1.7%	94.6% ± 1.8%	NS

Mean preischemic control values and postischemic functional recovery (expressed as a percentage of each individual preischemic value) and standard errors of percentage means in isolated rat hearts after 3-hour 10° C cardioplegic arrest. All hearts were protected with an initial 10 ml dose of Krebs-Henseleit cardioplegic solution (KC), with or without 11 mmol/L glucose (G), at the onset of the ischemic period. In the indicated groups, 6 ml boluses of multidose cardioplegic solution were infused every 30 minutes throughout the arrest period. AO, Aortic output; CO, cardiac output; SV, stroke volume; AP, maximum developed systolic aortic pressure; NS, not significant at 5% level.

*$p < 0.005$ compared with single-dose KC + G.

†$p < 0.005$ compared with single-dose KC.

‡$p < 0.025$ compared with single-dose KC + G.

Results

ANOVA suggested that the main effect of all three factors (cardioplegic solution, addition of glucose, and provision of multidose cardioplegia) were statistically significant for the variables AO, CO, and SV. Two-way

Table III. *St Thomas' Hospital cardioplegic solution*

	Single-dose cardioplegia		
	ST (n = 7)	*ST + G (n = 10)*	
Preischemic control values			
AO (ml/min)	47.0 ± 1.9	43.5 ± 2.3	
CO (ml/min)	65.6 ± 2.7	61.7 ± 2.1	
SV (ml)	0.24 ± 0.01	0.23 ± 0.01	
AP (mm Hg)	104 ± 2	104 ± 3	
Postischemic functional recovery			
AO	57.4% ± 5.2%	65.9% ± 2.9%	NS
CO	63.8% ± 4.9%	74.6% ± 3.8%	NS
SV	58.1% ± 4.0%	76.2% ± 3.2%	$p < 0.01$
AP	95.0% ± 1.7%	91.9% ± 1.8%	NS
Postischemic LDH loss (mU/gm/min)			
0-10 min	42.9 ± 8.0	66.4 ± 7.7	$p < 0.05$
	Multidose cardioplegia		
	ST (n = 9)	*ST + G (n = 8)*	
Preischemic control values			
AO (ml/min)	48.6 ± 2.0	46.3 ± 1.2	
CO (ml/min)	66.4 ± 1.3	63.8 ± 1.5	
SV (ml)	0.25 ± 0.02	0.21 ± 0.01	
AP (mm Hg)	107 ± 3	101 ± 3	
Postischemic functional recovery			
AO	74.6% ± 1.9%*	87.4% ± 1.9%†	$p < 0.005$
CO	80.0% ± 1.6%*	88.7% ± 2.2%‡	$p < 0.01$
SV	89.4% ± 2.6%§	93.8% ± 5.0%†	NS
AP	93.7% ± 1.2%	99.9% ± 2.3%‡	$p < 0.025$
Loss in coronary sinus effluent with each reinfusion of cardioplegic solution			
LDH (mU/gm/dose)	72.6 ± 3.6	82.2 ± 6.6	NS
Lactate (μmol/gm/dose)	1.86 ± 0.11	2.49 ± 0.16	$p < 0.05$
Postischemic LDH loss (mU/gm/min)			
0-10 min	148.0 ± 18.1§	109.5 ± 18.8‖	NS

Mean preischemic control values and postischemic functional and metabolic recovery (expressed as a percentage of each individual preischemic value) and standard errors of percentage means in isolated rat hearts after 3-hour 10° C cardioplegic arrest. Hearts were protected with St Thomas' Hospital cardioplegic solution (ST), with or without 11 mmol/L glucose (G), and multidose reinfusions of cardioplegic solution every 30 minutes throughout the ischemic period in the groups indicated. AO, Aortic output; CO, cardiac output; SV, stroke volume; AP, maximum developed systolic aortic pressure; LDH, lactate dehydrogenase); NS, not significant at 5% level.

*$p < 0.05$ compared with single-dose ST.

†$p < 0.005$ compared with single-dose ST + G.

‡$p < 0.025$ compared with single-dose ST + G.

§$p < 0.005$ compared with single-dose ST.

‖$p < 0.05$ compared with single-dose ST + G.

interaction between cardioplegic solution and either glucose or multidose infusion was found with AO, and between glucose and multidose infusion for aortic pressure.

Effect of glucose with single-dose cardioplegia. Hearts were subjected to a single coronary infusion of cardioplegic solution at the onset of the 3-hour ischemic period. The initial groups were protected with KC cardioplegic solution without glucose ($n = 6$). The mean recovery of AO was 50.6% ± 1.5%, and for CO, 56.5% ± 1.8%. The addition of glucose (11 mmol/L) ($n = 7$) did not improve recovery of AO (51.7% ± 1.7%) or CO (60.9% ± 2.7%) (Table II).

A similar protocol was followed when protecting hearts with either ST ($n = 7$) or ST + G ($n = 10$). The mean recovery with ST for AO was 57.4% ± 5.2% and for CO, 63.8% ± 4.9%. The addition of glucose again did not significantly improve AO (65.9% ± 2.9%) or CO (74.6% ± 3.8%), but did improve SV from 58.1% ± 4.0% to 76.2% ± 3.2%; $p < 0.01$ (Table III). However, postischemic lactate dehydrogenase loss, a measure of damage to membrane integrity, increased with the addition of glucose from 42.9 ± 8.0 mU/gm/min to 66.4 ± 7.7 mU/gm/min; $p < 0.05$.

Hence the effect of adding glucose to ST with single-dose cardioplegia was questionably beneficial, and in fact possibly harmful.

Effect of glucose with multidose cardioplegia. Hearts received the initial cardioplegic solution bolus at the onset of the ischemic period, together with reinfusions every 30 minutes throughout the 3-hour hypothermic 10° C period. The addition of glucose, 11 mmol/L, to KC improved AO recovery, from 57.0% ± 1.8% ($n = 6$) to 65.8% ± 2.2% ($n = 7$); $p < 0.025$. CO and SV were unchanged (see Table II).

The addition of glucose, 11 mmol/L, to ST improved recovery of AO from 74.6% ± 1.9% ($n = 9$) to 87.4% ± 1.9% ($n = 8$); $p < 0.005$, and CO from 80.0% ± 1.6% to 88.7% ± 2.2%; $p < 0.01$ (see Table III). The postischemic leakage of lactate dehydrogenase was unchanged (ST 148.02 ± 18.09 mU/gm/min and ST + G 109.50 ± 18.84 mU/gm/min). Including glucose in this cardioplegic solution increased end products

Table IV. *High-energy phosphate content taken at end of reperfusion*

	N	*ATP*	*CP*
Control	6	3.91 ± 0.25	5.05 ± 0.46
KC	6	2.67 ± 0.23*	5.19 ± 0.64
KC + G	7	2.90 ± 0.09*	4.98 ± 0.31
ST	9	3.22 ± 0.14*†	4.99 ± 0.34
ST + G	8	3.39 ± 0.08‡§	4.90 ± 0.28

Mean concentrations of adenosine triphosphate (ATP) and creatine phosphate (CP) expressed as μmol/gm wet weight after 10-minute recovery following 3-hour 10° C cardioplegic arrest, protected with 30-minute multidose reinfusions of cardioplegic solution. Hearts were protected with Krebs-Henseleit cardioplegic solution (KC) ± 11 mmol/L glucose (G) or St Thomas' Hospital cardioplegic solution (ST) ± 11 mmol/L G. Preischemic control values for ATP and CP were obtained by freeze clamping six hearts before the ischemic period in the experimental protocol.

*$p < 0.005$ compared with control.

†$p < 0.02$ compared with KC.

‡$p < 0.05$ compared with KC + G.

§$p < 0.05$ compared with control.

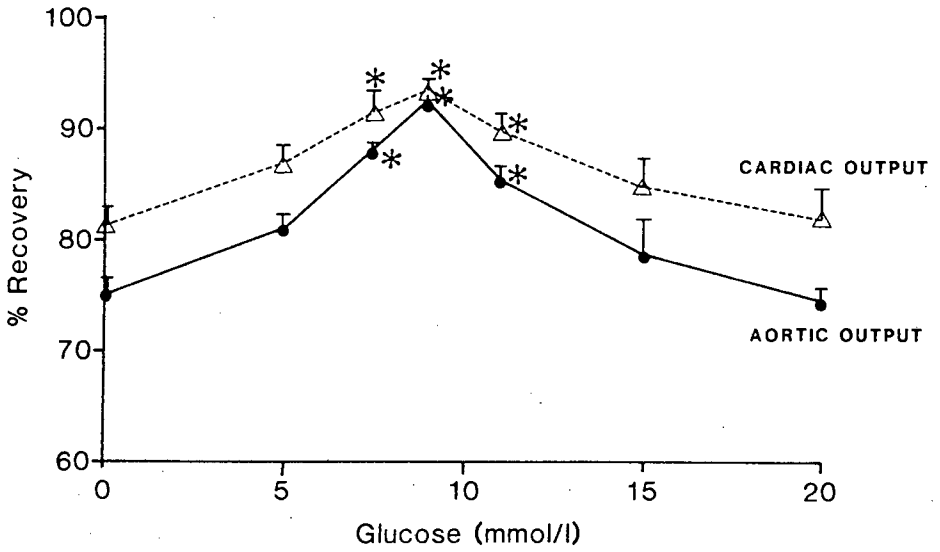

Fig. 2. Composite dose response graph for glucose added to ST. Additional values were superimposed on earlier studies; thus at glucose concentration 0 mmol/L ($n = 13$), 5 mmol/L ($n = 4$), 7.5 mmol/L ($n = 4$), 9 mmol/L ($n = 4$), 11 mmol/L ($n = 27$), 15 mmol/L ($n = 9$), 20 mmol/L ($n = 17$). Multidose reinfusions of cardioplegic solution were given every 30 minutes throughout a 3-hour 10° C ischemic period. Values represent the mean postischemic recovery of both AO and CO, expressed as a percentage of each individual preischemic value. *Vertical bars* represent standard errors of percentage means. *$p < 0.005$ compared with 0 mmol/L and 20 mmol/L concentrations of glucose.

of glycolysis, as lactate efflux (released during the ischemic period in the coronary sinus effluent, with each reinfusion of cardioplegic solution) increased from 1.86 ± 0.11 μmol/gm/dose to 2.49 ± 0.16 μmol/gm/dose of cardioplegic solution; $p < 0.05$, in the presence of glucose.

Tissue adenosine triphosphate content of all groups given multidose cardioplegia was statistically lower after reperfusion than the preischemic level; $p < 0.05$ (Table IV). Recovery of adenosine triphosphate was greater after hearts were protected with ST + G, 3.39 ± 0.08 μmol/gm, when compared with KC + G, 2.90 ± 0.09 μmol/gm; $p < 0.05$. However, the addition of glucose to KC or ST did not significantly improve postreperfusion adenosine triphosphate content. There was no statistical difference in the creatine phosphate content between any of the groups, including the control group.

If multidose cardioplegia was provided, addition of glucose to either cardioplegic solution was beneficial in terms of functional recovery.

Dose-response curve for glucose. With the same experimental model with regular multidose reinfusions, a dose-response graph for incremental amounts of glucose (0 mmol/L, 5 mmol/L, 7.5 mmol/L, 9 mmol/L, 11 mmol/L, 15 mmol/L, 20 mmol/L) was constructed for the ST + G cardioplegic solution. No adjustments were made to correct for osmolarity differences. Additional values for 0 mmol/L, 11 mmol/L, and 20 mmol/L were superimposed from previous and later studies. The observed percentage means of the preceding seven dose levels are shown in Fig. 2. A bell-shaped dose-response curve was seen for both AO and CO, and the appropriate statistical test rejected absence of pattern; $p < 0.05$. Each of the medial dose levels (7.5 mmol/L, 9 mmol/L, 11 mmol/L) gives a higher response than each of the extreme dose levels ($p < 0.005$) for AO and CO. Averages of responses for these medial dose levels exceed averages for the 5 mmol/L and 15 mmol/L dose levels; $p < 0.05$. Furthermore, although a 9 mmol/L concentration of glucose was associated with the optimal postischemic CO recovery of 93.6% ± 0.8%, n was only 4. Therefore we conclude that glucose was beneficial in the designated concentration range of 7 to 11 mmol/L.

Effect of multidose cardioplegia either with or without glucose. The independent beneficial effect on functional recovery associated with multidose cardioplegia is clear, both with and without added glucose. Providing multidose cardioplegia with KC improved the recovery of CO from 56.5% ± 1.8% to 70.5% ± 3.0%; $p < 0.005$. Furthermore, with KC + G, apart from aortic pressure, all indices of mechanical recovery improved; $p < 0.025$ (see Table II).

Multidose cardioplegia with either ST or ST + G increased postischemic recovery of AO, CO, and SV; $p < 0.025$ (see Table III). However, the provision of

multidose cardioplegia increased postischemic lactate dehydrogenase loss with ST from 42.9 ± 8.0 mU/gm/min to 148.0 ± 18.1 mU/gm/min; $p < 0.005$, and with ST + G from 66.4 ± 7.7 mU/gm/min to 109.5 ± 18.8 mU/gm/min; $p < 0.05$.

Furthermore, ST + G gave superior protection to that of KC + G with respect to both AO, CO, and SV; $p < 0.005$. The solution providing the best overall myocardial protection was the ST + G cardioplegic solution when multidose infusions were given.

Discussion

We studied the effect of adding glucose to two different cardioplegic solutions, in the absence or presence of multidose reinfusions of cardioplegic solution.

The primary function of a cardioplegic solution is to maximize energy conservation. This conservation is achieved by using hypothermia and manipulating electrolyte concentrations to halt all electromechanical activity. We demonstrated the superiority of ST ± G over KC ± G, thereby establishing the importance of using a properly formulated cardioplegic solution that optimizes all ion concentrations, not just potassium.

The second function of a cardioplegic solution is to prevent ischemically induced damage. A balance must be established between energy conservation and the maintenance of essential basal metabolic requirements, to control ionic homeostasis. Thus a milieu for ongoing energy production should be provided. Anaerobic metabolism is efficient in neonatal myocardium and can supply most of the energy requirements.[11, 12] In the mature heart anaerobic metabolism is not as efficient, but can contribute to energy needs, provided glycolysis is not inhibited by metabolic end products.

The addition of glucose (11 mmol/L) to KC cardioplegic solution was beneficial only in the presence of multidose reinfusions. The addition of glucose to ST cardioplegic solution, a "well-formulated" solution, was of questionable benefit with single-dose cardioplegia, improving only SV but also increasing postischemic lactate dehydrogenase loss and therefore possibly indicating some cellular membrane damage. However, in the presence of multidose cardioplegia, glucose improved mechanical recovery and did not alter postischemic lactate dehydrogenase leakage. Furthermore, we showed that glucose was beneficial only in the concentration range 7 to 11 mmol/L. Higher or lower concentrations had no effect. Multidose reinfusions of either cardioplegic solution during the 3-hour ischemic period also improved functional recovery, independently of any glucose effect, but were associated with increased lactate dehydrogenase loss.

Is glucose beneficial? In the study by Hearse, Stewart, and Braimbridge[2] the addition of glucose (10 mmol/L) to an ST-like solution was detrimental, and this deleterious effect increased progressively with higher concentrations. Their experiments were conducted at 28° C with a 70-minute ischemic period and single-dose cardioplegia, and were therefore without washout of metabolic end products. In total ischemia in the isolated rat heart there is no noncoronary collateral flow; thus there will be progressive accumulation of inhibitory end products of metabolism.[10, 13] Increases of both lactate and hydrogen ions independently produce feedback inhibition of glycolysis and other metabolic reactions.[9] However, in contrast to Hearse, Stewart, and Braimbridge,[2] we did not find a diminished functional recovery with single-dose ST cardioplegia and a glucose concentration of 11 mmol/L, although postischemic lactate dehydrogenase loss was increased.

The solution tested by Hearse, Stewart, and Braimbridge[2] contained a higher HCO_3 and lower sodium concentration than the standard ST solution we tested. The provision of more HCO_3 buffer was probably not an important variable in their model because lactate is the greater inhibitory factor. However, their study was conducted at 28° C, which could have contributed to the discrepant findings, because metabolic rate is proportional to temperature. We selected 10° C in our experimental protocol because the optimum temperature for myocardial preservation during cardioplegic arrest is less than 18° C.[14, 15] Moreover, in the clinical situation it is extremely difficult to achieve a myocardial temperature below 10° C.[14, 16] Glycolysis does continue at this temperature because we demonstrated increased lactate efflux in the multidose cardioplegia group with the addition of glucose. However, it is possibly less than at 28° C.

We would concur with Hearse, Stewart, and Braimbridge[2] that in total ischemia glucose is of questionable benefit and possibly harmful. However, we have now demonstrated that if multidose cardioplegia was provided, the addition of glucose (11 mmol/L) improved postischemic recovery and did not alter lactate dehydrogenase leakage. In the human clinical situation, because of noncoronary collateral flow, total ischemia occurs rarely even if single-dose cardioplegia is administered. This produces variable washout of both metabolic end products and the cardioplegic solution. Therefore our multidose model probably parallels the clinical situation more closely than the totally ischemic single-dose isolated rat heart model.

We were unable to demonstrate a beneficial effect of glucose on postreperfusion high-energy phosphate content. However, adenosine triphosphate and creatine phos-

phate were measured after 20-minute postischemic reperfusion, and therefore we had allowed for possible repletion of energy stores. Only severe cellular damage affecting continued postischemic metabolism would have been detected. However, glycolysis derived adenosine triphosphate could be important, because of possible adenosine triphosphate compartmentation and the need for glycolytic adenosine triphosphate for membrane function.[17] We showed a 34% increase of lactate efflux with each multidose reinfusion of cardioplegic solution if glucose was included. Glycolysis derived adenosine triphosphate was increased. Furthermore, as the cardioplegic solution was aerated (mean oxygen tension 150 mm Hg) and not anoxic, one must postulate that some aerobic metabolism was taking place, consequent to each reinfusion. Aerobic metabolism would have used additional lactate as substrate, and the total amount of energy derived from the inclusion of glucose was probably even greater than is suggested by calculations from the reported data. In addition, Rosenkranz and coworkers[18] have shown the poor correlation of postischemic adenosine triphosphate levels and functional recovery. Thus we can conclude that glucose (11 mmol/L) is beneficial in ST provided metabolic end products are removed.

Is multidose cardioplegia beneficial? The removal of inhibitory end products of anaerobic metabolism, by multidose reinfusions of cardioplegic solution with or without glucose, independently improved functional recovery in our study, but it also increased postischemic release of lactate dehydrogenase. Loss of the intracellular enzyme lactate dehydrogenase is thought to reflect membrane damage. Hence reinfusions of cardioplegic solution may have affected membrane integrity, even though accumulating end products of metabolism were removed and postischemic recovery improved.

It is possible that multidose reinfusions of a crystalloid solution such as ST, which does not balance colloid oncotic pressure, might increase protein flux from the interstitium with each reinfusion. In addition, improved mechanical function could also lead to improved washout of lactate dehydrogenase. Furthermore, the lost lactate dehydrogenase might be derived from the endothelium and not the myocyte, which would explain these findings that appear discrepant with the improved functional recovery. Alternatively, harmful free radicals are possibly generated with each reinfusion of cardioplegic solution. Further work would be necessary to explain these observations. However, the policy of periodically reinfusing cardioplegic solution throughout the arrest interval has generally been accepted by cardiac surgeons in the clinical situation. This strategy counteracts intraoperative problems of constant rewarming of the in situ heart and variable washout of cardioplegic solution by noncoronary collateral flow.

A dose-response graph was established to determine the concentration range within which glucose was beneficial in ST. Other studies have used a variety of concentrations in different cardioplegic solutions. Our results indicate that glucose in the range 7 to 11 mmol/L is beneficial in ST cardioplegic solution. Concentrations lower or higher had no significant effect. Although the therapeutic range is narrow, glycolytic adenosine triphosphate is essential for sarcolemmal function if ionic homeostasis is to be maintained[17]; thus inhibition of glycolysis either pharmaceutically or by lack of substrate would be detrimental. At lower concentrations there is perhaps insufficient glycolysis, and at higher glucose concentrations there is possibly insufficient washout of harmful products, which may outweigh any initial beneficial effects. It would therefore appear that the beneficial effect of glucose is dependent on the balance between the rate of glycolysis and efficiency of removal of end products. Any imbalance would be harmful; Hearse, Stewart, and Braimbridge[2] showed that total ischemia (metabolites not removed) or addition of insulin (glycolysis stimulated) in the presence of glucose (10 mmol/L) was harmful.

These experiments were performed in the isolated rat heart, which is not an equivalent physiologic model for the heart in human beings, although we believe our multidose procedure more closely parallels the clinical situation. Therefore we must caution direct clinical extrapolation. Studies in the dog and rabbit, however, have also demonstrated improved recovery when glucose is included in a variety of multidose cardioplegic solutions.[3,4] We have shown that the beneficial effect of including glucose at a concentration of 7 to 11 mmol/L in ST is seen only when intermittent multidose reinfusions of cardioplegic solution are provided to wash out end products of metabolism. Although one can argue that the beneficial effect of glucose is minor in clinical effects, it was statistically significant in our multidose model. In addition, in the clinical situation noncoronary collateral flow would not only assist with washout of metabolic end products, but also would supply glucose at a similar concentration to that which we tested. However, one should not rely on noncoronary collateral flow to modulate the composition of either a good or bad cardioplegic solution, because of its inherent variability.

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Appendix A–3

Effect of oxygenation and consequent pH changes on the efficacy of St. Thomas' Hospital cardioplegic solution.

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Effect of oxygenation and consequent pH changes on the efficacy of St. Thomas' Hospital cardioplegic solution

The hypothesis tested is that shifts in pH, induced when a cardioplegic solution is oxygenated, can be detrimental. We added either 100% nitrogen, 95% nitrogen and 5% carbon dioxide, 100% oxygen, or 95% oxygen and 5% carbon dioxide to the cardioplegic solution (St. Thomas' Hospital No. 2 plus glucose 11 mmol/L), and determined postischemic recovery of isolated rat hearts after 3 hours of 10° C cardioplegic protected ischemia. Hearts were arrested and reinfused every 30 minutes throughout the ischemic period with cardioplegic solution. When 5% carbon dioxide was added to nitrogen, the pH of the cardioplegic solution decreased from 9.1 (100% nitrogen) to 7.0 (95% nitrogen: 5% carbon dioxide), a change associated with improved postischemic functional recovery. Aortic output improved from 52.3% ± 2.7% to 63.9% ± 2.8%, $p < 0.05$, and cardiac output from 60.8% ± 3.6% to 75.4% ± 3.3%, $p < 0.01$. This improvement was associated with diminished efflux of lactate during ischemia but increased postischemic release of lactate dehydrogenase. When nitrogen was replaced with oxygen, the addition of 5% carbon dioxide resulted in a similar decrease of pH, which again was associated with improved postischemic functional recovery. Aortic output improved from 66.3% ± 2.8% (100% oxygen) to 88.9% ± 3.7% (95% oxygen: 5% carbon dioxide), $p < 0.005$, and cardiac output from 75.3% ± 4.1% to 88.9% ± 2.4%, $p < 0.01$. The efflux of lactate during ischemia and the postischemic release of lactate dehydrogenase were similar in both groups. Furthermore, provision of additional oxygen with perfluorocarbons in an electrolyte solution identical to the St. Thomas' Hospital plus glucose solution and oxygenated with 95% oxygen: 5% carbon dioxide conferred no extra protection. In conclusion, the St. Thomas' Hospital No. 2 plus glucose cardioplegic solution should be oxygenated but with 95% oxygen: 5% carbon dioxide and not 100% oxygen because of the additive effect of a relatively "acidotic" pH.

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Cardioplegic solutions induce rapid electromechanical arrest of the heart and, in conjunction with hypothermia, substantially decrease myocardial cellular oxygen demand. However, there is still a basal metabolic requirement of the cardioplegia arrested heart, approximately 0.3 ml oxygen per 100 gm/min in the dog heart at 22° C.[1] In the absence of oxygen anaerobic metabolism continues during ischemia, despite the use of cold cardioplegia. This anaerobic metabolism can be lessened by supplying oxygen, either bound to hemoglobin in blood cardioplegic solution or as dissolved oxygen in a crystalloid cardioplegic solution. The beneficial effect of oxygenating crystalloid cardioplegia, specifically St. Thomas' Hospital (ST) solution, has already been demonstrated.[2, 3] However, the introduction of any gas into a solution containing bicarbonate alters the partial pressure of carbon dioxide

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Table I. *Composition of solutions*

Composition	*Solution*		
	K-H buffer	*ST + G cardioplegia*	*FC cardioplegia*
NaCl (mmol/L)	118	110	110
$NaHCO_3$ (mmol/L)	25	10	10
KCl (mmol/L)	4.8	16	16
$MgSO_4$ (mmol/L)	1.2	—	—
$MgCl_2$ (mmol/L)	—	16	16
$CaCl_2$ (mmol/L)	1.2	1.2	1.2
Glucose (mmol/L)	11.1	11.0	11.0
KH_2PO_4 (mmol/L)	1.2	—	—
FC-43 (weight/volume)	—	—	20%

K-H buffer, Krebs-Henseleit buffer used for perfusion of isolated rat hearts; FC cardioplegia, a cardioplegic solution containing Fluosol-43 (FC-43). (An emulsion of the perfluorocarbon perfluorotributylamine and emulsifying agent Pluronic F-68, Green Cross Corporation, Osaka, Japan.)

(P_{CO_2}) in the solution, thereby producing a change in hydrogen ion concentration and thus pH. Altering the pH of a cardioplegic solution can affect functional recovery. Alkalosis in an acalcemic cardioplegic solution can result in the calcium paradox,[4] and consequently poor recovery. In addition, as the dissociation constant for any solution is temperature dependent,[5] a change in temperature alters the hydrogen ion concentration. Thus when a gas is introduced into a cardioplegic solution, both the temperature at which the gas is added and the temperature at which the solution is delivered interact to determine the final pH.

The object of this study was to determine the effect of pH on postischemic recovery with a calcium-containing cardioplegic solution. We added glucose (11 mmol/L) to ST cardioplegia (ST + G), because glucose at this concentration is beneficial, provided the end products of glycolytic metabolism are removed.[5a] To examine the effect of pH, independent of oxygen, initial experiments used the ST + G gassed with either 100% nitrogen or 95% nitrogen : 5% carbon dioxide and thereafter 100% oxygen or 95% oxygen : 5% carbon dioxide. A mixture containing 97% oxygen : 3% carbon dioxide was also investigated. Finally, we tested whether a perfluorocarbon, with its greater oxygen-carrying capacity, would be of benefit in ST + G cardioplegic solution.

Materials and methods

Preparation of hearts. The isolated perfused working rat heart model described by Neely and colleagues[6] was used. Hearts were obtained from male Long-Evans rats (250 to 400 gm). The care of the animals was in accordance with the "Principles of Laboratory Animal Care" of the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH Publication No. 80-23, revised 1978).

The animals were anesthetized with diethyl ether, the femoral vein exposed, and 200 IU of heparin injected. The hearts were then rapidly excised and placed into 4° C Krebs-Henseleit buffer solution (Table I). The aorta was cannulated within 1 minute and perfusion initiated at a constant pressure of 100 cm H_2O by the Langendorff method. Krebs-Henseleit buffer solution gassed with 95% oxygen: 5% carbon dioxide and maintained at a constant temperature of 37° C was used.

During this period the left atrium was cannulated and the pulmonary artery incised to ensure adequate ejection of coronary effluent. The hearts were stabilized for 10 minutes by Langendorff aortic retrograde perfusion before conversion to a working preparation. The left atrial filling pressure was then 20 cm H_2O, and the left ventricle ejected against a hydrostatic pressure of 100 cm H_2O. The aortic pressure was measured by a Statham P23dB pressure transducer (Spectramed Inc., Critical Care Division, Oxnard, Calif.) connected to a side arm from the aortic cannula. During the 10-minute atrial perfused working period control values for aortic and coronary flow rate, systolic aortic pressure, and heart rate were recorded. Any heart that did not achieve a stable level of function was rejected.

At the end of the working period the atrial and aortic cannulas were closed, and 10 ml of 10° C cardioplegic solution was infused at a pressure of 60 mm Hg by means of a side arm in the aortic cannula. Simultaneously the hearts were placed into a 10° C water-jacketed chamber to maintain 10° C hypothermia during ischemia.

Cardioplegic arrest and recovery. Cardioplegic arrest was maintained over a 3-hour ischemic period by infusing 6 ml of cardioplegic solution at 10° C every 30 minutes. At the end of the ischemic period each heart was reperfused for 10 minutes at 37° C in the nonworking Langendorff mode. The coronary sinus effluent during each reinfusion of cardioplegic solution and during the first and second 5-minute reperfusion periods was collected, and the oxygen tension (P_{O_2}), pH, and lactate dehydrogenase values were determined. Thereafter the heart was switched over to the working mode for another 10 minutes.

Postischemic mechanical recovery was compared with preischemic values. At the end of the experiment the hearts were freeze-clamped with Wollenberg tongs for enzymatic analysis of high-energy phosphates, expressed as micromoles per gram (wet weight). Control values for adenosine triphosphate and creatine phosphate were obtained in six hearts by freeze-clamping before the ischemic period in the experimental protocol.

Cardioplegic solutions. The composition of all solutions used is shown in Table I. The induced pH shift, produced by the con-

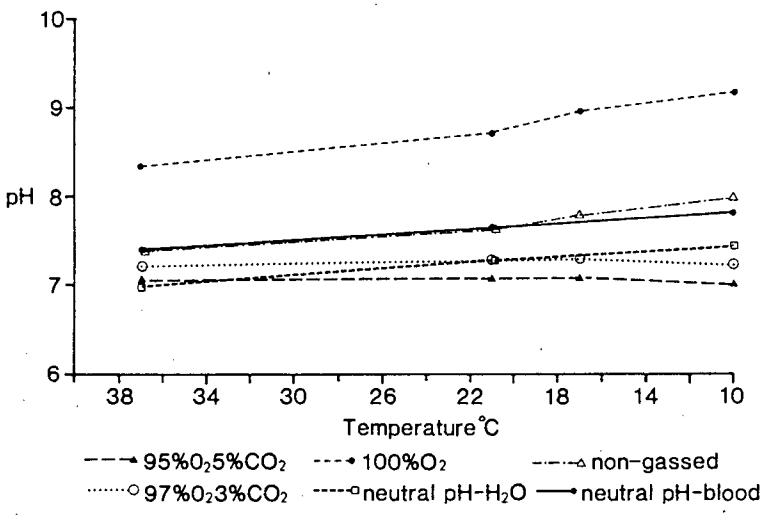

Fig. 1. The effect of temperature and oxygenation on the pH of the ST + G cardioplegic solution with varying concentrations of oxygen and carbon dioxide. Measurements made with IL system 1302 pH/blood gas analyzer (Instrumentation Laboratory System, Milan, Italy). Measurements were automatically corrected for temperature by the analyzer. In addition, the pH of intracellular neutrality and for blood with respect to temperature was calculated with the following equations: $pH/°C\ H_2O = -0.017$, and $pH/°C = -0.0147$ (Rosenthal factor), respectively.

tinuous gassing of the ST + G solution with 100% oxygen, 97% oxygen : 3% carbon dioxide, or 95% oxygen : 5% carbon dioxide was studied at different temperatures, in the range of 4° C to 37° C. Gas was added to the cardioplegic solutions for a minimum period of 30 minutes before the pH was measured with an IL system 1302 pH/blood gas analyzer (Instrumentation Laboratory System, Milan, Italy), which automatically corrected all pH measurements for temperature. The control "nongassed" ST + G solution was gassed with 95% oxygen : 5% carbon dioxide when initially prepared, and thereafter it was exposed and equilibrated to atmospheric partial pressures for at least 24 hours.

The buffering capacity of these solutions at 10° C was examined by adding incremental amounts of 1N hydrochloric acid (HCl) and recording the pH change. Titration curves were plotted and the buffering capacity (mmol HCl/L/U pH change) calculated as the instantaneous (three-point derivative) slope of the titration curve.

The perfluorocarbon cardioplegic solution tested was a solution containing Fluosol-43,[7] modified to have an electrolyte concentration identical to the ST + G solution. Bubble oxygenation of this solution produces foam, and it was therefore oxygenated and defoamed with a Bentley Bio 2 bubble oxygenator (Baxter Healthcare Corporation, Bentley Laboratories Inc., Irvine, Calif.).

Calculations and expression of results

The following indices of cardiac mechanical function were measured: aortic output (milliliters per minute), coronary flow (milliliters per minute), and heart rate (beats per minute). Cardiac output (milliliters per minute) was aortic output plus coronary flow, and stroke volume (milliliters) was cardiac output/heart rate.

Oxygen content of the cardioplegic solutions and oxygen uptake by the hearts were calculated with these formulas:

$$O_2 \text{ content (ml)} = P_{O_2} \cdot \text{Solubility coefficient}/760 \text{ mm Hg}$$

$$O_2 \text{ uptake (ml)} = (O_2 \text{ content of cardioplegic solution} - O_2 \text{ content of coronary sinus effluent})$$

The solubility coefficient for oxygen in 0.16N saline at 10° C is 0.037 ml of oxygen dissolved per milliliter of solution at 760 mm Hg pressure,[7] and for Fluosol-43 it is 0.077.[8]

Exclusion criteria. In the preischemic control period the following values were used to discard hearts: aortic output less than 30 ml/min, heart rate less than 200 beats/min or irregular rhythm, and coronary sinus flow greater than 22 ml/min.

In the postischemic period any heart with a coronary flow that increased significantly (>50% preischemic value) was excluded because of the probability of a left atrial leak.

Extreme observations or "outliers," based only on postischemic aortic output, were tested by Dixon's criteria with a significance of <5%. The entire heart was then excluded from further analysis so that the mean was not distorted by inflating the error variance as a result of major experimental errors. Values obtained during the postischemic working period were expressed as a ratio of each individual preischemic control value.

Results are presented as the mean ± the standard error of the mean. The statistical test used was the two-way analysis of variance, and if overall statistical significance was obtained, pairwise comparisons of means were done by the f test. Statistical significance was taken as $p < 0.05$.

Results

pH Shifts induced by temperature and oxygenation of a cardioplegic solution. Decreasing the temperature of the ST + G cardioplegic solution, as well as adding either 100% oxygen, 97% oxygen : 3% carbon dioxide, or 95% oxygen : 5% carbon dioxide, altered the pH of the

Table II. *Postischemic recovery of hearts and the effects of cardioplegic solution pH induced by adding gas mixtures*

	100% N_2 (n = 6)	*95% N_2: 5% CO_2 (n = 7)*	*100% O_2 (n = 8)*	*95% O_2: 5% CO_2 (n = 9)*
Cardioplegic solution				
10° C pH	9.1 ± 0.1	7.0 ± 0.01	9.3 ± 0.1	7.0 ± 0.02
Functional recovery				
Aortic output (%)	52.3 ± 2.7	63.9 ± 2.8[b]	66.3 ± 2.8[a]	88.9 ± 3.7[c, e]
Cardiac output (%)	60.8 ± 3.6	75.4 ± 3.3[a]	75.3 ± 4.1[a]	88.9 ± 2.4[c, e]
Stroke volume (%)	59.9 ± 4.6	74.2 ± 2.7[b]	74.7 ± 4.0[b]	88.2 ± 5.1[d, f]
Postischemic LDH loss (mU/min)				
0-5 min	57.6 ± 12.6	184.6 ± 15.6[a]	77.0 ± 10.9	90.0 ± 10.6[c]
5-10 min	37.5 ± 6.0	103.0 ± 10.1[a]	50.8 ± 5.7	52.0 ± 10.8[c]

Postischemic recovery expressed as mean ratios of individual preischemic values and standard error of the mean of isolated rat hearts after 3 hours of 10° C cardioplegic arrest. Hearts were protected with ST + G cardioplegic solution gassed with one of the above gases. LDH, Lactate dehydrogenase.

[a] $p < 0.01$ compared with 100% N_2 group.

[b] $p < 0.05$ compared with 100% N_2 group.

[c] $p < 0.01$ compared with 95% N_2 and 5% CO_2 group.

[d] $p < 0.05$ compared with 95% N_2 and 5% CO_2 group.

[e] $p < 0.01$ compared with 100% O_2 group.

[f] $p < 0.05$ compared with 100% O_2 group.

solution (Fig. 1). Reducing the temperature of the "ungassed" ST + G solution (aerated on preparation but thereafter allowed to equilibrate with the atmosphere) resulted in an increase in pH. At 37° C the pH was 7.4 but at 10° C it increased to 8.0, which is only marginally more alkalotic than biologic neutrality for blood (pH 7.4 at 37° C and pH 7.8 at 10° C, as ΔpH/° C for blood is −0.047, Rosenthal factor[5]).

In addition to the temperature effect, oxygenating this solution with gas mixtures containing carbon dioxide progressively decreased the pH at 10° C from 8.0 (ungassed) to 7.25 with 97% oxygen : 3% carbon dioxide and to 7.0 with 95% oxygen : 5% carbon dioxide, which is relatively acidotic with respect to intracellular neutrality (pH 7.46 at 10° C[5]). In contrast, gassing with 100% oxygen accentuated the hypothermia-induced alkalosis to pH 9.2 at 10° C (Fig. 1). Similar results were obtained with 100% nitrogen and 95% nitrogen : 5% carbon dioxide having a pH of 9.1 and 7.0, respectively (Table II).

The maximum buffering capacity of ST + G cardioplegic solution was found in the range pH 6 to 7 (Fig. 2), with the pK of the bicarbonate buffer being ±6.5. Consequently, the relatively "acidotic" solution, that is, gassed with 95% oxygen : 5% carbon dioxide would be the better buffered solution, inasmuch as its 10° C pH of 7.0 is closer to the observed pK of ±6.5 compared with the solution gassed with 100% oxygen having a 10° C pH of 9.2.

Recovery of cardioplegic arrested rat hearts

Effect of pH in the absence of oxygen. Hearts were protected with ST + G cardioplegic solution gassed with either 100% nitrogen or 95% nitrogen : 5% carbon dioxide, to determine the effect of altering pH without the addition of oxygen (Tables II and III).

The preischemic control values for the 100% nitrogen group were as follows: aortic output 47.5 ± 4.0 ml/min, cardiac output 64.5 ± 5.1 ml/min, heart rate 270 ± 9 beats/min. Two extreme observations were excluded from the original eight hearts—one highest and one lowest—and further analysis was done on the remaining six hearts. The postischemic aortic output recovery was 52.3% ± 2.7% and cardiac output 60.8% ± 3.6% with this "alkalotic" (pH = 9.1) solution.

With 95% nitrogen: 5% carbon dioxide the control values were as follows: aortic output 39.6 ± 3.62 ml/min, cardiac output 57.4 ± 3.9 ml/min, heart rate 276 ± 22 beats/min. Again two outliers were excluded from the original group of nine hearts—one highest and one lowest—and therefore seven hearts remained. Postischemic recovery of aortic output was 63.9% ± 2.8% and cardiac output 75.4% ± 3.3%. This relatively "acidotic" (pH = 7.0) cardioplegic solution (95% nitrogen : 5% carbon dioxide) was associated with significantly improved functional recovery, $p < 0.05$.

The pH of the coronary sinus effluent decreased from 9.1 ± 0.1 (arterial value) to 8.1 ± 0.1 in the group gassed with 100% nitrogen. This large pH change of 1.0 unit indicated that protons were produced and that the cardioplegic solution was poorly buffered. In contrast, when 95% nitrogen : 5% carbon dioxide were added, the pH of the coronary sinus effluent (7.1 ± 0.01) was relatively unaltered compared with the arterial pH of

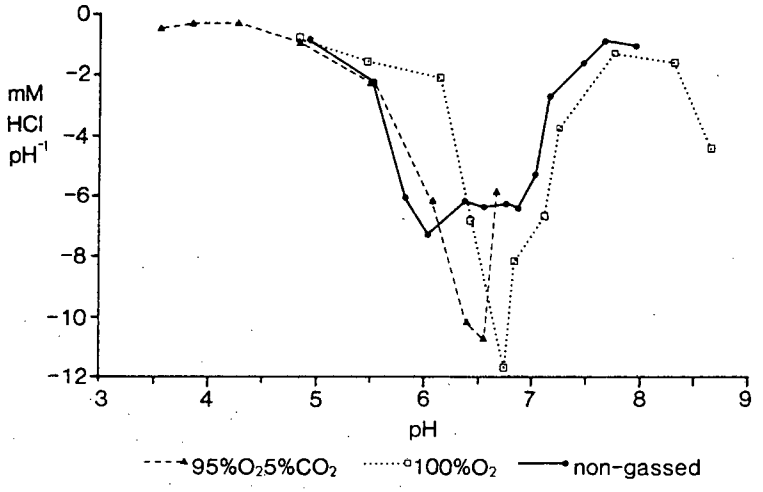

Fig. 2. The buffering capacity of the nongassed ST + G cardioplegic solution and alternatively ST + G oxygenated with either 100% oxygen or 95% oxygen and 5% carbon dioxide is shown. Buffering capacity is expressed as the instantaneous (three-point derivative) slope of the titration curve. Solution was titrated with 1N HCl at 10° C.

Table III. *Metabolic effects of cardioplegic solution pH induced by adding gas mixtures*

	100% N_2 (n = 6)	*95% N_2: 5% CO_2 (n = 7)*	*100% O_2 (n = 8)*	*95% O_2: 5% CO_2 (n = 9)*
Cardioplegic solution				
10° C pH	9.1 ± 0.1	7.0 ± 0.01	9.3 ± 0.1	7.0 ± 0.02
10° C Po_2 (mm Hg)			629 ± 11	640 ± 26
O_2 content (ml O_2/6 ml)			0.184 ± 0.003	0.187 ± 0.008
Coronary sinus effluent during 180 min ischemia				
pH	8.1 ± 0.1	7.1 ± 0.01	8.4 ± 0.1	7.1 ± 0.02
LDH (mU/ml)	9.6 ± 1.3	10.9 ± 0.9	11.9 ± 0.9	7.3 ± 0.5[a,c]
Lactate (μmol/ml)	0.80 ± 0.08	0.30 ± 0.02[b]	0.35 ± 0.02[b]	0.25 ± 0.02
O_2 uptake[d] (ml O_2/dose)			0.080 ± 0.002	0.097 ± 0.004[c]

Loss of metabolites during 3 hours of 10° C cardioplegic arrest expressed as mean and standard error of the mean of isolated rat hearts protected with ST + G cardioplegic solution gassed with one of the above gases. The solution was infused at the onset of ischemia and every 30 minutes throughout this period. Po_2, O_2 tension; LDH, lactate dehydrogenase.

[a] $p < 0.01$ compared with 95% N_2 and 5% CO_2 group.

[b] $p < 0.01$ compared with 100% N_2 group.

[c] $p < 0.01$ compared with 100% O_2 group.

[d] Expressed as mean volume of O_2 (milliliters) taken up by the hearts from each 6 ml reinfusion dose of maintenance cardioplegia.

7.0 ± 0.01, indicating that either fewer protons were formed or that there was better buffering at pH 7.0.

Effect of pH in the presence of oxygen. Similar pH changes were seen when nitrogen was replaced with oxygen. Hearts were protected with ST + G cardioplegic solution gassed with either 100% oxygen or 95% oxygen : 5% carbon dioxide (Tables II and III).

Preischemic control values for the 100% oxygen group were as follows: aortic output 43.3 ± 2.5 ml/min, cardiac output 60.4 ± 2.7 ml/min, heart rate 263 ± 12 beats/min. Three outliers were excluded from the original group of 11 hearts—two lowest and one highest—and therefore eight hearts remained. Postischemic recovery of aortic output was 66.3% ± 2.8% and cardiac output 75.3% ± 4.1%.

For the 95% oxygen : 5% carbon dioxide group, control values were as follows: aortic output 43.1 ± 2.4 ml/min, cardiac output 60.3 ± 2.5 ml/min, heart rate 273 ± 17 beats/min. No outliers were detected, and all nine hearts were analyzed. Postischemic aortic output recovery was 88.9% ± 3.7% and cardiac output 88.9% ± 2.4%. This relatively "acidotic" (pH = 7.0) cardioplegic solution (95% oxygen : 5% carbon dioxide) produced a significantly better postischemic functional recovery than the "alkalotic" (pH = 9.3) solution (100% oxygen), $p < 0.01$.

Table IV. *Postischemic recovery of hearts and effects of cardioplegic solution pH and oxygen content*

	ST + G with 97% O_2: 3% CO_2 (n = 7)	*ST + G with 95% O_2: 5% CO_2 (n = 11)*	*FC cardioplegia with 95% O_2: 5% CO_2 (n = 6)*
Cardioplegic solution			
10° C pH	7.25 ± 0.03	7.0 ± 0.02	7.1 ± 0.01
10° C Po_2 (mm Hg)	618 ± 20	620 ± 15	490 ± 14
O_2 content (ml O_2/100 ml)	3.01 ± 0.10	3.02 ± 0.07	4.96 ± 0.14[a]
Preischemic control values			
AO (ml/min)	38.9 ± 1.7	46.2 ± 1.1	47.2 ± 2.4
CO (ml/min)	55.4 ± 2.0	63.1 ± 1.4	65.5 ± 3.2
HR (beats/min)	262 ± 15	261 ± 8	278 ± 13
Postischemic functional recovery (%)			
AO (%)	84.0 ± 1.6	92.1 ± 1.2[b]	91.2 ± 0.8
CO (%)	87.3 ± 1.5	91.3 ± 1.2[c]	90.2 ± 0.9
SV (%)	89.2 ± 4.8	93.3 ± 4.5	91.4 ± 0.5
Preservation of high-energy phosphates			
ATP (μmol/gm)	3.38 ± 0.11	3.51 ± 0.09	3.24 ± 0.20
CP (μmol/gm)	4.54 ± 0.23	5.17 ± 0.24	3.98 ± 0.37[d]

Functional and metabolic recovery expressed as mean ratios of individual preischemic values and standard error of the mean of isolated rat hearts after 3 hours of 10° C cardioplegic arrest. Hearts were protected with ST + G cardioplegic solution oxygenated with O_2 and either 3% or 5% CO_2 or with a perfluorocarbon containing cardioplegic solution having an electrolyte concentration identical to ST + G. Control values for ATP and CP were obtained by freeze-clamping six hearts before the ischemic period in the experimental protocol. Controls (n = 6), ATP 3.91 ± 0.25 μmol/gm, CP 5.05 ± 0.46 μmol/gm. FC, Perfluorocarbon; Po_2, O_2 tension; AO, aortic output; CO, cardiac output; SV, stroke volume; ATP, adenosine triphosphate; CP, creatine phosphate.

[a] $p < 0.001$ compared with ST + G and 95% O_2 and 5% CO_2.

[b] $p < 0.01$ compared with ST + G and 97% O_2 and 3% CO_2.

[c] $p < 0.05$ compared with ST + G and 97% O_2 and 3% CO_2.

[d] $p < 0.02$ compared with ST + G and 95% O_2 and 5% CO_2.

In the group with 100% oxygen the arterial pH of 9.3 ± 0.1 decreased to 8.4 ± 0.1 in the coronary sinus effluent, whereas with 95% oxygen : 5% carbon dioxide the arterial pH of 7.0 ± 0.02 was relatively unaltered (Table III).

Providing oxygen with or without carbon dioxide, thus independent of any alterations in pH, resulted in improved functional recovery of all indices of mechanical recovery of aortic output, cardiac output ($p < 0.01$), and stroke volume ($p < 0.05$) compared with the anoxic (nitrogen) pH equivalent groups (Table II).

A separate group of experiments with an identical protocol, and therefore tabulated separately, was performed to further define the optimal pH range (Table IV). The pH was altered when the cardioplegic solution was gassed with either 97% oxygen : 3% carbon dioxide ($n = 7$) to pH 7.25 or again with 95% oxygen : 5% carbon dioxide ($n = 11$) to pH 7.0 at 10° C. The preischemic control values were similar to the previous groups, and no outliers were detected (Table IV).

The more acidotic solution (95% oxygen : 5% carbon dioxide) again gave the best postischemic recovery, an aortic output of 92.1% ± 1.2% and cardiac output of 91.3% ± 1.2% compared with the solution to which 97% oxygen : 3% carbon dioxide was added, with an aortic output of 84.0% ± 1.6% and cardiac output of 87.3% ± 1.5%, $p < 0.01$ and $p < 0.05$, respectively. This confirmed our initial data that pH 7.0 at 10° C is the optimal pH for the oxygenated ST + G cardioplegic solution.

Lactate and lactate dehydrogenase loss associated with cardioplegic pH. The loss of lactate in the absence of oxygen in the coronary sinus effluent during each infusion of cardioplegic solution gives an indication of the rate of glycolysis. The "acidotic" pH (95% nitrogen : 5% carbon dioxide) had a decreased efflux of lactate, 0.30 ± 0.02 μmol/ml as opposed to 0.80 ± 0.08 μmol/ml with the "alkalotic" cardioplegic solution (100% nitrogen), $p < 0.01$.

In contrast, in the presence of oxygen, and thus possible lactate-consuming aerobic metabolism, the lactate efflux was equivalent in both the "acidotic" (95% oxygen : 5% carbon dioxide) and "alkalotic" (100% oxygen) groups (Table III). The loss of lactate dehydrogenase (an indicator of damage to membrane integrity) during the cardioplegic arrest period was no different in the absence of oxygen. However, in the presence of oxygen the "acidotic" (pH 7.0) solution (95% oxygen : 5% carbon dioxide) was associated with diminished loss (Table III).

In the postischemic period the total 10 minutes of lactate dehydrogenase release in the absence of oxygen was increased with the "acidotic" solution (95% nitrogen : 5% carbon dioxide) to 143.8 ± 14.4 mU/min, as opposed to the "alkalotic" cardioplegic solution (100% nitrogen) of

47.6 ± 7.4 mU/min, $p < 0.01$. However, when nitrogen was replaced with oxygen this elevated lactate dehydrogenase loss seen with 95% nitrogen : 5% carbon dioxide decreased to 71.0 ± 9.2 mU/min with 95% oxygen : 5% carbon dioxide, $p < 0.01$, which was no different from the "alkalotic" (100% oxygen) group. In all groups the postischemic lactate dehydrogenase loss was highest in the first 5 minutes, which then decreased by ± 40% in the next 5 minutes.

Oxygen uptake during cardioplegic arrest. The oxygen content of the coronary sinus effluent, produced during each 6 ml reinfusion of cardioplegic solution, was measured to determine the oxygen uptake of the myocardium during hypothermic cardioplegic arrest. More oxygen was taken up by the hearts oxygenated with 95% oxygen : 5% carbon dioxide, 0.097 ± 0.004 ml oxygen per dose, as opposed to 0.080 ± 0.002 ml oxygen per dose if gassed with 100% oxygen, $p < 0.01$. This occurred despite the fact that the oxygen content of the infused "arterial" cardioplegic solution was similar in both groups (Table III). Specifically noteworthy is the observation that only 44% to 52% of available oxygen was actually taken up.

Addition of perfluorocarbon to the ST + G cardioplegic solution. We protected a final group of hearts with similar control preischemic values and no outliers with perfluorocarbons added to the cardioplegic solution ($n = 6$) (Table IV). Oxygenation of this solution with 95% oxygen : 5% carbon dioxide resulted in a 10° C pH of 7.1 ± 0.01 and a Po_2 of 490 ± 14 mm Hg. Despite this lower Po_2, the perfluorocarbon solution contained more oxygen, 4.96 ± 0.14 ml oxygen per 100 ml solution, than the oxygenated ST + G solution containing 3.02 ± 0.07 ml oxygen per 100 ml solution, $p < 0.001$. Perfluorocarbon cardioplegia with its greater oxygen content did not improve functional recovery of either aortic output, cardiac output, or stroke volume. In addition, although the postischemic creatine phosphate content was lower (3.98 ± 0.37 μmol/gm) compared with the ST + G cardioplegia (5.17 ± 0.24 μmol/gm, $p < 0.02$), the adenosine triphosphate and creatine phosphate contents of both groups were no different from the control concentrations (Table IV). The addition of perfluorocarbon to the ST + G cardioplegic solution conferred no extra benefit.

Discussion

We studied the effect of the pH of a cardioplegic solution on postischemic recovery in the isolated rat heart. In the absence of oxygen a relatively acidotic cardioplegic solution, induced by adding 95% nitrogen : 5% carbon dioxide, improved functional recovery. However, at this "acidotic" pH (10° C pH = 7.0) glycolysis was less, as evidenced by a diminished efflux of lactate. Either energy demand was lower or alternatively glycolysis was inhibited, as previous studies have demonstrated that a pH of 6.8 at 37° C (10° C pH <7.25) inhibits glycolysis.[9]

In the anaerobic heart the primary source of energy is from glycolysis, and inhibition could result in sarcolemma injury, manifested by the observed increased postischemic loss of lactate dehydrogenase in the 95% nitrogen : 5% carbon dioxide group. Our results further suggest that sarcolemma injury occurred on reperfusion after protection with an "acidotic" anoxic solution, inasmuch as lactate dehydrogenase loss during ischemia was similar in both groups. However, other advantageous "acidotic" pH effects contributed to an overall improved postischemic recovery, despite these observed detrimental effects with the anoxic cardioplegic solution.

Hearts protected with a cardioplegic solution gassed with oxygen with or without carbon dioxide as opposed to nitrogen with or without carbon dioxide showed improved recovery. The high lactate efflux seen when 100% nitrogen was added to the cardioplegic solution diminished when 100% oxygen ($p < 0.01$), was added, which suggested that lactate was now being utilized by oxidative phosphorylation. We also demonstrated that the increased postischemic lactate dehydrogenase loss, associated with possible diminished glycolysis seen in the anoxic "acidotic" (95% nitrogen : 5% carbon dioxide) group, was also reversed by the addition of oxygen (95% oxygen : 5% carbon dioxide). Oxygen, by allowing more efficient aerobic metabolism, was therefore beneficial in a cardioplegic solution.

In the presence of oxygen the "acidotic" cardioplegic solution pH, induced by gassing with 95% oxygen : 5% carbon dioxide, improved functional recovery and diminished lactate dehydrogenase loss during ischemia. This beneficial effect was not caused by a difference in Po_2 inasmuch as both the acidotic (95% oxygen : 5% carbon dioxide) and alkalotic (100% oxygen) solutions had equivalent oxygen content. The full beneficial effect of oxygenating a cardioplegic solution can thus be altered by factors such as an induced pH shift, consequent to the oxygenating gas mix used.

The oxygen consumption for an average 1 gm rat heart during 30 minutes of 10° C cardioplegic arrest, based on a basal metabolic demand of 0.3 ml oxygen/100 gm/min,[1, 10] would be ± 0.09 ml oxygen/30 min. We demonstrated that the oxygen taken up by the hearts on each reinfusion of cardioplegic solution approximated this value and was only ± 50% of the available oxygen dissolved in the cardioplegic solution. Previous studies[11, 12] have shown perfluorocarbon cardioplegia to be superior to crystalloid and blood cardioplegia. However, as these crystalloid solutions were not oxygenated and had differ-

ent electrolyte compositions, these studies only demonstrated the beneficial effect of supplying oxygen. We were able to show that the increased oxygen-carrying capacity of perfluorocarbon cardioplegia, with an identical electrolyte composition to that of a good oxygenated crystalloid solution (ST + G), conferred no extra protection. Therefore, as in the study by Tabayashi and colleagues,[13] we cannot recommend the use of perfluorocarbon in a cardioplegic solution. In addition, it has the risk of adverse reactions and requires a more complex oxygenation and delivery system.

The "correct" systemic pH during hypothermic cardiopulmonary bypass has been a contentious point in the literature.[5, 14] Ectotherms ("cold-blooded" animals) tend to maintain biologic neutrality, defined as $OH^-/H^+ = 1$, in relation to temperature. Biologic neutrality at 37° C is pH 7.4; however, as the dissociation constant for water decreases with diminishing temperature, thus reducing the concentration of hydrogen ions, the neutral pH at 10° C will be 7.8.[5] This optimizes enzymatic reactions[15] and helps maintain constant cellular volume.[14] In contrast, hibernating endotherms ("warm-blooded" animals) maintain a blood pH 7.4 *regardless of temperature.* Hence a pH of 7.4 at 10° C is relatively acidotic with respect to biologic neutrality (pH of 7.8 at 10° C), with consequent inhibition of metabolism and therefore possible conservation of energy.[16] Although biologic neutrality and its advantages might be appropriate for hypothermic systemic pH, the aim of cardioplegia-induced ischemic arrest is to diminish metabolic demand maximally. Therefore the "metabolic inhibitory" effect of a relatively acidotic pH might be more appropriate for a cardioplegic solution.

Intracellular pH can be rapidly modulated by the free sarcolemma passage of carbon dioxide and therefore a so-called respiratory acidosis has a more pronounced effect than a "metabolic" acidosis.[17] A reduction of intracellular pH markedly decreases sarcolemma calcium transport by sodium/calcium exchange[18] and decreases potassium efflux.[17] Furthermore, calcium binding to the phospholipid of sarcolemma sites is pH dependent: At a 37° C pH of 5.5 there is 0% calcium binding; at pH 7.0, 50%; and at pH 8.5, 100% binding.[19] In contrast, extracellular alkalosis could promote intracellular calcium accumulation by sodium/hydrogen and calcium/sodium exchange.[20] These pH-dependent ionic effects would support the use of a slightly acidotic cardioplegic solution.

Our cardioplegic solution ST + G contained glucose (11 mmol/L), which has been postulated to be damaging because of glycolytic lactate and proton formation.[21] In the anoxic groups the diminished functional recovery of pH 9.1 (100% nitrogen)–protected hearts was associated with increased lactate efflux. Therefore the superior postischemic function associated with pH 7.0 (95% nitrogen : 5% carbon dioxide) might have been caused by inhibition of glycolysis at this pH. However, in the oxygenated groups the lower recovery of pH 9.3 (100% oxygen)–compared with pH 7.0 (95% oxygen : 5% carbon dioxide)–protected hearts, was not associated with increased lactate formation. Therefore we do not believe the presence of glucose accounted for the poor recovery at high pH in the oxygenated solutions.

Reservations

These experiments were performed on the isolated rat heart model, which differs physiologically from the human. In particular, the presence of noncoronary collateral flow in the human would provide some glucose in addition to washout of metabolic end products between multidose cardioplegia. Hence care must be taken when extrapolating to the clinical situation.

Conclusion

A relatively acidotic pH of the ST + G cardioplegic solution at 10° C was superior to a more alkalotic pH. Improved postischemic recovery was shown in a 3-hour isolated rat heart model with intermittent reinfusions of cardioplegic solution given throughout the ischemic period. Providing oxygen, dissolved in the cardioplegic solution, was beneficial, and furthermore, oxygenation with 95% oxygen : 5% carbon dioxide (which induces an "acidotic" pH shift) was superior to 100% oxygen. The addition of a perfluorocarbon, an oxygen-carrying vehicle, to the optimally gassed ST solution confers no additional protection.

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Appendix A—4

Effect of pH shifts induced by oxygenating crystalloid cardioplegic solutions.

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I. Experimental

Effect of pH Shifts Induced by Oxygenating Crystalloid Cardioplegic Solutions

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Oxygenation of a bicarbonate-containing crystalloid cardioplegic solution alters the partial pressure of both oxygen (O_2) and carbon dioxide (CO_2). Therefore, oxygenating St. Thomas' Hospital II plus glucose (11 mmol/L) cardioplegic solution with 95% O_2 + 5% CO_2 induces a pH shift to 7.0 (10°C) as opposed to pH 9.3 with 100% O_2. In an isolated working rat heart model, we show that pH 7.0 (10°C) improves mechanical postischemic recovery in the absence or presence of O_2. However, in the absence of O_2, pH 7.0 appears to inhibit glycolysis and diminish the stability of cellular membranes. The provision of O_2 independently improved mechanical recovery and at pH 7.0, improved the preservation of the sarcolemma. Increasing the O_2 content by including a perfluorocarbon (FC-43) in the oxygenated St. Thomas' plus glucose cardioplegia is not additionally beneficial. St. Thomas' Hospital plus glucose cardioplegic solution should be oxygenated, but with 95% O_2 + 5% CO_2 and not 100% O_2.

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Oxygenating St. Thomas' Hospital II cardioplegic solution has previously been shown to improve postischemic myocardial recovery [1]. The more efficient aerobic metabolic pathways are able to produce a greater amount of high-energy phosphate, which can then be used to meet the ongoing basal metabolic requirements of the arrested myocardium [2].

The partial pressures of gases in a solution are dependent on those to which the solution is exposed. Oxygenating a crystalloid cardioplegic solution will increase the partial pressure of oxygen but must also alter the partial pressures of the other gases contained in the solution, as the total pressure of a mixture of gases cannot exceed ambient pressure (Dalton's law) [3]. Thus oxygenation will alter the partial pressure of O_2 and, also of specific interest, partial pressure of carbon dioxide. In turn, the total CO_2 content will be dependent on the partial pressure of CO_2 as well as its temperature-dependent solubility coefficient for that solution (Henry's law) [3, 4]. Furthermore, if the primary buffer in the cardioplegic solution is HCO_3, any change in the total CO_2 content will change the pH, which is the negative log[H^+], according to the following equation:

$$H_2O + CO_2 \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

Therefore, the pH of a bicarbonate-containing cardioplegic solution will be altered by both a change in temperature and a change in oxygenation.

We examined the effect of oxygenating St. Thomas' Hospital II plus glucose (11 mmol/L) cardioplegic solution (ST+G), which has the following composition:

Na 120 mmol/L
K 16 mmol/L
Mg 16 mmol/L
Ca 1.2 mmol/L
HCO_3 10 mmol/L
Glucose 11 mmol/L

This solution has a pH of 7.8 at room temperature. However, oxygenating with 100% O_2 displaced CO_2 and therefore shifted the pH to 9.3 (10°C). However, when 95% O_2 + 5% CO_2 was used, the addition of CO_2 induced a shift in pH to 7.0 (10°C). Oxygenation with either 100% O_2 or 95% O_2 + 5% CO_2 produced similar O_2 tensions of ± 635 mm Hg (at 10°C).

To examine the effect of pH independent of O_2, ST+G solution was also gassed with either 100% N_2 or 95% N_2 + 5% CO_2, which induced pH shifts similar to those just mentioned [5]. Finally, we also determined the effect of increasing the O_2 content of the oxygenated ST+G solution by including an O_2 carrier, 20% wt/vol perfluorocarbon (FC-43) [6], in an otherwise identical solution [5].

Material and Methods

The experimental model used was the isolated, perfused, working rat heart as previously described [5]. In brief, hearts were removed from anesthetized male Long-Evans

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rats, cannulated, and perfused retrogradely with Krebs-Henseleit buffer for 10 minutes. In the following 10-minute working period (left atrial pressure, 20 cm H_2O; afterload, 100 cm H_2O), control values for cardiac output and stroke volume were determined. The hearts were then arrested with 10 mL of the appropriately gassed ST+G cardioplegic solution (infusion pressure, 60 mm Hg). During the ensuing 3-hour ischemic period, the hearts were maintained at 10°C, and reinfusions of cardioplegia (6 mL) were given every 30 minutes. Lactate levels, lactate dehydrogenase (LDH) levels, and partial pressure of O_2 in the coronary sinus effluent during each reinfusion of cardioplegia were measured. A 10-minute Langendorff reperfusion period followed, during which coronary sinus LDH washout in each 5-minute interval was again determined. Assessment of hemodynamic recovery was made at the end of a subsequent 10-minute working period.

Postischemic hemodynamic values were expressed as a ratio of each individual preischemic control value. Percentage means and standard errors of means were analyzed by two-way analysis of variance, with pairwise comparisons by acceptance intervals of means by the *F* test. Significance was taken as a *p* value of less than 0.05.

Results and Comment

Effect of pH on Mechanical and Metabolic Postischemic Recovery

The preischemic control values were similar between the four groups (95% O_2 + 5% CO_2, n = 9; 100% O_2, n = 8; 95% N_2 + 5% CO_2, n = 7; 100% N_2, n = 6) and ranged from 57.4 ± 3.9 mL/min to 64.5 ± 5.1 mL/min for cardiac output and from 0.212 ± 0.012 mL to 0.238 ± 0.015 mL for stroke volume [5]. A pH of 7.0 (10°C) in the ST+G cardioplegic solution, induced by gassing with 5% CO_2, was associated with superior postischemic recovery of both stroke volume ($p < 0.05$) and cardiac output (95% N_2 + 5% CO_2, 75.4% ± 3.3%; 95% O_2 + 5% CO_2, 88.9% ± 2.4%) compared with pH 9.1 (100% N_2, 60.8% ± 3.6%; $p < 0.01$) or pH 9.3 (100% O_2, 75.3% ± 4.1%; $p < 0.01$) (Fig 1). Thus a cardioplegic solution with a pH of 7.0, which is comparatively acidotic to intracellular pH 7.46 (corrected for temperature 10°C), improved mechanical recovery in the absence or presence of O_2. In addition, the use of O_2 as opposed to N_2 also improved functional recovery ($p < 0.05$) independent of any pH effect (see Fig 1).

A number of mechanisms can explain the observed beneficial effect of a relatively acidotic cardioplegic solution other than inhibition of enzyme-dependent energy-consuming metabolic processes and therefore conservation of energy [7]. A respiratory acidosis rapidly alters intracellular pH as CO_2 passes freely across the sarcolemma, and slight acidosis decreases K^+ efflux, inhibits Na^+/Ca^{2+} exchange, and diminishes sarcolemmal calcium transport [8, 9]. These ionic effects would be beneficial during cardioplegic arrest, when attempts must be made to prevent the redistribution of ions and specifically to prevent intracellular calcium accumulation [10]. In contrast, extracellular alkalosis promotes intracellular calcium

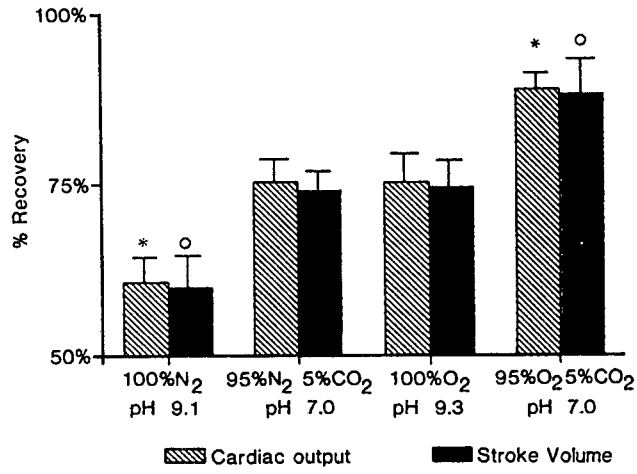

Fig 1. Effect of cardioplegic solution pH on postischemic mechanical recovery, expressed as mean ratios of individual preischemic values, of isolated rat hearts after 3 hours of 10°C cardioplegic arrest. Hearts were protected with multidose St. Thomas' Hospital plus glucose (11 mmol/L) cardioplegic solution gassed with 100% N_2 (n = 6), 95% N_2 + 5% CO_2 (n = 7), 100% O_2 (n = 8), or 95% O_2 + 5% CO_2 (n = 9), which induced the indicated pH shifts. The vertical bars represent the standard errors of the means. (= $p < 0.01$ compared with 95% N_2 + 5% CO_2 and 100% O_2; o = $p < 0.05$ compared with 95% N_2 + 5% CO_2 and 100% O_2.)*

accumulation by Na^+/H^+ and Na^+/Ca^{2+} exchange [11]. Furthermore, in contrast to ST+G, if a crystalloid cardioplegic solution is calcium free, the pH must be acidotic, as alkalosis increases the propensity for the calcium paradox to occur [12].

Comparisons in Anoxia

In the absence of O_2 the efflux of lactate from the coronary sinus, consequent to each reinfusion of cardioplegia, approximates the rate of glycolysis. We observed that the "acidotic" pH 7.0 (95% N_2 + 5% CO_2) was associated with a diminished mean efflux of coronary sinus lactate (0.30 ± 0.02 μmol/mL, n = 35 samples) during the cardioplegic arrest period compared with pH 9.1 (0.80 ± 0.08 μmol/mL, n = 29 samples, 100% N_2; $p < 0.01$), thus implying inhibition of glycolysis at pH 7.0 at 10°C. This is in accord with a previous study [13] that demonstrated that a pH of 6.8 at 37°C (pH < 7.25 corrected for temperature 10°C) inhibits hypoxic-accelerated glycolysis.

Hearse and co-workers [14] suggested that the inclusion of glucose in a cardioplegic solution is harmful because of accumulation of inhibitory metabolites, lactate, and protons. Therefore, it could be postulated that in the absence of O_2 (95% N_2 + 5% CO_2), the inhibition of glycolysis by pH 7.0 partly accounted for its associated improved functional recovery by preventing lactate accumulation in addition to possible inhibition of calcium entry into the cell. However, we observed increased postischemic washout of LDH, 143.8 ± 14.4 mU/min (n = 14 samples), with pH 7.0 (95% N_2 + 5% CO_2) as opposed to 47.6 ± 7.4 mU/min (n = 10 samples) ($p < 0.01$) with pH 9.1 (100% N_2). This increased postischemic LDH loss associated

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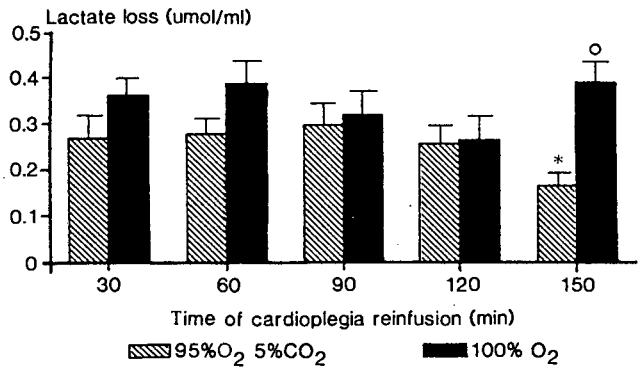

Fig 3. Effect of cardioplegic solution pH on washout of lactate with each reinfusion of cardioplegia. The mean washout in the coronary sinus effluent consequent to each multidose reinfusion of St. Thomas' plus glucose cardioplegic solution administered every 30 minutes during a 3-hour 10°C cardioplegic arrest period is indicated. The cardioplegic solution was oxygenated with either 95% O_2 + 5% CO_2 (pH 7.0) or 100% O_2 (pH 9.3). The vertical bars *represent the standard errors of the means. (* = $p < 0.01$ compared with 100% O_2 at that time interval; o = $p < 0.05$ compared with the respective 120-minute interval.)*

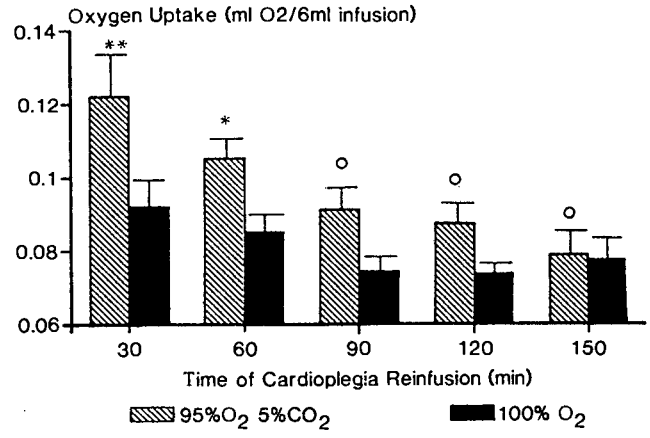

Fig 4. Effect of cardioplegic solution pH on myocardial oxygen (O_2) uptake from each reinfusion of cardioplegia. The mean amount of O_2 taken up by isolated rat hearts during each reinfusion of St. Thomas' Hospital plus glucose cardioplegic solution is shown. The cardioplegic solution was oxygenated with either 95% O_2 + 5% CO_2 (pH 7.0) or 100% O_2 (pH 9.3) and was reinfused every 30 minutes throughout the 3-hour ischemic arrest period. The vertical bars *represent the standard errors of the means. The initial O_2 content of both cardioplegic solutions was 0.18 mL O_2/6 mL solution before infusion. (* = $p < 0.05$ compared with 100% O_2 at that time interval; ** = $p < 0.01$ compared with 100% O_2 at that time interval; o = $p < 0.01$ compared with the respective 30-minute interval.)*

appear that the inclusion of glucose was no longer harmful at pH 7.0 in the presence of O_2. Our study therefore supports the use of a relatively acidotic pH for the cardioplegic solution. Furthermore, the presence of O_2 was synergistic to the relatively acidotic pH in improving postischemic mechanical myocardial recovery.

Utilization of O_2 in Oxygenated Crystalloid Cardioplegic Solutions

The O_2 contents of both oxygenated solutions (95% O_2 + 5% CO_2 and 100% O_2) were similar at 3 mL O_2/100 mL solution. The mean O_2 uptake for pH 7.0 (95% O_2 + 5% CO_2) was 0.097 ± 0.004 mL O_2/6 mL dose (n = 39 samples), which was greater than for pH 9.3 (100% O_2), 0.08 ± 0.002 mL O_2/6 mL dose (n = 39 samples) ($p < 0.01$). However, the amount of O_2 taken up by the myocardium was only approximately 50% of the available O_2 in the solution. Early in ischemia, more O_2 was taken up by the myocardium when it was oxygenated with 95% O_2 + 5% CO_2 ($p < 0.01$), but this O_2 uptake diminished with each subsequent multidose reinfusion of cardioplegia (Fig 4). However, the O_2 uptake with 95% O_2 + 5% CO_2 tended to remain higher at each reinfusion compared with 100% O_2 (pH 9.3) (see Fig 4). Thus oxidative metabolism decreased over time with 95% O_2 + 5% CO_2, but there did not appear to be any compensatory increase in anaerobic glycolysis with pH 7.0 (95% O_2 + 5% CO_2), as lactate washout did not increase during the ischemic period (see Fig 3). At pH 9.3 (100% O_2), O_2 uptake at the end of the ischemic period was no different from the initial O_2 uptake, and similarly lactate efflux remained constant.

Our observations suggest that a defect in O_2 utilization occurs in the cardioplegia-protected ischemic period and may occur more readily at pH 9.3. These abnormalities in mitochondrial O_2 metabolism may be similar to those previously identified in the postischemic reperfusion period [16]. It is possible that these mitochondrial defects are due to lactate, as lactate ions can produce morphological mitochondrial abnormalities within 10 minutes (37°C) of exposure [17]. Furthermore, in our study, oxygenated cardioplegia was infused only intermittently (every 30 minutes) during the ischemic period, at which time lactate loss was measured. Therefore it is possible that in the intervening periods, transient lactate fluxes similar to those observed with the anoxic groups occurred. The tendency for lactate loss to be higher with pH 9.3 (see Fig 3) might be an important correlation.

The addition of perfluorocarbon (FC-43), an O_2 carrier that releases O_2 linearly with changes in partial pressure of O_2, increased the O_2 content of the oxygenated ST+G solution (95% O_2 + 5% CO_2) from 3 mL O_2/100 mL solution to 5 mL O_2/100 mL solution. The ST+G + FC-43 cardioplegic solution (n = 6, pH 7.1) gave a postischemic recovery of cardiac output of 90.2% ± 0.9% and stroke volume of 91.4% ± 0.5%, which were not significantly different from the cardiac output of 91.3% ± 1.2% and stroke volume of 93.3% ± 4.5% with the ST+G solution (n = 11, pH 7.0) [5]. This finding may indicate that sufficient O_2 was contained in the standard ST+G solution gassed with 95% O_2 + 5% CO_2. Previous studies [18, 19] suggested that cardioplegic solutions containing perfluorocarbons are superior; however, the crystalloid cardioplegic solutions in those studies were not oxygenated, and the electrolyte compositions of the test solutions with or without perfluorocarbons were not kept constant. We have shown that the addition of perfluorocarbons to an otherwise identical solution is not beneficial.

with the "acidotic" solution suggests that sarcolemmal injury became manifest during the reperfusion period, as LDH is a measure of cellular membrane integrity. It has been suggested that the cell membrane is maintained by glycolytic-derived adenosine triphosphate (ATP) because of functional compartmentation [15] and that inhibition of energy production in the presence of continued energy requirements might therefore be detrimental. Thus anoxia and inhibition of anaerobic-accelerated glycolysis by this acidotic pH 7.0 (10°C) were possibly harmful to the cellular membrane, despite the improved postischemic functional recovery observed.

Comparisons at pH 9

In the presence of O_2, a large amount of the pyruvate produced would be oxidized by aerobic metabolism, thus removing the inhibitory effects of protons and lactate on glycolysis. Consequently, the efflux of lactate will grossly underestimate the rate of glycolysis in the presence of ongoing aerobic metabolism.

At pH 9, we observed that the addition of O_2 reduced the mean lactate efflux by more than 50% from 0.8 ± 0.08 μmol/mL (n = 29 samples, 100% N_2) to 0.35 ± 0.02 μmol/mL (n = 38 samples, 100% O_2) ($p < 0.01$). Assuming similar rates of glycolysis at pH 9, if not higher in the presence of O_2, as inhibitory effects of glycolysis are being removed by aerobic metabolism, then the total amount of ATP being generated in the aerobic hearts would be considerably higher. (For each molecule of extracellular glucose metabolized through glycolysis, a net number of two ATP molecules are generated, and aerobic metabolism produces an additional 36 ATP molecules.) Lactate dehydrogenase loss during ischemia (9.6 ± 1.3 mU/mL, 100% N_2; 11.9 ± 0.9 mU/mL, 100% O_2) and reperfusion (47.6 ± 7.4 mU/min, 100% N_2; 66.5 ± 7.9 mU/min, 100% O_2) was similar in both anaerobic and aerobic conditions at pH 9, a finding possibly indicating sufficient glycolytic ATP to maintain sarcolemmal integrity in both cases. Furthermore, the additional improved recovery in the presence of O_2 at this pH was probably due to increased ATP production as well as removal of potentially harmful end-products of glycolysis by aerobic metabolism.

Comparisons at pH 7

At pH 7.0, lactate efflux was similar for both anaerobic (0.30 ± 0.02 μmol/mL, n = 35 samples, 95% N_2 + 5% CO_2) and aerobic (0.25 ± 0.02 μmol/mL, n = 44 samples, 95% O_2 + 5% CO_2) conditions. However, the true rate of glycolysis was probably considerably higher in the presence of O_2, as glycolysis is less susceptible to inhibition by a low pH in the presence of O_2 as opposed to anoxia and has been previously shown to be inhibited only below a pH of 6.5 at 37°C (pH 6.95 at 10°C) if O_2 is present [13]. Aerobic metabolism would remove glycolytic end-products and consequently their inhibitory effects, as already mentioned. Thus it is also reasonable to assume increased ATP production at pH 7.0 in the presence of O_2.

Furthermore, LDH loss during ischemia was diminished at pH 7.0 in the presence of O_2, 7.3 ± 9.2 mU/mL (n = 45 samples, 95% O_2 + 5% CO_2) as opposed to 10.9 ± 0.9 mU/mL (n = 35 samples, 95% N_2 + 5% CO_2) ($p < 0.01$). Moreover, during reperfusion, LDH release was also diminished in the aerobic hearts, 71.0 ± 9.2 mU/min (95% O_2 + 5% CO_2, n = 12 samples) compared with 143.8 ± 14.4 mU/min (95% N_2 + 5% CO_2, n = 14 samples) ($p < 0.01$). Thus sarcolemmal integrity improved at pH 7.0 in the presence of O_2, possibly indicating that in the presence of O_2, there was sufficient glycolytic ATP at this pH 7.0 in contrast to anoxia.

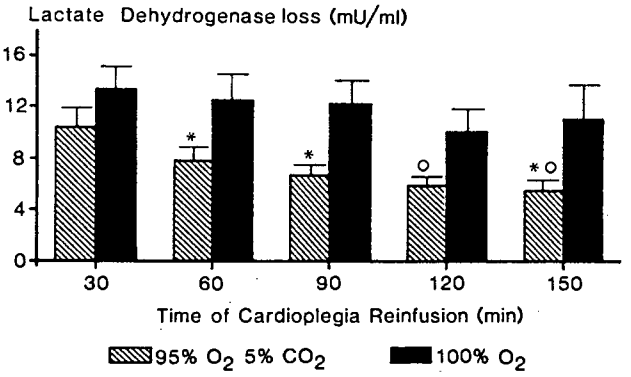

Fig 2. Effect of cardioplegic solution pH on washout of lactate dehydrogenase with each reinfusion of cardioplegia. The mean washout in the coronary sinus effluent consequent to each multidose reinfusion of St. Thomas' plus glucose cardioplegic solution administered every 30 minutes during a 3-hour 10°C cardioplegic arrest period is indicated. The cardioplegic solution was oxygenated with either 95% O_2 + 5% CO_2 (pH 7.0) or 100% O_2 (pH 9.3). The vertical bars *represent the standard errors of the means. (* = $p < 0.05$ compared with 100% O_2 at that time interval; o = $p < 0.05$ compared with the respective 30-minute interval.)*

Comparisons of pH 7 and pH 9 in the Presence of Oxygen

In aerobic conditions, pH 7.0 as opposed to pH 9.3 was associated with the better postischemic mechanical recovery (see Fig 1). In addition, LDH loss in the coronary sinus effluent during the ischemic period was less with pH 7.0 (95% O_2 + 5% CO_2) as opposed to pH 9.3 (100% O_2) ($p < 0.01$) for the majority of multidose reinfusions of oxygenated cardioplegia (Fig 2). Postischemic LDH loss was similar at both pHs (71.0 ± 9.2 mU/min, n = 12 samples; and 66.5 ± 7.9 mU/min, n = 10 samples, for pH 7.0 and 9.3, respectively). Consequently, sarcolemmal integrity was better maintained in the presence of O_2 at pH 7.0 as opposed to pH 9.3. The lactate washout during the cardioplegic arrest period tended to be lower with pH 7.0 (95% O_2 + 5% CO_2) at every reinfusion of cardioplegia compared with pH 9.3 (100% O_2) (Fig 3). However, the mean washout of lactate (0.25 ± 0.02 μmol/mL, n = 44 samples) at pH 7.0 did not attain significance compared with pH 9.3 (0.35 ± 0.02 μmol/mL, n = 38 samples). Therefore, although we could not show increased glycolysis at pH 9.3 in the presence of O_2, it is possible that the inclusion of glucose in our cardioplegic solution accounted for some of the diminished mechanical recovery with this alkalotic pH (100% O_2). However, if so, it would

Conclusion

St. Thomas' Hospital II plus glucose (11 mmol/L) crystalloid cardioplegic solution should be oxygenated. However, it is recommended that 95% O_2 + 5% CO_2 and not 100% O_2 be used with multidose cardioplegia administration because of the synergistic beneficial effect of a relatively acidotic induced pH shift and the provision of O_2. The induced shift in pH to 7.0 (10°C) observed when oxygenating with 95% O_2 + 5% CO_2 is associated with increased myocardial O_2 uptake, better maintenance of membrane integrity, and ultimately, improved postischemic functional recovery. Although the presence of glucose in our cardioplegic solution may have accounted for some of our observations, in the clinical situation, noncoronary collateral flow would supply some glucose in this physiological range. Therefore, we believe extrapolation to the standard St. Thomas' solution may be justified. A perfluorocarbon O_2 carrier is of no additional benefit in a well-formulated oxygenated crystalloid cardioplegic solution.

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Appendix A–5

Endothelial cell cytotoxicity of cardioplegic solutions used in South Africa.

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Cardiovascular Topics

Endothelial cell cytotoxicity of cardioplegic solutions used in South Africa

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Abstract

The hypothesis tested is that cardioplegic solutions, used to protect the myocardium during open-heart surgery, could be cytotoxic to endothelial cells. Vascular endothelial damage would be detrimental to postoperative myocardial function. Cultured monolayers of human endothelial cells were exposed for 12 hours at room temperature (22° C) to crystalloid cardioplegic solutions currently in use in South Africa, and to two internationally used solutions.

The international St Thomas' Hospital No. 2 (ST) and Bretschneider (B-HTK) cardioplegic solutions produced 24-hour post-exposure endothelial cell survivals of 81,0 ± 1,9% and 76,5 ± 4,1% respectively. The ST + glucose (10 mM) cardioplegic solution used at Groote Schuur Hospital produced a similar survival — 78,8 ± 2,9%. The best endothelial survival of 87,1 ± 2,2% was obtained with a modified ST plus glucose solution containing histidine (50 mM), the more powerful buffer included in B-HTK, which was significantly better than ST and B-HTK ($P < 0,05$).

In contrast, crystalloid cardioplegic solutions used elsewhere in South Africa were all cytotoxic to endothelial cells. SABAX cardioplegia was associated with a 24-hour survival of 0,6 ± 0,3%, which was significantly worse than that obtained with ST ($P < 0,001$). Plasmalyte B-based formulations produced no survival, and were also significantly worse than ST ($P < 0,001$). The cytotoxicity of these solutions was due to acalcaemia, excessively high potassium concentrations and too little magnesium.

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Today after prolonged periods of ischaemia cardiac surgeons expect near-normal postoperative recovery of myocardial function, if protective cardioplegic solutions were used. These solutions induce diastolic myocardial arrest and in conjunction with hypothermia, dramatically reduce myocardial energy needs.[1] Therefore, they have been primarily formulated to protect the myocyte, and little attention has been given to their effect on other cells.

However, the vascular endothelium is an extremely important integral component of all organs, and in the heart endothelial injury could be detrimental to myocardial function. Vascular endothelial cells form the first element in the blood barrier to the interstitium, and modulate a variety of biological functions.[2] The normal endothelial surface is antithrombogenic,[3] and also provides mediators that act on the underlying vascular smooth muscle to modulate vascular tone and blood flow.[4] Ischaemia alone damages the endothelium, and results in increased vascular permeability, interstitial oedema,[5] and release of endothelial enzymes.[6] These enzymes (e.g. xanthine oxidase) are associated with the production of toxic oxygen free radicals[7] that in turn damage adjacent myocytes. Therefore, isolated injury to the endothelium will predispose to thrombosis, vasoconstriction and myocardial infarction and adversely affect myocardial function. Furthermore, these harmful effects occur even in the absence of cell death.[3]

Cardioplegic solutions, by virtue of being infused intravascularly, should therefore not only protect the

myocyte but also the endothelium. However, previous studies have shown that these solutions can in fact be cytotoxic to endothelial cells.[8,9] Endothelial cells are attachment-dependent cells, and cytotoxicity in monolayer cultures can be observed with light microscopy as loss of cellular attachment and cellular disruption.

This study was undertaken to determine the endothelial cytotoxic potential of crystalloid cardioplegic solutions currently in use in South Africa. Although these solutions are usually at a temperature of 4°C when infused into the heart, during clinical cardiac surgery the heart is continually rewarmed by transmitted heat and the myocardial temperature therefore varies between 14°C and 20°C. We therefore elected to examine the effect of these solutions at room temperature (22°C). Furthermore, the cardioplegic solution is maintained in the coronary circulation throughout the surgical cross-clamp period of 1 - 3 hours by intermittent reinfusions, and in transplantation units hearts are stored in these solutions for up to 6 hours. Therefore, an extended exposure period of 12 hours was used in this study.

Finally, in order to establish the components that were either harmful or beneficial, modifications of these solutions were tested.

The majority of endothelial functions stated above occur universally throughout the vascular tree, although various vascular beds might be associated with special characteristics, e.g. an increased rate of prostacyclin production by the internal mammary artery endothelium.[10] However, the majority of these studies were performed in different animal models which in turn may be associated with differences between species. Therefore, we elected to study human cells and were thus limited to venous endothelial cells.

Materials and methods

Endothelial cells were harvested from unused segments of saphenous veins obtained from patients undergoing coronary artery bypass graft surgery, as described by Zilla *et al.*[11] The venous segment was flushed with incomplete culture medium (Medium 199 — Earle's salts (Flow Laboratories; Irvine, Scotland), with neither additional serum nor endothelial growth factor (ECGF)), then distended with 0,1% collagenase (Cooper Biomedical; Malvern, Pennsylvania) for approximately 15 minutes, which was then inactivated by flushing with culture medium containing 30% fetal calf serum (Delta Bioproducts; Johannesburg). This fluid was collected and centrifuged at 800 rpm for 10 minutes; the resultant cellular pellet was then resuspended in 2 ml complete culture medium (Medium 199 plus 20% fetal calf serum and ECGF (Collaborative Research Inc.; Lexington, Mass.)). This suspension was placed into a 9,6 cm^2 culture plate well, precoated for 2 hours with human fibronectin (Inotech; Zurich, Switzerland). All cultures were kept in a 37°C, 90% humidity, 5% CO_2 incubator, and were always handled sterilely under a laminar flow hood.

The primary culture was rinsed after 24 hours with Dulbecco's phosphate-buffered saline (Flow Laboratories) and thereafter 'fed' twice weekly by replacing 50% of the supernatant with fresh complete culture medium. Endothelial cells were passaged at 80% preconfluence, designated by a microgrid technique. Monolayer confluence was usually obtained within 2 - 4 weeks of the endothelial cell harvest. Primary cultures were then split at a ratio of 1:22, using trypsin EDTA (Flow Laboratories) at 37°C for cell detachment. The cells were then seeded into a 175 cm^2 flask precoated for 12 hours with gelatin (Difco Laboratories, Detroit, Michigan), or onto 9 mm cover-slips for immunohistochemical staining of von Willebrand factor (factor VIII-related antigen), which is a typical cell marker for endothelial cells. This culture was managed as before, and confluence obtained within ± 10 days.

The cells were again passaged, but now in a 1:1 or 1:2 ratio, into 12-well culture plates (3,5 cm^2 per well) precoated with gelatin. Complete mixing of the cellular suspension was obtained by agitation and by using a bulb syringe in order to ensure uniform seeding of all wells in this tertiary culture. Confluence of 1:1 passage was obtained after 2 - 3 days.

Cardioplegic solutions

Our unit has previously documented the variety of crystalloid cardioplegic solutions used in South Africa and the paucity of reported objective comparisons regarding their effectiveness.[12] These include the SABAX cardioplegic solution (SBX) (SABAX Ltd, Samuel Evans Rd., Aeroton, Johannesburg), Plasmalyte B plus 15 ml 15% KCl per litre (PB-1), Plasmalyte B plus 15 ml 15% KCl, 10 ml 8,5% $NaHCO_3$ and 20 ml 50% dextrose per litre (PB-2), and at Groote Schuur Hospital the St Thomas' Hospital No. 2 solution plus 3,6 ml 50% dextrose per litre (ST + G). The SBX cardioplegia tested was their multidose solution that has a K^+ concentration of 12,0 mM, as opposed to their induction solution that has a K^+ content of 24,1 mM. These solutions were compared with two internationally accepted cardioplegic solutions: the St Thomas' Hospital No. 2 (ST) and the European Bretschneider HTK (B-HTK) (Dr F. Koehler, Chemie GmbH, Alsbach, West Germany). ST should not be confused with the St Thomas' Hospital No. 1 (Macarthy Medical, John Bell and Croyden, London, UK) solution, made up by adding a 20 ml ampoule containing MgCl, KCl and procaine to Ringer's solution; this is a different formulation and has been shown to be an inferior solution.[13]

All solutions were gassed with 95% O_2, 5% CO_2, and then allowed to equilibriate with the atmosphere for 12 hours before being filtered (0,22 U) before use. Osmolarities were measured with a crystalloid osmometer (Osmomat 030; Gonotec, Berlin, West Germany) and pH (after gassing) with a blood gas analyser (IL System 1302 pH/blood gas analyser; Instrumentation Laboratory System, Milan, Italy). The compositions of these cardioplegic solutions are given in Table I.

TABLE I. COMPOSITION OF CARDIOPLEGIC SOLUTIONS USED IN SOUTH AFRICA

	ST	*ST+G*	*SBX*	*PB-1*	*PB-2*	*B-HTK*
Na	120	120	140,7	130	140	15
K	16	16	12,0	34	34	10
HCO_3	10	10	38,3	27	37	—
Mg	16	16	1,5	1,5	1,5	4,0
Ca	1,2	1,2	0,9	—	—	—
Cl	160	160	119,3	140	140	50
SO_4						
PO_4						
Glucose	—	10	50,5	—	56	—
Mannitol	—	—	68,4	—	—	30
Histidine	—	—	—	—	—	198
Tryptophan	—	—	—	—	—	2
KH-2-oxygluterate	—	—	—	—	—	1
Osmolarity (mOsml/l)	293	302	416	302	357	302
pH (22°C)	7,1	7,1	7,7	7,7	7,8	7,0

All values are given in mmol/l, and pH was measured after gassing with 95% O_2, 5% CO_2.

In order to further define any specific electrolyte concentration associated with endothelial cytotoxicity, we examined modifications of PB-1 and ST. In addition, a modified ST + G solution with a lower sodium content of 100 mM (to allow for osmotic space), plus histidine 50 mM (an integral component of B-HTK), was examined.

Experimental protocol

Cultures of confluent monolayers of human endothelial cells that had not been 'fed' in the preceding 24 hours were used (Fig. 1). A baseline grid count of each well was done using an inverted phase contrast microscope (ID 02 MT; Zeiss, West Germany). The cell count in '20 grid squares' was the total number of cells seen in each four corner squares of a marker grid, and counted in five different areas of each well.

The culture medium was then removed and the cardioplegic solution applied initially as a rinse to ensure removal of all medium, and then a 1 ml aliquot reintroduced. The initial temperature of the cardioplegic solutions was 10°C, and after application the cell cultures were kept at room temperature (22°C) for 12 hours. The morphological appearance of the cells was then examined by light microscopy before removing the cardioplegic solution. Thereafter the cultures were rinsed with incomplete culture medium, and 1 ml complete medium but without ECGF was introduced. All cell cultures were now placed back in the 37°C 5% CO_2 incubator to allow for a period of continued metabolism and for any delayed expression of cell injury.

The post-exposure cell survival was determined after rinsing the cells with incomplete culture medium to remove debris and dead cells at 24 hours and again at 36 hours after exposure.

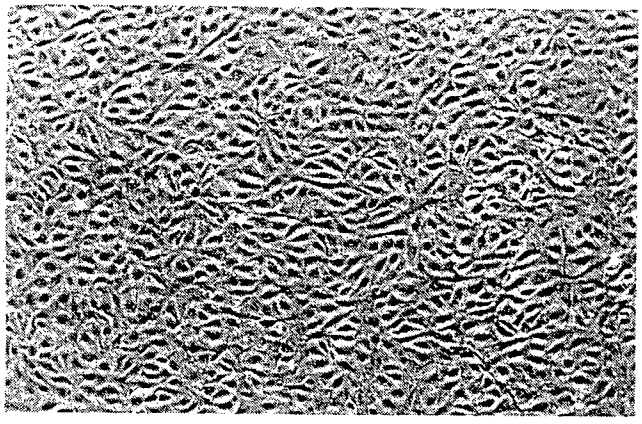

Fig. 1. Appearance on light microscopy of a confluent monolayer culture of human venous endothelial cells. A typical 'cobblestoned' surface with no intercellular gaps is observed (× 100).

Expression of results

The cell count/cm^2 was calculated by multiplying the '20 grid square count' by 125. The percentage of surviving cells at each time period was expressed as a ratio of each individual post-exposure grid count to the respective baseline grid count. Values are presented as percentage means and standard errors of percentage means.

The total number of wells tested is given as *N*. In addition, to ensure reproducibility each solution was tested in a number of different culture plates (P), and on cells obtained from different primary cultures (C).

Student's *t*-test was used to compare differences on the assumption of equal variance. Appropriate tables were then used to determine *P* values. Statistical significance was assumed when the *P* value was $< 0,05$. Confidence intervals are reported.

Results

A confluent monolayer of cultured human endothelial cells seen on light microscopy is shown in Fig. 1, and exhibits a typical 'cobblestoned' appearance. These cells

TABLE II. ENDOTHELIAL CELL SURVIVAL AFTER 12 HOURS' EXPOSURE AT 22°C TO CARDIOPLEGIC SOLUTIONS USED IN SOUTH AFRICA

Solution	*N*	*Baseline count (cells/cm²)*	*% survival at 24 h*	*% survival at 36 h*
ST	16	71 890 ± 5 299	81,0 ± 1,9	77,1 ± 1,7
B-HTK	12	58 010 ± 3 946	76,5 ± 4,1	73,8 ± 4,2
ST+G	12	62 750 ± 2 746	78,8 ± 2,9	75,3 ± 2,2
SBX	15	61 042 ± 5 536	0,6 ± 0,3*	0,2 ± 0,2*
PB-1	15	62 375 ± 7 003	0*	0*
PB-2	12	46 938 ± 5 251	0*	0*

Cell survival at 24 and 36 hours after exposure is presented, as a ratio to each individual baseline pre-exposure cell count/cm². Results are presented as means and standard errors of means.
*$P < 0,001$ compared with ST or B-HTK, at respective post-exposure times.

were then exposed to cardioplegic solutions for 12 hours at room temperature (22°C). The internationally used cardioplegic solutions ST and B-HTK produced similar endothelial cell survivals 24 hours after exposure.

Exposure to ST cardioplegia (N = 16, P = 6, C = 4) caused the cells to contract and a more granular cytoplasm was seen (Fig. 2). However, this appearance was reversible after removal of the cardioplegic solution and an 81,0 ± 1,9% survival was recorded at 24 hours, which was no different to the 36-hour survival. When exposed to B-HTK (N = 12, P = 4, C = 3) the cellular morphology did not differ significantly from the pre-exposure baseline appearance, and a 24-hour survival of 76,5 ± 4,1% and similar 36-hour survival was obtained. These survivals were no different from those obtained with ST.

Cardioplegic solutions used in South Africa

The inclusion of glucose (10 mM) in the St Thomas solution (N = 12, P = 4, C = 3) produced a similar morphological effect on the endothelial cells after 12 hours of exposure as seen with ST, and a similar 24-hour survival of 78,8 ± 2,9%. Likewise, the 36-hour survival was no different (Table II).

In contrast, all the other crystalloid cardioplegic solutions used in South Africa produced extremely poor endothelial cell survivals. The SBX solution (N = 15, P = 5, C = 3) caused the cells to lose adherence, become round and swollen and disrupt (Fig. 3). The 24-hour survival of 0,6 ± 0,3% was markedly inferior to those obtained with ST, ST + G and B-HTK; $P <$ 0,001. Both the plasmalyte B-based solutions, PB-1 (N = 15, P = 5, C = 3) and PB-2 (N = 12, P = 4, C = 2) caused loss of cellular adherence, and floating round,

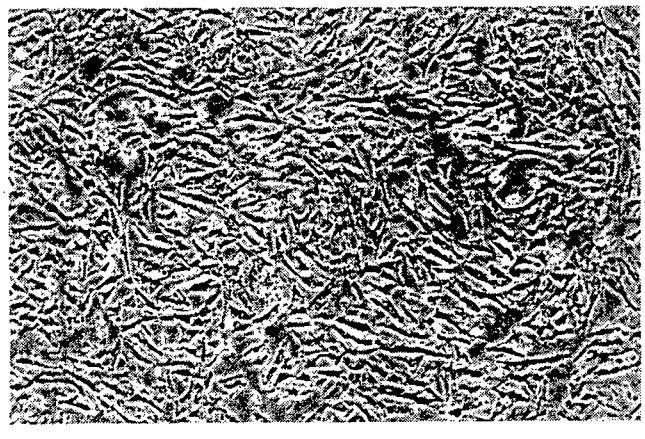

Fig. 2. Appearance on light microscopy of endothelial cells after 12 hours' exposure at 22°C to ST cardioplegic solution. The cells have contracted and intercellular gaps are now seen (× 100).

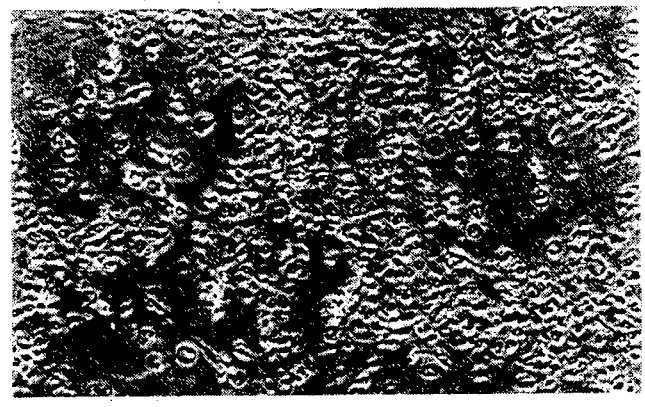

Fig. 3. Appearance on light microscopy of endothelial cells after 12 hours' exposure at 22°C to SBX cardioplegic solution. The cells have lost adherence and are round and swollen, and some have fragmented (× 100).

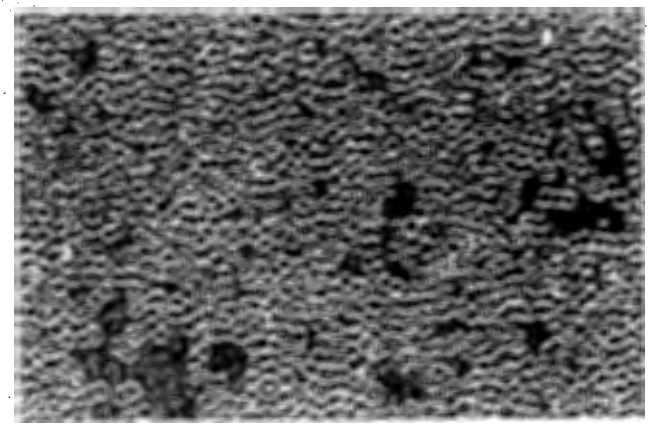

Fig. 4. Appearance on light microscopy of endothelial cells after 12 hours' exposure at 22°C to a Plasmalyte B plus potassium (30 mM) formulation (PB-1). There is complete loss of cellular adherence and all cells are round and swollen (× 100).

swollen cells could be seen on light microscopy (Fig. 4). There was no survival 24 hours after exposure with both PB-1 and PB-2, in contrast to the two international solutions; $P < 0{,}001$.

Experimental modifications of Plasmalyte B

The addition of calcium (1,2 mM) to the acalcaemic PB-1 solution (N = 15, P = 5, C = 2) resulted in a mixed appearance of the cells after 12 hours' exposure. Portions of the culture showed loss of adherence with round swollen cells, while in other areas dense contracted cells were seen. In contrast to the 0% survival observed with PB-1, the 24-hour survival now increased to 31,7 ± 7,3%; $P < 0{,}005$. In another experimental solution the potassium concentration of PB-1 was decreased to 16 mM (N = 6, P = 2, C = 1) and similar to PB-1, no survival was obtained (Fig. 5).

However, if both of the above modifications were combined (N = 9, P = 3, C = 1), i.e. PB-(K = 16 mM, Ca = 1,2 mM) then endothelial cell survival increased to 50,5 ± 6,6% at 24 hours, although in comparison with PB-(Ca = 1,2 mM) it just failed to achieve significance ($P = 0{,}07$). This solution was also still inferior to ST ($P < 0{,}005$), which has a higher concentration of magnesium (16 mM).

Experimental modifications of St Thomas' solution

In contrast to ST, both SBX and PB-2 contain a high concentration of glucose (± 50 mM) and sodium (± 140 mM) (Table I). However, in ST the addition of 50 mM glucose (N = 11, P = 4, C = 2) was not

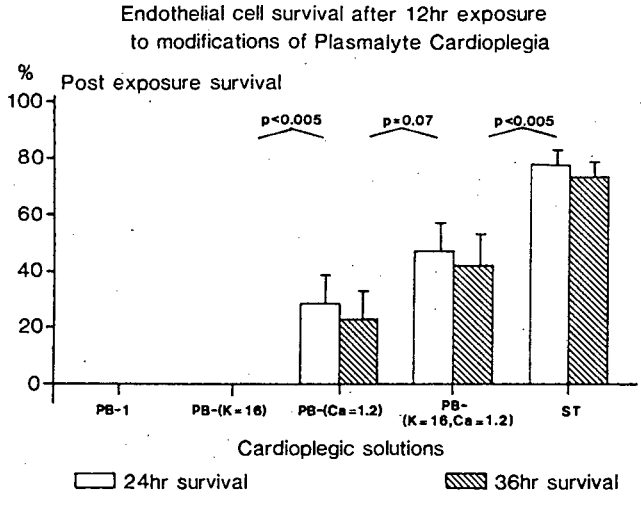

Fig. 5. Mean percentage survival of cultured human endothelial cells 24 hours and 36 hours after 12 hours' exposure at 22°C to experimental modifications of Plasmalyte B cardioplegic solutions. The vertical bars represent standard errors of means. PB-1 — Plasmalyte B with a K^+ concentration of 34 mM; PB-(K = 16) — Plasmalyte B with a K^+ concentration of 16 mM; PB-(Ca = 1,2) — Plasmalyte B with a K^+ concentration of 34 mM and Ca^{2+} of 1,2 mM; PB-(K = 16, Ca = 1,2) — Plasmalyte B with a K^+ concentration of 16 mM and Ca^{2+} of 1,2 mM.

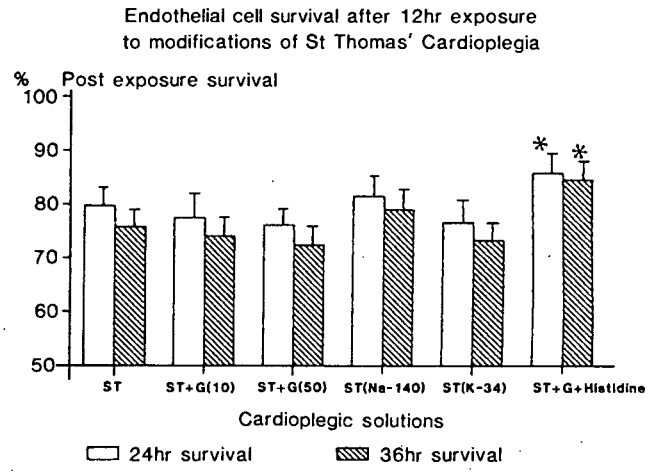

Fig. 6. Mean percentage survival of cultured human endothelial cells 24 hours and 36 hours after 12 hours' exposure at 22°C to experimental modifications of cardioplegic solutions. The vertical bars represent standard errors of means. ST + G (10) — ST plus 10 mM glucose; ST + G (50) — ST plus 50 mM glucose; ST (Na-140) — ST plus 20 mM NaCl (therefore has an Na^+ concentration of 140 mM); ST (K-34) — ST plus 18 mM KCl (therefore has a K^+ concentration of 34 mM); ST + G + histidine — ST + G plus 50 mM histidine, but has an Na^+ concentration of only 100 mM. (*$P \times 0{,}05$ compared with either ST or ST + G, at both 24 and 36 hours after exposure.)

detrimental, and resulted in a 24-hour post-exposure endothelial cell survival of 77,4 ± 1,7%. Similarly, increasing the sodium content of ST to 140 mM (N = 10, P = 4, C = 2) by adding 20 mM NaCl also did not decrease survival (82,8 ± 2,6% at 24 hours) (Fig. 6).

Although a lower potassium concentration tended to be beneficial in the calcium-containing plasmalyte B solution (PB-(K = 16 mM, Ca = 1,2 mM)), if the potassium concentration of ST was increased to 34 mM (N = 12, P = 4, C = 2) by adding 18 mmol KCl, the 24-hour survival of 77,9 ± 2,8% was no different to that obtained with the standard ST, which has a potassium concentration of 16 mM.

Finally, 12 hours' exposure of endothelial cells to a modified ST + G (10 mM) solution containing 50 mM histidine (the more powerful buffer included in B-HTK) but with an Na^+ concentration of only 100 mM (N = 15, P = 5, C = 4), produced less retraction and a more normal appearance on light microscopy. The 24-hour cell survival of 87,1 ± 2,2% and 36-hour survival of 86,0 ± 2,1% were better than those obtained with both ST and ST + G cardioplegic solutions, at both post-exposure periods ($P < 0{,}05$) (Fig. 6).

Discussion

When confluent monolayers of cultured endothelial cells were exposed to various cardioplegic solutions for 12 hours at room temperature (22°C), the two internationally used solutions, ST and B-HTK, were associated with acceptable 24-hour post-exposure cell sur-

vivals of 81% and 76,5% respectively. The cardioplegic solution in use at Groote Schuur Hospital, ST + G, which contains 10 mM glucose and has been shown to be superior to ST in a multidose isolated rat heart model,[25] produced a similar cell survival. In contrast, the other crystalloid cardioplegic solutions used in six other units in South Africa,[12] namely SBX and Plasmalyte B-based solutions (PB-1, PB-2), were cytotoxic to endothelial cells. This would be detrimental to postoperative myocardial function.

We have shown that in terms of endothelial cell survival, the most important omission in the composition of PB-1 and PB-2 is that of calcium. Acalcaemia has been previously reported to be harmful in cardioplegic solutions, by predisposing to the calcium paradox.[14] The calcium paradox is the phenomenon of severe morphological, functional and biochemical cellular damage that occurs when hearts are reperfused with a fluid containing calcium ions after a period of calcium-free perfusion.[15] Furthermore, this phenomenon has also been noted in canine kidney perfusions, and produced changes in the vessel walls and cell membranes.[16] Therefore, it is not specific to the myocyte and could affect other cells. We observed that the acalcaemic PB-1 produced loss of cellular adherence before calcium was reintroduced, and therefore cannot be interpreted as the calcium paradox. However, since endothelial cells are attachment-dependent,[17] this loss can be interpreted as cytotoxicity. The addition of calcium 1,2 mM to PB-1 improved survival. In contrast, the acalcaemic B-HTK solution was not cytotoxic, possibly because it has a low sodium content, which is beneficial in an acalcaemic solution.[18]

In addition, although we were unable to show that a high potassium concentration of 34 mM was detrimental in ST, a high potassium has been associated with endothelial damage[19] and appeared to contribute to some of the observed cytotoxicity of PB-1. It is also relevant to note that the experimental protocol of Follette *et al.*, which closely resembled the clinical situation (i.e. 500 ml crystalloid cardioplegia infused over 5 minutes, every 20 minutes for 2 hours), also induced endothelial damage.[19] Unfortunately they did not document the entire composition of their cardioplegic solution.

Clinically, non-coronary collateral flow would moderate this observed toxicity since it not only reintroduces calcium but also continually washes out the cardioplegia.[20] However, one should not rely on this variable to avert cytotoxicity.

In this study ST + G (10 mM) produced similar endothelial survivals to those of ST, and increasing the glucose content to 50 mM was not detrimental to the endothelial cells. Furthermore, an increased sodium concentration (140 mM) was also not detrimental when tested in an ST-based solution. Therefore, it would appear that neither the high glucose content nor the high sodium content of SBX and PB-2 were major contributory factors in their observed cytotoxicity. The major reason for endothelial damage by SBX is probably its low magnesium content, although we have not examined the possible cytotoxic potential of high osmolarity. This interpretation would support the findings of Hearse *et al.*[21] with regard to the optimum concentration of magnesium in a cardioplegic solution.

Cardioplegic solutions have been primarily formulated in isolated rat heart and *in situ* dog heart preparations by measuring post-ischaemic functional and metabolic recovery. Dose-response curves for each individual electrolyte finally resulted in the present formulation of ST.[22] Our observations on endothelial cytotoxicity support these findings. However, in contrast to B-HTK, ST initially altered the morphological appearance of the cells. Although this change was reversible and resulted in similar post-exposure survivals, it could well be deleterious *in vivo*. Minor reversible injurious alterations of the endothelial surface can increase vascular permeability, and release activating enzymes,[6,23] and furthermore has been postulated to accelerate atherogenesis.[24] The experimental modification to ST, i.e. ST + G + histidine (50 mM), improved both the cellular morphology and post-exposure survival, and might well be a significant enhancement. It would appear that the greater buffering capacity provided by histidine was beneficial, since endothelial cells are known to be high lactate producers.[6] However, further experimental work would have to be done to confirm these observations in other models.

We recognise that venous endothelial cells differ from coronary artery endothelial cells. However, our model examined universal characteristics of endothelial cells, namely cell death, attachment dependence and morphological changes such as cellular contraction, in response to exposure to physiological solutions. Therefore, we believe extrapolation to the arterial system is justifiable.

In conclusion, the only crystalloid cardioplegic solution presently in use in South Africa that is not toxic to endothelial cells is the St Thomas' Hospital No. 2 solution with or without glucose (10 mM). All other solutions, i.e. the SABAX cardioplegic solution and various Plasmalyte B formulations, are cytotoxic to endothelial cells.

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Appendix A–6

Endothelial cell toxicity of solid-organ preservation solutions.

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Endothelial Cell Toxicity of Solid-Organ Preservation Solutions

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Endothelial cell damage caused by myocardial cardioplegic solutions (Bretschneider HTK and St. Thomas' Hospital No. 2) or renal and hepatic cold storage solutions (modified Collins and University of Wisconsin solution) was assessed in monolayer cultures of adult human venous endothelial cells at 4° to 10°C with phase-contrast microscopy. St. Thomas' Hospital solution caused the cells to contract, resulting in disruption of monolayer integrity and opening of intercellular gaps, and resulted in a 24-hour postexposure survival of 51.0% ± 2.4%. Bretschneider HTK solution altered cellular morphology less and produced the best postexposure survival (80.2% ± 2.6%; $p < 0.001$). Although morphology was altered the least with University of Wisconsin solution, postexposure survival with this solution, which was similar to that with modified Collins solution, was superior to that with St. Thomas' ($p < 0.01$) but inferior to that with Bretschneider HTK ($p < 0.05$). The superior protection provided by Bretschneider HTK was due to its additives histidine, tryptophan, and KH-2-oxygluterate ($p < 0.005$), and to its low chloride content ($p < 0.005$). Furthermore, modifying St. Thomas' solution by decreasing its chloride content improved cell survival to 71.2% ± 2.3% ($p < 0.001$). Normothermic (37°C) exposure to Bretschneider HTK, modified Collins, and University of Wisconsin solution was cytotoxic, whereas normothermic exposure to St. Thomas' cardioplegia was not. In conclusion, the preservation solution that is the least harmful to endothelial cells at hypothermia is Bretschneider HTK cardioplegic solution.

(Ann Thorac Surg 1990;50:902–10)

Techniques used for solid-organ preservation primarily involve decreasing basal cellular energy requirements by selectively manipulating specific energy-consuming processes in conjunction with hypothermia. Thus the majority of centers store kidneys and livers at 4° to 10°C and flush these organs intravascularly with "intracellular" electrolyte equivalent preservation solutions to abolish energy-consuming transmembranous electrolyte gradients. Simple hypothermic storage periods of 12 to 24 hours are now clinically achievable for the liver and kidney.

In contrast, the myocardium has a much higher rate of energy consumption because of myocyte electromechanical activity. Thus cardioplegic solutions have been primarily formulated to produce diastolic arrest by increasing the extracellular concentration of potassium and magnesium, or alternatively decreasing the concentration of sodium and calcium. Despite electromechanical arrest the heart is only viable clinically for 4 to 8 hours with simple hypothermic storage. Such periods have been achieved with both intracellular and extracellular electrolyte equivalent cardioplegic solutions.

The endothelium, which is common to all organs, is possibly even more vulnerable to ischemia than cardiomyocytes [1]. Endothelial cells form the primary interface between the blood and interstitium, and modulate a variety of biologic functions [2]. The normal endothelial surface is antithrombogenic and also provides mediators that modulate vascular tone [3]. Ischemia and intravascular infusion of solutions can cause endothelial damage [4–6]. Injury can result in a procoagulant endothelium, vasoconstriction [7, 8], release of enzymes associated with the production of toxic free radicals, increased vascular permeability which predisposes to interstitial edema [9], and accelerated atherogenesis [10]. Furthermore, gradual cell damage leads to the manifestation of these deleterious effects before total loss of the endothelium occurs [11]. Therefore, it is extremely important that organ preservation solutions express minimal toxicity to endothelial cells, and they should in fact protect this structure. The preservation of the endothelium might well be a dominant factor in lung storage, if one considers the extent of the pulmonary vascular bed.

To date, few studies have determined to what extent commonly used preservation solutions influence the endothelium. Furthermore, electrolyte concentrations in cardioplegic solutions are specific for the myocyte and are not necessarily suitable for the preservation of endothelial cells, which do not have inward voltage-dependent sodium or calcium currents [12]. Thus modifications that additionally diminish basic membrane energy requirements, improve buffering, or diminish cellular edema might be of more direct benefit to the endothelium.

This study was undertaken to examine the effects of

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prolonged endothelial cell contact with various standard solutions used for myocardial, renal, and hepatic preservation. Endothelial cells are attachment-dependent cells, and cytotoxicity can be observed in monolayer cultures with light microscopy as loss of cellular attachment and cellular disruption. We elected to study human venous endothelial cells as they were easily obtainable and the majority of endothelial functions stated herein occur universally throughout the vascular tree.

We examined the effect of these solutions both at 4° to 10°C and at 37°C, as the majority of centers infuse these solutions at a temperature of 4°C into the normothermic (37°C) organ to induce hypothermia (4° to 10°C). In addition, modified cardioplegic solutions are also used at normothermia as reperfusion solutions. Finally, in an attempt to establish the primary components that were either harmful or beneficial, modifications to these solutions were developed and tested.

Material and Methods

Endothelial Cell Culture Techniques

Endothelial cells were harvested from human saphenous vein segments obtained from adult patients undergoing coronary artery bypass grafting or from organ transplantation donors as previously described [13]. After cannulation, the venous segment was flushed with incomplete culture medium (Medium 199—Earle's salts; Flow Laboratories, Irvine, Scotland), then filled with 0.1% collagenase (CLS II; Cooper Biomedical, Malvern, PA) and incubated at 37°C for 15 minutes. The collagenase-cell suspension was then inactivated by flushing with culture medium containing 30% fetal calf serum (Delta Bioproducts, Johannesburg, South Africa), collected, and centrifuged for 10 minutes at 120 *g*. The pellet was resuspended in 2 mL of complete culture medium (Medium 199 plus 20% fetal calf serum and endothelial cell growth factor; Collaborative Research Inc, Lexington, MA) and plated into a 9.6-cm^2 well of a six-well culture plate precoated with human fibronectin (Inotech, Zurich, Switzerland; 18 μg/mL). All cultures were incubated at 37°C in a 5% CO_2 atmosphere.

To remove erythrocytes and cell debris the primary culture was rinsed after 24 hours with Dulbecco's phosphate-buffered saline (Flow Laboratories). Thereafter, complete culture medium (2 mL) was added, and 50% of the supernatant was replaced twice weekly with fresh complete culture medium. Endothelial cells were passaged at 80% partial cell coverage, designated by a microgrid technique [13]. This partial cell coverage was usually obtained 10 days after endothelial cell harvest.

To reduce the cell-damaging effect of multiple passages [14], primary cultures were passaged at a ratio of 1:22, using trypsin ethylenediaminetetraacetic acid (Flow Laboratories). These first-passage endothelial cells were either seeded into a 175-cm^2 flask precoated for 12 hours with gelatin (Difco Laboratories, Detroit, MI; 10 ng/mL in 0.1 mol/L $NaHCO_3$ buffer) or onto 9-mm glass cover-slips for immunohistochemical staining with von Willebrand factor (factor VIII–related antigen), which is a typical cell marker for endothelial cells [15]. Cultures were managed as before, and confluence was again obtained within 10 days.

The cells were passaged once more, but in a 1:1 ratio into 12-well culture plates (3.5 cm^2 per well) precoated with gelatin. Confluence of second-passage monolayers was obtained after 2 to 3 days. Individual wells were assessed microscopically for completeness and homogeneity of cell coverage, and unevenly covered wells were excluded.

Table 1. Composition of Preservation Solutions[a]

Component	ST	COL	UW-CSS	B-HTK
Na	120	10	20	15
K	16	115	140	10
HCO_3	10	10	. . .	. . .
Mg	16	3	5	4
Ca	1.2	. . .	. . .	. . .
Cl	159	15	. . .	50
Glucose · H_2O		139		
Histidine HCl · H_2O				18
Histidine				180
Tryptophan				2
KH-2-oxygluterate				1
Mannitol				30
Lactobionic acid			100	
Raffinose · $5H_2O$			30	
Adenosine			5	
Glutathione (reduced)			3	
Allopurinol			1	
Pentafraction			50 g/L	
Osmolarity (mosmol/L)	300	300	320	298
pH (37°C)[b]	7.26	7.27	7.27	7.33

[a] Concentrations are given in mmol/L, unless otherwise indicated.
[b] The pH was measured after gassing with 95% O_2 and 5% CO_2.

B-HTK = Bretschneider HTK; COL = modified Collins solution; ST = St. Thomas' Hospital No. 2; UW-CSS = University of Wisconsin cold storage solution.

Preservation Solutions

Preservation solutions tested were the St. Thomas' Hospital No. 2 (ST; Sabax Ltd, Johannesburg, South Africa) and Bretschneider HTK (B-HTK; Dr F Koehler Chemie GmbH, Alsbach, Federal Republic of Germany) cardioplegic solutions, the modified Collins (COL) kidney preservation solution, made up by adding 50 mL of 50% dextrose and 1.2 mL of 50% $MgSO_4$ to the standard Collins solution (Baxter Health Care Corporation, Deerfield, IL), and the University of Wisconsin cold storage solution (UW-CSS; Du Pont Pharmaceuticals, Wilmington, DE), but without the recommended additional additives (penicillin, insulin, dexamethasone). The compositions of these solutions are given in Table 1.

Modifications of these primary preservation solutions

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Table 2. Composition of Modified Experimental Solutions[a]

Component	ST	ST-Low Cl	ST-Ca (?)	Incell High Cl	No HTK
Na	120	120	120	10	15
K	16	16	16	126	10
HCO_3	10	10	10	10	. . .
Mg	16	16	16	16	4
Cl	159	34	158	158	50
Ca	1.2	1.2	0	. . .	. . .
			0.05		
			0.6		
Gluconate		126			
Mannitol					30
Sucrose					208
Osmolarity (mosmol/L)	300	276	300	288	312
pH (37°C)[b]	7.26	7.28	7.23	7.27	7.27

[a] Concentrations are given in mmol/L, unless otherwise indicated.
[b] The pH was measured after gassing with 95% O_2 and 5% CO_2.

Incell-High Cl = an experimental intracellular electrolyte equivalent solution with a high chloride ion content. No-HTK = Bretschneider HTK cardioplegic solution, but without histidine, tryptophan, and KH-2-oxygluterate. Sucrose was used to maintain equivalent osmolarity. ST = St. Thomas' Hospital No. 2 cardioplegic solution, and modifications as indicated.

were made up in our laboratory to examine the specific effect of chloride ion concentration and to examine B-HTK in more detail. St. Thomas' cardioplegia with a low chloride concentration (ST-Low Cl) was made with sodium and potassium gluconate instead of the respective chloride salt. However, gluconate binds calcium and therefore affects the free ionized calcium ion content, thus ST with varying calcium concentrations (ST-Ca 0 mmol/L, 0.05 mmol/L, 0.6 mmol/L) were also tested. The "free" ionized calcium of these solutions was measured using a calcium-ion–selective electrode (Ciba Corning 288 Blood Gas System, Ciba Corning Diagnostics Corporation, Medfield, MA). However, because of the high magnesium concentration of ST, an estimate of the magnesium ion interference in the calcium ion measurements had to be subtracted from the initial values. The estimate was obtained by measuring the "ionized calcium" in two acalcemic solutions that did or did not contain magnesium (16 mmol/L) but were otherwise identical to the corresponding modified ST solution. This "corrected" estimate of the free ionized calcium is the reported value. In addition, an acalcemic "intracellular" solution with a high chloride content (Incell-High Cl) was prepared. Apart from a substantially higher chloride concentration this solution was not markedly dissimilar to COL (Tables 1, 2). Finally, a solution matching the electrolyte content of B-HTK but without the additives histidine, tryptophan, or KH-2-oxygluterate (No-HTK) was prepared. Sucrose was used in No-HTK to maintain equivalent osmolarity. The compositions of these experimental solutions are given in Table 2.

All solutions were gassed with 95% O_2 and 5% CO_2 30 minutes before use to ensure pH stability and uniform oxygen content. Gassing with 95% O_2 and 5% CO_2 will induce a slight acidotic pH shift if the primary buffer in the solution is bicarbonate (ST, COL). The pH of the ST cardioplegic solution was therefore slightly acidotic (pH, 7.26 at 37°C) compared with the value reported for clinical use, but was similar to that of the other solutions (Tables 1, 2). However, we have previously shown that this acidotic shift in pH improves postischemic recovery of the isolated rat heart after protection with an oxygenated ST plus glucose (11 mmol/L) cardioplegic solution [16].

Experimental Protocol

A baseline in situ grid count of each well was performed [13], using an inverted-phase contrast microscope (ID 02 MT, Zeiss, Oberkochen Federal Republic of Germany). The cell count in "20 grid squares" was the total number of cells seen in each of the four corner squares of a marker grid and counted in five different areas of each well. The culture medium was then removed and the preservation solution applied initially as a rinse to ensure removal of all medium, and then a 1-ml aliquot was reinserted.

In the normothermic experiments, preservation solutions at room temperature were introduced onto the cells, which were then incubated at 37°C in a 5% CO_2 atmosphere. Preservation solutions for the hypothermic experiments were cooled to 10°C before application, and the cultures were then stored in a refrigerator at 4° to 10°C in a normal atmosphere.

The cells were exposed to the preservation solutions for 12 hours, and the morphology of the cells was then examined by light microscopy. Thereafter the solution was removed and the cells were rinsed with incomplete culture medium; and 1 mL of complete medium, but without endothelial cell growth factor, was reapplied. All cell cultures were again placed in a 37°C, 5% CO_2 incubator to assess cell survival and allow for any delayed expression of cell damage. A postexposure cell count was performed after the cells were rinsed with incomplete culture medium, to remove debris and dead cells, at 24 hours and again at 36 hours.

Expression of Results

The cell count per square centimeter was calculated at each of three time periods from in situ microgrid counts as previously described [13]. The percentage of surviving cells in each well at the 24-hour and 36-hour postexposure time periods was derived from the ratio of each individual postexposure count to the corresponding initial preexposure count for that well. Values are presented as survival percentage means and standard errors of survival percentage means.

The total number of wells tested is given as N, and to ensure reproducibility each solution was tested in a number of different culture plates (P). Cells were obtained from three separate human donors, and we attempted to test each solution on cells from at least two different primary cultures (C), where these were available. Student's *t* test was used to compare differences on the

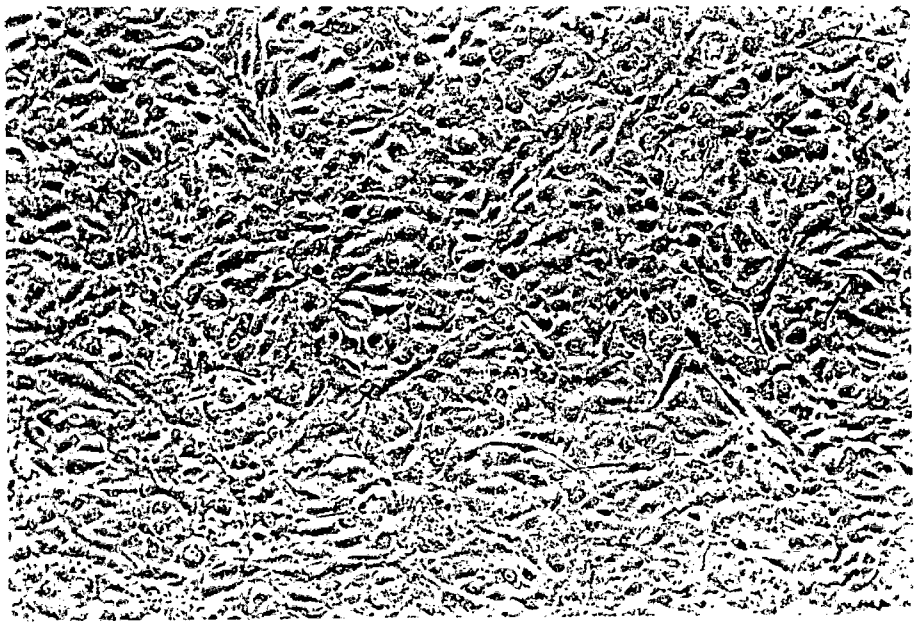

Fig 1. Phase contrast microscopic appearance of a confluent monolayer of adult human saphenous vein endothelial cells. Typical cobblestone morphology and monolayer growth of a pure endothelial cell culture are shown. (×100 before 25% reduction.)

assumption of equal variances. Appropriate tables were then used to determine p values. Statistical significance was assumed when the p value was less than 0.05. Several comparisons were of a priori interest, and the stated level of significance was used without multiple comparison adjustments on the basis of the a priori intentions. Many of the p values of interest are exceptionally small, and even the crudest adjustment for multiple comparisons will leave the conclusion of significant differences unchanged. The wells were treated as though they demonstrably generated independent observations. Although this is not theoretically optimal, the low variation between primary cultures in the same treatment regimen ensures that the approximate method generates valid conclusions for these data.

Results

The phase contrast microscopy appearance of a confluent monolayer of cultured human endothelial cells is shown in Figure 1, which shows the typical "cobblestone" morphology.

Hypothermic Exposure (10°C)

Exposure at 10°C for 12 hours to ST (N = 16, P = 5, C = 3) caused the cells to contract, and denser cytoplasmic granulations and intercellular gaps were seen (Fig 2). However, this appearance was reversible after removal of the cardioplegic solution and reincubation in culture medium, and the 24-hour postexposure survival was 51.0% ± 2.4%. The 36-hour survival was no different (Table 3). In contrast, exposure to B-HTK (N = 13, P = 4, C = 3) altered the morphological appearance of the endothelial cells less; contraction of the cells was less but intercellular gaps still appeared. The 24-hour postexposure endothelial cell survival was 80.2% ± 2.6%; after 36 hours it was slightly lower at 73.5% ± 1.6% ($p < 0.05$). This was superior to ST ($p < 0.001$).

Exposure to UW-CSS did not alter the cells' appearance and preserved their morphology the best, whereas COL induced prominent nuclei with otherwise minimal cellular changes. Collins solution (N = 9, P = 3, C = 2) was associated with a 24-hour endothelial cell survival of 62.4% ± 3.1%, which was no different than the 69.7% ± 2.9% survival of cells exposed to UW-CSS (N = 13, P = 4, C = 3). Cell survival with both these alternative solid-organ preservation solutions was better than with ST ($p < 0.01$), but inferior to that with B-HTK ($p < 0.05$ at both postexposure periods) (see Table 3).

MODIFICATIONS TO BRETSCHNEIDER HTK CARDIOPLEGIC SOLUTION. When endothelial cells were exposed for 12 hours at 10°C to No-HTK (see Table 1), the 24-hour cell survival was 69.0% ± 2.3% (N = 9, P = 3, C = 2). This survival was inferior to that with the standard B-HTK formulation ($p < 0.01$) and no different than that with COL (see Table 3). The morphological appearance of the cells was similar to those treated with B-HTK.

MODIFICATIONS TO INTRACELLULAR PRESERVATION SOLUTIONS. Incell-High Cl (N = 11, P = 3, C = 2) is an intracellular electrolyte equivalent solution with a high chloride concentration, and produced a diminished 24-hour endothelial cell survival of 43.6% ± 4.5% ($p < 0.005$), in contrast to COL (see Table 3). This high concentration of chloride caused the cells to contract, and intercellular gaps were now easily seen, similar to the morphology observed with ST.

MODIFICATIONS TO ST. THOMAS' CARDIOPLEGIC SOLUTION. Endothelial cells were exposed for 12 hours at 10°C to ST-Low Cl (N = 11, P = 4, C = 2) and 24-hour survival increased to 71.2% ± 2.3% ($p < 0.001$ compared with ST, which has a high chloride content [see Tables 2, 3]), but ST-Low Cl was still inferior to B-HTK ($p < 0.05$). Although the postexposure morphological appearance of cells ex-

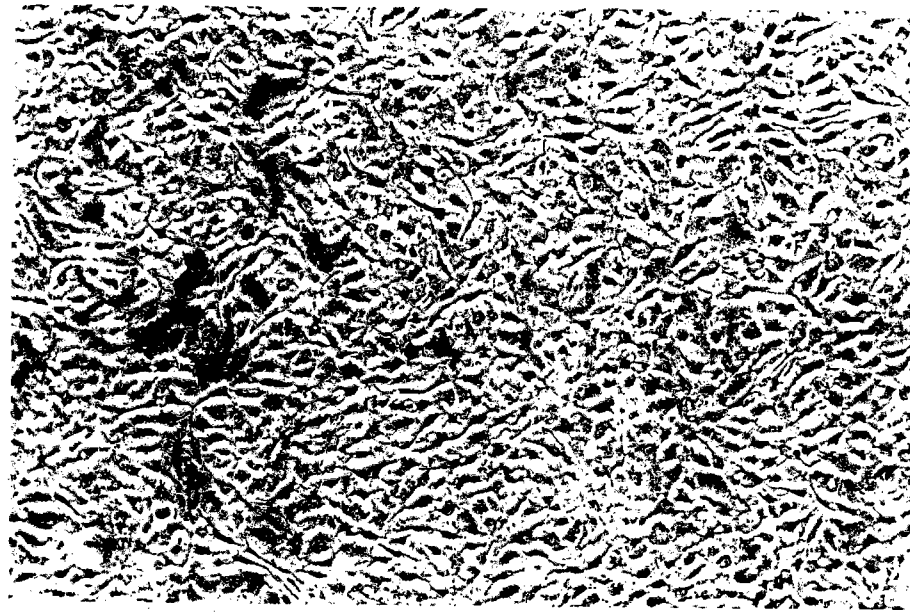

Fig 2. Phase contrast microscopic appearance of a monolayer of adult human endothelial cells after 12-hour exposure at 4° to 10°C to St. Thomas' Hospital No. 2 cardioplegic solution. Endothelial cells show condensed cytoplasmic granulations and have contracted, causing disruption of monolayer integrity and the opening of wide intercellular gaps. (×100 before 25% reduction.)

posed to ST-Low Cl showed some cellular contraction with intercellular gaps, this was less than that seen after exposure to ST.

The anion gluconate (used to replace chloride ions in ST-Low Cl) binds calcium, and the estimated free ionized calcium of ST (0.9 mmol/L) decreased to 0.16 mmol/L in ST-Low Cl. Therefore, to independently examine the effect of a lowered Ca^{2+} concentration in ST, we tested a hypocalcemic ST-Ca 0.6 mmol/L (N = 6, P = 2, C = 2) having a free Ca^{2+} of 0.5 mmol/L, and ST-Ca 0.05 mmol/L solution (N = 7, P = 2, C = 2) with a free Ca^{2+} of less than 0.05 mmol/L. Cell survivals of 60.4% ± 4.2% and 59.9% ± 4.3%, respectively, were obtained 24 hours after exposure, and these hypocalcemic ST solutions were not statistically superior to ST (Fig 3), in contrast to ST-Low Cl. The estimated free ionized calcium of ST-Low Cl (0.16 mmol/L) fell between those of the hypocalcemic ST solutions.

However, an "acalcemic" ST solution, ST-Ca 0 mmol/L (N = 8, P = 3, C = 2) improved 24-hour cell survival to 64.8% ± 2.1% ($p < 0.001$) and 36-hour cell survival ($p < 0.001$) compared with ST. These cells also showed less contraction with smaller intercellular gaps; however, cellular contraction was least with ST-Low Cl. A note of

Table 3. Endothelial Cell Survival After 12-Hour Hypothermic Exposure[a]

Solution	N	Baseline Count (cells/cm²)	% Survival at 24 h	% Survival at 36 h
Primary preservation solutions				
B-HTK	13	21,231 ± 1,780	80.2% ± 2.6%[b]	73.5% ± 1.6%[b]
UW-CSS	13	21,510 ± 2,328	69.7% ± 2.9%[b,c]	65.5% ± 1.5%[b,d]
COL	9	26,486 ± 2,139	62.4% ± 3.1%[d,e]	61.1% ± 2.3%[d,e]
ST	16	20,758 ± 1,545	51.0% ± 2.4%	49.6% ± 2.5%
Modifications				
ST-Low Cl	11	25,557 ± 2,341	71.2% ± 2.3%[b,c]	67.0% ± 2.2%[b,c]
Incell-High Cl	11	26,330 ± 2,423	43.6% ± 4.5%	41.3% ± 4.4%
No-HTK	9	26,306 ± 2,418	69.0% ± 2.3%[b,d]	66.3% ± 1.6%[b,d]

[a] Cultured human endothelial cell survival after exposure to organ preservation solutions for 12 hours at hypothermia (4° to 10°C). The cell survival at 24 h and 36 hours after exposure is presented as a percentage of each individual baseline preexposure cell count per square centimeter. Results are presented as survival percentage means and standard errors of percentage means. [b] $p < 0.001$ compared with ST at corresponding time periods. [c] $p < 0.05$ compared with B-HTK at corresponding time periods. [d] $p < 0.005$ compared with B-HTK at corresponding time periods. [e] $p < 0.01$ compared with ST at corresponding time periods.

B-HTK = Bretschneider HTK cardioplegic solution; COL = modified Collins solution; Incell-High Cl = an experimental intracellular electrolyte equivalent solution with a high chloride ion content; No-HTK = B-HTK without histidine, tryptophan, and KH-2-oxygluterate; ST = St. Thomas' Hospital No. 2; UW-CSS = University of Wisconsin cold storage solution.

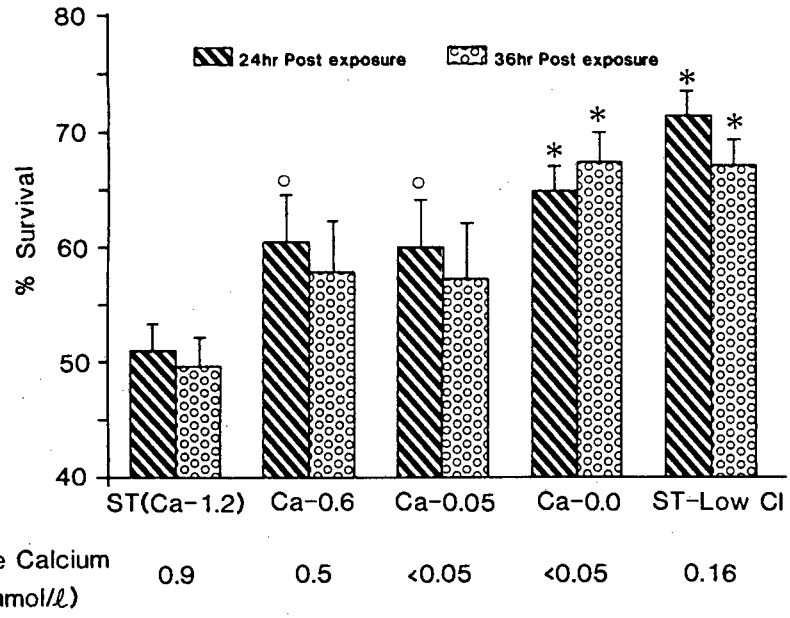

Fig 3. Mean percentage survival of cultured adult human endothelial cells 24 and 36 hours after 12-hour hypothermic (4° to 10°C) exposure to St. Thomas' Hospital No. 2 cardioplegic solution (ST). The effect of varying calcium (Ca) concentrations (0, 0.05, and 0.6 mmol/L) compared with the standard ST (Ca = 1.2 mmol/L) and with an ST solution with a low chloride content (also containing Ca = 1.2 mmol/L) is shown. The vertical bars *represent standard errors of percentage means. The estimated free ionized calcium of each solution is indicated separately. (*p < 0.001 compared with ST(Ca-1.2); °p < 0.05 compared with ST-Low Cl.)*

caution must be mentioned though, as the measured free ionized calcium of ST-Ca 0 mmol/L was not zero; even deionized distilled water contains trace amounts of calcium.

Normothermic Exposure (37°C)

For the normothermic experiments the endothelial cells were maintained for 12 hours at 37°C in a buffered 5% CO_2 atmosphere. In contrast to the hypothermic experiments, ST (N = 9, P = 3, C = 3) was now associated with the best 24-hour endothelial survival of 72.0% ± 2.3% ($p <$ 0.001 for all comparisons) (Table 4). In addition, these cells showed only prominent nuclei with otherwise minimal changes in the immediate postexposure period, in contrast to the marked changes observed with hypothermic exposure.

The intracellular preservation solutions, ie, COL (N = 6, P = 2, C = 1), UW-CSS (N = 6, P = 2, C = 2), and B-HTK (N = 9, P = 3, C = 3), all produced immediate loss of adherence and cellular disruption (Fig 4), and 24-hour postexposure endothelial cell survivals were less than 20% (see Table 4). However, the high-chloride intracellular solution Incell-High Cl (N = 6, P = 1, C = 1) was associated with a 24-hour survival of 51.1% ± 3.9% ($p <$ 0.001 compared with COL, UW-CSS, B-HTK) (see Table 4).

Table 4. Endothelial Cell Survival After 12-Hour Normothermic Exposure[a]

Solution	N	Baseline Count (cells/cm²)	% Survival at 24 h	% Survival at 36 h
Primary preservation solutions				
B-HTK	9	25,472 ± 3,762	19.1% ± 3.5%[b,c]	18.3% ± 3.4%[b,c]
UW-CSS	6	17,146 ± 825	7.7% ± 1.3%[b,c]	6.7% ± 1.3%[b,c]
COL	6	26,771 ± 952	6.1% ± 0.4%[b,c]	2.8% ± 0.4%[b,c]
ST	9	25,097 ± 3,612	72.0% ± 2.3%	70.1% ± 2.1%
Modifications				
ST-Low Cl	8	27,313 ± 2,410	74.6% ± 1.6%[d]	64.3% ± 1.8%[c]
Incell-High Cl	6	29,333 ± 696	51.1% ± 3.9%[b]	45.8% ± 3.3%[b]
ST-Acalcemic	8	28,828 ± 2,332	66.4% ± 2.0%[d]	56.1% ± 2.1%[b,d]

[a] Cultured human endothelial cell survival after exposure to organ preservation solutions for 12 hours at normothermia (37°C). The cell survival at 24 and 36 hours after exposure is presented as a percentage of each individual baseline preexposure cell count per square centimenter. Results are presented as survival percentage means and standard errors of percentage means. [b] $p < 0.001$ compared with ST at corresponding time periods. [c] $p < 0.001$ compared with Incell-High Cl at corresponding time periods. [d] $p < 0.05$ compared with Incell-High Cl at corresponding time periods.

Abbreviations as in Table 3.

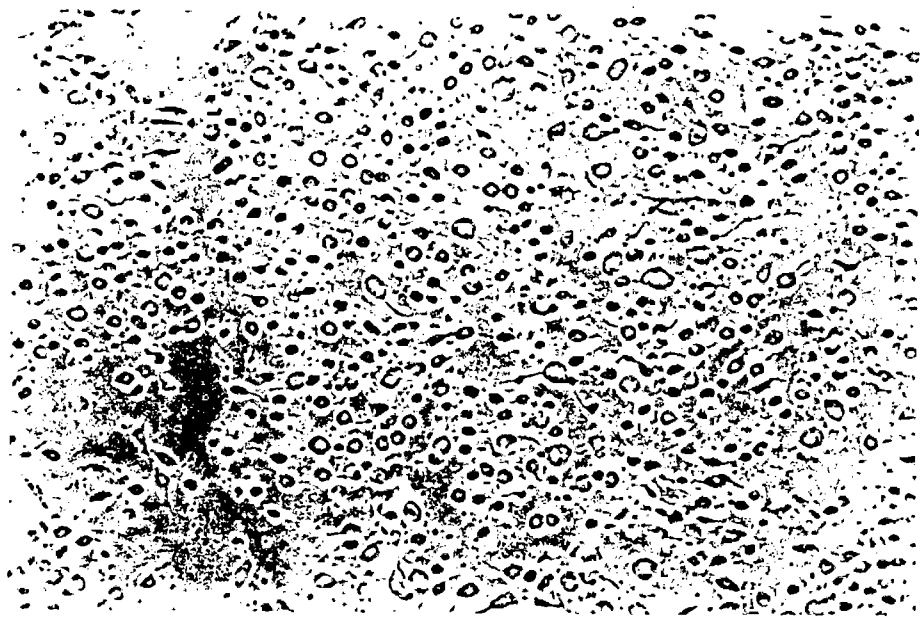

Fig 4. Phase contrast microscopic appearance of a monolayer of adult human endothelial cells after 12-hour exposure at 37°C to modified Collins preservation solution. All endothelial cells are detached from the surface of the culture plate, and the majority show swelling, membrane disruption, or karyorrhexis. (×100 before 27% reduction.)

Finally, exposure to ST-Low Cl (N = 8, P = 3, C = 2) for 12 hours at normothermia produced a postexposure survival of 74.6% ± 1.6%, which was similar to that seen with ST. In addition, the 24-hour survival with ST-Ca 0 mmol/L (N = 8, P = 3, C = 2) of 66.4% ± 2.0% was also no different than that with ST, although at 36 hours it was inferior ($p < 0.001$) (see Table 4). Postexposure morphology was similar to that seen with ST.

Comment

We studied the effect of various internationally used organ preservation solutions on cultured adult human venous endothelial cells. We recognize that arterial endothelial cells differ from venous endothelial cells; however, similarities also exist [17]. Furthermore, many reported differences between cells derived from distinct vascular beds have been observed in various culture conditions and animal models, which in turn may be associated with interspecies differences [18]. We based our model on the assessment of morphological alterations and evaluation of cell detachment, as indicators of cell damage and ultimately cell death. Both these criteria are common to arterial and venous endothelium. Therefore, we believe extrapolation to the arterial system is justifiable.

When confluent monolayers of endothelial cells were exposed to these solutions for 12 hours at hypothermia (4° to 10°C), Bretschneider HTK cardioplegic solution was associated with the best 24-hour postexposure cell survival (80%), although cellular morphology was initially altered by the solution. Bretschneider HTK provided better endothelial preservation than St. Thomas' Hospital No. 2 cardioplegic solution (51%). In addition, both the modified Collins kidney preservation solution and the University of Wisconsin liver and pancreatic storage solution, although superior to ST, were inferior to B-HTK.

Hence the intracellular electrolyte equivalent solutions (B-HTK, COL, UW-CSS) designed to abolish transmembranous electrolyte gradients provided better hypothermic endothelial protection than an extracellular solution (ST). However, the solution that preserved morphology the best (UW-CSS) was not associated with the best postexposure survival.

Nevertheless, if the cells were exposed to these solutions for 12 hours at normothermia (37°C), the intracellular electrolyte equivalent preservation solutions were cytotoxic, with less than 20% 24-hour postexposure survival. St. Thomas' cardioplegia solution was now superior, producing 72% endothelial cell survival.

An atmosphere of 5% CO_2 was used for the normothermic experiments in order to ensure pH stability, because of the substantially lower solubility coefficient of CO_2 at this higher temperature. In our opinion this would have improved normothermic cell survival, and therefore might have accounted for the apparent superior endothelial protection afforded by ST at 37°C, in contrast to 10°C. However, we infer that the observed diminished survival responses with the intracellular solutions at normothermia were determined by the interaction of solution composition and temperature. These contrasting findings possibly support the hypothesis of Kohno and associates [19], who suggest that two types of solutions are required for optimal myocardial preservation, a solution for normothermic arrest and then another for hypothermic preservation. In addition, it would imply that standard cardioplegic solutions (eg, B-HTK) should not necessarily be used as the convenient basic composition for normothermic reperfusion solutions.

To further define the differences between these two groups of solutions we examined various modifications. Primary disparities between intracellular electrolyte equivalent solutions and extracellular solutions are acalcemia, low sodium concentration, and low chloride concentration of the former.

Calcium and Sodium

A sodium-poor solution must essentially be calcium-free (B-HTK, UW-CSS, COL) for it to be effective [20], as low extracellular sodium concentrations open calcium channels, which could increase cytosolic calcium and thus activate injurious reactions [21]. Thus acalcemia appears to be a prerequisite for an intracellular formulation. However, in extracellular-formulated cardioplegic solutions acalcemia is reported to be harmful by predisposing to the calcium paradox [20], a phenomenon of severe cellular damage that occurs when hearts are reperfused with a fluid containing calcium ions after a period of calcium-free perfusion [22]. Furthermore, this phenomenon is not specific to the myocyte as it has been noted in canine kidney perfusions, where it produced changes to the vessel walls and cell membranes [23].

Although we demonstrated a beneficial effect of an acalcemic ST solution on endothelial cells at hypothermia, this solution was possibly not truly acalcemic as it probably contained trace amounts of calcium as mentioned previously. However, survival decreased at normothermia with the acalcemic ST in contrast to ST, possibly as the likelihood of the calcium paradox increases with high extracellular sodium concentration and elevated temperature [24].

Chloride

We have shown that the low chloride content of intracellular solutions is an important feature in their composition, as Incell-High Cl, which had a higher chloride concentration than COL, resulted in diminished hypothermic endothelial cell survival. However, at normothermia Incell-High Cl produced better endothelial cell survival (51%) than the other intracellular solutions (<20%). Thus a low chloride content was beneficial at *hypothermia* in terms of endothelial cell survival and was associated with diminished morphological alterations. In contrast, at *normothermia* a low chloride concentration appeared to contribute to the observed cytotoxicity of the intracellular solutions.

In the extracellular solution ST, a low chloride concentration improved endothelial cell survival at hypothermia (71%) and was not detrimental at normothermia. However, the improved hypothermic endothelial cell survival provided by ST-Low Cl might have been partly due to a lower free ionized calcium as a result of calcium binding by gluconate [25]. Nevertheless, ST-Ca 0.05 mmol/L and ST-Ca 0.6 mmol/L, with free ionized calciums of less than 0.05 mmol/L and 0.5 mmol/L, respectively, thus bracketing the free calcium content of ST-Low Cl (free Ca^{2+} = 0.16 mmol/L), did not themselves significantly improve ST.

Chloride is a freely permeable extracellular anion, and ischemia-induced intracellular acidosis or elevated sodium level will encourage chloride to enter the cell, down its electrochemical gradient [26]. This entry would promote intracellular edema, as impermeate intracellular anions cannot leave the cell. Decreasing the chloride content of organ preservation solutions, by replacement with high-molecular-weight impermeate anions, helps regulate cellular edema [27]. Furthermore, these anions may bind calcium and magnesium and thus alter their free ionized concentrations, as we have demonstrated. Nevertheless a low chloride concentration was beneficial in both intracellular and extracellular solutions.

Additives: Buffers, Substrates

To determine why B-HTK was superior to the other intracellular solutions we examined No-HTK, a basic electrolyte formulation without the additives of B-HTK (histidine, tryptophan, KH-2-oxygluterate). This was inferior to B-HTK, and no better than COL or UW-CSS at both 24 hours and 36 hours after exposure. The beneficial effect of the HTK additives might have been wholly or in part due to the greater buffering capacity of histidine, as endothelial cells are known to be high lactate producers [1].

Potassium

The No-HTK solution has a potassium concentration of 10 mmol/L, in contrast to the otherwise similar COL solution (potassium concentration, 115 mmol/L) (see Tables 1, 2). Because both solutions gave similar results, we infer that the high potassium concentration of 115 mmol/L in the intracellular COL solution did not contribute to hypothermic cytotoxicity. However, a high potassium concentration has been reported to be harmful in extracellular solutions [25]. Furthermore, the observed cytotoxicity of the standard intracellular preservation solutions at normothermic exposure was not wholly due to a high potassium level, as B-HTK (normothermic survival <20%) only contains a potassium concentration of 10 mmol/L.

Temperature

In a previous study ST solution and the original Bretschneider-Procaine solution showed similar cytotoxicity, which increased equally when the temperature was raised from 19°C to 37°C [6]. However, this study evaluated endothelial cell suspensions by a method of dye exclusion (trypan blue). Furthermore, the Bretschneider-Procaine solution differs from the B-HTK solution now used internationally and in our study. In contrast, we show increased survival and adherence of the cells at normothermic (as opposed to hypothermic) exposure with ST. However, this increase might have been due to the improved buffering provided by the 5% CO_2 atmosphere used in all the normothermic groups.

As discussed in this report, the observed normothermic cytotoxicity of the intracellular solutions was related more to a low concentration of sodium, as well as a low concentration of chloride, than to a high concentration of potassium. These observations may be important when considering using cardioplegic solutions as reperfusion solutions at 37°C.

Conclusion

This study used a basic index, morphology and cell survival, to assess the endothelial response to preservation solutions, and more sensitive endothelial functions should also be assessed even if indirectly [7, 8], to highlight differences between less toxic solutions. Fur-

thermore, although we do not suggest altering the composition of cardioplegic solutions on the basis of these findings, in a similar endothelial study assessing cardioplegic solutions used in South Africa [28], we showed poor endothelial preservation with some cardioplegic solutions being used clinically. This in turn correlated with another study that assessed postischemic myocardial function in an in vivo baboon heart model after protection with similar cardioplegic solutions [29]. We did not examine blood cardioplegia, which is not routinely used for long-term organ storage, because of the possibility of immunological incompatibility if not autologous. However, Harjula and associates [30] showed good endothelial preservation after 2 hours of exposure to blood cardioplegia at 22°C.

The best available solution for hypothermic endothelial cell preservation is the B-HTK cardioplegic solution; however, it should not be used at normothermia. Its superiority at hypothermia appears to be due to the low chloride content of intracellular solutions and to the additives histidine, tryptophan, and KH-2-oxygluterate in B-HTK. Furthermore, we were able to improve the efficacy of the extracellular ST cardioplegic solution in terms of endothelial cell survival by replacing chloride ions with gluconate.

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Appendix A-7

Practicalities of delivering crystalloid cardioplegic solutions: I. Oxygenation

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Cardiovascular Topics

Practicalities of delivering crystalloid cardioplegic solutions (I)

Oxygenation

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Abstract

Oxygenation of crystalloid cardioplegic solutions has been shown to improve post-ischaemic myocardial recovery by supplying oxygen dissolved in solution. We compared the oxygen content of 'unoxygenated' St Thomas' Hospital No. 2 cardioplegic solution (0,45 - 0,84 ml O_2/100 ml solution) with the oxygen contents obtained by various oxygenating methods at different gas flow rates. Oxygenating St Thomas' cardioplegia with 95% O_2, 5% CO_2 at 1,0 l/min at hypothermia (4°C) in a standard 1 litre glass vacolitre kept in ice, produced an oxygen content of 3,48 ± 0,14 ml O_2/100 ml solution within 30 minutes. This oxygen content was no different to that obtained by means of a more complex hypothermic recirculation oxygenating method.

In the clinical situation these saturated cardioplegic solutions could rewarm in the cardioplegic delivery tubing in between each dose, and thus cause gas to come out of solution as bubbles. We therefore oxygenated St Thomas' cardioplegia with 95% O_2, 5% CO_2 at 1,5 l/min at room temperature (19°C - 24°C) either in an oxygenating reservoir or in its 1 litre glass vacolitre. Both produced similar oxygen contents — 2,32 ± 0,16 and 2,07 ± 0,11 ml O_2/100 ml solution respectively — representing 60% of that obtained with hypothermic oxygenation. Clinically, the solution would then have to be cooled to 4°C - 6°C before infusion. However, there would be no potential for bubble formation if rewarming occurred during clinical use.

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Cardioplegic solutions are used to protect the myocardium during routine open-heart surgery while the aorta is cross-clamped. The chemical composition of these solutions in conjunction with hypothermia stops electromechanical activity, and thereby conserves energy.[1,2] However, the arrested hypothermic myocardium still has a basal metabolic requirement, and therefore cardioplegic solutions should contain substrates that can be metabolised to meet these ongoing energy needs.[3] Furthermore, if a cardioplegic solution is oxygenated the more efficient aerobic metabolic pathways will produce greater amounts of high-energy phosphates, despite the fact that these solutions are only infused intermittently during the ischaemic period.[4]

There are two primary categories of cardioplegic solutions, viz. blood and crystalloid cardioplegic solutions, both of which can carry oxygen.[5] Blood cardioplegia, a combination of a crystalloid *concentrate* and oxygenated blood withdrawn from the cardiopulmonary bypass circuit, will supply oxygen bound to haemoglobin. The delivery system for blood cardioplegia has been fairly well standardised, and depends upon the ratio in which the crystalloid *concentrate* is added to the blood component, either as a 1:2 or 1:4 dilution. The international gold standard is the 1:4 blood cardioplegia developed by Buckberg,[6] and delivered using tubing of differing internal diameters passed through the same

'roller-pump head' (Fig. 1). One revolution of a standard roller-pump using 1:4 delivery would provide 15 ml of 'blood cardioplegia' by combining 3 ml concentrate (1/8" tubing) and 12 ml oxygenated blood (1/4" tubing). However, only a limited amount of oxygen is actually available, since blood cardioplegia has a low haematocrit and at low temperatures less oxygen is released because of a leftward shift of the oxyhaemoglobin dissociation curve.[7] This more complex blood cardioplegia delivery system must also contain a heat exchanger that can adjust the temperature of the final mixed cardioplegic solution to 4°C - 10°C before infusion.

On the other hand, crystalloid cardioplegic solutions can supply oxygen dissolved in solution, and oxygenation of these solutions has previously been shown to improve post-ischaemic myocardial recovery.[4,8,9] In order to supply the maximum amount of dissolved oxygen, the crystalloid cardioplegic solution should be oxygenated at a low temperature (4°C - 6°C), because of increased solubility of gases at low temperatures.[10] Therefore, complex hypothermic recirculation systems have been designed to ensure maximal oxygenation of crystalloid cardioplegic solutions (Fig. 2). However, a hypothermic maximally oxygenated cardioplegic solution has the potential to form gaseous emboli if the solution rewarms in the delivery tubing before infusion. The solubility coefficient for oxygen diminishes as temperature increases, and thus oxygen will escape from the now supersaturated solution and form bubbles. These bubbles are potentially harmful if then infused into the coronary circulation. This danger could be avoided by oxygenating the cardioplegic solution at a temperature equal to or higher than that it is likely to be rewarmed to, but this would possibly imply accepting a lower oxygen content.

We therefore compared the oxygen content of the St Thomas' Hospital No. 2 cardioplegic solution (ST; Sabax Ltd, Johannesburg) when oxygenated by three different methods. The advantages, disadvantages and precautions that should be taken when oxygenating crystalloid cardioplegic solutions by these three methods are discussed.

Methods

Oxygen was supplied as 95% O_2, 5% CO_2 for all cardioplegic oxygenating systems and gas flow was kept constant with a gas flow meter (Air-Shields Vickers; Hatboro, Pennsylvania, USA) during each evaluation. The oxygen tension (Po_2) was measured at 5-minute intervals for 30 minutes from the onset of oxygenating ST, with a Ciba Corning 288 blood gas analyser (Ciba Corning Diagnostics Corporation; Medfield, Massachusetts, USA). Each method was evaluated ($N = 6$) using a gas flow rate of 1,0 l/min. Thereafter alternative gas flow rates (0,5, 1,5 or 2,0 l/min) were tested.

Oxygenation at hypothermia

The first method evaluated was the aforementioned hypothermic recirculation system (CCAS-OC oxygenated crystalloid cardioplegic administration system, American Bentley; Irvine, California, USA). The cardioplegic solution is oxygenated in a hard shell reservoir (OxyHi reservoir) and continuously recirculated through an aluminium cooling

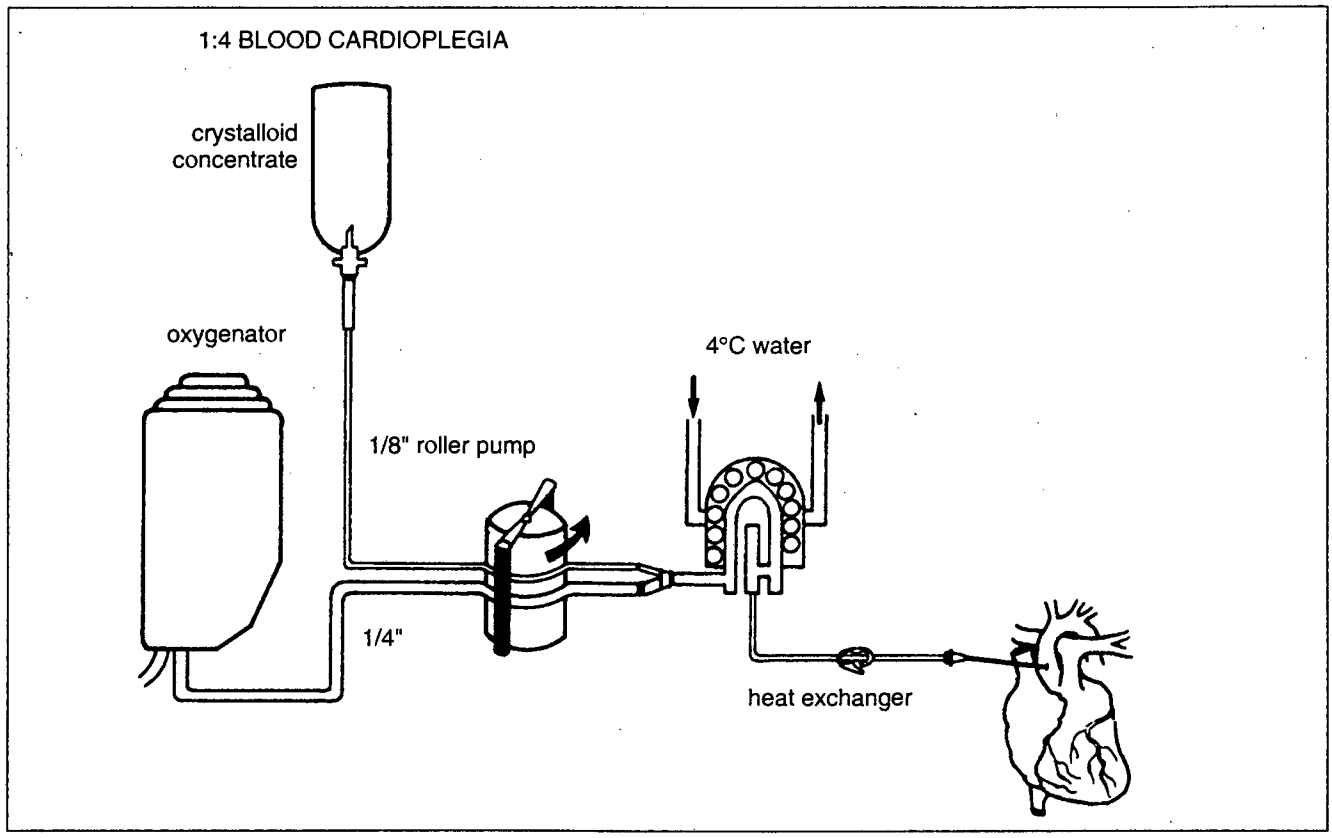

Fig. 1. A blood cardioplegia delivery system. One part crystalloid concentrate and four parts blood taken from the cardiopulmonary bypass oxygenator are combined and then cooled by a heat exchanger, before infusion into the coronary circulation. The composition of the crystalloid concentrate would depend upon the intended delivery ratio.

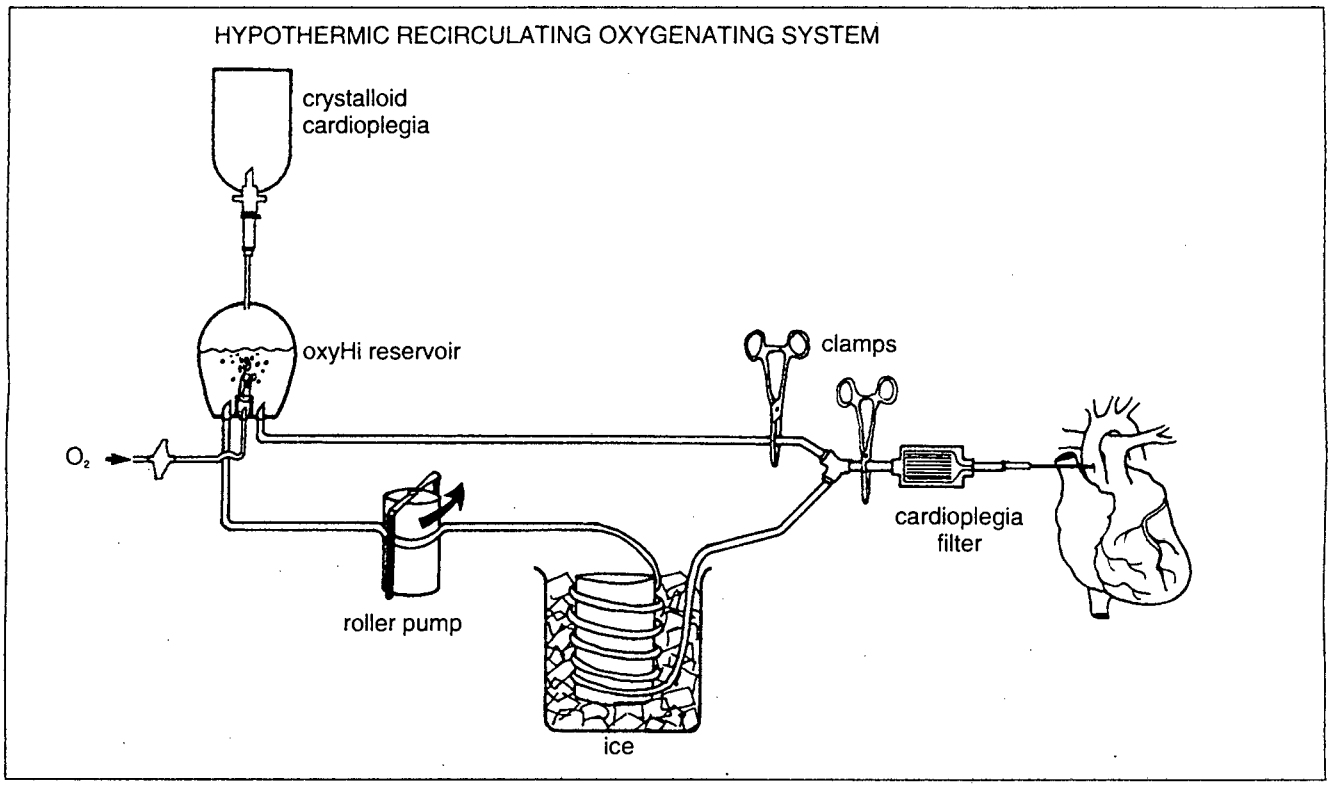

Fig. 2. A delivery system for crystalloid cardioplegia that ensures cold (4°C) cardioplegia and maximal oxygenation thereof, by continually recirculating the solution through a cooling coil placed in ice. The removal/application of the indicated clamps allows the cardioplegic solution to be recirculated continuously or alternatively delivered to the patient.

coil placed in ice, thus assuring continuous maximal hypothermic oxygenation (Fig. 2). In order to standardise the experiments, oxygenation was only commenced once the temperature of the ST had been decreased to less than 4°C by recirculation through the coil in ice.

The second method evaluated was simple bubble oxygenation of the cardioplegic solution in its original manufacturer's container (1 litre glass bottle). Cold ST that had been stored in the fridge had a mean temperature of 13°C, and was therefore kept in a bucket of ice for 30 minutes to ensure a temperature of less than 4°C before use. The cardioplegic solution was then oxygenated in its container while kept in ice. Oxygen was introduced into the 1 litre ST glass vacolitre through the 'venting port', thus the gas was introduced into the bottom of the upright container, bubbled through the solution and exhausted via two needles inserted in the 'injection port'.

Oxygenation at room temperature

The third method evaluated consisted of oxygenating ST at room temperature (19°C - 24°C) either in an OxyHi reservoir or its 1 litre glass vacolitre. In clinical practice the cardioplegic solution would thereafter have to be cooled to 4°C - 6°C before infusion, by either a cooling coil or heat exchanger.

Calculations and statistics

Blood gas analysers measure pH and Po_2 at an electrode temperature of 37°C. These values were therefore corrected to the temperature of the sample (T), either automatically by the analyser or by using the formulae described by Kelman and Nunn:[11]

pH (corrected) = pH - 0,015 * (T - 37)

Po_2 (corrected) = Po_2 * 10 ^ (0,0052 + 0,027 * (1 - 10 ^ (- 0,13 * (100 - % O_2 saturation))) * (T - 37))

These corrections are not precise since the above formulae were devised for blood as opposed to physiological crystalloid solutions. However, they will approximate the true value more closely than if coefficients for pure water were used.[12] Oxygen content was then calculated from the corrected Po_2 (mmHg) by using the formula: oxygen content = Po_2 (corrected)/760 mmHg * solubility coefficient (see Table I).

Means and standard error of means for $N = 6$ observations are presented, and were analysed by two-way analysis of variance, with pairwise comparisons by acceptance intervals of means using the F test. Statistical significance was taken as $P < 0,05$.

Results

The Po_2 of 'unoxygenated' St Thomas' Hospital No. 2 cardioplegic solution, exposed to the atmosphere and thus equilibrated to ambient partial pressure, was approximately 150 mmHg. This corresponded to an oxygen content of 0,45 - 0,84 ml O_2/100 ml solution, depending on the temperature of the solution. The pH of 'unoxygenated' ST (corrected to a temperature of 10°C) ranged from pH 7,6 to pH 8,3, again depending upon temperature and thus CO_2 content. The oxygen content and pH of ST were then measured during oxygenation at different gas (95% O_2 5% CO_2) flow rates with $N = 6$ for each evaluation.

Oxygenation at hypothermia

When ST was oxygenated at a gas flow of 1,0 l/min at hypothermia (temperature less than 4°C) with the CCAS-OC recirculation system, the oxygen content increased from the above baseline values to 3,35 ± 0,22 ml O_2/100 ml solution within 20 minutes, $P < 0,01$. This was not significantly different to the final maximum oxygen content of 3,47 ± 0,14 ml O_2/100 ml solution obtained after 30 minutes of oxygenation. The oxygen content produced by oxygenating at a lower gas flow rate of 0,5 l/min was no different (Table II).

Alternatively, cold ST (temperature less than 4°C) was simply oxygenated (1,0 l/min gas flow) in its original 1 litre glass vacolitre. The oxygen content increased to a maximum of 3,48 ± 0,14 ml O_2/100 ml within 30 minutes. This was no different to the above more complex recirculation system, either in time taken to stabilise or final oxygen content (Table II).

The final pH of ST (corrected to a temperature of 10°C) after oxygenating with 95% O_2, 5% CO_2 was similar with both methods, namely 7,03 ± 0,03 and 7,06 ± 0,02, respectively.

Oxygenation at room temperature

Oxygenating ST in the OxyHi reservoir (no recirculation) at room temperature at a gas flow rate of 1,0 l/min produced an oxygen content of 2,39 ± 0,12 ml O_2/100 ml solution within 30 minutes. This oxygen content was lower than when ST was oxygenated at hypothermia ($P < 0,01$). Furthermore, the major increase in oxygen content was obtained within 15 minutes of commencing oxygenation ($P < 0,01$), and there was no significant increase thereafter (Table III). The pH (corrected to a temperature of 10°C) of ST after oxygenation at room temperature was 7,32 ± 0,03, which was more alkalotic than that observed with the above hypothermic oxygenation methods ($P < 0,01$). Increasing the gas flow rate to 1,5 l/min did not alter either the interim or final oxygen contents (Table III). However, increasing the gas flow further to 2 l/min resulted in higher initial values in the first 15 minutes of oxygenation, but the final 30 minutes oxygen content of 2,63 ± 0,04 ml O_2/100 ml solution was no different to the final values observed with the lower gas flow rates. Similarly, the final pHs (corrected to a temperature of 10°C) when oxygenating with either 1,5 or 2,0 l/min were identical at pH 7,27 ± 0,01, and no different to that observed at a flow rate of 1,0 l/min.

Oxygenating ST at 1,5 l/min (95% O_2, 5% CO_2) at room temperature in its 1 litre glass vacolitre as opposed to the OxyHi reservoir resulted in a more gradual increase in oxygen content ($P < 0,05$), but the final 30-minute value of 2,07 ± 0,11 ml O_2/100 ml solution was no different to the values observed with the same gas flow rate in the OxyHi reservoir (Table III). The final pH of 7,27 ± 0,03 was also no different.

These oxygen contents obtained by oxygenating the cardioplegic solution at room temperature were approximately 60% of that observed when ST was oxygenated at hypothermia (4°C).

TABLE I. SOLUBILITY COEFFICIENT: ml OF OXYGEN DISSOLVED PER 100 ml OF 0,155 N NaCl AT 760 mmHg PRESSURE [10]

4,256 (4°C)	2,989 (20°C)	2,821 (23°C)
3,689 (10°C)	2,931 (21°C)	2,768 (24°C)
3,048 (19°C)	2,875 (22°C)	2,273 (37°C)

Maintenance of oxygen dissolved in solution

Hypothermic oxygenation of ST using the CCAS-OC recirculation system ensures not only a cardioplegic solution temperature of less than 4°C, but also that maximal oxygen content is maintained over any time period. In contrast, after oxygenating a cardioplegic solution in a glass bottle, it must be vented with a large-bore needle to the atmosphere when used clinically, thus potentially allowing contained gases to equilibrate to atmospheric partial pressures. ST was therefore fully oxygenated in its original 1 litre glass vacolitre for 30 minutes at a temperature of less than 4°C. The glass bottle was then kept in ice (4°C) but vented to the atmosphere without further oxygenation, and after 2 hours the oxygen content was still 3,51 ± 0,12 ml O_2/100 ml solution. Thus we observed no significant loss of oxygen from cold (4°C) ST in the 2-hour period following full oxygenation (Fig. 3). In addition, the pH of ST after 2 hours was 7,07 ± 0,02, which was no different to the initial pH on completion of oxygenation (pH 7,06 ± 0,02).

Similarly, initially oxygenating ST for 30 minutes at room

TABLE II. OXYGEN CONTENT OF CARDIOPLEGIA OXYGENATED AT HYPOTHERMIA

	Duration of oxygenation (min)						
Method	*0*	*5*	*10*	*15*	*20*	*25*	*30*
CCAS-OC 0,5 l/min	0,78 ± 0,16	2,10 ± 0,10*	2,76 ± 0,10*	3,41 ± 0,14*	3,58 ± 0,11	3,55 ± 0,14	3,69 ± 0,01
CCAS-OC 1,0 l/min	1,05 ± 0,04	2,11 ± 0,18*	2,88 ± 0,10*	3,24 ± 0,17	3,35 ± 0,22**	3,45 ± 0,15	3,47 ± 0,14
GL-B 1,0 l/min	0,60 ± 0,12†	1,66 ± 0,30*†	2,62 ± 0,13*	2,98 ± 0,18	3,23 ± 0,18**	3,48 ± 0,14	3,48 ± 0,14

GL-B = 1 litre glass bottle.
*$P < 0,01$ compared to the preceding time interval.
**$P < 0,05$ compared to the 10-minute time interval.
†$P < 0,05$ compared to CCAS-OC 1,0 l/min at the corresponding time interval.

(ml O_2/100 ml solution)

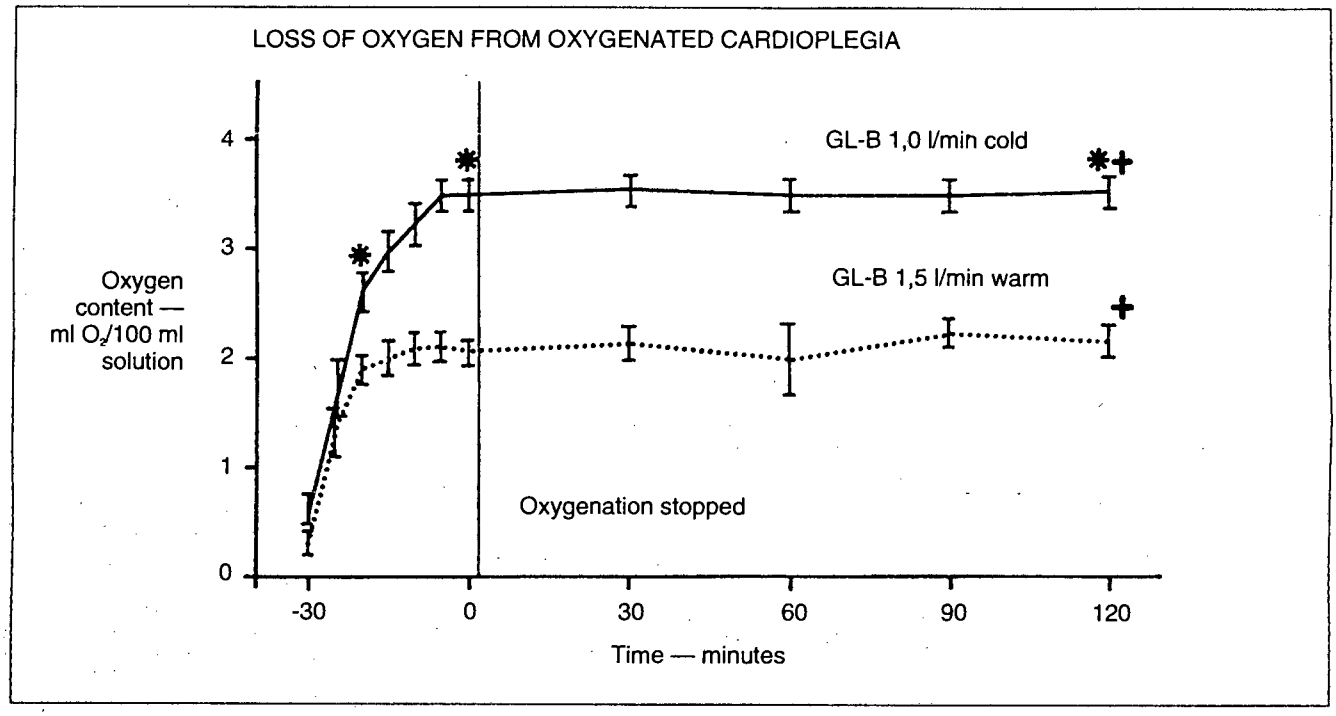

Fig. 3. Loss of oxygen from oxygenated cardioplegia. The ST was fully oxygenated with 95% O_2, 5% CO_2 for 30 minutes, at either hypothermia (4°C; at 1,0 l/min) or room temperature (20°C - 22°C; at 1,5 l/min) in its original 1 litre glass vacolitre (GL-B). Thereafter, oxygenation was discontinued and the glass bottle was vented with a large-bore needle to the atmosphere, and oxygen content monitored. Means for 6 evaluations, with the vertical bars representing standard error of means, are presented. (*P < 0,01 compared to GL-B 1,5 l/min warm, at the respective time period; † P = NS compared to time = 0.)

temperature and thereafter maintaining ST at room temperature, in its 1 litre glass vacolitre vented with a needle to the atmosphere, also did not result in any significant loss of oxygen content in the subsequent 2 hours (Fig. 3).

Discussion

If a crystalloid cardioplegic solution contains bicarbonate (viz. St Thomas' Hospital No. 2 cardioplegic solution), then oxygenation should be with 95% O_2, 5% CO_2 and not 100% O_2.[9] The addition of 5% CO_2 stabilises the pH optimally at 7,0 (corrected to 10°C), without any drop in Po_2. We therefore used 95% O_2, 5% CO_2 to oxygenate ST in each system being evaluated, and in addition monitored pH.

We have shown that a maximum oxygen content in ST (3,5 ml O_2/100 ml solution) could be obtained by means of a hypothermic (4°C) recirculation oxygenating system (CCAS-OC) at a gas flow rate of either 0,5 or 1,0 l/min. However, simply oxygenating cold ST (4°C) in its original 1 litre glass vacolitre for 30 minutes with 95% O_2 5% CO_2 at 1,0 l/min while immersed in ice, also resulted in a similar final oxygen content. After initially oxygenating the cardioplegic solution for 30 minutes with the latter system and

TABLE III. OXYGEN CONTENT OF CARDIOPLEGIA OXYGENATED AT ROOM TEMPERATURE

	Duration of oxygenation (min)						
Method	*0*	*5*	*10*	*15*	*20*	*25*	*30*
OxyHi 1,0 l/min	0,72 ± 0,07	1,50 ± 0,25*	1,82 ± 0,26	2,14 ± 0,22**	2,24 ± 0,17	2,18 ± 0,13	2,39 ± 0,12
OxyHi 1,5 l/min	0,38 ± 0,15	1,89 ± 0,14*	2,23 ± 0,16	2,26 ± 0,18	2,36 ± 0,14**	2,40 ± 0,13	2,32 ± 0,16
OxyHi 2,0 l/min	0,79 ± 0,07†	2,14 ± 0,14*‡	2,49 ± 0,07‡	2,63 ± 0,07**‡	2,54 ± 0,09	2,60 ± 0,07‡	2,63 ± 0,04
GL-B 1,5 l/min	0,35 ± 0,08	1,40 ± 0,20*†	1,91 ± 0,09*	1,99 ± 0,13	2,10 ± 0,13	2,11 ± 0,14	2,07 ± 0,11

GL-B = 1 litre glass bottle.
*P < 0,01 compared to the preceding time interval.
**P < 0,05 compared to the 5-minute time interval.
†P < 0,05 compared to OxyHi 1,5 l/min at the corresponding time interval.
‡P < 0,05 compared to OxyHi 1,0 l/min at the corresponding time interval.

(ml O_2/100 ml solution)

then discontinuing oxygenation (e.g. to administer the initial dose of cardioplegia), the oxygen content of ST (exposed to the atmosphere but still kept in ice) did not decrease significantly during the subsequent 120 minutes before subsequent doses of cardioplegia). In addition, the final pH of ST (pH 7,06 at 10°C) was also maintained at this previously determined optimal value.[9] Therefore, the more complex recirculation system for oxygenating crystalloid cardioplegia does not appear to be necessary.

If a solution is fully saturated with a gas at a low temperature, rewarming of the solution results in the gas coming out of the now supersaturated solution as bubbles. In the clinical situation, cardioplegic solution contained in the cardioplegic delivery line rewarms to the prevailing theatre environment temperature of 19°C - 24°C, in the period between each reinfusion of cardioplegia. If gaseous emboli are formed, they would be harmful to the myocardium if infused with subsequent doses of cardioplegia. Therefore, if a crystalloid cardioplegic solution is maximally oxygenated the delivery system should incorporate a means of removing these bubbles before successive reinfusions. Bubbles can be removed by either a microfilter (CAS-filter; American Bentley, Irvine, California, USA) inserted immediately before the ascending aorta or alternatively the 'warm cardioplegic solution containing gaseous emboli in the line' can be removed through an aortic vent, before administering each subsequent reinfusion. Hearse *et al.*[13] advise routine use of an 0,8 μm microfilter in order to ensure removal of potentially harmful microparticles, contained in the majority of crystalloid cardioplegic solutions[13] as well as microbubbles. However, as there will always be a potential site for bubble formation in the short length of tubing connecting the filter to the aorta, the use of only a microfilter to prevent infusion of formed microbubbles will not be 100% effective. In contrast, venting the cardioplegic solution contained in the delivery line leading to the operating table to waste before each reinfusion, removes all bubbles and also removes cardioplegic solution that has potentially rewarmed after the previous dose of cardioplegia. This volume of cardioplegia could, depending upon the patient's weight, be a significant percentage of the next dose of cardioplegia. Infusion of warm cardioplegia would itself decrease the protective effect of multidose cardioplegia. However, this method requires a special cardioplegic delivery cannula (Aortic root cannula with vent line, 12GA: DLP, Grand Rapids, Michigan, USA), as well as the removal/ reapplication of appropriate clamps before and after each bolus of cardioplegia. Nevertheless, one should consider using both of these methods routinely when administering oxygenated crystalloid cardioplegia. In addition, an 0,2 μm bacterial filter (Pall Biomedical Products Corporation, Glen Grove, New York, USA) should be interposed in the 95% O_2 5% CO_2 gas line to prevent possible contamination of the solution.

Alternatively, if the cardioplegic solution is oxygenated at a temperature equal to or higher than it is likely to be rewarmed to, the solution would not become supersaturated and therefore gas bubbles would not form. Thus, if the cardioplegic solution is oxygenated at room temperature and only then cooled to 4°C before infusion, the tendency for subsequent bubble formation would be diminished. However, oxygenating at room temperature (19°C - 24°C) produces an oxygen content of only 2,1 ml O_2/100 ml solution, which is 60% of the maximal obtainable oxygen content, and also does not induce a shift in pH (pH 7,27; corrected to 10°C) to the previously determined optimal value (pH 7,0 at 10°C) for oxygenated ST.[9] Whether an oxygen content of 2,1 ml O_2/100 ml solution will produce equivalent beneficial effects, as previously shown with maximal hypothermic (4°C - 10°C) oxygenated cardioplegic solutions (oxygen content 3,0 - 4,0 vol %),[4,7,9] would still have to be determined. As yet we can only postulate that this 'submaximal' oxygen content might be sufficient, since in an isolated rat heart model we previously showed that only approximately 50% of available oxygen is in fact taken up upon each reinfusion of a well oxygenated ($Po_2 \pm 550$ mmHg at 10°C) cardioplegic solution.[9] Oxygen dissolved in solution is released in a linear fashion, and theoretically all contained oxygen is available to the myocyte.[7] Furthermore, lower oxygen contents might be beneficial since high partial pressure of oxygen can increase the formation of oxygen free radicals,[15] which are potentially harmful to the myocardium. However, other studies have shown that oxygen contents that are lower than those obtained by oxygenating cardioplegic solutions with 95% O_2, 5% CO_2 at 20°C are associated with diminished myocardial protection.[14]

Other alternative techniques that have been proposed in order to obtain high cardioplegic solution oxygen contents include overnight cold pressurisation of the solution with oxygen, after displacement of nitrogen by prior warming of the solution.[16] However, the sterility 'seal' of the vacolitre would have been broached for more than 12 hours at the time of use, and this would therefore be a point of concern. Alternatively, Daggett[17] suggested that the amount of oxygen dissolved in solution could be increased by hyperbaric oxygenation; a 15 cm H_2O PEEP bottle is connected to the vent of the container in which the cardioplegic solution is oxygenated. We have not evaluated either of these methods.

In conclusion, simple oxygenation of cold (4°C) St Thomas' Hospital No. 2 cardioplegic solution with 95% O_2, 5% CO_2 at 1,0 l/min in its original 1 litre glass vacolitre is as effective as more complex hypothermic recirculation oxygenating delivery systems, with respect to oxygen content. However, with both of these methods measures should be taken to ensure that gaseous emboli formed in the cardioplegic delivery line are not infused into the coronary circulation, with subsequent doses of cardioplegia.

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Appendix A-8

Practicalities of delivering crystalloid cardioplegic solutions: II. Temperature.

Von Oppell U O,

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Cardiovasc J South Afr 1992; (In press).

PRACTICALITIES OF DELIVERING CRYSTALLOID CARDIOPLEGIC SOLUTIONS. II. TEMPERATURE.

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ABSTRACT

Profound cardiac hypothermia ($10^{0}C$ - $15^{0}C$) is an essential component of most techniques used to provide myocardial protection when the aorta is cross-clamped during open heart surgery. Cardiac hypothermia is induced and maintained primarily by infusing cold cardioplegic solutions into the heart. We evaluated whether crystalloid cardioplegic delivery systems do indeed deliver the solution to the heart at a temperature of less than $6^{0}C$.

Crystalloid cardioplegic solutions stored in a standard fridge are often too warm ($11.1 \pm 0.6^{0}C$). Therefore, solutions stored in a fridge should additionally be immersed in ice for at least 30 min before use, to ensure a sufficiently low temperature ($3.0 \pm 0.6^{0}C$, $p < 0.01$). Alternatively, if cardioplegic solutions are stored at room temperature then a cooling mechanism must be used in the delivery system. However, a cooling coil immersed in ice may not be adequate at cardioplegic flow rates greater than 200 ml / min ($p < 0.01$).

In addition, cardioplegic solution contained in the delivery lines leading to the heart rewarms towards ambient room temperature ($19^{0}C$ - $23^{0}C$) during the interval (10 min - 30 min) in between each reinfusion of cardioplegia ($14.1 \pm 0.8^{0}C$ and $20.1 \pm 0.6^{0}C$ respectively, $p < 0.01$). This volume of "warm cardioplegia" (temperature of greater than $6^{0}C$) subsequently infused varied from 66 ± 7 ml to 129 ± 4 ml, and was proportional to the total volume of cardioplegia contained in the lines (72 - 105 ml). Infusion of a significant percentage of "warm cardioplegia" ($16 \pm 2\%$ to $51 \pm 1\%$ of the total dose of cardioplegic solution reinfused), would decrease the efficacy of myocardial protection.

INTRODUCTION

Cardiac surgeons rely primarily on two fundamental principles to ensure adequate intra-operative myocardial protection during open heart surgery whilst the aorta is cross clamped; uniform delivery of a cardioplegic solution throughout the myocardium, and profound *cardiac* hypothermia [1,2]. Energy demands are thus drastically diminished

during the ischaemic aortic cross clamp period. Optimal protection is attained by arresting the myocardium in diastole with a cold cardioplegic solution, and maintaining the in situ heart at a temperature of 10°C - 15°C [3]. Although myocardial temperatures should be kept as low as possible though not below zero [4], in the clinical situation it is difficult to maintain the nonisolated heart at temperatures below 10°C [5]. Noncoronary collateral flow, surgical handling, the theater environment (room temperature, operating room lights) and conducted heat from adjacent organs (liver, descending thoracic aorta) constantly rewarms the myocardium [6]. Therefore, the majority of cardiac surgeons reinfuse cold cardioplegic solution intermittently throughout the aortic cross clamp period. This not only maintains cardioplegic arrest, but is also the primary means of maintaining the heart hypothermic. Nevertheless, other modalities (systemic and topical hypothermia) are also used to assist in keeping the myocardial temperature below 15°C whilst the aorta is cross clamped [4,7].

Systemic hypothermia implies cooling the entire body to a temperature of 22°C - 32°C after commencing cardiopulmonary bypass, by means of a heat exchanger in the extracorporeal circulation. Energy requirements for all organs are thus diminished, and cardiopulmonary bypass is safer as the period the brain can withstand total circulatory arrest (without sustaining neurological dysfunction) is prolonged from 3 min at 37°C to 45 min at 18°C [8], should the need arise. However, of specific importance to the cardiac surgeon is that the gradient along which the myocardium is constantly being rewarmed by adjacent organs is decreased, and flow rates can be decreased thus diminishing noncoronary collateral flow. Nevertheless, prolonging cardiopulmonary bypass because of the time required for induction and reversal of systemic hypothermia can be harmful. Topical hypothermia improves epicardial cooling and reduces conducted heat, but can also be harmful; phrenic nerve palsy, increased postoperative respiratory complications, and epicardial frostbite injury. Thus, the infusion of cold cardioplegic solution remains the most important means of producing and maintaining uniform myocardial hypothermia, in addition to producing diastolic cardiac arrest.

Hypothermia is an essential synergistic factor when crystalloid cardioplegic solutions are used. The majority of cardioplegic solutions rely on a high potassium concentration to induce diastolic arrest but too high a concentration (greater than 20 mmol/L) can be detrimental, as this concentration of potassium then promotes intracellular calcium influx which is harmful during ischaemia [9,10]. However, if one assumes that the myocardium will always be maintained hypothermic ($10^{o}C$ - $15^{o}C$), then the lowest possible concentration of potassium can be used, as hypothermia potentiates potassium arrest by itself inducing partial membrane depolarization [11,12]. Therefore, it is essential that optimally formulated crystalloid cardioplegic solutions should be infused into the heart at temperatures of $4^{o}C$ - $6^{o}C$. This is achieved by either maintaining the vacolitre of cardioplegia below $4^{o}C$ in ice, before and in between each multidose reinfusion, or alternatively the delivery system must include a device that cools the solution; viz a heat exchanger or cooling coil immersed in ice. We determined whether methods of infusing crystalloid cardioplegic solutions do indeed deliver the solution to the myocardium at the correct temperature, that is less than $6^{o}C$.

We also evaluated the degree and amount of cardioplegic solution contained in the delivery line leading to the heart, that rewarms towards ambient theater temperature ($19^{o}C$ - $23^{o}C$) in between each reinfusion of cardioplegia. The efficacy of multidose cardioplegia is diminished if a significant volume of warm cardioplegia is inadvertently infused with subsequent reinfusions of cardioplegia.

METHODS

A) Assessment of means used to ensure a cardioplegic solution temperature of less than $6^{o}C$.

Two primary means of delivering crystalloid cardioplegic solutions were evaluated:-

i) **Infusion of cardioplegic solutions stored in the fridge.**

The temperature of cold St Thomas' Hospital No 2 cardioplegic solution (Sabax

Laboratories; Aeroton, Johannesburg) stored in a fridge can vary considerably. The cardioplegic solution removed from the fridge was therefore additionally immersed in ice for 30 min (to ensure a temperature of less than 4°C) prior to infusion. We also evaluated whether this cold cardioplegic solution (less than 4°C) should thereafter in addition be infused by a single pass through a cooling coil (1/4", 65 ml aluminium coil: American Bentley; Irvine, California, USA) immersed in ice.

ii) **Infusion of warm cardioplegia stored at room temperature.**

The cardioplegic solution was either infused after a single pass through the American Bentley cooling coil, or through a 3/16", 150 ml plastic coil (Sarns: 3M Health Care; Ann Arbor, Michigan, USA).

The temperature of the "delivered" cardioplegic solution was measured with an electronic temperature probe (B-A-Therm-A Bentley OXYG; American Bentley), inserted into the aortic end of the cardioplegic delivery line, 0.5 min and 1 min after starting the infusion which was kept at a constant flow rate by an extracorporeal roller pump (Sarns: 3M Health Care). In order to assess the efficiency of each system this was repeated at different flow rates:- 100 ml/min, 200 ml/min, 300 ml/min, 400 ml/min, and 500 ml/min. N = 6 for each flow rate, and thus N = 12 for recorded observations at each flow rate.

B) <u>Assessment of volume of "warm cardioplegic solution" infused during subsequent reinfusions of cardioplegia.</u>

The cardioplegic solution delivery lines were positioned similar to the clinical situation, exposed to the operating theater lights and ambient temperature. Cold (less than 4°C) cardioplegic solution was infused through a 1/4", 3.0 meter, cardioplegic delivery set incorporating a CAS-filter (American Bentley), and the "delivered temperature" of the solution at the "aortic end" was recorded every 10 seconds.

The clinical situation was simulated by initially flushing the cardioplegic solution through the lines immediately before infusion of an "induction dose" of cardioplegia. An appropriate interval was then designated before two reinfusions of cardioplegia, without removal / flushing of the cardioplegic solution contained in the delivery lines. Each "dose" of cardioplegia was infused for one minute at the predetermined rate of delivery. The volume of infused "warm cardioplegic" solution having a temperature of greater than $6^{o}C$ was then calculated.

Variables examined were:-

i) The effect of increasing the time interval in between each multidose reinfusion of cardioplegia, from 10 min to 30 min.

ii) The effect of increasing the infusion flow rate of the cardioplegic solution from 250 ml/min to 400 ml/min. Hence, each "dose" of cardioplegia was either 250 ml or 400 ml.

iii) The effect of decreasing the diameter of the tubing of the cardioplegic infusion set, from 1/4" to 3/16". The volume of cardioplegic solution contained in the 1/4" set was 105 ml, and in the 3/16" set 72 ml.

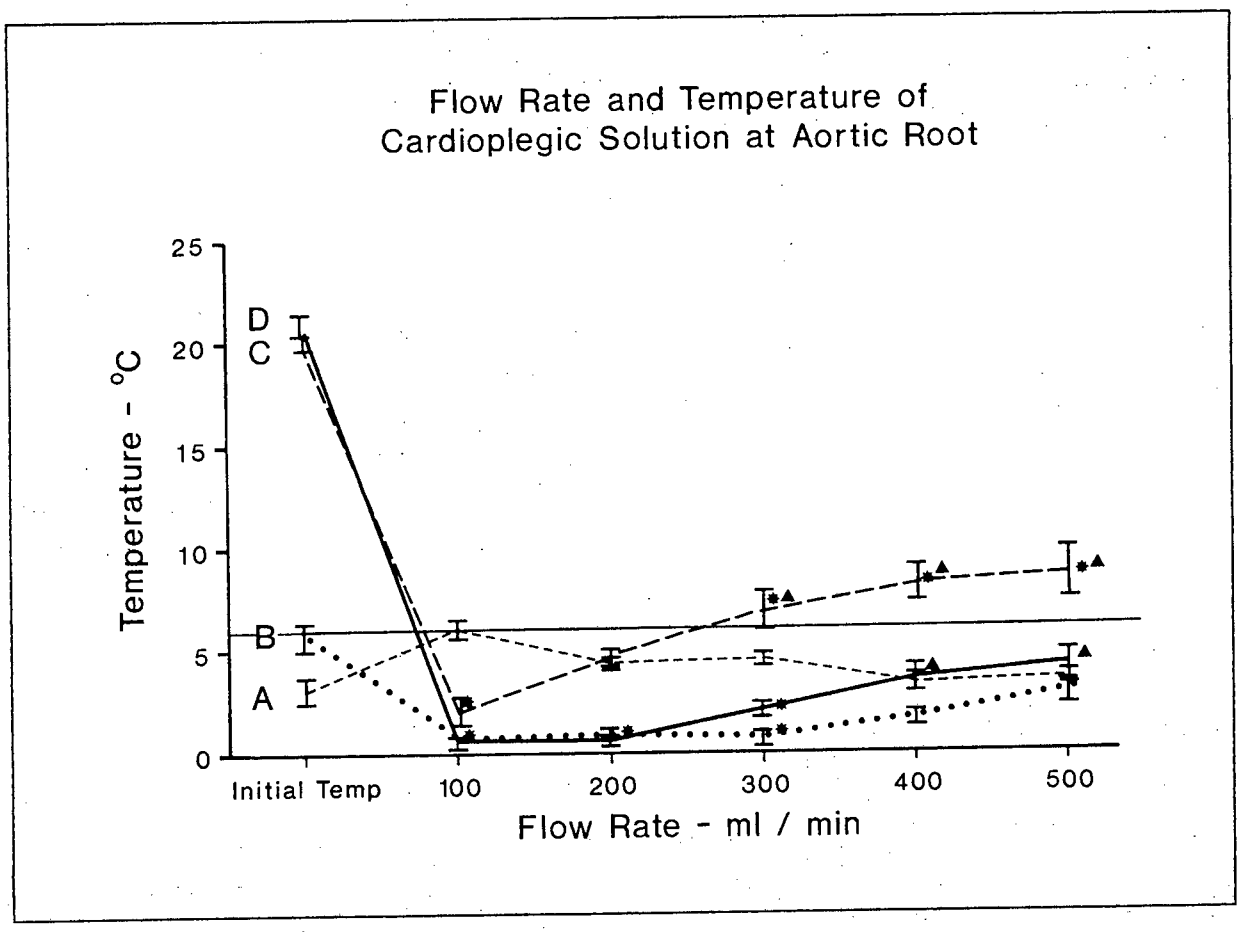
STATISTICAL ANALYSIS

Means and standard errors of means were analysed by the Anova one or two way analysis of variance, with pair wise comparisons of means by acceptance intervals with the f-test. Statistical significance was taken as $p < 0.05$.

RESULTS

A) Assessment of means used to ensure a cardioplegic solution temperature of less than 6°C (Figure 1).

Figure 1.



Legend:

Cardioplegic solution removed from the fridge was infused, after being kept in ice for 30 min (A), or in addition infused through a cooling coil immersed in ice (B) to ensure a temperature of less than 6°C. Alternatively, cardioplegic solution that was initially at room temperature (19°C - 23°C) was infused through either an American Bentley (C) or Sarns (D) cooling coil immersed in ice. The temperature of the cardioplegic solution at the "aortic end" of the cardioplegic delivery line was measured 0.5 and 1.0 min after commencing the infusion, at different flow rates. The recommended temperature below which cold crystalloid cardioplegia should be infused (6°C) is indicated (solid straight line). Means and standard errors of means (vertical bars) for N = 12 observations are presented.

* - $p < 0.01$ compared to A at similar flow rate.

^ - $p < 0.01$ compared to 200 ml / min flow rate.

i) Infusion of cardioplegic solutions stored in the fridge.

Cardioplegic solutions removed from the fridge (stored for a minimum period of 12 hr) had temperatures of 11.1 ± 0.6°C. However, after immersion in ice for 30 min the temperature decreased to 3.0 ± 0.6°C, $p < 0.01$. When no additional cooling method

was used the "delivered temperature" of the cardioplegic solution was 4.2 ± 0.3°C, if infused at a rate of 200 ml/min. This did not vary significantly when the flow rate was increased to 500 ml/min (3.4 ± 0.3°C).

Alternatively, cold cardioplegic solution removed from the fridge and immersed in ice (temperature 5.7 ± 0.6°C) was in addition infused through a cooling coil immersed in ice. The "delivered temperature" was 0.8 ± 0.2°C at 200 ml/min flow rate, which was lower than the previous method ($p < 0.01$), but at 500 ml/min the "delivered temperature" was 2.9 ± 0.5°C, which was no different to the previous method.

ii) Infusion of cardioplegic solutions stored at room temperature.

In contrast, if the cardioplegic solution was initially at room temperature (20 ± 0.4°C) and cooling was only by means of a single pass through a cooling coil immersed in ice (American Bentley), then the "delivered temperature" varied significantly with flow rate. At an infusion flow rate of 200 ml/min the "delivered temperature" of the cardioplegic solution was 4.5 ± 0.5°C, which was no different to infusion of cold (less than 4°C) cardioplegia without ancillary cooling mechanisms. However, at a higher flow rate of 500 ml/min the "delivered temperature" increased to 8.6 ± 1.2°C ($p < 0.01$ compared to 200 ml/min flow rate), which was not only higher than the aforementioned method ($p < 0.01$), but also greater than the recommended temperature for delivering crystalloid cardioplegic solutions (6°C).

The plastic cooling coil (Sarns) was larger and thus more efficient; at 200 ml/min the delivered temperature was 0.5 ± 0.2°C, and at 500 ml/min it was 4.1 ± 0.7°C ($p < 0.01$, compared to the American Bentley coil). The "delivered temperature" produced by the Sarns coil at 500 ml/min was no different to the values obtained previously with the infusion of the aforementioned cold (less than 4°C) cardioplegia, when no additional cooling mechanism was used.

B) Assessment of volume of "warm cardioplegic solution" infused during subsequent reinfusions of cardioplegia.

Cardioplegic solutions were stored for at least 24 hr in either of two fridges. The mean temperature of the solutions when removed from the fridges were 14.9 ± 0.4°C and 11.5 ± 0.7°C respectively. Therefore, in order to ensure the correct infusion temperature (4°C - 6°C), the vacolitre of cardioplegia was then immersed in a bucket of ice for at least 30 min prior to each experiment. Thereafter, the temperature of the cardioplegic solutions were consistently below 4°C irrespective of the fridge in which stored, viz 2.6 ± 0.5°C.

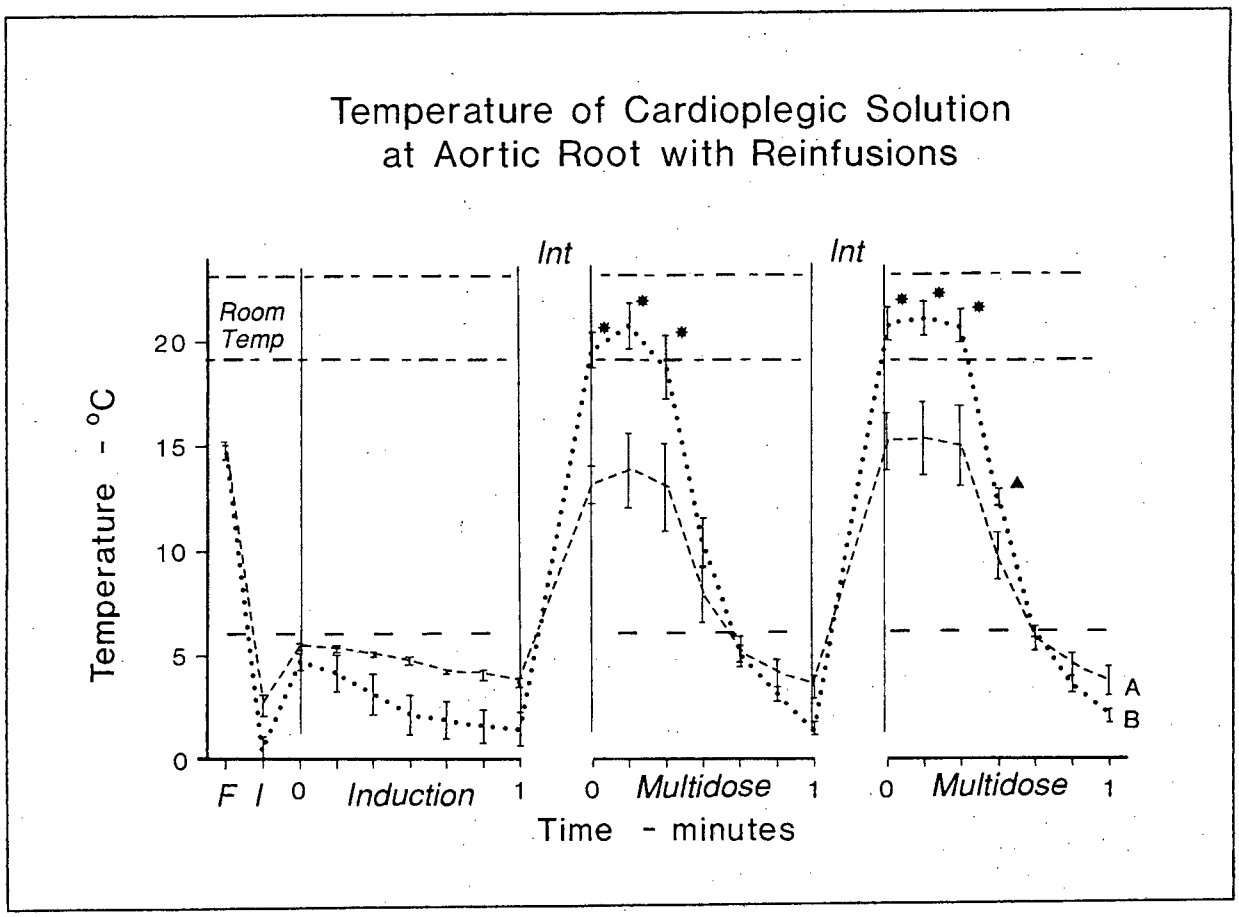
The cardioplegic delivery lines were flushed immediately before administering the "induction dose" of cardioplegia. This ensured that the "induction dose" was infused at a temperature of less than 6°C. However, after infusion of this "induction dose" the cardioplegic solution contained in the delivery lines rewarmed towards ambient room temperature (Figure 2). This resulted in a significant volume of "warm cardioplegia" (66 - 129 ml) being initially infused with each subsequent reinfusion of cardioplegia. This was effected by the following variables:-

i) Effect of time interval, in between each reinfusion of cardioplegia (Figure 2). An "induction dose" of cardioplegic solution at a temperature of 2.6 ± 0.5°C was infused through the 1/4", 3.0 m set (volume 105 ml), at a rate of 250 ml/min for one minute. Thereafter, during the following 10 minute interval the temperature of the cardioplegic solution in the delivery line increased to 13.1 ± 0.9°C ($p < 0.01$), and upon infusion of a second "dose" of cardioplegia (250 ml) a volume of 117 ± 21 ml was infused at a temperature of greater than 6°C. This was 46.8 ± 8.4 % of the 250 ml dose of cardioplegia. If the intervening interval between doses was increased to 30 min, then the solution rewarmed to a higher temperature of 19.5 ± 0.8°C ($p < 0.01$), which was no different to ambient room temperature (21.7 ± 0.3°C). The volume of "warm cardioplegic solution" subsequently infused was 125 ± 1 ml, which corresponded to 50 ±

0.4 % of the 250 ml dose, and was no different to the volume of "warm cardioplegia" infused after a 10 min interval.

Similar intervals were allowed, prior to administering another multidose of cardioplegia, and similar temperatures and volumes of "warm cardioplegia" were again recorded (Figure 2).

Figure 2



Legend:

The temperature of the cardioplegic solution at the "aortic end" of a 1/4", 3.0 m (plus CAS-filter) cardioplegic delivery line was measured during infusion of cardioplegia. Cardioplegic solution removed from the fridge (F) was kept in ice for 30 min (I) to ensure a temperature of less than 4°C. An "induction dose" was infused (250 ml/min for 1 min) immediately after flushing the lines. Thereafter, a second and third multidose of cardioplegia (250 ml/min for 1 min) was infused after either a 10 min (A) or 30 min (B) interval (Int). Means and standard errors of means (vertical bars) for N = 6 observations are presented. The recommended temperature at which crystalloid cardioplegic solutions should be infused (6°C), and the temperature range of operating theaters is indicated.

* - $p < 0.01$ compared to A at corresponding time. ^ - $p < 0.05$ compared to A at corresponding time.

ii) Rate of Administration.

The cardioplegic solution was again infused through the 1/4", 3.0 m, 105 ml set, an interval of 10 min was allowed in between each reinfusion, but the solution was now infused at a rate of 400 ml/min. Again during the 10 min interval the cardioplegic solution in the line rewarmed to a similar temperature 15.6 ± 0.4°C (Table I). The volume of "warm cardioplegic solution" then infused was also similar 111 ± 9 ml. However, because of the higher volume infused (400 ml) this formed only 28 ± 2% of the dose, a smaller percentage compared to the 250 ml dose ($p < 0.01$).

Table I

VOLUME OF WARM CARDIOPLEGIC SOLUTION INFUSED WITH SUBSEQUENT REINFUSIONS OF CARDIOPLEGIA.

VARIABLES.	A	B	C	D
Cardioplegic delivery set:	1/4"	1/4"	1/4"	3/16"
Time interval in between each reinfusion (min):	30	10	10	10
Flow rate of cardioplegia infused (ml/min):	250	250	400	400
Volume of cardioplegia contained in the delivery line (ml):	105	105	105	72
Temperature of cardioplegic solution in ice prior to each reinfusion (°C):	0.5 ± 0.5	0.9 ± 0.2	0.4 ± 0.3	1.1 ± 0.4
EFFECT OF VARIABLES.				
Temperature of cardioplegia at aortic end of delivery line before each reinfusion (°C):	20.1[a] ± 0.6	14.1 ± 0.8 ±	15.6 0.4	16.2 ± 0.4
Volume of cardioplegia reinfused which had a temperature of greater than 6°C (ml):	129 ± 4	117 ± 13	111 ± 9	66[c] ± 7
Expressed as percentage of total volume infused:	1% ± 1%	47% ± 5%	28%[b] ± 2%	16%[c] ± 2%
Expressed as percentage of volume of contained in delivery line:	122% ± 3%	112% ± 12%	106% ± 9%	91.0% ± 10%

Legend:

A 3.0 m cardioplegic infusion set consisting of either 1/4" or 3/16" tubing, plus CAS-filter was evaluated. The effect of altering the time interval (10 min or 30 min) in between each reinfusion of cardioplegia, or increasing the dose of cardioplegia (250 ml/min or 400 ml/min for 1 min), on the amount of "warm cardioplegic solution" subsequently infused was assessed. Means and standard errors of means for N = 12 observations are presented.

a - $p < 0.01$ comparing A with B, b - $p < 0.01$ comparing C with B c - $p < 0.01$ comparing D with C

iii) Volume of solution contained in the cardioplegic delivery line.

The cardioplegic solution was now infused through the 3/16", 3.0 m set which had a volume of 72 ml, at a rate of 400 ml/min. The interval in between each reinfusion of cardioplegia was limited to 10 min, and as before the solution rewarmed to $16.2 \pm 0.4^{o}C$ during this period. However, the total volume of warm cardioplegia now reinfused was less, 66 ± 7 ml ($p < 0.01$), which was only $16 \pm 2\%$ of the "dose" infused, $p < 0.01$ (Table I).

DISCUSSION

If crystalloid cardioplegic solutions are used to provide intra-operative myocardial protection, these solutions should be infused into the heart at temperatures of $4^{o}C$ - $6^{o}C$ (2,11). We have shown that if the temperature of the vacolitre of cardioplegia is less than $4^{o}C$, then simple infusion without any accessory cooling coils will deliver the cardioplegic solution at the recommended temperature over a flow rate of 100 - 500 ml/min. However, the temperature of a vacolitre of cardioplegic solution removed from a fridge can vary significantly, depending upon fridge temperature, duration of storage, or presence of additional insulating packaging material (eg. manufacturer's surrounding plastic bag). Therefore, either the cardioplegic solution that has been stored in the fridge should additionally be immersed in ice for a minimum period of 30 min prior to use, or a cooling coil should be used in the cardioplegic delivery circuit, in order to ensure infusion at the correct temperature.

If crystalloid cardioplegic solutions stored at room temperature are used, then it is essential that some method of cooling is provided prior to infusion. We have demonstrated that if cooling is by a single pass through an ice immersed cooling coil, this might not provide sufficient cooling at higher flow rates. This is a manifestation of the efficiency of the cooling coil or heat exchanger. In the clinical situation, the usual flow rate for prograde cardioplegia delivery is 250 - 400 ml/min, and may be higher if there is

any minor aortic incompetence. Therefore, a larger cooling coil (eg. Sarns) or more efficient heat exchanger would be necessary, in order to consistently ensure a "delivered temperature" of less than 6^{o}C.

Although the vacolitre of cardioplegic solution can be kept in ice, in between each reinfusion of cardioplegia, the solution contained in the delivery line leading to the heart will rewarm towards ambient room temperature (19^{o}C - 23^{o}C). We have shown that within a 10 minute interval, the cardioplegic solution contained in the delivery line rewarms significantly, and that by 30 min approximates room temperature. This in turn implies that with subsequent reinfusions of cardioplegia, a significant volume of "warm cardioplegia" (i.e. cardioplegia with a temperature of greater than 6^{o}C) would be infused. This may be as much as 50% of the total dose of cardioplegic solution reinfused. This percentage of "warm cardioplegia" reinfused was similar whether there was a 10 min or 30 min interval in between reinfusions, and is dependant upon the volume of cardioplegic solution contained in the delivery line, and the total dose reinfused each time. This is especially important in paediatric cardiac surgery where small volumes (5 - 10 ml/kg) of cardioplegic solution are reinfused. If the dose of cardioplegia is equal or less than the volume of cardioplegic solution contained in the delivery line, then it is possible that the entire dose reinfused with each subsequent dose could be at temperatures approximating 20^{o}C as opposed to less than 6^{o}C.

In order to diminish this potentially detrimental reinfusion of "warm cardioplegia", the volume of cardioplegic solution that rewarms to room temperature should be reduced as much as possible. Thus the cardioplegic delivery lines should be short, narrow diameter tubing (3/16"), but should also not limit flow. In addition, to further diminish the potential volume of cardioplegic solution that rewarms, either a cooling coil can be incorporated in the line as close as possible to the operating table, or a recirculation type delivery system could be used. The more complex continuous hypothermic recirculation system will not only ensure that the cardioplegic solution is always at a sufficiently low temperature prior to infusion, but can also be designed to limit "dead space" where the

solution could rewarm. Alternatively, the "warm cardioplegic solution" contained in the delivery line should be vented to waste before each reinfusion; by using a special aortic cardioplegic infusion catheter (aortic root cannula with vent - DLP; Grand Rapids, Michigan, USA).

In conclusion, crystalloid cardioplegic solutions should be oxygenated [13,14], delivered through an 0.8 u filter [15], at a temperature of 4°C - 6°C, and sufficient pressure (greater than 50 mm Hg, but less than 150 mm Hg [16]) for prograde infusion, to ensure optimal myocardial preservation. If retrograde cardioplegia is to be used, to ensure uniform delivery in the presence of critical coronary stenoses [11], then apart from a maximum retrograde perfusion pressure of 40 mm Hg (measured in the coronary sinus) similar conditions would apply [17]. Crystalloid cardioplegic solutions stored in a fridge may not necessarily be at a sufficiently cold temperature. Therefore, it is advisable that they be additionally immersed in ice for 30 min prior to use, to ensure a vacolitre temperature of less than 4°C. The delivered temperature of the cardioplegic solution will then be acceptable (4°C - 6°C) over the flow range of 100 - 500 ml/min. However, a significant volume of cardioplegic solution contained in the delivery line rewarms to ambient room temperature (19°C -23°C) in the interval (10 - 30 min) in between each reinfusion of cardioplegia. This volume of "warm cardioplegia" would diminish the efficacy of intraoperative myocardial protection if infused, and should therefore be limited by the design of the administration set, or alternatively vented to waste prior to each reinfusion of cardioplegic solution.

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APPENDIX B—1

IN VIVO PRIMATE STUDY: THE EFFECT OF ADDING GLUCOSE TO THE ST THOMAS' CARDIOPLEGIC SOLUTION

INTRODUCTION

We previously showed that the addition of glucose to the St Thomas' Hospital No 2 cardioplegic solution was beneficial in the isolated rat heart model (see Appendix A-2). However, glucose was only beneficial in the concentration range 7 - 11 mmol/L and only if multidose reinfusions of cardioplegic solution were provided, in order to wash out end products of metabolism.

This study was therefore undertaken to investigate whether the addition of glucose (10 mmol/L) to the St Thomas' cardioplegic solution was also beneficial in the non-human primate model.

MATERIALS AND METHODS

The in vivo primate model as described in Chapter 2 was used. The chacma baboon (Papio ursinus) was selected because of its close evolutionary relationship to man. Animals weighing 15 - 24 kg were used, and six experiments were performed in each group.

Experimental Protocol

The animals were anaesthetised, intubated, and ventilated. A median sternotomy was performed and the heart cannulated for cardiopulmonary bypass. Control haemodynamic and metabolic variables were measured prior to commencing cardiopulmonary bypass. Systemic temperature was then lowered and maintained at 26^{o}C, the aorta was cross-clamped and cold (4^{o}C) cardioplegic solution (15 ml/kg;

Table I) was infused. In addition, intermittent iced saline was used for topical hypothermia, and maintenance cardioplegia (100 ml) was reinfused every 30 minutes throughout a 3-hour ischaemic cross-clamp period. Additional ventricular biopsies were taken prior to the release of the aortic cross-clamp. A 15-minute normothermic reperfusion period followed, and the animals were then weaned from cardiopulmonary bypass and postischaemic haemodynamic and metabolic variables measured 5 and 30 minutes after termination of cardiopulmonary bypass.

The dose of cardioplegic solution administered in these experiments approximated the clinical situation. On induction; 15 ml/kg body weight or 2.2 - 2.6 ml/gm heart weight, and with multidose reinfusions 100 ml; 4.2 - 6.7 ml/kg body weight or 0.6 - 1.14 ml/gm heart weight, was administered.

Table I

COMPOSITION OF CARDIOPLEGIC SOLUTIONS

mmol/L	St Thomas' No 2	St Thomas' + Glucose
Na^+	120	120
K^+	16	16
HCO_3^-	10	10
Ca^{2+}	1.2	1.2
Mg^{2+}	16	16
Glucose	--	10
Osmolarity (mOsm/L)	323.6	333.6
Osmolality (mOsm/kg H_2O)	293	304
pH (10^0C)	7.4	7.4

Legend:
Osmolarity was calculated assuming 100% ionic dissociation, whereas osmolality was measured by freezing point depression with a cryoscopic osmometer (Osmomat 030; Gonotec, Berlin, Germany).

Expression of results

Values of functional parameters obtained during the postischaemic working period were expressed as a percentage of each individual preischaemic control value. Tissue high

energy phosphate content was expressed in absolute values (umol/g wet weight). Results were then presented as percentage means and standard errors of percentage means.

Statistics

Statistical test used was the two way analysis of variance (ANOVA-2), and if overall statistical significance was obtained pair wise comparisons were then done by acceptance intervals of means with the *f* test. Statistical significance was taken as $p < 0.05$.

RESULTS

Postischaemic recovery of mechanical function

The preischaemic control values of both groups were similar, and are shown in Table II.

The addition of glucose (10 mmol/L) to the St Thomas' Hospital No 2 cardioplegic solution did not alter the postischaemic recovery at 5-min after termination of cardiopulmonary bypass (Table II). However, 30-min after termination of cardiopulmonary bypass, the presence of glucose in the cardioplegic solution was associated with a significant decrease in the postischaemic recovery of both CI, SVI and SWI ($p < 0.05$; Table II).

Table II

POSTISCHAEMIC MECHANICAL RECOVERY

	St Thomas' No 2	St Thomas' + Glucose
	N = 6	N = 6
Cardiac Index		
Preischaemic ($L/min/m^2$)	3.18 ± 0.54	3.33 ± 0.24
% Recovery 5-min	99.79 ± 15.19	77.08 ± 6.25
% Recovery 30-min	93.55 ± 11.05	60.8 ± 8.86[a]
Stroke Volume Index		
Preischaemic ($ml/beat/m^2$)	28.09 ± 4.13	31.5 ± 3.17
% Recovery 5-min	73.98 ± 12.64	71.58 ± 6.59
% Recovery 30-min	93.17 ± 10.13	60.02 ± 8.97[a]
Left Ventricular dp/dt		
Preischaemic (mm Hg/sec)	1044.4 ± 61.0	961.4 ± 69.8
% Recovery 5-min	118.2 ± 16.3	107.0 ± 16.7
% Recovery 30-min	103.4 ± 9.8	82.3 ± 10.7
Stroke Work Index		
Preischaemic ($gm.m/beat/m^2$)	43.58 ± 6.90	47.42 ± 4.76
% Recovery 5-min	75.50 ± 18.55	73.38 ± 14.84
% Recovery 30-min	93.10 ± 13.56	52.27 ± 12.11[a]

Legend:
Animals were subjected to a 3-hour hypothermic cross-clamp period protected with multidose reinfusions of indicated cardioplegic solutions. Haemodynamic variables were measured at a left atrial pressure of 5 mm Hg. Postischaemic recovery is expressed as mean percentage recoveries of each individual preischaemic value, and standard errors of percentage means.
a - $p < 0.05$ compared to St Thomas No 2.

Left ventricular function curves (CI & SWI) were determined both prior to and after the ischaemic cross-clamp period (30-minute post cardiopulmonary bypass), by measuring haemodynamic variables at different left atrial preloads. Although the preischaemic control values at a left atrial pressure of 5 mm Hg were similar between the two groups (St Thomas' with or without glucose), some of the preischaemic control values for the St Thomas' plus glucose group were greater at higher left atrial filling pressures (Table III). Nevertheless, the percentage recovery of both CI and SWI at the majority of the left

atrial filling pressures recorded, was significantly lower if glucose was added to the St Thomas' cardioplegic solution ($p < 0.05$).

Table III

LEFT VENTRICULAR FUNCTION CURVE

LAP	5	10	15	20 (mm Hg)
CI (L/min/m^2)				
St Thomas'				
Pre	3.18 ± 0.54	3.62 ± 0.38	4.07 ± 0.43	4.38 ± 0.52
% Rec	93.6 ± 11.1	81.5 ± 8.5	78.6 ± 6.9	59.9 ± 6.1
St Thomas' plus Glucose				
Pre	3.33 ± 0.24	4.45 ± 0.21[a]	5.30 ± 0.36[a]	4.84 ± 0.32
% Rec	60.8 ± 8.9[b]	56.8 ± 6.4[a]	41.9 ± 6.7[b]	44.4 ± 5.2
Data normalized to the integrated preischaemic control curve				
Cont.	3.25 ± 0.28	4.04 ± 0.24	4.69 ± 0.33	4.61 ± 0.30
ST	3.04 ± 0.36	3.29 ± 0.34	3.68 ± 0.32[c]	2.76 ± 0.28[d]
ST+G	1.98 ± 0.29[de]	2.29 ± 0.26[de]	1.96 ± 0.31[df]	2.04 ± 0.24[d]
SWI (gm·m/beat/m^2)				
St Thomas'				
Pre	43.58 ± 6.90	53.74 ± 4.28	55.61 ± 4.43	56.73 ± 4.41
% Rec	93.10 ± 13.56	74.87 ± 9.02	76.29 ± 8.68	56.89 ± 5.44
St Thomas' plus Glucose				
Pre	47.42 ± 4.76	63.01 ± 5.34	74.16 ± 9.28[a]	67.21 ± 8.38
% Rec	52.27 ± 12.1[b]	57.59 ± 9.05	43.14 ± 7.05[a]	42.16 ± 5.24
Data normalized to the integrated preischaemic control curve				
Cont.	45.50 ± 4.04	58.38 ± 3.55	64.88 ± 5.65	61.97 ± 4.78
ST	42.36 ± 6.17	43.71 ± 5.26[c]	49.50 ± 5.63[c]	35.25 ± 3.37[d]
ST+G	23.78 ± 5.51[df]	33.62 ± 5.28[d]	27.99 ± 4.6[df]	26.13 ± 3.25[d]

Legend:

A postischaemic left ventricular function curve was obtained 30 min after weaning from cardiopulmonary bypass at increasing left atrial pressures (LAP), and compared to a similar preischaemic curve. Mean percentage postischaemic recovery (% Rec) was calculated from each individual postischaemic and preischaemic ratio. An average preischaemic control function curve (Cont.) was derived by pooling the data from both curves, and postischaemic function for each group normalized to this curve for display purposes.

ST - St Thomas' cardioplegic solution (N = 6); ST+G - ST plus glucose (10 mmol/L) cardioplegic solution (N = 6).

a - $p < 0.05$; b - $p < 0.01$; compared to St Thomas',

c - $p < 0.05$; d - $p < 0.01$; compared to Cont.,

e - $p < 0.05$; f - $p < 0.01$; compared to ST.

These ventricular function curves were normalized for display purposes as described in Chapter 2. In summary, the preischaemic control values of both groups were pooled and thereafter the percentage recovery of each individual group normalized to this average preischaemic control curve. Statistical analysis of this normalized data showed that the postischaemic recovery of hearts protected with the St Thomas' cardioplegic solution was no different from control preischaemic values at atrial filling pressures of 5 - 10 mm Hg (Table III). However, at higher left atrial filling pressures (greater than 10 - 15 mm Hg) postischaemic recovery was depressed ($p < 0.05$). In contrast, postischaemic recovery associated with the St Thomas' plus glucose cardioplegia was depressed at all left atrial filling pressures compared to the preischaemic control ($p < 0.01$), and recovery was less than that associated with the standard St Thomas' Hospital No 2 cardioplegic solution.

Post ischaemic recovery of metabolic parameters

Myocardial ATP content decreased from a preischaemic control value of 2.98 ± 0.4 umol/gm wet heart weight to 1.49 ± 0.32 umol/gm at the end of the 3-hour ischaemic period (prior to release of the aortic cross-clamp), $p < 0.01$, when hearts were protected with St Thomas' Hospital No 2 cardioplegic solution. However, after reperfusion and termination of cardiopulmonary bypass the ATP levels had recovered to 2.38 ± 0.27 umol/gm ($p < 0.01$). Myocardial CP content showed a similar pattern, $p < 0.05$ (Table IV). In contrast, when hearts were protected with St Thomas' plus glucose cardioplegia ATP content at the end of the 3-hour ischaemic period (2.84 ± 0.17 umol/gm) was no different from the preischaemic value (2.26 ± 0.39). However, following the release of the aortic cross-clamp and after weaning from cardiopulmonary bypass the ATP content decreased to 1.62 ± 0.36 umol/gm ($p < 0.05$). No significant change in CP concentrations was detected (Table IV). In addition, no significant difference in tissue lactate was detected.

Table IV

METABOLIC PARAMETERS

Control	End Ischaemic	Post Ischaemic 5-min	Post Ischaemic 30-min
ATP (umol/gm wet heart weight)			
St Thomas'			
2.98 ± 0.4	1.49 ± 0.32[d]	2.38 ± 0.27[f]	2.67 ± 0.44
St Thomas' plus Glucose			
2.26 ± 0.39	2.84 ± 0.17[b]	1.62 ± 0.36[e]	1.77 ± 0.09[a]
CP (umol/gm wet heart weight)			
St Thomas'			
5.75 ± 0.49	2.49 ± 1.86[c]	5.41 ± 0.88[e]	5.37 ± 0.74
St Thomas' plus Glucose			
4.04 ± 0.3	2.49 ± 1.31	3.55 ± 0.67	3.96 ± 0.36
Lactate (umol/gm wet heart weight)			
St Thomas'			
10.92 ± 2.39	14.66 ± 2.28	12.26 ± 3.58	8.31 ± 1.76
St Thomas' plus Glucose			
5.28 ± 3.52	9.30 ± 2.25	7.75 ± 2.78	6.31 ± 2.06

Legend:
Myocardial concentrations of ATP, CP and lactate were measured prior to a 3-hour hypothermic ischaemic period protected with multidoses of either St Thomas' (N = 6) or St Thomas' plus glucose (10 mmol/L; N = 6) cardioplegic solutions (Control), at the end of the ischaemic period and 5 min and 30 min after weaning from cardiopulmonary bypass.
a - $p < 0.05$; b - $p < 0.01$; compared to St Thomas',
c - $p < 0.05$; d - $p < 0.01$; compared to control,
e - $p < 0.05$; f - $p < 0.01$; compared to End Ischaemic.

COMMENT

The addition of glucose (10 mmol/L) to the St Thomas' Hospital No 2 cardioplegic solution decreased postischaemic recovery following a 3-hour hypothermic ischaemic period protected with multidoses of cardioplegia. Nevertheless, the St Thomas' plus glucose cardioplegic solution resulted in better preservation of myocardial ATP during the ischaemic period. However, in the postischaemic period ATP content was depressed. This suggests that the presence of glucose in the cardioplegic solution augmented ATP production during the ischaemic period, but suppressed ATP

production and decreased myocardial function during reperfusion of the postischaemic myocardium.

APPENDIX B-2

ISOLATED RAT HEART STUDY: THE EFFECT OF ALTERING THE pH OF THE ST THOMAS' CARDIOPLEGIC SOLUTION

INTRODUCTION

This study was undertaken as a pilot study to determine the effect of altering the pH of a modified St Thomas' Hospital No 2 cardioplegic solution on postischaemic mechanical recovery. Glucose (11 mmol/L) was included in the cardioplegic solution and pH was altered by adding either 1 N HCl or NaOH to the cardioplegic solution.

MATERIALS AND METHODS

The isolated perfused working rat heart model as described in Chapter 2 was used. Hearts were removed from male Long Evans rats weighing between 250 gm - 350 gm.

Experimental Protocol

The aorta was cannulated and Langendorff perfusion with 95 % O_2 5 % CO_2 gassed Krebs Henseleit buffer commenced and maintained for 10 min, prior to conversion to the working preparation (left atrial filling pressure 20 cm H_2O, left ventricle afterload 100 cm H_2O). Haemodynamic variables were measured after 10 minutes of work. The atrial and aortic cannulae were then closed and 10 ml cardioplegic solution (10^oC) was infused at a pressure of 80 cm H_2O, and simultaneously the hearts were placed into a 10^oC chamber for a 3-hour period of global ischaemia. Multidose reinfusions of cardioplegic solution (6 ml) were administered every 30 minutes throughout the 3-hour ischaemic period. Hearts were then reperfused at 37^oC in the non working Langendorff mode for 10 min at 100 cm H_2O, and thereafter switched to the working mode for a further 10 min prior to measuring postischaemic functional recovery.

Cardioplegic solutions.

The "control" cardioplegic solution tested was a modified St Thomas' Hospital No 2 cardioplegic solution containing 11.0 mmol/L glucose (pH - 7.4). The experimental cardioplegic solutions were made up by adding either 1 N HCl or 1 N NaOH to the St Thomas' plus glucose (11.0 mmol/L) cardioplegic solution to obtain the indicated pH. pH was measured at 37°C with a Radiometer pH - M-83 autocal pH meter (Radiometer, Copenhagen, Denmark). Alterations of pH would therefore also have altered the osmolality, sodium concentration and buffering capacity of the cardioplegic solution slightly.

Expression of results

Values of functional parameters obtained during the postischaemic working period were expressed as a percentage of each individual preischaemic control value. Results are presented as percentage means and standard errors of percentage means.

Statistics

The statistical test used was the one way analysis of variance (ANOVA-1), and if overall statistical significance was obtained pair wise comparisons were then done by acceptance intervals of means with the *f* test. Statistical significance was taken as $p < 0.05$.

RESULTS

Postischaemic recovery of mechanical function

The preischaemic control values of all groups were similar, and are shown in Table I.

Table I

POSTISCHAEMIC MECHANICAL RECOVERY

	AO		CO		SV	
	Pre Ischaemic ml/min	Percentage Recovery	Pre Ischaemic ml/min	Percentage Recovery	Pre Ischaemic ml	Percentage Recovery
pH - 6.0	45.5 ± 1.5	0[b]	62.0 ± 1.4	0[b]	0.224 ± 0.014	0[b]
pH - 6.8	50.5 ± 1.9	9.5[b] ± 5.6	69.5 ± 2.2	18.8[b] ± 9.3	0.238 ± 0.012	26.4[b] ± 12.0
pH - 7.0 (N =7)	45.4 ± 2.5	70.1[b] ± 3.5	61.1 ± 2.8	76.3[a] ± 4.1	0.232 ± 0.013	81.5 ± 3.0
pH - 7.4	45.0 ± 3.7	85.8 ± 1.4	61.5 ± 4.3	90.2 ± 1.8	0.237 ± 0.017	95.9 ± 4.6
pH - 7.8	45.0 ± 3.7	91.5 ± 0.9	60.0 ± 4.5	94.5 ± 1.4	0.236 ± 0.012	90.2 ± 5.8

Legend:

Isolated rat hearts (N = 4, unless otherwise indicated) were subjected to 3-hour 10°C ischaemia protected with multidose cardioplegic solutions. The cardioplegic solution evaluated was the St Thomas' plus glucose (11.0 mmol/L) cardioplegic solution, and pH was altered by the addition of either 1 N HCl or NaOH.

a - $p < 0.05$ compared to pH - 7.4,

b - $p < 0.01$ compared to pH - 7.4.

The pH of the standard St Thomas' plus glucose cardioplegic solution was 7.4. Increasing the pH to 7.8 by the addition of NaOH tended to increase postischaemic recovery but significance was not obtained (Table I). However, decreasing the pH to 7.0 or less decreased postischaemic recovery of both AO and CO ($p < 0.05$). However, this might have also been as a result of the concomitant decrease in the buffering capacity of the cardioplegic solution.

APPENDIX B-3

ISOLATED RAT HEART STUDY: SODIUM AND OSMOLAL DOSE RESPONSE CURVES FOR THE ST THOMAS' CARDIOPLEGIC SOLUTION

INTRODUCTION

This study was undertaken as a pilot study to confirm the optimal sodium concentration and osmolality for the St Thomas' Hospital No 2 cardioplegic solution.

MATERIALS AND METHODS

The isolated perfused working rat heart model as described in Chapter 2 was used. Hearts were removed from male Long Evans rats weighing between 250 gm - 350 gm.

Experimental Protocol

The aorta was cannulated and Langendorff perfusion with 95 % O_2 5 % CO_2 gassed Krebs Henseleit buffer commenced and maintained for 10 min, prior to conversion to the working preparation (left atrial filling pressure 20 cm H_2O, left ventricle afterload 100 cm H_2O). Haemodynamic variables were measured after 10 minutes of work. The atrial and aortic cannulae were then closed and 10 ml cardioplegic solution (10^oC) was infused at a pressure of 80 cm H_2O, and simultaneously the hearts were placed into a 10^oC chamber for a 3-hour period of global ischaemia. Multidose reinfusions of cardioplegic solution (6 ml) were administered every 30 minutes throughout the 3-hour ischaemic period. Hearts were then reperfused at 37^oC in the non working Langendorff mode for 10 min at 100 cm H_2O, and thereafter switched to the working mode for a further 10 min prior to measuring postischaemic functional recovery.

Cardioplegic solutions.

The "control" cardioplegic solution tested was the St Thomas' Hospital No 2 cardioplegic solution. The experimental cardioplegic solutions were modified St Thomas'

cardioplegic solutions containing varying concentrations of sodium (osmolality kept constant with sucrose), or varying osmolality by the addition of sucrose (sodium kept constant). Osmolality was measured by freezing point depression with an automatic cryoscopic osmometer (Osmomat 030: Gonotec; Berlin, Germany).

Expression of results

Values of functional parameters obtained during the postischaemic working period were expressed as a percentage of each individual preischaemic control value. Results are presented as percentage means and standard errors of percentage means.

Statistics

The statistical test used was the one way analysis of variance (ANOVA-1), and if overall statistical significance was obtained pair wise comparisons were then done by acceptance intervals of means with the *f* test. Statistical significance was taken as $p < 0.05$.

RESULTS

Sodium concentration of the St Thomas' Hospital No 2 cardioplegic solution

Table I

POSTISCHAEMIC MECHANICAL RECOVERY

	AO		CO		SV	
	Pre Ischaemic ml/min	Percentage Recovery	Pre Ischaemic ml/min	Percentage Recovery	Pre Ischaemic ml	Percentage Recovery
Na - 120	49.0 ± 1.3	76.1 ± 2.2	67.0 ± 1.3	82.9 ± 2.5	0.262 ± 0.016	85.3 ± 6.9
Na - 110	42.0 ± 2.2	85.8[b] ± 1.5	53.5[b] ± 2.6	93.5[b] ± 2.3	0.228 ± 0.006	105.5[a] ± 5.8
Na - 100	43.5 ± 2.6	86.1[b] ± 1.1	61.0 ± 1.7	88.5 ± 1.0	0.226 ± 0.004	93.4 ± 7.2
Na - 90	36.5[b] ± 2.9	80.6 ± 1.3	50.5[b] ± 3.8	88.3 ± 2.5	0.200 ± 0.021	90.4 ± 6.6
Na - 80	50.0 ± 0.8	76.0 ± 1.5	66.0 ± 1.4	81.9 ± 0.9	0.234 ± 0.126	84.3 ± 3.8
Na - 60	49.5 ± 1.3	67.2[a] ± 2.4	66.5 ± 1.0	74.0[b] ± 1.7	0.251 ± 0.013	75.4 ± 2.7
Na - 40	45 ± 5.1	4.8[b] ± 4.8	64.5 ± 4.3	22.2[b] ± 3.1	0.246 ± 0.026	24.4[b] ± 1.5

Legend:

Isolated rat hearts (N = 4) were subjected to 3-hour 10°C ischaemia protected with multidose cardioplegic solutions, either St Thomas' or modificd St Thomas' cardioplegic solutions containing varying concentrations of sodium ions (osmolality kept constant by addition of sucrose).

a - $p < 0.05$ compared to Na - 120,

b - $p < 0.01$ compared to Na - 120.

The sodium concentration of the standard St Thomas' Hospital cardioplegic solution was decreased from 120 mmol/L to 110 mmol/L, and this modification improved the postischaemic recovery of both AO, CO and SV ($p < 0.05$; Table I). Thus, this pilot

study suggested that the optimal sodium content for the St Thomas' Hospital No 2 cardioplegic solution was 110 mmol/L. Furthermore, the sodium concentration of the standard St Thomas' cardioplegic solution could be decreased to a concentration of 80 mmol/L before postischaemic recovery diminished significantly.

Osmolality of the St Thomas' Hospital No 2 cardioplegic solution

Table II

POSTISCHAEMIC MECHANICAL RECOVERY

	AO		CO		SV	
	Pre Ischaemic ml/min	Percentage Recovery	Pre Ischaemic ml/min	Percentage Recovery	Pre Ischaemic ml	Percentage Recovery
Osmol-300	49.0 ± 1.3	76.1 ± 2.2	67.0 ± 1.3	82.9 ± 2.5	0.262 ± 0.016	85.3 ± 6.9
Osmol-350	47.3 ± 0.7	86.6[a] ± 2.1	62.7[a] ± 0.7	92.1[a] ± 2.3	0.244 ± 0.019	95.6 ± 5.4

Legend:
Isolated rat hearts (N = 4) were subjected to 3-hour 10°C ischaemia protected with multidose cardioplegic infusions, either St Thomas' or a modified St Thomas' cardioplegic solution containing a higher osmolality. The sodium concentration was kept constant at 120 mmol/L in both solutions. Osmol-300: standard St Thomas' Hospital No 2 cardioplegic solution that has a measured osmolality of 295 - 300 mOsm/kg H_2O. Osmol-350: modified St Thomas' with a measured osmolality of 350 mOsm/kg H_2O due to the addition of sucrose (50 mmol/L).
a - $p < 0.05$ compared to Osmol-300.

The St Thomas' Hospital No 2 cardioplegic solution is an isotonic solution with an osmolality of 295 - 300 mOsm/kg H_2O. Increasing the osmolality to 350 mOsm/kg H_2O improved postischaemic mechanical recovery of both AO and CO ($p < 0.05$; Table II).

Osmolality of a modified St Thomas' cardioplegic solution containing a sodium concentration of 100 mmol/L

Table III

POSTISCHAEMIC MECHANICAL RECOVERY

	AO		CO		SV	
	Pre Ischaemic ml/min	Percentage Recovery	Pre Ischaemic ml/min	Percentage Recovery	Pre Ischaemic ml	Percentage Recovery
Osmol-260	51.0 ± 4.0	81.5[a] ± 0.5	70.5 ± 5.3	83.2[a] ± 1.3	0.246 ± 0.016	91.9 ± 8.4
Osmol-310	43.5 ± 2.6	86.1 ± 1.1	61.0 ± 1.7	88.5 ± 1.0	0.226 ± 0.004	93.4 ± 7.2
Osmol-340 (N = 7)	47.1 ± 1.6	87.7 ± 1.3	64.9 ± 2.6	91.5 ± 1.0	0.243 ± 0.014	96.0 ± 1.9
Osmol-360	46.5 ± 1.9	81.8[a] ± 0.4	64.5 ± 1.3	86.1 ± 2.0	0.248 ± 0.017	85.2 ± 5.7

Legend:
Isolated rat hearts (N = 4, unless otherwise indicated) were subjected to 3-hour 10°C ischaemia protected with multidose infusions of modified St Thomas' cardioplegic solutions containing a sodium concentration of 100 mmol/L in all solutions. The osmolality was altered by the addition of sucrose and the measured osmolality (Osmol-; mOsm/kg H_2O) of the solution is indicated.
a - $p < 0.05$ compared to Osmol-310.

The sodium concentration of the St Thomas' cardioplegic solution was decreased to 100 mmol/L and osmolality altered by the addition of sucrose. Hypo-osmolality (less than 300 mOsm/kg H_2O) was associated with a decreased postischaemic recovery of both AO and CO ($p < 0.05$). However, moderate hyperosmolality (340 mOsm/kg H_2O) did not alter postischaemic mechanical recovery (Table III).

APPENDIX B-4

ISOLATED RAT HEART STUDY: DECREASING THE CHLORIDE CONTENT OF THE ST THOMAS' CARDIOPLEGIC SOLUTION

INTRODUCTION

This study was undertaken to determine whether decreasing the chloride concentration of the St Thomas' cardioplegic solution would alter postischaemic mechanical recovery. The chloride content of the solution was diminished by replacing the chloride salt of the sodium and potassium contained in the solution with the gluconate salts.

MATERIALS AND METHODS

The isolated perfused working rat heart model as described in chapter 2 was used. Hearts were removed from male Long Evans rats weighing between 270 gm - 400 gm.

Experimental Protocol

The aorta was cannulated and Langendorff perfusion with 95 % O_2 5 % CO_2 gassed Krebs Henseleit buffer commenced and maintained for 10 min, prior to conversion to the working preparation (left atrial filling pressure 20 cm H_2O, left ventricle afterload 100 cm H_2O). Haemodynamic variables were measured after 10 minutes of work. The atrial and aortic cannulae were then closed and 10 ml cardioplegic solution (10^oC) was infused at a pressure of 80 cm H_2O, and simultaneously the hearts were placed into a 10^oC chamber containing the relevant cardioplegic solution for a 3-hour period of global ischaemia. No reinfusions of cardioplegic solution were administered. Hearts were then reperfused at 37^oC in the non working Langendorff mode for 10 min at 100 cm H_2O, and thereafter switched to the working mode for a further 10 min prior to measuring postischaemic functional recovery.

Cardioplegic solutions.

The "control" cardioplegic solution tested was the St Thomas' Hospital No 2 cardioplegic solution (ST). The experimental solution evaluated was a modified St Thomas' solution containing a low concentration of chloride (ST-Low Cl; Table I).

Table I

COMPOSITION OF CARDIOPLEGIC SOLUTIONS

(mmol/L)	St Thomas' No 2	St Thomas' - Low Cl
Na^+	120	120
K^+	16	16
HCO_3^-	10	10
Ca^{2+}	1.2	1.2
Mg^{2+}	16	16
Cl^-	160.4	34.4
Gluconate	---	126

Expression of results

Values of functional parameters obtained during the postischaemic working period were expressed as a percentage of each individual preischaemic control value. Results are presented as percentage means and standard errors of percentage means.

Statistics

The statistical test used was the one way analysis of variance (ANOVA-1), and if overall statistical significance was obtained pair wise comparisons were then done by acceptance intervals of means with the *f* test. Statistical significance was taken as $p < 0.05$.

RESULTS

Postischaemic recovery of mechanical function

The preischaemic control values of all groups were similar, and are shown in Table II.

Table II

POSTISCHAEMIC MECHANICAL RECOVERY

	A O		C O		S V	
	Pre Ischaemic ml/min	Percentage Recovery	Pre Ischaemic ml/min	Percentage Recovery	Pre Ischaemic ml	Percentage Recovery
ST	50.43 ± 2.02	49.8 ± 5.1	68.43 ± 2.09	62.8 ± 6.9	0.228 ± 0.011	66.6 ± 8.0
ST-Low Cl	51.71 ± 1.82	55.5 ± 4.7	67.14 ± 2.24	66.7 ± 4.3	0.243 ± 0.009	63.7 ± 5.7

Legend:
Isolated rat hearts (N = 7) were subjected to 3-hour 10°C ischaemia after a single dose of either St Thomas' (ST) or a modified St Thomas' cardioplegic solution containing a low concentration of chloride (ST-Low Cl).
All comparisons p = NS.

The chloride concentration of the St Thomas' Hospital No 2 cardioplegic solution was decreased from 160 .4 mmol/L to 34.4 mmol/L by replacing chloride with gluconate. This modification did not alter the mechanical postischaemic recovery of hearts protected with this solution (Table II).

Metabolic indices of postischaemic recovery

Table III

POSTREPERFUSION METABOLIC CONCENTRATIONS

	ATP umol/gm	CP umol/gm	Percent H_2O %
ST	3.14 ± 0.12	6.04 ± 0.18	86.5 ± 0.2
ST - Low Cl	3.19 ± 0.10	6.50 ± 0.20	86.5 ± 0.3

Legend:
Isolated rat hearts (N = 7) were subjected to 3-hour 10°C ischaemia after a single dose of either St Thomas, or a modified cardioplegic solutions containing a low concentration of chloride. Post-ischaemic values of ATP, CP (umol/gm fresh weight) and percentage water content were measured by freeze-clamping at the end of the postischaemic reperfusion period after measuring the haemodynamic variables.
All comparisons p = NS.

The concentrations of the cellular high energy phosphates and percentage water content after 20 min reperfusion following the 3-hour ischaemic period were not altered by decreasing the chloride concentration of the St Thomas' Hospital No 2 cardioplegic solution (Table III).

APPENDIX B-5

ISOLATED RAT HEART STUDY: THE ADDITION OF HISTIDINE TO THE ST THOMAS' CARDIOPLEGIC SOLUTION

INTRODUCTION

The buffer contained in ST Thomas' Hospital No 2 cardioplegic solution is bicarbonate which has a weak buffering capacity, in contrast to the high buffering capacity of histidine contained in Bretschneider HTK4 cardioplegic solution (B-HTK4). However, the electrolyte composition of B-HTK4 is closer to that of an intracellular electrolyte equivalent solution (Table I), because of osmotic considerations due to the high concentration of histidine (198 mmol/L) used in this solution. This study was undertaken to investigate whether a modified St Thomas' cardioplegic solution with an increased buffering capacity, albeit lower than B-HTK4 but retaining an extracellular electrolyte equivalent composition, would improve postischaemic myocardial recovery.

We examined the effect of including histidine (50 mmol/L) in a modified ST solution containing 10 mmol/L glucose (to ensure that substrate for glycolysis was present) and a slightly lower sodium concentration of 100 mmol/L (in order to create osmotic space), in the isolated rat heart model.

MATERIALS AND METHODS

The isolated perfused working rat heart model as described in Chapter 2 was used. Hearts were removed from male Long Evans rats weighing between 220 gm - 420 gm.

Experimental Protocol

The aorta was cannulated and Langendorff perfusion with 95 % O_2 5 % CO_2 gassed Krebs Henseleit buffer commenced and maintained for 10 min, prior to conversion to the working preparation (left atrial filling pressure 20 cm H_2O, left ventricle afterload

100 cm H_2O). Haemodynamic variables were measured after 10 minutes of work. The atrial and aortic cannulae were then closed and 10 ml cardioplegic solution (10^oC) was infused at a pressure of 80 cm H_2O, and simultaneously the hearts were placed into a 10^oC chamber containing the relevant cardioplegic solution for a 3-hour period of global ischaemia. No reinfusions of cardioplegic solution were administered. Hearts were then reperfused at 37^oC in the non working Langendorff mode for 10 min at a pressure of 80 cm H_2O initially and thereafter for 10 min at 100 cm H_2O. Hearts were then switched to the working mode for a further 10 min, prior to measuring postischaemic functional recovery.

A similar protocol was followed with a second series of hearts, but each heart was freeze-clamped with Wollenburg tongs at the end of the 3-hour ischaemic period before reperfusion, for determination of myocardial content of ATP, CP and tissue lactate. Control values for ATP and CP were obtained in six hearts by freeze-clamping prior to the ischaemic period in the experimental protocol.

Cardioplegic solutions

The "control" cardioplegic solutions tested were the St Thomas' plus 10 mmol/L glucose (ST+G) and B-HTK4 cardioplegic solutions. The addition of 50 mmol/L histidine to this modified St Thomas' cardioplegic solution (ST+G+Histidine) was then evaluated. In order to create osmotic space for the inclusion of histidine in this experimental solution, the sodium concentration of the St Thomas' cardioplegic solution was decreased from 120 mmol/L to 100 mmol/L (see appendix B-3). Furthermore, to ensure that any beneficial effect was not consequent to this change in sodium concentration or altered osmolality, a further "control" solution identical to ST+G+Histidine but containing sucrose instead of histidine (ST+G+Sucrose) was tested (Table I). All cardioplegic solutions were gassed with 95% O_2 5% CO_2 when initially prepared, but thereafter allowed to equilibrate with atmospheric partial pressures prior to use.

Table I

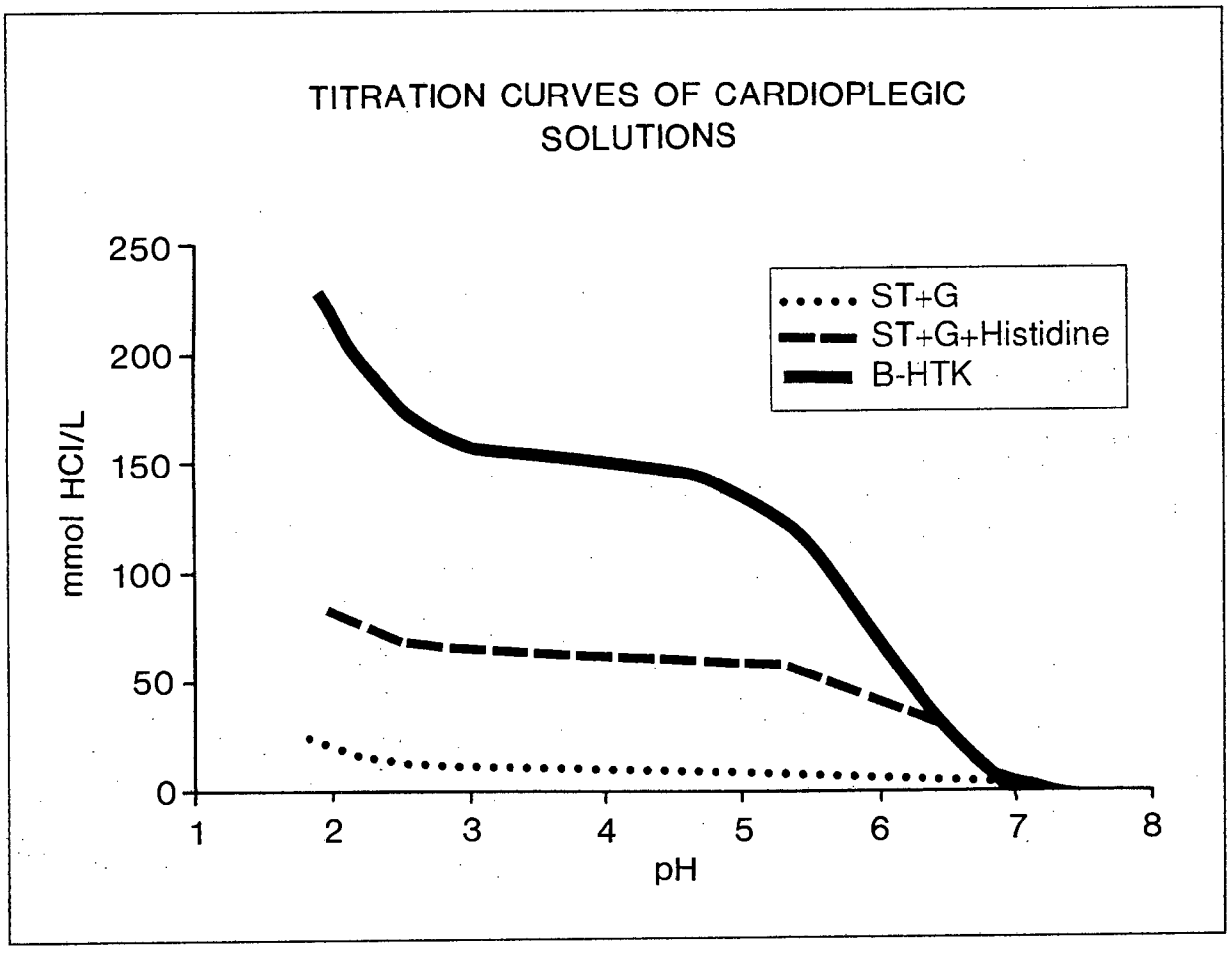
COMPOSITION OF CARDIOPLEGIC SOLUTIONS

(mmol/L)	ST+G	ST+G + Histidine	ST+G + Sucrose	B-HTK4
Na^+	120	100	100	15
K^+	16	16	16	10
HCO_3^-	10	10	10	--
Ca^{2+}	1.2	1.2	1.2	--
Mg^{2+}	16	16	16	4
Glucose	10	10	10	--
Histidine	--	50	--	198
Sucrose	--	--	50	--
Mannitol	--	--	--	30
KH-2-Oxyglutarate	--	--	--	1
Tryptophan	--	--	--	2
Osmolarity (mOsm/L)	333.6	343.6	343.6	310
Osmolality (mOsm/kg H_2O)	304	320	320	298
pH (10^oC)	7.4	7.2	7.4	7.0

Legend:
The composition of the St Thomas plus glucose (ST+G) and Bretschneider HTK4 (B-HTK4) cardioplegic solutions, as well as two experimental solutions containing either histidine (ST+G+Histidine) or sucrose (ST+G+Sucrose) is shown. Osmolarity was calculated by assuming 100% ionic dissociation and osmolality measured by freezing point depression (Osmomat 030 automatic cryoscopic osmometer: Gonotec, Berlin, Germany).

The titration curve of each of these cardioplegic solutions was determined by adding 0.5 ml aliquots of 1 N HCl to 500 ml of solution at 22^oC (continually stirred and gassed with 95 % O_2 5 % CO_2 to maintain CO_2 constant), and recording the pH change (Radiometer pH - M-83 autocal pH meter: Radiometer, Copenhagen, Denmark). The actual buffering capacity of the bicarbonate containing solutions when in the myocardium might well have been overestimated, as CO_2 cannot escape in the myocardium during ischaemia. Titration curves were plotted and thereafter the buffering capacity (mmol HCl/L/δ pH) calculated as the instantaneous three point derivative of the titration curve (Figure 1).

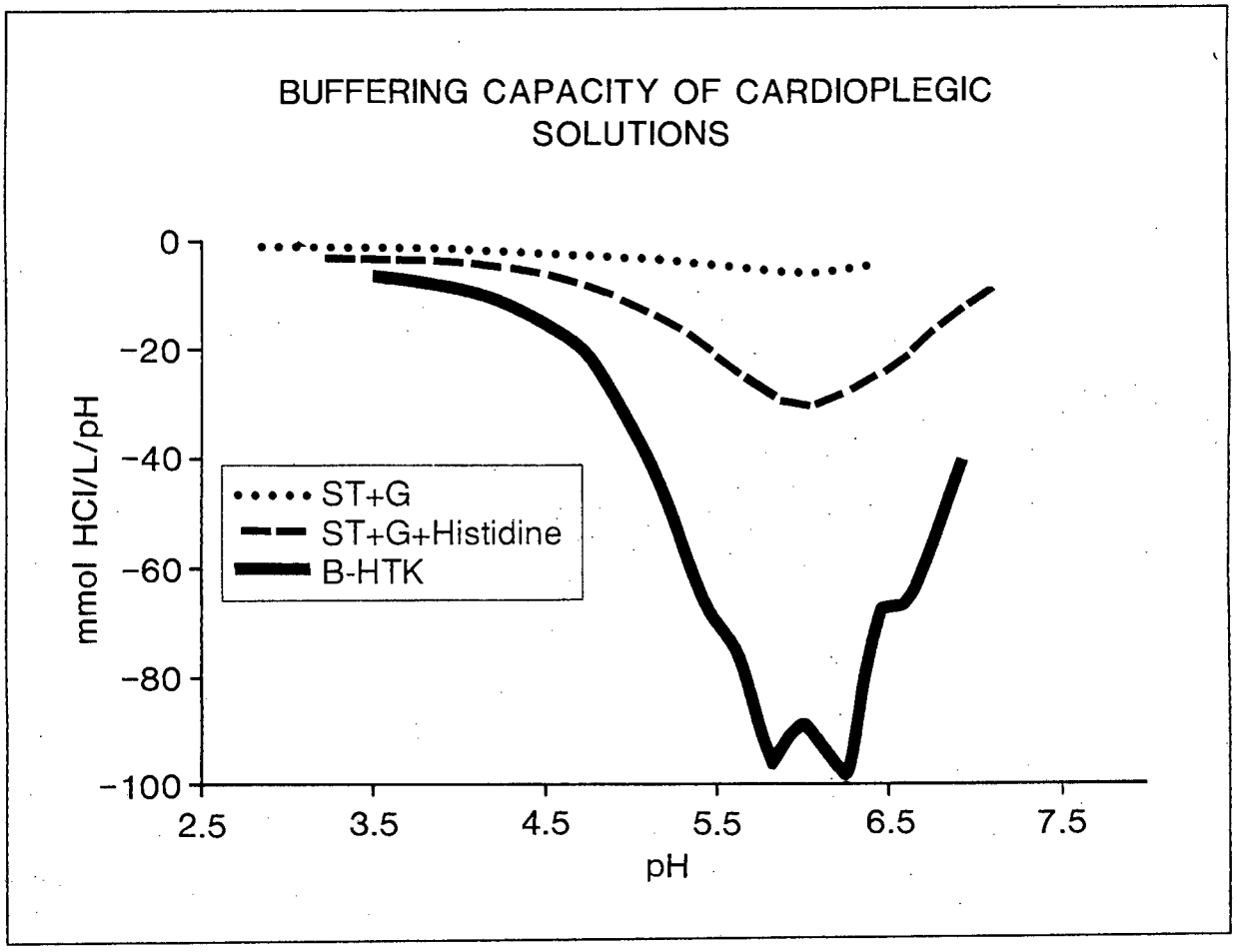
Figure 1 A



Legend:
Titration (A) curve of the St Thomas plus glucose (ST+G), Bretschneider HTK4 (B-HTK) and ST+G+Histidine cardioplegic solutions.

The maximum buffering capacity of the bicarbonate containing ST+G cardioplegic solution was 6 mmol HCl/L/δ pH at pH 6.0. The addition of 50 mmol/L histidine to ST+G increased the buffering capacity to 30 mmol HCl/L/δ pH over the pH range 6.0 - 6.3. In contrast, the highly buffered B-HTK4 cardioplegic solution containing approximately four times the concentration of histidine of our experimental solution, had a maximum buffering capacity of 100 mmol HCl/L/δ pH between pH 5.8 - 6.6.

Figure 1 B



Legend:
Buffering capacity (B) curve of the St Thomas plus glucose (ST+G), Bretschneider HTK4 (B-HTK) and ST+G+Histidine cardioplegic solutions. Buffering capacity is expressed as the instantaneous three point derivative slope of the titration curve (A).

Expression of results

Values of functional parameters obtained during the postischaemic working period were expressed as a percentage of each individual preischaemic control value. Results are presented as percentage means and standard errors of percentage means.

Statistics

The statistical tests used were either the one way or if appropriate the two way analysis of variance (ANOVA-1, ANOVA-2), and if overall statistical significance was obtained pair wise comparisons were then done by acceptance intervals of means with the *f* test. Statistical significance was taken as $p < 0.05$.

RESULTS

Postischaemic recovery of mechanical function

Hearts (N = 9) were protected with a single coronary infusion of cardioplegic solution at the onset of the 3-hour ischaemic period. One low "outlier" was excluded from further analysis in each group by Dixon's criteria (see Chapter 2), and further analysis was done on N = 8 for each group. The preischaemic control values of all groups were similar, and are shown in Table II.

Table II

POSTISCHAEMIC MECHANICAL RECOVERY

	A O		C O		S V	
	Pre Ischaemic ml/min	Percentage Recovery	Pre Ischaemic ml/min	Percentage Recovery	Pre Ischaemic ml	Percentage Recovery
ST+G	49.3 ± 3.7	61.8%[a] ± 2.3%	68.0 ± 4.9	71.9%[b] ± 2.2%	0.263 ± 0.016	70.0% ± 4.3%
ST+G+Histidine	45.8 ± 3.2	75.1% ± 3.7%	61.8 ± 3.5	82.5% ± 5.4%	0.258 ± 0.011	79.5% ± 4.8%
ST+G+Sucrose	53.4 ± 3.2	56.6%[a] ± 2.4%	71.0 ± 3.7	66.0%[a] ± 2.2%	0.247 ± 0.010	68.5% ± 6.3%
B-HTK4	50.5 ± 2.5	53.2%[a] ± 3.5%	70.3 ± 2.5	67.9%[a] ± 3.5%	0.245 ± 0.008	65.5%[b] ± 2.9%

Legend:
Isolated rat hearts were subjected to 3-hour 10°C ischaemia after a single dose of either St Thomas plus glucose (ST+G), or Bretschneider HTK4 (B-HTK4) crystalloid cardioplegic solutions, or the experimental solutions ST+G plus histidine or sucrose.
a - $p < 0.01$ compared with ST+G+Histidine
b - $p < 0.05$ compared with ST+G+Histidine

The addition of histidine to the modified ST+G solution improved postischaemic recovery of both aortic output from 61.8% ± 2.3% to 75.1% ± 3.7% ($p < 0.01$), and cardiac output from 71.9% ± 2.2% to 82.5% ± 5.4% ($p < 0.05$). Although stroke

volume also increased from 70.0 % ± 4.3 % to 79.5 % ± 4.8 %, statistical significance was not achieved. In contrast, Bretschneider HTK4 cardioplegic solution having a threefold higher buffering capacity than ST+G+Histidine, did not provide better myocardial protection than ST+G (Table II). Postischaemic recovery of aortic output was 53.2 % ± 3.5 %, cardiac output 67.9 % ± 3.5 % and stroke volume 65.5 % ± 2.9 %. ST+G+Histidine provided superior myocardial protection compared to B-HTK4 in all comparisons, $p < 0.05$ (Table II).

The improved recovery with ST+G+Histidine was due to the addition of the amino acid histidine and not because of any osmotic or low sodium effect, as a similar solution containing sucrose (ST+G+Sucrose) as opposed to histidine was not more effective than ST+G. Postischaemic recovery following protection with ST+G+Sucrose was inferior to ST+G+Histidine; aortic output 56.6 % ± 2.4 % ($p < 0.01$), cardiac output 66.0 % ± 2.2 % ($p < 0.01$) and stroke volume 68.5 % ± 6.3 %.

Metabolic indices of postischaemic recovery

Preservation of high energy phosphates

Preischaemic control values of ATP and CP were 3.96 ± 0.07 umol/gm fresh weight and 5.65 ± 0.19 umol/gm fresh weight respectively (Table III). ATP and CP content decreased in all groups by the end of the three hour ischaemic period ($p < 0.01$). However, hearts protected with ST+G+Histidine retained a significantly higher end-ischaemic concentration of ATP (2.17 ± 0.24 umol/gm) than those protected with ST+G (1.42 ± 0.15 umol/gm, $p < 0.01$), or B-HTK4 (0.42 ± 0.09 umol/gm, $p < 0.01$). The end-ischaemic CP contents were similarly decreased with both ST+G and ST+G+Histidine, but with B-HTK4 the decrease was further reduced ($p < 0.05$).

Table III

END-ISCHAEMIC METABOLIC CONCENTRATIONS

	ATP	CP	Lactate
Preischaemic Control	3.96 ± 0.07	5.65 ± 0.19	0.14 ± 0.04
ST+G	1.42 ± 0.15[a]	0.53 ± 0.07[a]	19.98 ± 0.84[a]
ST+G+Histidine	2.17 ± 0.24[abd]	0.50 ± 0.04[ae]	21.07 ± 0.56[ad]
B-HTK4	0.42 ± 0.09[ab]	0.18 ± 0.02[ac]	13.41 ± 1.31[ab]

Legend:
Isolated rat hearts (N = 6) were subjected to 3-hour 10°C ischaemia after a single dose of either St Thomas plus glucose (ST+G), or Bretschneider HTK4 (B-HTK4) crystalloid cardioplegic solutions, or the experimental solution ST+G+Histidine. End-ischaemic values of ATP, CP and lactate (umol/gm fresh weight) were measured by freeze-clamping at the end of the ischaemic period, prior to reperfusion. Control concentrations were measured in six hearts by freeze-clamping prior to the ischaemic period.

a - $p < 0.01$ compared with Control.
b - $p < 0.01$ compared with ST+G.
c - $p < 0.05$ compared with ST+G.
d - $p < 0.01$ compared with B-HTK4.
e - $p < 0.05$ compared with B-HTK4.

End-ischaemic lactate content

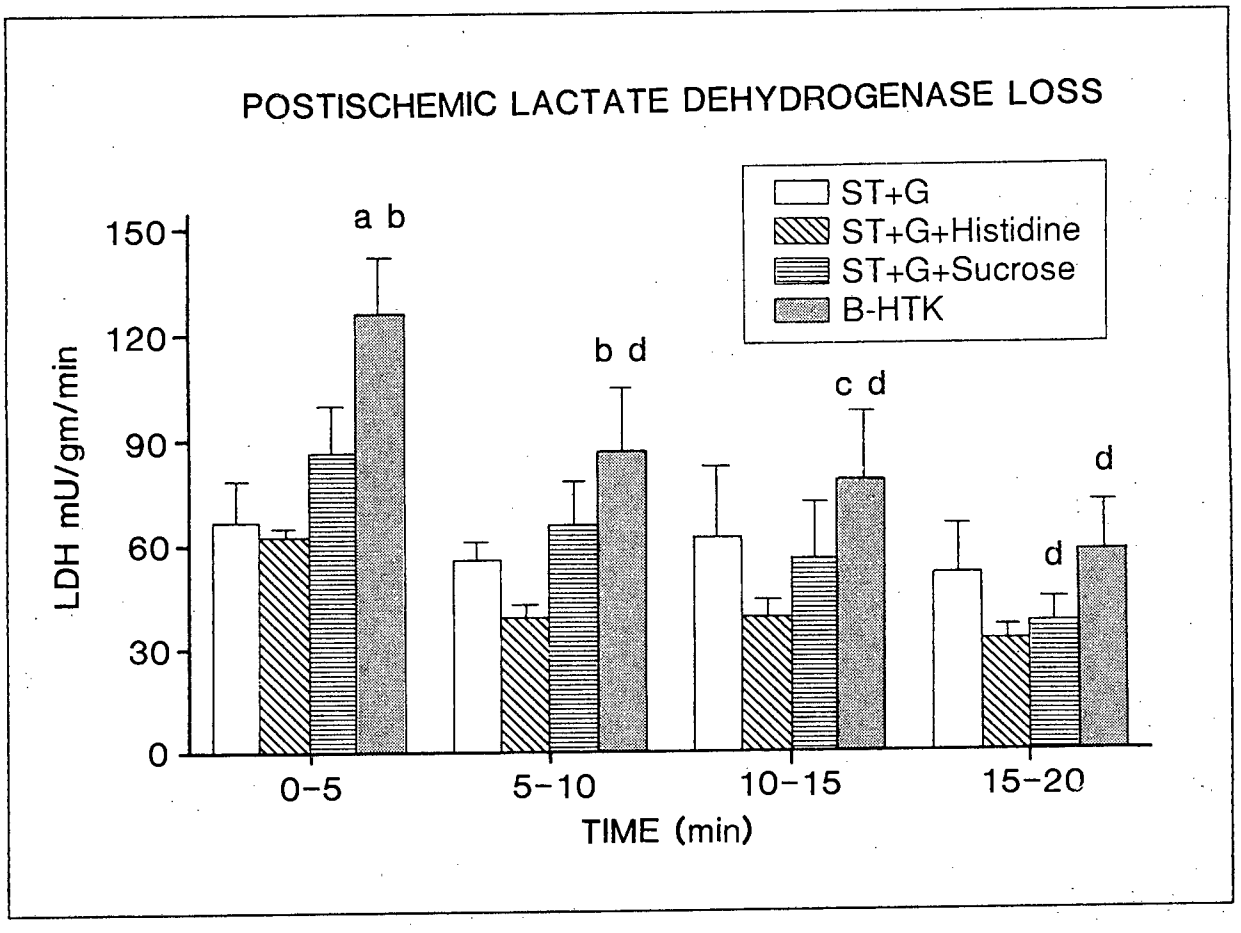
Continued anaerobic glycolysis during the 3-hour period of total ischaemia resulted in a significant increase of total tissue lactate (Table III). Lactate content increased from a preischaemic value of 0.14 ± 0.04 umol/gm fresh weight to 19.98 ± 0.84 umol/gm when hearts were protected with ST+G ($p < 0.01$). This was no different from ST+G+Histidine (21.07 ± 0.56 umol/gm). However, B-HTK4 which does not contain any glucose was associated with less lactate accumulation (13.41 ± 1.31 umol/gm, $p < 0.01$).

Postischaemic LDH washout

The mean washout of LDH, a measure of damage to membrane integrity, throughout the 20 min reperfusion period for ST+G was 58.55 ± 6.74 mU/gm/min which was no different from the washout associated with ST+G+Histidine, namely 42.79 ± 3.30 mU/gm/min. However, LDH washout increased significantly if hearts were protected

with B-HTK4, to 87.44 ± 11.96 mU/gm/min ($p < 0.05$). This was primarily due to an initial high washout of LDH in the first five minutes of reperfusion, which then decreased linearly during the subsequent reperfusion period, $p < 0.01$, (Figure 2).

Figure 2



Legend:

Isolated rat hearts were subjected to 3-hour 10°C ischaemia after a single dose of either St Thomas plus glucose (ST+G) or Bretschneider HTK4 (B-HTK) crystalloid cardioplegic solutions, or the experimental solutions ST+G plus Histidine or Sucrose (50 mmol/L). The mean and standard error of mean postischaemic washout of lactate dehydrogenase (LDH), during each 5 min interval of a 20 min postischaemic reperfusion period in the Langendorff mode, for each group are shown.

a - $p < 0.01$ compared with ST+G.

b - $p < 0.01$ compared with ST+G+Histidine.

c - $p < 0.05$ compared with ST+G+Histidine.

d - $p < 0.05$ compared with 0 - 5 min value.

COMMENT

We studied the effect of adding histidine (50 mmol/L) to a modified St Thomas' Hospital No. 2 cardioplegic solution that also contained glucose (10 mmol/L), to enhance ongoing anaerobic glycolysis during cardioplegic arrest. The addition of histidine to ST+G increased buffering capacity fivefold, increased end-ischaemic ATP content although end-ischaemic lactate content was unaltered, and improved postischaemic mechanical recovery. In contrast, the even more highly buffered Bretschneider HTK4 cardioplegic solution, having a buffering capacity threefold higher than ST+G+Histidine, but an electrolyte composition more closely approximating intracellular values, was associated with diminished end-ischaemic ATP, CP and lactate contents, increased LDH loss during reperfusion, and a mechanical recovery no better than ST+G.

APPENDIX B-6

IN VIVO PRIMATE STUDY: PILOT STUDIES ON THE EFFECT OF MODIFIED ST THOMAS' CARDIOPLEGIC SOLUTIONS CONTAINING HISTIDINE

INTRODUCTION

We previously showed that the addition of histidine (50 mmol/L) to a modified St Thomas' Hospital No 2 cardioplegic solution was beneficial in the isolated rat heart model (see Appendix B-5). In addition, we also showed that glucose (7 - 11 mmol/L) was beneficial in the isolated rat heart model (see Appendix A-2) but not in the in vivo primate model (see Appendix B-1). One postulate for this later discrepancy in results between the two models was an inadequate buffering power of the St Thomas' cardioplegic solution, which is possibly detrimental in the presence of glucose and therefore enhanced anaerobic metabolism.

A pilot study was therefore undertaken to evaluate the addition of histidine (50 mmol/L) and histidine plus glucose (10 mmol/L) to the St Thomas' cardioplegic solution, in the in vivo primate model.

MATERIALS AND METHODS

The in vivo primate model as described in Chapter 2 was used. The chacma baboon (Papio ursinus) was selected because of its close evolutionary relationship to man. Animals weighing 10 - 29 kg were used.

Experimental Protocol

The animals were anaesthetised, intubated, and ventilated. A median sternotomy was performed and the heart cannulated for cardiopulmonary bypass. Control haemodynamic and metabolic variables were measured prior to commencing

cardiopulmonary bypass. Systemic temperature was then lowered and maintained at 26°C, the aorta was cross-clamped and cold (4°C) cardioplegic solution (15 ml/kg; Table I) was infused. In addition, intermittent iced saline was used for topical hypothermia, and maintenance cardioplegia was reinfused every 30 minutes throughout a 3-hour ischaemic cross-clamp period. Additional ventricular biopsies were taken prior to the release of the aortic cross-clamp. A 15-minute normothermic reperfusion period followed, and the animals were then weaned from cardiopulmonary bypass and postischaemic haemodynamic and metabolic variables measured 5 and 30 minutes after termination of cardiopulmonary bypass.

Table I

COMPOSITION OF CARDIOPLEGIC SOLUTIONS

(mmol/L)	ST	ST + Histidine	ST + Glucose + Histidine
Na^+	120	100	100
K^+	16	16	16
HCO_3^-	10	10	10
Ca^{2+}	1.2	1.2	1.2
Mg^{2+}	16	16	16
Glucose	--		10
Histidine	--	50	50
Osmolarity (mOsm/L)	323.6	333.6	343.6
Osmolality (mOsm/kg H_2O)	293	310	320
pH (10°C)	7.4	7.2	7.2

Legend:
The composition of the St Thomas cardioplegic solution (ST), as well as two experimental solutions containing histidine (ST + Histidine) or histidine plus glucose (ST + Glucose + Histidine) is shown. Osmolarity was calculated by assuming 100% ionic dissociation and osmolality measured by freezing point depression (Osmomat 030 automatic cryoscopic osmometer: Gonotec, Berlin, Germany).

The addition of 50 mmol/L histidine to the St Thomas' cardioplegic solution increased the buffering capacity from 6 mmol HCl/L/δ pH, to 30 mmol HCl/L/δ pH (see Appendix B-5).

Expression of results

Values of functional parameters obtained during the postischaemic working period were expressed as a percentage of each individual preischaemic control value. Tissue high energy phosphate content was expressed in absolute values (umol/g wet weight). Results were then presented as percentage means and standard errors of percentage means.

Statistics

The statistical test used was the two way analysis of variance (ANOVA-2), and if overall statistical significance was obtained pair wise comparisons were then done by acceptance intervals of means with the *f* test. Statistical significance was taken as $p < 0.05$.

RESULTS

Postischaemic recovery of mechanical function

The preischaemic control values of all three groups were similar, and are shown in Table II. Three experiments were performed in each group. However, in the St Thomas' plus Histidine plus Glucose group one experiment was abandoned due to technical factors and one animal could not be weaned from cardiopulmonary bypass. In contrast, all animals in the other two groups could be successfully weaned from cardiopulmonary bypass.

In this pilot study only a few experiments were performed in each group, and although the addition of histidine to the St Thomas' cardioplegic solution tended to improve postischaemic recovery, significance was not obtained. However, the addition of glucose to the St Thomas' plus histidine solution caused profound depression of postischaemic recovery of all indices compared to both the standard St Thomas' and St Thomas' plus histidine cardioplegic solutions, $p < 0.05$ (Table II).

Table II

POSTISCHAEMIC MECHANICAL RECOVERY

	St Thomas' No 2	St Thomas' + Histidine	St Thomas' + Histidine + Glucose
	N = 3	N = 3	N = 2
Cardiac Index			
Preischaemic ($L/min/m^2$)	3.08 ± 0.22	3.34 ± 0.50	3.48 ± 0.85
% Recovery 5-min	79.56 ± 15.64	104.33 ± 13.12	19.86 ± 19.86[ac]
% Recovery 30-min	84.03 ± 9.28	94.59 ± 16.50	23.40 ± 23.40[ac]
Stroke Volume Index			
Preischaemic ($ml/beat/m^2$)	26.93 ± 4.35	25.94 ± 2.93	33.53 ± 11.59
% Recovery 5-min	78.27 ± 3.33	107.54 ± 16.14	15.28 ± 15.28[ac]
% Recovery 30-min	77.79 ± 7.51	96.61 ± 17.40	22.65 ± 22.65[ac]
Left Ventricular dp/dt			
Preischaemic (mm Hg/sec)	1092.8 ± 36.6	1107.0 ± 36.47	1162.0 ± 83.0
% Recovery 5-min	131.04 ± 38.19	142.44 ± 21.69	35.02 ± 35.02[ac]
% Recovery 30-min	119.0 ± 9.73	105.27 ± 15.80	28.35 ± 28.35[a]
Stroke Work Index			
Preischaemic ($gm.m/beat/m^2$)	42.95 ± 6.71	43.97 ± 5.91	47.40 ± 10.87
% Recovery 5-min	83.15 ± 13.21	114.75 ± 16.63	9.22 ± 9.22[bd]
% Recovery 30-min	82.14 ± 4.50	85.13 ± 16.06	11.32 ± 11.32[bd]

Legend:
Animals were subjected to a 3-hour hypothermic cross-clamp period protected with multidose reinfusions of indicated cardioplegic solutions. Haemodynamic variables were measured at a left atrial pressure of 5 mm Hg. Postischaemic recovery is expressed as mean percentage recoveries of each individual preischaemic value, and standard errors of percentage means.
a - $p < 0.05$ compared to St Thomas' No 2,
b - $p < 0.01$ compared to St Thomas' No 2,
c - $p < 0.05$ compared to St Thomas' plus Histidine,
d - $p < 0.01$ compared to St Thomas' plus Histidine.

Left ventricular function curves (CI & SWI) were determined both prior to and after the ischaemic cross-clamp period (30-minute post cardiopulmonary bypass), by measuring haemodynamic variables at different left atrial preloads. The preischaemic control curves were similar in all groups (Table III).

Table III

LEFT VENTRICULAR FUNCTION CURVE

LAP	5	10	15	20 (mm Hg)
CI (L/min/m^2)				
St Thomas'				
Pre	3.08 ± 0.22	3.86 ± 0.43	4.26 ± 0.49	4.59 ± 0.63
% Rec	84.03 ± 9.28	70.07 ± 9.90	62.54 ± 4.48	43.11 ± 2.50
St Thomas' plus Histidine				
Pre	3.34 ± 0.50	4.39 ± 0.27	4.61 ± 0.59	4.67 ± 0.77
% Rec	94.59 ± 16.50	73.91 ± 5.30	71.19 ± 4.50	64.31 ± 4.56
St Thomas' plus Glucose plus Histidine				
Pre	3.48 ± 0.85	3.62 ± 0.13	3.55 ± 0.65	3.44 ± 0.83
% Rec	23.4 ± 23.40^{bd}	21.39 ± 21.39^{bd}	23.87 ± 23.87^{ad}	17.86 ± 17.86^{c}
Data normalized to the integrated preischaemic control curve				
Cont.	3.28 ± 0.25	4.00 ± 0.21	4.21 ± 0.32	4.33 ± 0.41
ST	2.76 ± 0.3	2.80 ± 0.4	2.63 ± 0.19^{e}	1.87 ± 0.11^{f}
ST+H	3.10 ± 0.54	2.96 ± 0.21	3.00 ± 0.19	2.79 ± 0.20^{e}
ST+G+H	0.77 ± 0.77^{bdf}	0.86 ± 0.86^{bdf}	1.01 ± 1.01^{af}	0.77 ± 0.77^{acf}
SWI (gm·m/beat/m^2)				
St Thomas'				
Pre	42.95 ± 6.71	48.89 ± 10.33	54.05 ± 8.80	52.66 ± 9.69
% Rec	82.14 ± 4.50	74.94 ± 7.15	64.53 ± 7.19	47.58 ± 4.40
St Thomas' plus Histidine				
Pre	43.97 ± 5.91	66.17 ± 2.81	70.78 ± 9.63	64.80 ± 16.45
% Rec	85.13 ± 16.06	63.07 ± 5.01	66.17 ± 3.72	70.95 ± 16.08
St Thomas' plus Glucose plus Histidine				
Pre	47.40 ± 10.87	54.95 ± 10.91	51.80 ± 16.70	43.93 ± 13.62
% Rec	11.32 ± 11.32^{bd}	12.37 ± 12.37^{bd}	13.70 ± 13.70^{bd}	13.82 ± 13.82^{ad}
Data normalized to the integrated preischaemic control curve				
Cont.	44.44 ± 3.64	56.89 ± 4.97	59.76 ± 6.22	55.03 ± 7.45
ST	36.51 ± 2.0	42.63 ± 4.07	38.56 ± 4.3	26.18 ± 2.42^{e}
ST+H	37.84 ± 7.14	35.88 ± 2.85	39.54 ± 2.22	39.05 ± 8.85
ST+G+H	5.03 ± 5.03^{bdf}	7.03 ± 7.03^{bcf}	8.19 ± 8.19^{bdf}	7.60 ± 7.60^{df}

Legend:

A postischaemic left ventricular function curve was obtained 30 min after weaning from cardiopulmonary bypass at increasing left atrial pressures (LAP), and compared to a similar preischaemic curve. Mean percentage postischaemic recovery (% Rec) was calculated from each individual postischaemic and preischaemic ratio. An average preischaemic control function curve (Cont.; N = 8) was derived by pooling the data from all groups, and postischaemic function for each group normalized to this curve for display purposes. ST - St Thomas' cardioplegic solution (N = 3); ST+H - ST plus histidine (50 mmol/L) cardioplegic solution (N = 3); ST+G+H - ST+H plus glucose (10 mmol/L) cardioplegic solution (N = 2).

a - $p < 0.05$, **b** - $p < 0.01$; compared to St Thomas' No 2, **c** - $p < 0.05$, **d** - $p < 0.01$; compared to St Thomas' plus Histidine, **e** - $p < 0.05$, **f** - $p < 0.01$; compared to Cont.

The addition of histidine to the St Thomas' cardioplegic solution tended to improve postischaemic ventricular function, but significance was not obtained. Nevertheless, the addition of glucose to this cardioplegic solution depressed postischaemic ventricular function ($p < 0.05$), despite the low experimental numbers.

In conclusion, the addition of histidine to the St Thomas' cardioplegic solution tended to improve postischaemic recovery, although one cannot place full emphasis on the results of this pilot study because of the low experimental numbers. However, the addition of glucose to this modified St Thomas' cardioplegic solution which had a five-fold greater buffering capacity than the standard St Thomas' solution, resulted in significantly lower postischaemic myocardial recovery.

APPENDIX B-7

ISOLATED RAT HEART STUDY: PROVIDING COLLOID IN THE ST THOMAS' CARDIOPLEGIC SOLUTION

INTRODUCTION

This study was undertaken to determine whether the addition of colloid (Dextran-40) to the St Thomas' Hospital No 2 cardioplegic solution was beneficial.

MATERIALS AND METHODS

The isolated perfused working rat heart model as described in Chapter 2 was used. Hearts were removed from male Long Evans rats weighing between 280 gm - 380 gm.

Experimental Protocol

The aorta was cannulated and Langendorff perfusion with 95 % O_2 5 % CO_2 gassed Krebs Henseleit buffer commenced and maintained for 10 min, prior to conversion to the working preparation (left atrial filling pressure 20 cm H_2O, left ventricle afterload 100 cm H_2O). Haemodynamic variables were measured after 10 minutes of work. The atrial and aortic cannulae were then closed and 10 ml cardioplegic solution (10^oC) was infused at a pressure of 80 cm H_2O, and simultaneously the hearts were placed into a 10^oC chamber containing the relevant cardioplegic solution for a 3-hour period of global ischaemia. No reinfusions of cardioplegic solution were administered. Hearts were then reperfused at 37^oC in the non working Langendorff mode for 10 min at 100 cm H_2O, and thereafter switched to the working mode for a further 10 min prior to measuring postischaemic functional recovery.

Cardioplegic solutions.

The "control" cardioplegic solution tested was the St Thomas' Hospital No 2 cardioplegic solution (ST). The addition of either 2 gm % or 3 gm % Dextran-40 (Rheomacrodex) to

ST was evaluated. The appropriate quantity of Rheomacrodex 10% which contains NaCl 150 mmol/L was used, and the concentration of sodium chloride (110 mmol/L) in the final modified cardioplegic solution was kept constant (Table I).

Table I

COMPOSITION OF CARDIOPLEGIC SOLUTIONS

(mmol/L)	ST	ST + 2% Dextran-40	ST + 3% Dextran-40
Na^+	120	120	120
K^+	16	16	16
HCO_3^-	10	10	10
Ca^{2+}	1.2	1.2	1.2
Mg^{2+}	16	16	16
Dextran-40	--	2%	3%
Osmolality (mOsm/kg H_2O)	298	305	306
Colloid Oncotic Pressure (mm Hg)	0.2	15	25

Legend:
The composition of the St Thomas' Hospital No 2 cardioplegic solution (ST), as well as two experimental solutions containing either 2% or 3% Dextran-40 is shown. Colloid oncotic pressure was measured by a colloid osmometer (Osmomat 050: Gonotec, Berlin, Germany). Osmolality was measured by freezing point depression (Osmomat 030: Gonotec, Berlin, Germany).

Expression of results

Values of functional parameters obtained during the postischaemic working period were expressed as a percentage of each individual preischaemic control value. Results are presented as percentage means and standard errors of percentage means.

Statistics

The statistical test used was the one way analysis of variance (ANOVA-1), and if overall statistical significance was obtained pair wise comparisons were then done by acceptance intervals of means with the *f* test. Statistical significance was taken as $p < 0.05$.

RESULTS

Increasing the colloid oncotic pressure from 0 mm Hg to 25 mm Hg of the St Thomas' Hospital No 2 cardioplegic solution by the addition of 3 % Dextran-40 tended to improve postischaemic mechanical recovery, but significance was not obtained (Table II). Furthermore, postreperfusion high energy phosphate and water content were not altered (Table III).

Table II

POSTISCHAEMIC MECHANICAL RECOVERY

	A O		C O		S V	
	Pre Ischaemic ml/min	Percentage Recovery	Pre Ischaemic ml/min	Percentage Recovery	Pre Ischaemic ml	Percentage Recovery
ST	49.13 ± 1.75	54.4 ± 5.2	63.0 ± 2.16	63.8 ± 5.4	0.247 ± 0.008	63.7 ± 4.3
ST + 2% Dextran-40	43.75 ± 2.31	51.4 ± 3.6	57.0 ± 2.28	59.8 ± 3.3	0.249 ± 0.011	63.2 ± 3.4
ST + 3% Dextran-40	50.88[a] ± 2.40	58.9 ± 3.9	67.0[a] ± 2.87	71.0 ± 4.2	0.244 ± 0.10	70.7 ± 2.8

Legend:

Isolated rat hearts (N = 8) were subjected to 3-hour 10°C ischaemia after a single dose of either St Thomas' or modified solutions containing Dextran-40.

a - $p < 0.05$ compared to ST + 2 % Dextran-40,

All other comparisons p = NS.

Table III

POSTREPERFUSION METABOLIC CONCENTRATIONS

	ATP umol/gm	CP umol/gm	Percent H_2O %
Preischaemic Control	-	-	86.1 ± 0.3
ST	3.04 ± 0.06	5.94 ± 0.17	86.5 ± 0.2
ST + 2% Dextran-40	3.03 ± 0.08	6.15 ± 0.23	86.3 ± 0.2
ST + 3% Dextran-40	3.08 ± 0.09	6.18 ± 0.25	86.5 ± 0.2

Legend:

Isolated rat hearts (N = 8) were subjected to 3-hour 10°C ischaemia after a single dose of either St Thomas, or modified cardioplegic solutions containing Dextran-40. Post-ischaemic values of ATP, CP (umol/gm fresh weight) and percentage water content were measured by freeze-clamping at the end of the postischaemic reperfusion period after measuring the haemodynamic variables. Control concentrations of water content were measured in seven hearts by freeze-clamping prior to the ischaemic period.

All comparisons p = NS.

APPENDIX B-8

PRACTICALITIES OF DELIVERING CARDIOPLEGIC SOLUTIONS

III. PRESSURE

METHODS

We compared the delivered ascending aortic pressure, measured with a 9 Ga (11 French) cardioplegic delivery catheter containing an integral pressure monitoring port (aortic root cannula - Cat. 23009: DLP; Grand Rapids, Michigan, USA), during clinical administration of cardioplegia, with proximally monitored cardioplegic line pressures. The St Thomas' Hospital No 2 crystalloid cardioplegic solution was used, and infusion was at constant pressure. Each of three possible delivery systems; viz gravity (N = 6; solution infused from a height of ± 178 cm; 133 mm Hg), pressure bag placed around the vacolitre of cardioplegic solution (N = 6; inflated to 300 mm Hg) or roller-pump infusion method (N = 13), was assessed during routine cardiac surgery. With the roller pump method, the perfusionist monitored the ascending aortic root pressure and altered the infusion rate of the cardioplegia accordingly, in order to obtain an ascending aortic root pressure of at least 80 mm Hg during all infusions. Therefore, the speed of the pump was progressively increased to maximum till this aortic root pressure was obtained. The maximal pressure that could be generated was a proximal cardioplegic line pressure of 300 mm Hg.

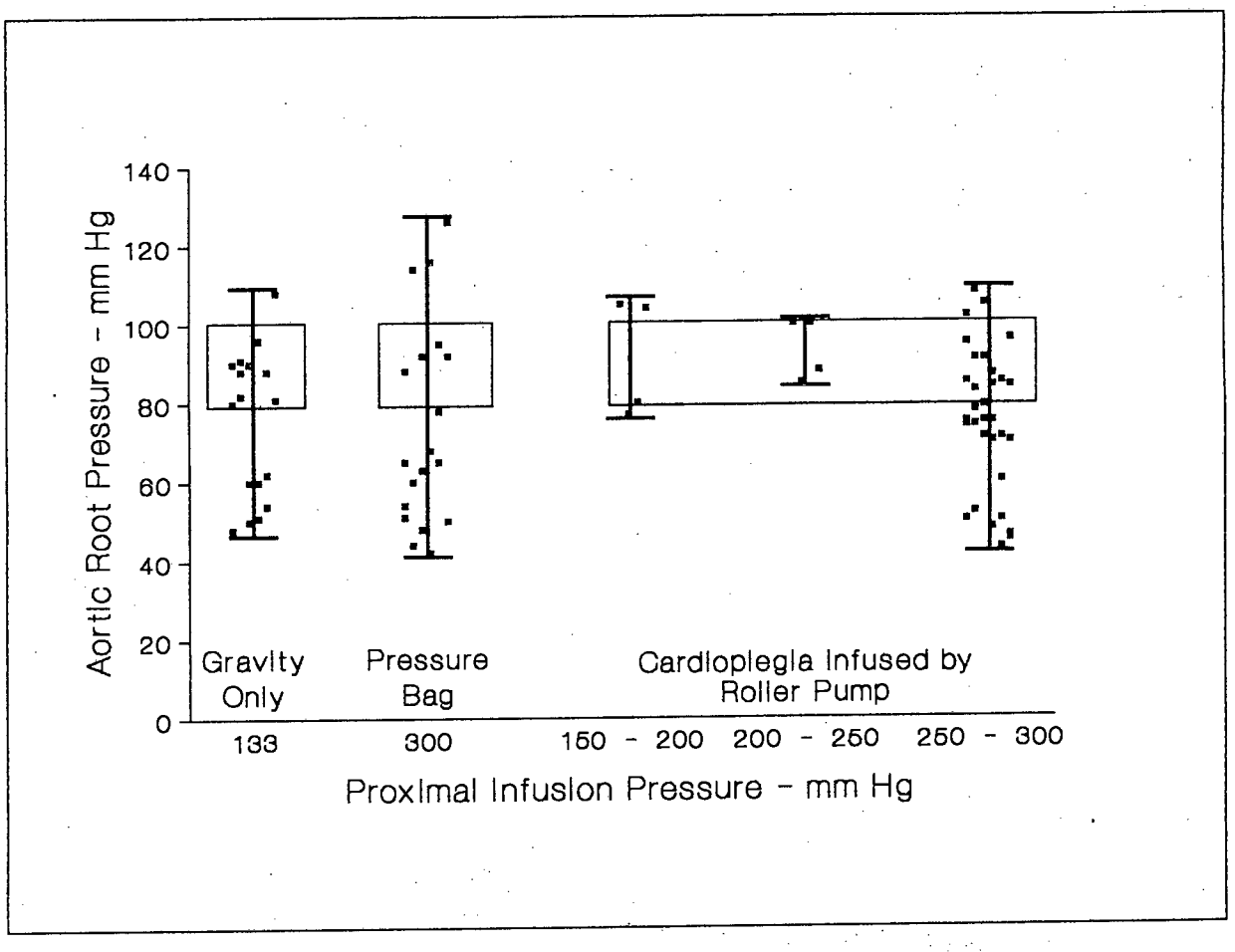
The cardioplegic infusion set used for the gravity infusions with or without external pressurization was a 1.5 m length of 3/16" polyvinyl tubing. Proximal line pressure was assumed to be equivalent to either the gravitational height or degree of pressurization. With the roller pump system, a 3.5 m length of 1/4" polyvinyl tubing incorporating an 0.2 um pore size cardioplegic filter was used. The proximal line pressure was measured 4 m from the distal end of the tubing.

All three of the above methods of infusing cardioplegic solutions were used routinely at Groote Schuur Hospital by different perfusionists and surgeons at the time of this study. These observations were therefore recorded during routine surgery, and there was no randomization of patients, as the method of infusing the cardioplegia was chosen by individual surgeons. During each infusion of cardioplegia, both proximal and distal aortic root pressures, and duration of cardioplegic infusion were recorded by an independent observer.

RESULTS

Figure 1.

AORTIC PRESSURE DURING INFUSION OF CARDIOPLEGIC SOLUTIONS.



Legend:

The measured aortic root pressure during administration of cardioplegia by three methods was compared. When infusing cardioplegia by gravity (N = 6), the height of the vacolitre from the level of the right atrium was 178 cm H_2O (133 mm Hg). If a pressure bag was used (N = 6), this was inflated to 300 mm Hg. Alternatively, a roller pump was used (N = 12), in which case the perfusionist attempted to maintain the measured aortic root pressure between 80 - 100 mm Hg.

Gravity Infusion

Infusion of crystalloid cardioplegia by gravity alone, from a height of approximately 178 cm (above the right atrium) produces a proximal infusion "pressure" of ± 133 mm Hg. This method was used in 6 patients undergoing coronary artery bypass grafting (N = 5) or closure of a traumatic ventricular septal defect (N = 1). Ascending aortic root pressures of 86.6 ± 7.4 mm Hg were produced with this method, and rate of infusion of

the cardioplegic solution was calculated to be 298.7 ± 17.9 ml/min, on induction of cardioplegia. With subsequent multidoses of cardioplegia (N = 11) the aortic root pressure was 69.0 ± 5.1 mm Hg and infusion rate 372.9 ± 32.9 ml/min (Figure 1).

Pressure Bag Infusion

In 6 patients undergoing coronary artery bypass graft (N = 3), or mitral valve replacement (N = 3) infusion of cardioplegic solution was achieved by pressurizing the vacolitre of cardioplegia, by an external pressure bag (inflated to 300 mm Hg). This method produced an aortic root infusion pressure of 75.7 ± 11.0 mm Hg at a calculated flow rate of 430.1 ± 19.0 ml/min on induction, and again was no different with subsequent multidoses (73.6 ± 7.3 mm Hg at 387.7 ± 26.5 ml/min). On induction, although this "pressure bag" method produced a higher cardioplegic flow rate than simple gravity infusion ($p < 0.05$), the infusion pressure at the ascending aorta was not increased.

Roller Pump System

A roller pump was used to infuse the cardioplegic solution in 13 patients undergoing coronary artery bypass grafting. the cardioplegic flow rate was progressively increased by the perfusionist till an aortic pressure of 80 - 100 mm Hg, or maximal pump speed was achieved. Even if maximal flow was necessary, the distally measured aortic infusion pressures varied from 43 - 108 mm Hg (Fig 1). The mean pressure on induction of cardioplegic arrest was 86.1 ± 3.4 mm Hg at a calculated flow rate of 378.0 ± 51.0 ml/min and was no different with subsequent multidoses (74.0 ± 3.8 mm Hg at a flow rate of 329.9 ± 18.6 ml/min).

COMMENT

The measured pressure in the ascending aorta when infusing cardioplegic solutions varied from 42 - 126 mm Hg. This pressure range was similar with each of the three clinically used methods.

However, when simply infusing cardioplegia by gravity (height 178 cm; 133 mm Hg) the maximum obtainable pressure is limited. Only one patient dose was infused at a recorded aortic pressure of greater than 100 mm Hg, but a number of infusions were at possibly suboptimal pressures. The external pressure bag method (inflated to 300 mm Hg) was also associated with a wide range of measured aortic root pressures; some excessively high and others too low. In contrast, with the roller pump delivery system, both high and low pressures can too a certain extent be prevented. Low aortic root pressures during infusion of cardioplegia may be due to mild aortic regurgitation, which can be compensated for by increasing flow rate. Therefore, we believe that the preferred method of infusing cardioplegia is by means of a roller pump.

The aortic root pressure also did not correlate with the proximally measured "line pressure". The same aortic root cannula, which is also a potentially flow limiting factor was used in all groups, although a cardioplegic line filter was only included in the roller pump groups. This poor correlation is due to resistance in the cardioplegic delivery line tubing, between the proximal measuring port and the ascending aorta, varying with flow. Thus, cardioplegic infusion pressures should be monitored directly in the ascending aorta, as the proximal cardioplegic "line pressure" is unreliable.

APPENDIX B-9

IN VIVO PRIMATE STUDY: THE EFFECT OF A MODIFIED ST THOMAS' CARDIOPLEGIC SOLUTION CONTAINING HISTIDINE

INTRODUCTION

We previously showed that the addition of histidine (50 mmol/L) to a modified St Thomas' Hospital No 2 cardioplegic solution was beneficial in the isolated rat heart model (see Appendix B-5). In vivo non-human primate model pilot studies also suggested a beneficial effect of histidine (see Appendix B-6). However, in this model the standard St Thomas' solution already provided good protection to the myocardium, and larger numbers in each group would have been necessary to achieve statistical significance. We therefore elected to change the experimental model by introducing an initial global ischaemic period of ventricular fibrillation prior to cardioplegic arrest, in order to assess the effects of our experimental cardioplegic solutions on energy depleted hearts.

In addition, two further modifications of the St Thomas' Hospital cardioplegic solution were introduced; decreasing the calcium content to 0.6 mmol/L (see section 3.1), and addition of adenosine 1 mmol/L (Boehm D: unpublished data). The composition of the experimental cardioplegic solution; St Thomas' plus Histidine plus Adenosine (ST-HA) is shown in Table 1.

MATERIALS AND METHODS

The in vivo primate model as described in Chapter 2 was used. The chacma baboon (Papio ursinus) was selected because of its close evolutionary relationship to man. Animals weighing 15 - 34 kg were used.

Experimental Protocol

The animals were anaesthetised, intubated, and ventilated. A median sternotomy was performed and the heart cannulated for cardiopulmonary bypass. Control haemodynamic and metabolic variables were measured prior to commencing cardiopulmonary bypass. Cardiopulmonary bypass was commenced and systemic temperature maintained at 32°C. The hearts were then depleted of energy reserves by cross-clamping the aorta for 20 min, in addition to inducing fibrillation (spontaneous fibrillation after initial electrical induction). Thereafter cold (4°C) cardioplegic solution (15 ml/kg; Table I) was infused into the coronary circulation, and systemic temperature lowered and maintained at 26°C.

Table I

COMPOSITION OF CARDIOPLEGIC SOLUTIONS

(mmol/L)	St Thomas' No 2	St Thomas' + Histidine + Adenosine
Na^+	120	100
K^+	16	16
HCO_3^-	10	10
Ca^{2+}	1.2	0.6
Mg^{2+}	16	16
Histidine	--	50
Adenosine	--	1
Osmolarity (mOsm/L)	323.6	332.8
Osmolality (mOsm/kg H_2O)	293	310
pH (10°C)	7.4	7.2
Buffer capacity (mmol HCl/L/δ pH)	6	30

Legend:
The composition of the St Thomas cardioplegic solution, as well as an experimental solution containing histidine, adenosine and a lower concentration of calcium is shown. Osmolarity was calculated by assuming 100% ionic dissociation and osmolality measured by freezing point depression (Osmomat 030 automatic cryoscopic osmometer: Gonotec, Berlin, Germany).

Intermittent iced saline was used for topical hypothermia with each administration of cardioplegic solution, and maintenance cardioplegia was reinfused every 30 minutes

throughout a 3-hour ischaemic cross-clamp period. Additional ventricular biopsies were taken prior to the release of the aortic cross-clamp. A 15-minute normothermic reperfusion period followed, and the animals were then weaned from cardiopulmonary bypass and postischaemic haemodynamic and metabolic variables measured 5 and 30 minutes after termination of cardiopulmonary bypass.

Expression of results

Values of functional parameters obtained during the postischaemic working period were expressed as a percentage of each individual preischaemic control value. Tissue high energy phosphate content was expressed in absolute values (umol/g wet weight). Results were then presented as percentage means and standard errors of percentage means.

Statistics

The statistical test used was the two way analysis of variance (ANOVA-2), and if overall statistical significance was obtained pair wise comparisons were then done by acceptance intervals of means with the *f* test. Statistical significance was taken as $p < 0.05$.

RESULTS

Postischaemic recovery of mechanical function

The preischaemic control values of the groups were similar, and are shown in Table II. In the St Thomas' group (N = 8) three animals could not be weaned from cardiopulmonary bypass. In contrast, all animals in the experimental ST-HA group (N = 6) could be successfully weaned from cardiopulmonary bypass.

Table II

POSTISCHAEMIC MECHANICAL RECOVERY

	St Thomas' No 2	St Thomas' + Histidine + Adenosine
	N = 8	N = 6
Cardiac Index		
Preischaemic ($L/min/m^2$)	4.82 ± 0.47	4.48 ± 0.41
% Recovery 5-min	42.09 ± 13.39	92.10 ± 12.53[a]
% Recovery 30-min	38.68 ± 12.07	86.64 ± 10.93[a]
Stroke Volume Index		
Preischaemic ($ml/beat/m^2$)	40.79 ± 4.62	33.64 ± 3.55
% Recovery 5-min	30.87 ± 9.83	83.81 ± 8.16[b]
% Recovery 30-min	29.52 ± 9.08	82.49 ± 9.05[b]
Left Ventricular dp/dt		
Preischaemic (mm Hg/sec)	1027.25 ± 70.45	1017.00 ± 111.73
% Recovery 5-min	72.70 ± 15.33	117.89 ± 10.08[a]
% Recovery 30-min	56.30 ± 17.10	103.30 ± 9.3[a]
Stroke Work Index		
Preischaemic ($gm.m/beat/m^2$)	68.31 ± 9.03	53.59 ± 7.90
% Recovery 5-min	21.91 ± 7.54	92.05 ± 12.62[b]
% Recovery 30-min	20.67 ± 6.42	78.11 ± 10.62[b]

Legend:

Animals were subjected to a 20-min period of ventricular fibrillation with global ischaemia at 32°C prior to 3-hour hypothermic cardioplegic arrest with multidose reinfusions of indicated cardioplegic solutions. Haemodynamic variables were measured at a left atrial pressure of 5 mm Hg. Postischaemic recovery is expressed as mean percentage recoveries of each individual preischaemic value, and standard errors of percentage means.

a - $p < 0.05$ compared to St Thomas' No 2, b - $p < 0.01$ compared to St Thomas' No 2.

The experimental ST-HA cardioplegic solution improved the postischaemic recovery of all indices of mechanical recovery, both 5-min and 30-min after weaning from cardiopulmonary bypass (Table II). Furthermore, there was no difference between either the 5-min or 30-min postischaemic values. Thirty minutes after weaning from cardiopulmonary bypass percentage recovery of cardiac index increased from 38.68 ±

12.07 % with ST to 86.64 ± 10.93 % with ST-HA ($p < 0.05$), and stroke work index increased from 20.67 ± 6.42 % to 78.11 ± 10.62 % ($p < 0.01$).

Left ventricular function curves were determined both prior to and after the ischaemic cross-clamp period (30-minute post cardiopulmonary bypass), by measuring haemodynamic variables at different left atrial preloads. The preischaemic control values at all left atrial filling pressures were similar in both groups (Table III). The experimental ST-HA cardioplegic solution was associated with better recovery of postischaemic left ventricular function as assessed by cardiac index at both 5 mm Hg and 10 mm Hg left atrial filling pressures ($p < 0.01$), and stroke work index at both 5 mm Hg, 10 mm Hg ($p < 0.01$) and 15 mm Hg ($p < 0.05$) filling pressures.

The percentage recovery of both groups was then normalized for display purposes to an integrated preischaemic control curve as discussed in chapter 2. The recovery of hearts protected with St Thomas' cardioplegic solution was depressed at all left atrial filling pressures compared to the integrated control curve ($p < 0.01$). In contrast, with ST-HA recovery was depressed at 10 mm Hg, 15 mm Hg and 20 mm Hg filling pressures ($p < 0.01$), but the no different to control at 5 mm Hg.

In conclusion, the experimental modified St Thomas' cardioplegic solution containing histidine and adenosine was associated with better recovery of myocardial mechanical function following 3-hour cardioplegic arrest of energy depleted hearts.

Table III

LEFT VENTRICULAR FUNCTION CURVE

LAP	5	10	15	20 (mm Hg)
CI ($L/min/m^2$)				
St Thomas' No 2				
Pre	4.82 ± 0.47	5.71 ± 0.27	6.58 ± 0.51	7.18 ± 0.48
% Rec	38.68 ± 12.07	37.85 ± 11.98	30.60 ± 10.38	25.47 ± 10.40
St Thomas' plus Histidine plus Adenosine				
Pre	4.48 ± 0.41	6.04 ± 0.90	7.08 ± 0.82	7.27 ± 0.90
% Rec	86.64 ± 10.93[b]	79.57 ± 12.26[b]	53.44 ± 5.28	42.19 ± 5.70
Data normalized to the integrated preischaemic control curve				
Cont.	4.68 ± 0.31	5.85 ± 0.40	6.79 ± 0.44	7.22 ± 0.45
ST	1.81 ± 0.04[d]	2.22 ± 0.05[d]	2.08 ± 0.05[d]	1.84 ± 0.05[d]
ST-HA	4.05 ± 0.03[b]	4.66 ± 0.05[bc]	3.63 ± 0.02[bd]	3.05 ± 0.03[bd]
SWI ($gm \cdot m/beat/m^2$)				
St Thomas' No 2				
Pre	68.31 ± 9.03	75.48 ± 6.69	83.90 ± 9.40	78.43 ± 8.50
% Rec	20.67 ± 6.42	23.67 ± 7.60	21.68 ± 7.65	18.98 ± 7.95
St Thomas' plus Histidine plus Adenosine				
Pre	53.59 ± 7.90	82.90 ± 16.41	86.68 ± 14.26	79.76 ± 13.64
% Rec	78.11 ± 10.62[b]	64.94 ± 11.89[b]	48.44 ± 4.57[a]	40.90 ± 7.43
Data normalized to the integrated preischaemic control curve				
Cont.	62.00 ± 6.28	78.66 ± 7.69	85.09 ± 7.80	79.00 ± 7.28
ST	12.82 ± 0.40[d]	18.62 ± 0.58[d]	18.45 ± 0.60[d]	14.99 ± 0.58[d]
ST-HA	48.43 ± 0.67[b]	51.08 ± 0.91[bd]	41.22 ± 0.36[ad]	32.31 ± 0.54[d]

Legend:

A postischaemic left ventricular function curve was obtained 30 min after weaning from cardiopulmonary bypass at increasing left atrial pressures (LAP), and compared to a similar preischaemic curve. Mean percentage postischaemic recovery (% Rec) was calculated from each individual postischaemic and preischaemic ratio. An average preischaemic control function curve (Cont; N = 14) was derived by pooling the data from all groups, and postischaemic function for each group normalized to this curve for display purposes.

ST - St Thomas' cardioplegic solution (N = 8); ST-HA - ST plus histidine (50 mmol/L) plus Adenosine (1 mmol/L) cardioplegic solution (N = 6).

a - $p < 0.05$, b - $p < 0.01$; compared to St Thomas' No 2,

c - $p < 0.05$, d - $p < 0.01$; compared to Cont.

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CURRICULUM VITAE

Born in Pretoria, South Africa on the 11 September 1954. Matriculated in 1971 at St David's College, Marist Brothers Inanda, Johannesburg with a first class pass and distinctions in mathematics and physics. A bachelor degree in medicine and surgery (MB ChB) was then obtained at the University of the Witwatersrand, Johannesburg in 1977, and the Abelheim medal awarded for the most distinguished final year medical student in obstetrics.

Postgraduate training in cardiothoracic surgery was then pursued at the teaching hospitals attached to the University of the Witwatersrand. The Fellowship of the College of Surgeons of South Africa (FCS (SA) - Cardiothoracic) was obtained in 1986, and awarded the Brebner medal for the most distinguished student in part 2A of the fellowship.

Appointed as a consultant cardiothoracic surgeon with clinical responsibilities incorporating thoracic surgery, transplantation, adult and paediatric cardiac surgery at Groote Schuur Hospital, Cape Town, and lecturer at the University of Cape Town in February 1987. Specific interests include both adult cardiac and arrhythmia surgery, and research in the field of myocardial protection.

Hobbies include board sailing, horse riding, hiking, snorkel and scuba diving.