New Solutions for the Heart

An Update in Advanced Perioperative Protection

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Fundamentals of the Past: Cardioplegia: The First Period Revisited

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2.1 Historical Overview

Techniques for controlling the heart during cardiopulmonary bypass (CPB) and aortic occlusion have occupied and still occupy a centre stage in cardiac surgical research. The first 25–30 years started with diverse attempts at cardiac arrest and subsequently at myocardial protection and, after initial failures, this became a successful period with development of concepts and techniques that still apply. The period thereafter is in the authors' opinion to a large extent characterized by continuous reassessment and refinement of principles and of consolidation of techniques. Notwithstanding, major new developments have taken place in molecular biology, physiology and pharmacology as well as in surgical practice. Within this context not only intraoperative but truly perioperative myocardial protection with improvements in diagnostic techniques, drug treatment, anesthesia and intensive care has come into focus and allowed cardiac surgery to take on more seriously ill patients. This chapter is written with the intention to provide insight in the thinking and problem solving during the early period until 1980–1985. It presents the history of what is well known as "cardioplegia" and thereafter presents some concepts and issues considered at the time. Only briefly are comments made that relate to current principles and practice.

2.1.1 Background and Definitions

When open heart surgery took off after the development of heart-lung machines (Gibbon 1954; Lillehei et al. 1956; Rygg and Kyvsgaard 1958), a partly unforeseen challenge was that

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the unloaded heart on CPB continued to beat and had to be arrested in order to provide conditions for anatomic repair (Melrose 1980). Soon thereafter a further discovery was that surgical needs conflicted with myocardial demands. The needs of the surgeon include rapid induction, maintenance and easy reversal of cardiac arrest, a relaxed heart to allow for mobilization and traction, a preferably bloodless and unobscured field, and sufficient time for adequate correction of cardiac or coronary defects. A fulfillment ideally implies reversible induction of diastolic arrest, that coronary perfusion with blood may be avoided or minimized, and that periods of myocardial ischemia might be well tolerated.

Myocardial demands, on the other hand, requires that the cell machinery remains intact with rapid restoration of metabolism and function after cardiac arrest. An optimal procedure for the heart thus indicates that ischemia should preferably be avoided or that harmful effects induced by any inherent ischemia can be delayed and that a consequent reperfusion results in an uneventful recovery. In spite of all improvements made with time, the need-demand contradiction still represents a potential conflict in the everyday practice of cardiac surgery, not least when considering the state of the patient being operated upon.

Early techniques that were widely used to arrest and/or protect the heart were based on single or combined modalities such as:

- Hypothermia, systemic and/or supplied by topical cooling (Bigelow et al. 1950; Shumway et al. 1959; Swan 1973)
- Global ischemia with continuous or intermittent aortic occlusion (Cooley et al. 1962) and
- Aortic root or intracoronary perfusion with blood (Kay et al. 1958) and, when needed, electively induced ventricular fibrillation (Senning 1952).

Whereas these modalities alone or combined have survived up to the present day, during the 1970s they were mostly supplemented with or replaced by arresting the heart by "*cardioplegia*" based on chemical means (Bleese et al. 1978; Bretschneider et al. 1975; Follette et al. 1978a; Gay and Ebert 1973; Hearse et al. 1976a; Roe et al. 1977; Tyers et al. 1977). In general terms *cardioplegia* can be defined as a technique involving single or repeated injections, infusions or perfusions into the aortic root or into the coronary vasculature of a hypo- or normothermic solution (primarily) designed to arrest the heart (stricter sense) and (secondarily) also to protect the myocardium (wider sense) during aortic cross-clamping with global ischemia.

How the overall procedure of cardioplegia provided myocardial protection appeared as a very complex issue and many sophisticated explanations were forwarded. In more pragmatic terms, however, the salient properties of what was to become *cold chemical cardioplegia* could be divided into *three additive components* (Hearse et al. 1981a):

- *Chemical arrest* or the sparing of cell energy through rapid induction of arrest in diastole.
- *Hypothermia* or slowing the rate of cellular reactions thereby delaying energy decay and other deleterious processes during ischemia.
- *Additional protection* related to protective agents that prevent or reverse unfavourable ischemia-induced cellular changes.

Energy-sparing obtained by chemically induced diastolic arrest and by simultaneously added hypothermia at 20°C reduces myocardial oxygen demands (MVO_2) by almost 95%

and is therefore an obvious mutual factor explaining the efficacy of cold cardioplegic techniques. That hypothermia not only retards energy consumption but also degenerative cellular reactions during ischemia underpins its central role as the second component of cardioplegia (Bretschneider 1964; Hearse et al. 1976a). The third component may be more difficult to define, but, as was subsequently shown, the incorporation into coronary infusates of magnesium (Hölscher 1967; Hearse et al. 1976a), a buffering agent such as histidine (Bretschneider 1980; Preusse et al. 1979), and of metabolic substrates and of oxygen (Bleese et al. 1976; Bretschneider 1980; Follette et al. 1978a; Buckberg 1979) combats ischemia and adds further to the efficacy of cardioplegic procedures.

The early history of cardioplegia can be divided into three distinctly different phases with shifting opinions and practice: the birth and burial 1955–1960; the survival years 1960–1970; and the rediscovery and international acceptance during the 1970s. Each of these periods were dominated by eminent surgeons and scientists making their individual imprints upon the ongoing developments.

2.1.2 Birth and Burial

At the birth of open heart surgery the search for agents to stop the heart to improve conditions for surgical repair focused on a wide variety of pharmaceuticals (Lam et al. 1955; Melrose 1980) such as acetylcholine, muscle relaxants, antihistamines, and local anesthetics. Stimulated by Ringer's work (Ringer 1883) on the opposing effects of calcium and potassium upon the heart beat, Melrose and associates (Melrose et al. 1955) investigated the cardioplegic properties of potassium. After gaining experience in dogs they applied aortic root injections of potassium citrate for arrest of the human heart. The potassium citrate, maintained highly concentrated in glass vials prior to use, was dissolved in 30 ml batches of blood giving an injectate concentration in excess of 200 mM. The injection provided immediate cardiac arrest and conditions for short-lasting anatomic repair. Accordingly, Melrose's report in 1955 led to international recognition and adoption of his technique. In these very pioneering days of cardiac surgery bypass time was kept to an absolute minimum, ventricular fibrillation was feared and safe coronary perfusion techniques were yet to come. In this situation potential hazards of the Melrose solution were not easily detected. However, in 1959 it was reported (Helmsworth et al. 1959) that myocardial necrosis could always be found in dog hearts after potassium citrate arrest, and in 1960 this finding was confirmed by evidence in patients (McFarland et al. 1960). These two papers then led to the abandonment of chemically induced cardioplegia as it was now known. In retrospect, it is easy to forget how close Melrose came, in animal experiments, to establish a more rational use of potassium, and also that he was an early predecessor of modern blood cardioplegia.

2.1.3 Survival and Success

During the 1960s German scientists started research on cardioplegia and laid the basis for its future principles and general management. Hölscher observed the arresting and protective properties of magnesium and the local anesthetic procaine (Hölscher 1960, 1967) and

proposed that the toxicity of the Melrose solution was due to citrate chelation of endogenous magnesium and calcium. Some years later, Kirsch (Kirsch et al. 1972) described the efficacy of injecting a highly concentrated solution of magnesium aspartate (161 mM) and procaine (11 mM) into the aortic root. The Kirsch *injection cardioplegia* combined with systemic hypothermia and topical cooling was introduced clinically in 1969 and gained popularity not least due to its simplicity.

2.1.3.1 Bretschneider and Söndergaard

The most influential German group of the 1960s, headed by Bretschneider, advocated hypothermia combined with very thorough myocardial equilibration by a single infusion of a cold (4°C) cardioplegic solution, ie *cold chemical cardioplegia* (Bretschneider 1964; Reidemeister et al. 1967; Nordbeck et al 1974; Bretschneider et al. 1975) , this to be assisted by systemic hypothermia (15–20°C). Important observations mainly in ex vivo and in vivo dog hearts were:

- Energy-consuming ventricular fibrillation in hearts arrested by deep hypothermia without cardioplegia
- The considerable time required for complete equilibration of temperature between the coronary infusate and the myocardium during cooling
- The relationship between different forms of chemical arrest and myocardial oxygen demand
- The role of tissue ATP as a predictor of myocardial recovery from ischemia.

During the 1960s the Bretschneider group developed a number of "intracellular" solutions employing sodium plus calcium depletion for inducing a nondepolarized cardiac arrest thus minimizing transmembrane gradients of these ions at end of infusion and preferably also during subsequent ischemia. The solutions, as represented by no 3 in Table 1, were sodium-poor (5–12 mM) and calcium-free, included procaine (7.4–11.0 mM or finally zero), and contained almost normal or moderately elevated potassium (5–10 mM) and magnesium (1–9 mM).

Söndergaard in Denmark introduced the Bretschneider solution no. 3 into clinical use in 1964 and may be regarded as the surgical pioneer of modern cardioplegia. In 1975 he reported (Söndergaard et al. 1975) on 100 aortic valve operations with aortic occlusions of 80–120 min and with a low 6% mortality. Indirectly Søndergaard was also one of the early innovators of blood cardioplegia since the Bretschneider procedure advocated at the time involved two steps:

- Firstly, the infusion of a cold mixture of blood, glucose, mannitol and procaine in order to keep the heart oxygenated and rapidly arrested during the initial cooling phase; and
- Secondly, the infusion with myocardial equilibration of the cardioplegic solution itself.

In retrospect, it is fair to state that the total work by Bretschneider and associates remained underrated for an unfortunately long time. Contributing to this were the international neglect of cardioplegia in the 1960s and the apparent complexity of proposed principles and techniques.

Table 1 The composition of the Bretschneider (BR) and the St. Thomas' Hospital (STH) cardioplegic solutions

Component (mM)	BR no 3	BR-HTK	STH-1	STH-2
NaCl	12	18	144	120
$NaHCO_3$				10
KCL	10	10	20	16
$MgCl_2$	2	4	16	16
$CaCl_2$		0.02	2.2	1.2
Procaine–HCl	7.4		1	
Mannitol	239	33		
Histidine		180		
Histidine–HCl		18		
Tryptophan		2		
α-ketoglutarate		1		
pH	5.5–7.0	7.1 (25°C)	5.5–7.0	7.8
Osmolality (mOsm/Kg H_2O)	290 (320)	280 (302)	300–320	285–300

2.1.3.2 International Reawakening

During the 1970s the international interest in chemical arrest of the heart reawakened, and by the end of the decade the procedure of cold chemical cardioplegia had gained close to universal acclaim as the most useful approach to an adequate surgical and metabolic handling of the heart. In USA Gay, Levitsky, Roe, Tyers and Buckberg reassessed the use of potassium below 40 mM (Table 2) for inducing cardiac arrest (Gay and Ebert 1973; Levitsky 1977; Roe et al 1977; Tyers et al. 1977).

Whereas Buckberg initially advocated crystalloid solutions (Nelson et al. 1976), he (Follette et al. 1978a; Buckberg 1979) soon changed to blood as the vehicle for potassium induced cardioplegia (Table 3) and advocated intermittent perfusion with cold cardioplegic blood plus systemic hypothermia during aortic occlusion. Buckberg also proposed that the myocardium should be kept in a diastolic arrested state with warm cardioplegic blood during the initial reperfusion phase (Follette et al 1978b) and thereafter recommended warm blood cardioplegia during the induction phase (Rosenkranz et al 1986). Also the Buckberg group assessed quantitatively noncoronary collateral flow in the washout of cardioplegic solutions (Brazier et al 1975) and confirmed the necessity of repeated administration or *multi-dose cardioplegia* as also advocated by Engelmann et al. (1978).

In Germany Bleese and Döring (Bleese et al. 1976; Bleese et al. 1978) developed and applied clinically intermittent or nearly continuous perfusion with a cold oxygenated crystalloid cardioplegic solution (Table 2), this as an improvement upon their previous practice with the Kirsch injection technique. Also the Bretschneider group reassessed their formulations (Bretschneider 1980; Gebhard et al. 1983; Preusse et al. 1979, 1980; Preusse 1993) and while still keeping to sodium and calcium withdrawal for chemical arrest, they

Table 2 The composition of four different crystalloid cardioplegic solutions

Components (mM)	Hamburg	Tyers	Gay-Ebert	Roe
NaCl	25	88	38	27
$NaHCO_3$	25		10	
Na-acetate		27		
Na-gluconate		23		
KCl	5	20	40	20
$KHCO_3$		10		
$MgCl_2$		1.5		
Mg-aspartate	2			1.5
$CaCl_2$	0.5	0.5		
Procaine–HCl	4			
Glucose	10		277	278
Mannitol	200			
Hydroxyethyl starch	6%			
Special additives	Oxygen Methylprednisolone Gentamycin	Heparin		Tris
Osmolality (mOsm/Kg)	320	275	365	347
Oncotic pressure (mm Hg)	35 mm Hg			
Oxygen tension	>600 mm Hg			

Observe that the Hamburg and the Tyers solutions are based on a largely extracellular ionic matrix containing calcium and sodium whereas the Gay-Ebert and Roe solutions are formulated intracellularly with zero calcium and low to intermediate levels of sodium. The oxygenated Hamburg solution is applied for intermittent or subcontinuous perfusion whereas the other solutions are administered by infusion. All solutions are applied under hypothermic conditions

Table 3 Blood cardioplegia solution (Follette et al. 1978a)

Component	Concentration
Blood from CPB circuit	1,000 ml
KCl	26 mM
CPD* from standard blood storage bag	20 ml
Tris (0.3 M)	20 ml
Final cardioplegic solution	
K^+	30 mM
Ionized Ca^{2+}	0.30 mM
Hematocrit	20%
Osmolarity	355 mOsm/KgH_2O
pH	7.7

CPD citrate-phosphate-dextrose

filled the available osmolal gap with the amino acid histidine. This was based on the group's finding of interstitial (reflecting intracellular) pH as an important marker for survival as

ATP. The histidine/histidine–Cl buffer pair (180–18 mM) possesses an ideal temperature profile (Rahn et al. 1975) for preservation of optimal pH during hypothermia, and histidine may scavenge reactive oxygen species (ROS) like singlet oxygen and hydroxyl radicals (Kukreja et al. 1991). In the new solution procaine was omitted in favour of a rise in magnesium. With minor changes this latter formulation has survived to the current date, now in the form (Table 1) of the Bretschneider histidine-tryptophane-α-ketoglutarate (HTK) solution (Custodiol™, Dr. F. Köhler Chemie, Alsbach-Hähnlein, Germany).

2.1.3.3 British Rediscoveries

During the 1970s cardioplegia regained the interest of British scientists. In 1972 Proctor, working on design and performance of oxygenators for CPB, reported on his experience (Proctor 1972) in long term donor heart preservation. By applying continuous coronary perfusion with an oxygenated hypothermic (4°C) extracellular-type crystalloid solution containing procaine and colloids, dog hearts were kept alive for up to 4 days. However, the main forward drive in cardioplegia research was the group headed by Hearse and Braimbridge at St. Thomas' Hospital in London. Thus by applying isolated hearts from small animals, mainly rats, for screening of mechanisms and agents the group (Hearse et al. 1974, 1975, 1976a, b, 1978a, b) characterized individually and together multiple factors involved in cardiac arrest and in myocardial protective interventions during ischemia and reperfusion. These studies were accompanied by in-depth analyses into the very complex pathophysiology of myocardial ischemia and reperfusion. Principal findings from small animal hearts were confirmed in dogs on CPB (Rosenfeldt et al. 1980; Jynge et al. 1981). In conclusion the group emphasized pragmatically the importance of a careful approach to the formulation of coronary infusates. Thus they should preferably deviate as little as possible from the extracellular fluid they were to replace and they should be based on a close dose-response characterization of main ionic constituents like potassium and magnesium and of potential additives (Hearse et al. 1981c; Chambers and Braimbridge 1993).

A St. Thomas' Hospital solution no 1 (STH-1) with moderately elevated potassium (20 mM) and magnesium (16 mM) and a small additive of procaine (1 mM) in an *extracellular* ionic matrix was introduced clinically by Braimbridge in 1975 and he reported (Braimbridge et al. 1977) encouraging initial experience in 1977. Thus, a comparison of patients undergoing valve replacements using STH-1 and hypothermia (1975–1976) with his previous (1972–1975) practice of coronary perfusion with blood, demonstrated a substantial benefit. The obvious advantages of working in a bloodless field were also acknowledged.

Further preclinical studies (Jynge et al. 1978, 1981; Jynge 1980) confirmed the importance of maintaining near to normal extracellular concentrations of calcium and sodium avoiding major fluctuations in these key ions during and following coronary infusion of cardioplegic solutions. On the basis of the accumulated experience from ex vivo rat and in vivo dog studies an isosmolal St. Thomas' Hospital solution no 2 (STH-2) was formulated with moderate elevations of potassium (16 mM) and magnesium (16 mM) together with near to normal sodium (120 mM) and calcium (1.2 mM) and a minor content of bicarbonate (10 mM) for initial pH control. This

purely ionic and crystalloid solution can, without constraints concerning its administration, be applied for single-dose or multi-dose cardioplegia depending on the duration of aortic occlusion and on the washout by noncoronary collateral blood flow. Commercially available STH-2 (Plegisol™, Hospira Inc., Lake Forest, Illinois, USA) and STH-1 made up by local hospital pharmacies are still in broad clinical use, mostly for its simplicity of application.

2.1.4 Status After 25 Years

At the end of the first 25 years with diverging opinions some main principles were acknowledged and already applied clinically with apparent success. The recommended cardioplegic solutions were largely crystalloid, whether based on an *intracellular* or "Bretschneider-like" ionic formulation or on an *extracellular* and "St. Thomas' Hospital-like" ionix matrix. Furthermore, *blood cardioplegia* with potassium-enriched and low-calcium containing blood was underway, especially in USA where Buckberg (Buckberg 1979) now predicted an end to the "cardioplegic controversy" concerning type of solution to use. The pro's and con's of hypothermia and the importance of avoiding a reperfusion injury were also recognized. The status at the time cannot be better presented than in the summing up by Kirklin (1979) at the first transatlantic workshop on cardioplegia held in New York in 1979: "The technique of cold cardioplegia has without question enormously improved surgical exposure and ease of operating during cardiac surgery. Properly done, it has severely reduced the incidence of myocardial necrosis, and thereby improved postoperative cardiac performance. It has thus made care of the patient in the early postoperative period much simpler than it was in earlier times. Most patients now convalesce easily, do not need special interventions, and are dismissed from hospital on the fifth or sixth day following the operation. We do not have proof that late myocardial failure will be reduced through the use of this technique, but it seems likely that this will be the case".

Kirklin further stated from a multifactorial analysis of risk factors and his team's clinical success that only one negative predictive factor was not improved by cold cardioplegia, namely preoperative myocardial failure with patients being NYHA functional classes III or IV. This statement from 1979 is appropriate to cite since we still, despite major later developments, are dealing with the problem of providing adequate protection to the failing heart, particularly in the elderly.

2.2 Concepts and Issues

The above, apparently successful, story from the first period of cardioplegia would not have been possible without major developments made in close collaboration between surgeons and basic scientists. Below five main concepts and issues have been selected for brief presentation as important lessons that had to be understood at the time.

2.2.1 Energy, Metabolism and Recovery from Ischemia

A most obvious key to myocardial protection was found in cell energetics and in the content and function of ATP. However, with mitochondrial respiration already discovered and the ATP producing proton circuit recently characterized (Mitchell 1966), still the diverse roles of ATP as the life-giving molecule and mechanisms of its transport and its translation to work were only partly known at the start of the 1970s. In cardioplegia and ischemia research two energy related concepts became subject to considerable debate: time and temperature limits for expected survival from surgical ischemia; and mechanisms and causes of ischemia-induced contracture.

2.2.1.1 Rescucitation Time

Bretschneider's main approach was to accept an uninterrupted long period of total ischemia which should be well tolerated by applying cold chemical cardioplegia (Bretschneider 1964; Reidemeister et al. 1967; Preusse 1993). He established an early focus of attention on conservation of cell energy and proposed that chemical arrest spared cell ATP stores, that hypothermia delayed ATP depletion and that an appropriate buffering system might provide anaerobic production of a minor, but important, amount of ATP. He also formulated the concept of *practical resuscitation time* or *t-ATP* as the time required for myocardial ATP to fall from about 6 mmol/g wet weight to 4 mmol/g wet weight. This level was shown to be critical for functional recovery from ischemia and rapid weaning from CPB. As shown from isolated dog hearts during total global ischemia at 25°C (Fig. 1), t-ATP increased from 60 min in control hearts to 200 min with the Bretschneider solution no 3 and further to 280 min with a high concentration of the extracellular histidine buffer in the HTK solution (Bretschneider et al. 1975; Bretschneider 1980).

In other experiments the Bretschneider group showed that the slower decay of tissue ATP by HTK was closely paralleled by a gradual consumption of cellular glycogen stores and a rise in tissue lactate (Nordbeck et al. 1974; Preusse et al. 1979; Gebhard et al. 1983). In later studies interstitial pH measurements showed that conservation of interstitial pH (critical limit 6.0), or more precisely avoidance of severe acidosis was instrumental for subsequent recovery of function (Preusse 1993).

Experiments by the Hearse group were in accordance with Bretschneider's findings on relationships between ATP conservation and cardiac function (Hearse et al. 1974). Thus, recovery of cardiac output in isolated working rat hearts depended greatly on the content of ATP at the end of ischemia: 12 μmol/g dry weight (50% of normal) formed a lower level for expectancy of any recovery; and above this level an almost linear correlation was found between ATP and cardiac output. Altogether, these studies by leading scientists documented the very crucial role of cellular ATP levels in the coupling between metabolism and function. In subsequent studies on how to provide anaerobic energy production during ischemia, the Hearse group showed that stimulation of glycolysis in zero flow conditions

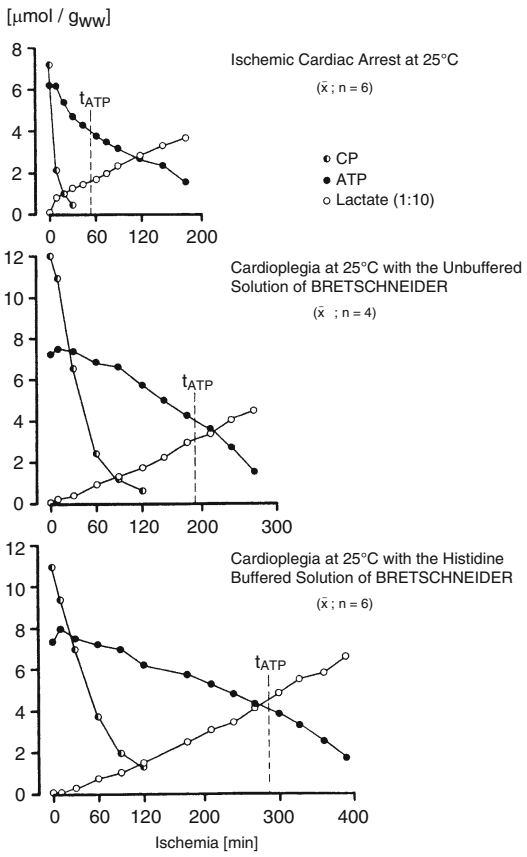

Fig. 1 *Myocardial content of ATP, creatine phosphate (CP) and lactate during ischemia.* Control hearts and hearts receiving initial perfusion with nonbuffered Bretschneider solution no 3 and buffered Bretschneider HTK solution. Results were obtained from isolated dog hearts kept ischemic at a myocardial temperature of 25°C. (Reproduced from Preusse 1993 by permission of Kluwer Academic Publishers)

was deleterious and hence glucose (Hearse et al. 1978b) or lactate (Hearse et al. 1978b) were not recommended as additives to the STH solutions.

2.2.1.2 The Stone Heart Challenge

In 1967 and 1969 the first reports appeared (Taber et al. 1967; Najafi et al. 1969) which documented transmural or subendocardial (Cooley et al. 1972) myocardial necrosis and low cardiac output after valve replacements. In 1972 Cooley, the proponent of normothermic ischemic arrest (Cooley et al. 1962), described the "*stone heart*" (Cooley et al. 1972); this was an irreversible contracture that occurred occasionally during and following nonprotected aortic occlusions, particularly in hypertrophied hearts. At the time this was taken up as a challenge for biochemists to become involved in surgical research (Katz and Tada 1972). Evidence that ATP depletion was the etiological factor behind stone heart formation

was first provided by Hearse and associates in 1977 (Hearse et al 1977). Using isolated rat hearts with left ventricular pressure recordings and tissue ATP measurements at brief intervals, they showed that the onset of contracture (rise in resting tension) during ischemia started when ATP was reduced by approximately 50%, and that ATP-hydrolysis and contracture accelerated thereafter. The time to onset and also the extent of contracture could be delayed or diminished by interventions that reduced myocardial energy demands including potassium arrest, magnesium elevation, calcium antagonists and hypothermia. Conversely, metabolic poisoning had the opposite effect. From further experiments it was concluded that ischemic contracture was the result of ATP depletion forming rigor complexes between actin and myosin, that the onset of contracture was a purely ATP related event, and, that only the final extent of contracture was influenced by calcium-sensitive mechanisms. The stone heart paper contributed to our knowledge of the pathophysiology of ischemia and was of importance in turning surgeons towards new techniques for myocardial protection.

2.2.2 Hypothermia: The Second Component of Protection

2.2.2.1 Hypothermia on Its Own

In spite of more recent sceptical attitudes (Salerno 2007), hypothermia has followed cardiac surgery from the earliest until the most recent days. The first intracardiac operations with inflow occlusion and total circulatory arrest were undertaken with moderate hypothermia induced by body surface cooling (Bigelow et al. 1950). During this phase it was shown (Swan 1973) that systemic hypothermia slowed both heart rate and myocardial oxygen demands. Thus at 26°C oxygen consumption was reduced by 75% but at lower temperatures the rate of decline was less. Also below this level ventricular fibrillation was frequently observed. It was concluded that moderate hypothermia with a systemic temperature between 32°C and 29°C would reduce myocardial energy requirements substantially without imposing any hazard to cardiac function. Somewhat later, an "ice age" started with systemic hypothermia supplemented with topical cooling and use of saline slush (Shumway et al. 1959). Also intermittent ischemic arrest combined with systemic and topical hypothermia became a common technique.

In experimental studies of hypothermia as a separate modality for arrest and protection two particularly important observations were made. The first was that whereas heart rate fell almost linearly with temperature (Archie and Kirklin 1973), the force of each contraction increased (Coraboeuf and Weidmann 1954). The second was that myocardial oxygen consumption, although being gradually lowered together with temperature, actually increased when calculated for each heart beat (Archie and Kirklin 1973). On further cooling ventricular fibrillation also raised myocardial oxygen requirements. Accordingly, hypothermia, however effective in slowing basal metabolism, which accounts for 15–20% of normal myocardial oxygen demand (Braunwald 1969; Challoner 1968), was less efficient in reducing electromechanical activity accounting for the remaining 80–85%.

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2.2.2.2 Hypothermia Plus Cardioplegia

The above problem was solved with the arrival of chemical arrest in diastole. Thus chemical cardioplegia reduces oxygen consumption from 6 to 8 ml/100g/min in the empty beating heart to 0.5–1.5 ml/100g/min at normothermia (Buckberg et al. 1977) and, when applied at 20°C or 15°C, a further reduction to 0.30 ml/100 g/min and 0.15 ml/100 g/min respectively can be expected (Preusse 1993). In line with this, hypothermia fulfils the role as the second component of myocardial protection by complementing the first, i.e., chemical arrest.

The efficacy of hypothermia is apparent from Fig. 2 showing the results from isolated working rat hearts protected by preischemic infusion of a cardioplegic solution (Hearse 1982). When temperature of cardioplegic infusion (2 min) and ischemia (60 min) was kept at 37°C, the hearts were unable to recover any cardiac output during normothermic reperfusion (15 min). In contrast, at or below 20°C the recovery was 90% or higher. An important observation at the time was that the dose-response curve of hypothermia was nonlinear with a marked inflection between 32°C and 24°C. The inflection was explained by lipid phase transitions in cell membranes (Inesi et al. 1973; Gordon et al. 1978) conferring an extra slowing of membrane embedded transporters and enzymes. An interpretation of the above rat heart study would be that cardioplegia with moderate hypothermia is safe for shortlasting aortic occlusions with temperature in the range of 24–28°C, but safer still when kept closer to 20°C.

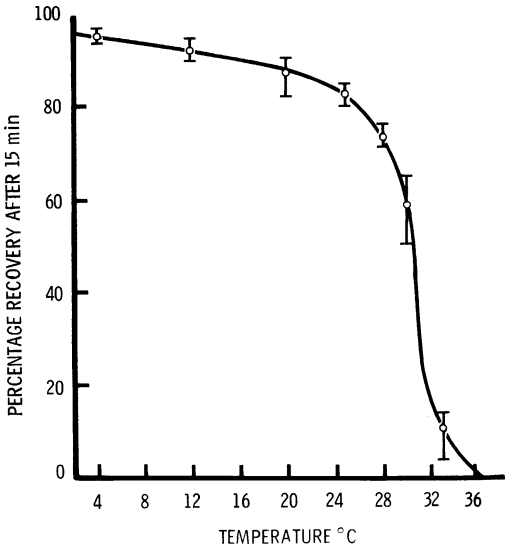

Fig. 2 *The second component of protection: hypothermia*. Isolated working rat hearts were subjected to 60 min of ischemia after receiving preischemic infusion of St. Thomas' Hospital solution no 1 enriched with ATP and creatine phosphate. Temperature of infusion and ischemia as indicated. Hearts were reperfused for 15 min with recording of aortic flow rate. Observe the sharp inflection between 33 and 24°C, and that below 20°C a full recovery is obtained. (Reproduced from Hearse 1982 by permission of Editrice CLUEB, Bologna)

2.2.2.3 Hypothermic Injury

Hypothermia induces a complex mixture of both beneficial and detrimental effects in homeothermic species (Hearse et al. 1981b) and optimal temperature levels for the entire body on CPB and for the arrested heart are still questioned. A particular issue that was heavily debated in the 1970s was the potential induction of a more advanced hypothermic injury with a major loss of protection. Careful reanalysis of a large number of studies (Hearse et al. 1981b) showed that hypothermic injury with loss of protective properties from a progressive decrease in myocardial temperature resulted when three main factors coincided: rapid and extensive cooling by coronary infusion or perfusion; use of noncardioplegic solutions; and presence of myocardial ischemia. The immediate consequence of the two first factors was cold shock induced release of calcium from sarcoplasmic reticulum (SR) (Endo 1977) accompanied by a slow reuptake into the SR (Katz and Repke 1967). Elevated intracellular calcium then activates energy-wasting and unfavourable cellular reactions. While these latter changes might be overcome by maintaining perfusion and energy production, the simultaneous presence of ischemia with further inhibition of metabolism acts to exacerbate cold-induced changes. Apparently, canine hearts were able to survive deep hypothermic perfusion for days (Proctor 1972) when the perfusion medium was formulated with an immediately arresting agent.

In essence, it seems that the immediate induction phase of cardioplegia is most important and adds a partial confirmation to the practice subsequently proposed by the Buckberg group (Buckberg 1979) with warm induction prior to cooling when using blood cardioplegia.

2.2.3 Calcium Control

2.2.3.1 Calcium Takes Centre Stage

In parallel with the history of cardioplegia major discoveries on cell calcium regulation saw the light of day. Important observations were made on the sarcolemmal sodium–calcium exchange (Langer 1977), on the SR for intracellular calcium storage-release (Ebashi and Lippman 1962; Katz and Repke 1967; Endo 1977), and on a new class of negative inotropic drugs and vasodilators with properties as antagonists of sarcolemmal slow calcium channels (Fleckenstein 1971). In addition, models of acute myocardial infarction in dog hearts demonstrated how cell calcium control might be at stake during ischemia and more so during reperfusion (Jennings et al. 1960; Jennings and Reimer 1979). Altogether, the growing acceptance of cell calcium as crucial in myocardial injury and protection had great impact upon the development of cardioplegia as an applied science. The accumulated knowledge at the end of the 1970s thus revealed multiple roles of calcium when formulating cardioplegic solutions and when applying them in combination with hypothermia for myocardial protection during aortic occlusion.

2.2.3.2 Cardioplegia and the Calcium Paradox

A controversial issue was the use of calcium-free coronary infusates and the possibility that an extensive calcium washout might induce a *calcium paradox*. Thus when calcium is reintroduced to a myocardium with a sarcolemma depleted of a critical high affinity fraction of calcium maintaining its integrity (Frank et al. 1977), cell calcium overload with contracture and membrane injury is immediate and massive (Zimmerman et al. 1967).

Some studies indicated that the calcium-free Bretschneider solutions might induce a calcium paradox (Jynge et al. 1977; Jynge et al. 1978; Jynge 1982; Ruigrok et al 1983) as indicated in Fig. 3. However, these solutions were apparently safe since they were sodium-poor and applied under deep hypothermia (Jynge 1980; Gebhard et al. 1983; Jynge 1983) which seemed to stabilize the sarcolemma against removal of a critical fraction of calcium. Furthermore, in the clinical situation with aortic occlusion noncoronary collateral flow is likely to ensure the presence of at least some calcium in the sarcolemma. When studying different formulations and situations in isolated rat hearts (Jynge 1983), trace calcium (25 μM), magnesium elevation, low sodium and simultaneous hypothermia during calcium-free perfusion prevented or delayed the paradox. Conversely, normal sodium, elevated potassium, calcium-binding anions like phosphate and citrate, ischemia and a warm heart were provocative factors.

In retrospect, the myocardial injuries inflicted by the Melrose solution (Melrose 1980) might be interpreted as the consequences of citrate induction of a calcium paradox. It is of interest to note that the most recent version of the Bretschneider HTK solutions (Custodiol) is formulated with a minor calcium additive (20 μM).

2.2.3.3 Ionic Interactions and Calcium Control

As already indicated, the relationship between calcium and sodium was shown to be essential for optimal formulation of cardioplegic solutions. Thus in the bidirectional sodium–calcium exchange of the sarcolemma three sodium ions are exchanged with one calcium ion for calcium influx (during depolarization) and efflux (during repolarization). A practical consequence is that lowering of extracellular sodium during coronary infusion of a sodium-poor cardioplegic solution has to be matched by a far more extensive reduction in extracellular calcium in order to induce diastolic arrest. Thus, the window for incorporating calcium in the Bretschneider solution No. 3 was marginal in normothermic rat hearts (Fig. 3) (Jynge 1982) and below 50 μM in hypothermic dog hearts (Gebhard et al. 1983). In addition it was found that myocardial equilibration with this sodium-poor and calcium-free solution might go through intermediate stages due to sodium–calcium interactions prior to achieving the lowest oxygen requirement.

According to recommendations the Bretschneider HTK solution has to be infused in a single-dose manner over 6–8 min requiring a volume of 2–3 L in adult patients. Most intriguingly, experiments in hypothermic ischemic dog hearts (Warnecke et al. 1981) documented that electromechanical reactivation required less washout of cardioplegic

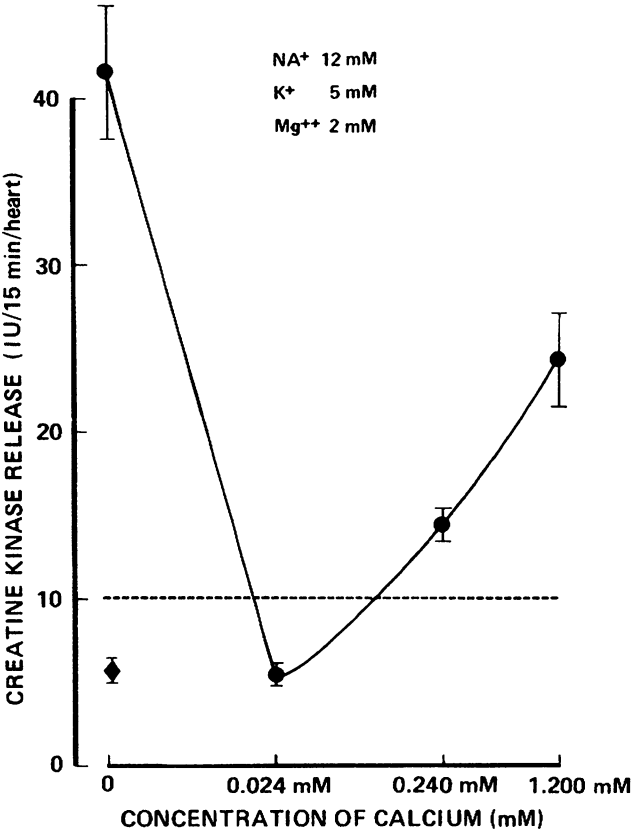

Fig. 3 *Sodium-poor coronary infusates and optimal calcium*. The accumulated creatine kinase (CK) release during reperfusion (15 min) after ischemia (30 min) in isolated normothermic rat hearts is shown. Hearts (●) received preischemic infusion for 2 min (8 ml) of Bretschneider solution no 3 devoid of or supplemented with calcium. Hearts (♦) receiving preischemic infusion over 2 min with less voume (4 ml) of Bretschneider solution devoid of calcium. The dotted line shows the level of CK release on reperfusion of control hearts. (Reproduced from Jynge 1982 by permission of Editrice CLUEB, Bologna)

solution after arrest with the HTK solution than with the STH-2 solution. As a consequence of gradual noncoronary collateral inflow of blood, myocardial protection with the otherwise superior BR-HTK was reduced but slightly improved with STH-2. It is difficult to explain these differences except by differences in reequlibration patterns with the extracellular ionic environment of inflowing blood. The STH-2 solutions containing near to normal sodium showed a broad calcium window in ischemic rat hearts (Yamamoto et al. 1984) with concentrations optimal for postischemic recovery in the range 0.6–1.2 mM. Furthermore, the tolerance to preischemic sodium depletion in the presence of normal calcium was also considerable (Jynge 1982) as documented by postischemic CK release in rat hearts (Fig. 4). Intriguingly, elevated potassium (16 mM) was more sensitive to sodium reduction than

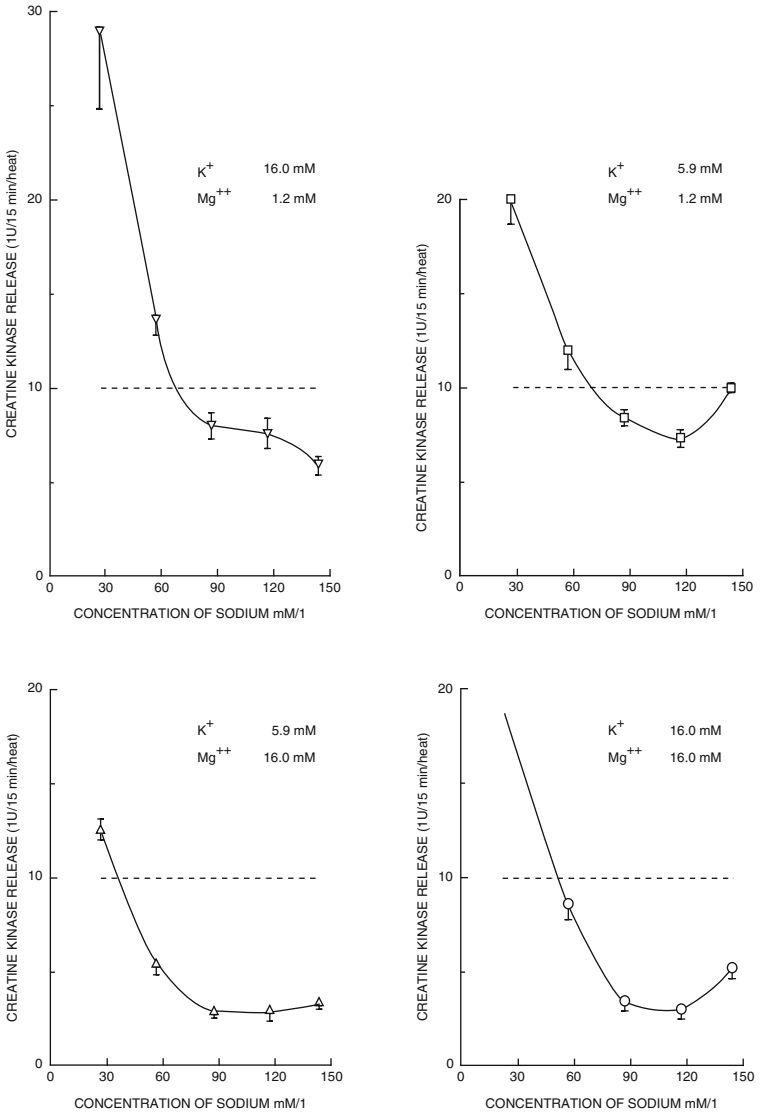

Fig. 4 *Calcium-containing coronary infusates and optimal sodium*. The accumulated creatine kinase (CK) release during reperfusion (15 min) after ischemia (30 min) in isolated normothermic rat hearts is shown. Hearts received preischemic infusion (2 min and 8 ml) of solutions with a fixed concentration of calcium (1.2 mM) but different concentrations of sodium (30–150 mM) . Sodium chloride was substituted with mannitol. Substitution with choline chloride revealed similar results. The *dotted line* shows the level of CK release on reperfusion of control hearts. (Reproduced from Jynge 1982 by permission of Editrice CLUEB, Bologna)

elevated magnesium (16 mM) whereas the combined elevations revealed an intermediate sodium sensitivity with an optimal level of 90–120 mM. These results contributed to the formulation of the STH-2.

Altogether, it appeared that the main advantages with an extracellular type ionic formulation as applied in the crystalloid STH-2 solution, as well as in composite blood cardioplegia solutions, were less complex equilibration with the extracellular ionic environment and less rigorous infusion requirements except for multi-dose administration to counteract washout by noncoronary inflow of blood. Conversely, the major and outweighing advantage with intracellular solutions as examplified by Bretschneider HTK was the large osmolal space available for inclusion of a high concentration of the beneficial histidine buffer.

2.2.4 Reperfusion

In 1960 Danforth and Bing reported detailed metabolic studies (Danforth et al 1960) on glycolytic fluxes during global myocardial ischemia and reperfusion while the Jennings' group (Jennings et al. 1960) simultaneously described functional and structural consequences associated with reperfusion in regional ischemia. In an anticipated reversal to normal conditions after aortic cross-clamping three phases of reperfusion were then identified (Hearse 1982). During the initial seconds to minutes of reperfusion the return of oxygen causes an immediate resumption of oxidative metabolism and reactivation of electromechanical function. The second phase, occurring over the next few minutes, leads to the reversal of ischemia-induced cell swelling and restoration of normal patterns of ion regulation and of metabolites and cofactors. The third phase requiring hours to days involves the repair of damaged organelles like mitochondria and cell membranes and the restoration of normal metabolic pathways including de novo synthesis of adenine nucleotides.

In surgical practice it was soon discovered that securing the myocardial transition from an almost hibernating to a fully working state is a complex process. Thus reperfusion pressure had preferably to be lowered to prevent edema formation (Engelman et al. 1978; Lindal et al. 1995), ventricular fibrillation had to be rapidly converted (Buckberg 1977) and residual air within heart chambers and coronary arteries had to be taken care of (Goldfarb and Bahnson 1963). A further obstacle to circumvent was the "no reflow" phenomenon (Leaf 1973; Brachfeld 1974; Fabiani 1976) induced by swelling of endothelial cells with venular trapping of neutrophils attracted by inflammation and compounded by the compressive forces of contracture and cellular plus interstitial edema. This fitted well with both earlier (Jennings et al. 1960) and later (Jennings and Reimer 1979) observations from the Jennings' group of specific reperfusion injuries that were initiated on return of blood and oxygen to a previously ischemic myocardium. It was also shown that reperfusion injury might express itself in reperfusion arrhythmias, in prolonged myocardial recovery with metabolic and contractile dysfunction (later to become known as "stunning"), and in an accelerated necrosis in tissue already doomed by ischemia. However, the fourth entity of a lethal reperfusion injury potentially amenable to therapeutic interventions (Hausenloy and Yellon 2008) was not yet recognized, as were neither preconditioning (Murry et al. 1986) nor postconditioning (Zhao et al. 2003) both potent endogenous syndromes in prevention or amelioration of reperfusion injuries.

When shedding light upon basic mechanisms the Hearse group compared three "re-admission" syndromes of relevance for events taking place on a cellular level during postischemic reperfusion (Hearse et al. 1979). In studies in isolated rat hearts myocardial creatine kinase (CK) release to the asanguineous perfusion medium was rapid with a maximum within 2 min of readmission. Three levels of peak CK release were observed: far the highest on readmission of calcium after calcium-free perfusion (>400 IU/min/g dry wt.); intermediate on reoxygenation after hypoxia (>50 IU/min/g dry wt.); and the lowest (~10 IU/min/g dry wt.) during reperfusion with oxygenated and calcium-containing medium after severe ischemia. In the readmission syndromes both calcium and reactive oxygen species (ROS) were identified as main culprits inducing immediate contracture and severe mechanical stress to injured cell and mitochondrial membranes with cell death and a graded CK release as the end result. In retrospect, it is of interest to note that the two factors, initial calcium overload and initial ROS release, together activate mitochondrial permeability transition pores and lethal reperfusion injury which may be inhibited or ameliorated by postconditioning procedures and drugs (Hausenloy and Yellon 2008).

Attempts to utilise antioxidants, whether pure scavengers or catalytic agents, were in their infancy at the start of the 1980s, but already some interest was on using key antioxidant enzymes (Fridovich 1974) like superoxide dismutase (SOD) and catalase (CAT). Thus, in 1982 Shlafer found that supplementation of SOD plus CAT to a cardioplegic solution gave superior protection during hypothermic ischemia than afforded by the solution itself and concluded that cytotoxic ROS contributed to a separate reperfusion injury (Shlafer et al. 1982). These findings were later both confirmed (Gardner et al. 1983; Ambrosio et al. 1987; Ytrehus et al. 1987) and negated (Gallagher et al. 1986; Uraizee et al. 1987) by other investigators. Apparently, shortcomings of native SOD or CAT enzymes, not least by being macromolecules, can be overcome by applying synthetic small molecular mimetics that are more likely to enter target cells and act intracellularly. Accordingly, more recent studies have shown that salen mangenese complexes (Tanguy et al. 1996) and manganese-dipyridoxyl diethethylene diamide (Karlsson et al. 2001) were effective in reducing reperfusion arrhythmias and myocardial infarct size.

2.2.5 Blood as Cardioplegic Vehicle

At the end of the 1970s influential new ideas came from protagonists of blood cardioplegia (Buckberg 1979; Barner et al. 1979). Thus Buckberg (as the foremost protagonist) claimed to have found a solution to discrepancies between different crystalloid "schools" of thought and practice. Based on the early experience of Melrose (Melrose et al. 1955) he proposed the use of blood as vehicle for an extracellular type, elevated-potassium and reduced-calcium based cardioplegia (Table 3). A number of apparent advantages were proposed, such as regular intermittent administrations to maintain aerobic metabolism, buffering by blood at an optimal pH, potential scavenging of ROS by blood constituents, and oncotic prevention of cell swelling and improvement in capillary distribution.

In a pioneering study published in 1978 Follette et al. (1978b) compared the efficacy of three protective procedures in dogs on CPB: continuous normothermic perfusion in the

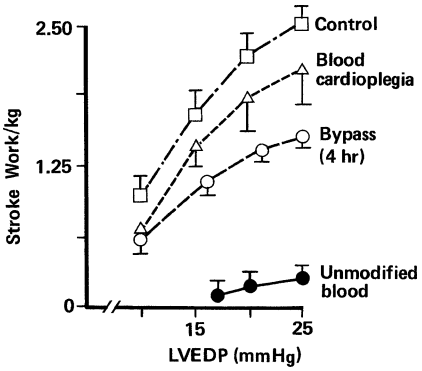

Fig. 5 *Blood cardioplegia and myocardial protection in dogs*. Stroke work during progressive volume loading after continuous normothermic perfusion (bypass) and hypothermic ischemia with unmodified blood or blood cardioplegia. For detailse, see text. (Reproduced from Follette et al. 1978a by permission of the American Heart Association, New York)

empty beating heart (continuous bypass); hypothermic ischemia with multidose infusion of cold (22°C) blood (unmodified blood); and hypothermic ischemia with multidose administration of cold (22°C) cardioplegic blood (blood cardioplegia). Infusions were given at 20 min intervals during 120 min of ischemia and before aortic declamping. As shown in Fig. 5, the recovery of stroke work, after 30 min of reperfusion, following cardioplegia was improved compared to continuous bypass and not significantly reduced below control values. However, the unmodified blood group of hearts hardly recovered any function at all. Myocardial ATP and creatine phosphate were well maintained with continuous bypass and intermittent ischemia with multi-dose blood cardioplegia but fell by 45% with unmodified blood. Clearly this study demonstrated the efficacy of blood cardioplegia, but for eventual comparison it lacked a control group with crystalloid cardioplegia. In retrospect, however, it is to be noted that in high risk patients undergoing CABG (Ibrahim et al. 1999) significantly enhanced myocardial protection with positive effects upon arrhythmias, recovery of function and myocardial high energy phosphates was provided with blood-based compared to crystalloid-based St. Thomas' Hospital solution (STH-1).

A particular question at the time was the efficacy of cold blood as a vehicle for oxygen delivery. Thus impairment of capillary flow by sludging of erythrocytes was partly feared. Another concern was that blood oxygen delivery might prove ineffective due to enhancement of oxygen–haemoglobin affinity by hypothermia and a high pH. As seen in Fig. 6 from Follette's initial study (Follette et al. 1978a) myocardial oxygen uptake in dog hearts receiving intermittent infusions with unmodified cold blood was 1.8 ml O_2/min/100 g while hearts arrested by blood cardioplegia consumed only 0.75 mlO_2/min/100 g. This approximate 2.5 fold higher value in hearts arrested solely by cold most probably resulted from a higher wall tension and ventricular fibrillation but might also be attributed to a lower pH of the infusate releasing more oxygen from haemoglobin. While these results undoubtedly showed that oxygen was released from cold blood, some might also have come from that physiologically dissolved in plasma. By undertaking oxygen debt calculations of the data presented it seemed likely that oxygen delivery might have had two almost equally important sources. In the search for other salient effects, it was later shown that the high buffering capacity with the histidine-imidazole-bicarbonate system of erythrocytes and the

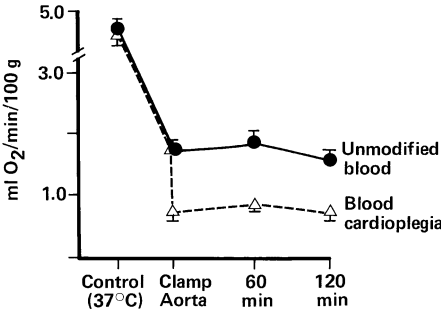

Fig. 6 *Blood cardioplegia and myocardial oxygen consumption in dogs*. Observe the higher oxygen uptake in hearts receiving unmodified cold blood during prolonged aortic clamping than in hearts receiving cold cardioplegic blood. For details-see text. (Reproduced from Follette et al. 1978a by permission of the American Heart Association, New York)

high content of scavenger and catalytic antioxidants (Illes et al. 1989a, b) were important contributing factors behind the efficacy of blood cardioplegia.

A convincing aspect of blood cardioplegia procedures has been optimal reanimation of hearts following aortic occlusion thus bridging the gap between ischemic and normal metabolism. Evidence was soon provided (Follette et al. 1978b; Kirklin 1990) that postischemic recovery of function could be improved considerably through keeping the heart arrested by perfusion with warm cardioplegic blood for the first few minutes after ischemia but before aortic declamping. This diverts energy production away from early reactivation of ionic pumps and towards early cellular repair. In parallel, unfavourable resumption of contractile function with mechanical stress upon weakened cell membranes is avoided in this critical phase. A further benefit of warm cardioplegic blood reperfusion beside that of electromechanical unloading, acid buffering and antioxidant effects, was the observation that addition (Lazar et al. 1980a) of amino acid substrates like glutamate and aspartate enhanced the recovery of metabolism.

The overall progress in reperfusion strategy (Buckberg 1995) was further extended by applying an extra period of warm blood cardioplegic perfusion (secondary cardioplegia) in failing hearts (Lazar et al. 1980b), to improve metabolism and function prior to weaning from CPB. The experience from reperfusion with warm cardioplegic blood was in 1983 (Rosenkranz et al. 1986) logically transferred to the introduction side of cardiac operations. The new concept of warm cardioplegic induction was based on the realization that in ischemic and energy-depleted hearts the induction of cardioplegia in reality represents an essentially first phase of reperfusion, and that a potential activation of cellular defence might improve myocardial tolerance to the forthcoming ischemia. Another positive effect of warm induction beside optimal oxygen delivery and energy metabolism is the effective arrest in diastole before start of cooling.

2.3 Summary and Closing Remarks

After an exciting period of experimentation and early clinical application cardioplegia has in the last 20–25 years undergone further developments, first of all based on thorough

analyses of its practical adaptation both in general and as applied in different intraoperative situations. Especially, the necessity of providing homogenous distribution of both arrest and cooling has led to the rediscovery (Lillehei et al. 1956; Menasché et al. 1982) of retrograde coronary sinus perfusion, and combined antegrade and retrograde delivery has become a familiar technique for administration of both blood- and crystalloid-based solutions. More recently, the advent of warm heart surgery (Barner 1995; Salerno 2007) has reopened an old debate of optimal temperature of the myocardium as well as other tissues during CPB.

Blood cardioplegia with major advances in surgical application now seems to be the choice of a majority of surgical centres but crystalloid cardioplegia, as it was conceived 30 years ago, is still in broad clinical use. Whereas meta-analyses based on minor clinical studies indicate that blood cardioplegia is superior (Guru et al 2006; Jacob et al. 2008), this has been more difficult to prove in larger groups of patients (Ovrum et al. 2004). In closing this review on selected issues and concepts from the first period of cardioplegia research, we are still left with pro's and con's concerning techniques to be used, and it is apparent that an in-depth knowledge of both surgical procedures and the pathophysiology of myocardial ischemia is imperative. Of particular interest for the early future will be to see how surgical protection can be combined with pre- and postconditioning with regard to reperfusion and long term recovery. By invoking these endogenous and general responses cardioplegia can no longer be seen as a separate entity but as an integral part of perioperative myocardial protection.

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