St. Thomas' hospital cardioplegia versus intermittent aortic cross-clamping in aorto-coronary bypass surgery

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ST. THOMAS' HOSPITAL CARDIOPLEGIA VERSUS INTERMITTENT
AORTIC CROSS-CLAMPING IN AORTO-CORONARY BYPASS SURGERY

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PROEFSCHRIFT

ter verkrijging van de graad van doctor in de
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Prof. Dr. F.I.M. Bonke,
volgens het besluit van het College van Dekanen,
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aan mijn ouders,

voor Astrid, Nina en Lara.

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SYMBOLS AND ABBREVIATIONS

AC	Aortic cross-clamping
A-CS	Arterial-coronary sinus (difference)
ACT	Actual clotting time
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
cCa^{2+}	Ionized calcium
CI	Cardiac index
CK-MB	Creatine kinase isoenzyme MB
CPB	Cardiopulmonary bypass
dPlv/dt max,pos	Positive, maximum first derivative of left ventricular pressure
d.w.	dry weight
ECG	Electrocardiogram
e_c	Natural tangential strain
e_z	Natural axial strain
e_{zc}	Shear angle
$e_{endo,est}$	Estimated subendocardial fiber shortening
FCR	Fractional catabolic rate constant
Hb	Hemoglobin
HBDH	α -hydroxybutyrate dehydrogenase
Ht	Hematocrit
i.m.	intramuscular
i.v.	intravenous
k_d	Apparent enzyme disappearance constant
LAICA	Left anterior interventricular coronary artery
LCCA	Left circumflex coronary artery
LVSWI	Left ventricular stroke work index
MDH	Malate dehydrogenase
MFGC	Magnetic field generating coil
min	minutes
n	number of observations
Pao,sys	Systolic aortic blood pressure
Pao,dia	Diastolic aortic blood pressure

Pao,mea	Mean aortic blood pressure
Plv	Systolic left ventricular blood pressure
Plv,ed	end-diastolic left ventricular blood pressure
PCO ₂	Partial carbon dioxide tension
PLV	Plasma volume
PO ₂	Partial oxygen tension
PWP	Pulmonary artery wedge pressure
s	seconds
SC	Sensor coil
SD	Standard deviation
SEM	Standard error of the mean
TER	Transcapillary escape rate constant.

Patient groups

N	Normothermia (34°C) and intermittent aortic cross-clamping
H	Hypothermia (25°C) and intermittent aortic cross-clamping
C	Cardioplegia using continuous aortic cross-clamping in combination with ice-cold St Thomas Hospital cardioplegia

Animal groups

N	Normothermia (34°C) and intermittent aortic cross-clamping (4 times 10 minutes)
H	Hypothermia (25°C) and intermittent aortic cross-clamping (4 times 10 minutes)
C	Hypothermic potassium cardioplegia (St Thomas Hospital) with continuous aortic cross-clamping (60 min)
NB	Normothermia with intermittent aortic cross-clamping and multiple left ventricular biopsies
HB	Hypothermia with intermittent aortic cross-clamping and multiple left ventricular biopsies
NC	Normothermia without intermittent aortic cross-clamping
HC	Hypothermia without intermittent aortic cross-clamping
CON	Control group with complete instrumentation, but without cardiopulmonary bypass
EC	Enzyme release during control conditions without cardiac manipulation
HPLV	Hypothermia with intermittent aortic cross-clamping and multiple plasma volume determinations

1. INTRODUCTION

1.1 Open-heart surgery

Congenital and acquired heart diseases have been known for a long time, but it was not until about 30 years ago that the combined efforts of clinicians, surgeons, biochemists and engineers enabled the performance of a successful open-heart operation. Intracardiac operations are complex because of the requirement of circulatory arrest during the performance of the correction. Initially short periods of systemic circulatory arrest have been used for minor interventions, but in this procedure the time available for surgery is 8-10 min and therefore of limited use. Adequate correction of most cardiac diseases requires a quiet and bloodless heart for surgical reasons. Numerous efforts and subsequent discoveries in the past, as summarized in table 1.1, finally resulted in the knowledge and experience necessary to sustain the patients systemic blood circulation during the course of the operation. This was achieved with cardiopulmonary bypass (CPB), using an oxygenator and an artificial pump (fig 1.1). After connection of the extracorporeal circuit to the patient, a flow of 3 to 5 liters per min can be maintained, which is adequate to support the peripheral circulation.

The highly important oxygenator was designed to exchange oxygen and carbon dioxide in the venous blood of the extracorporeal circuit. From the many types of oxygenators constructed, nowadays the bubble oxygenator, supplied with a heat exchanger, is commonly used in open-heart surgery. Membrane oxygenators are used in pediatric patients and during long lasting operations.

Once the heart-lung machine could replace heart and lung function, it became possible to arrest the heart and to stop the blood circulation within the heart after cross-clamping of the aorta. Meanwhile peripheral circulation was artifactually maintained. Cross-clamping of the aorta, however, induces periods of global ischemia, limiting the time available for cardiac repair. Extension of the ischemic period was found to be allowed when cardioprotective measures were taken.

Starting with the repair of congenital heart and valvular diseases in the early years of cardiac surgery, we are now facing the widespread incidence of

Table 1.1 Historical events in cardiopulmonary bypass (After Ream and Fogdall, 1982).

1928	Pulsatile perfusion of dog's head, using piston pump.
1930	Organ perfusion, bacterial filtering.
1934	Roller pump for blood infusion (DeBakey).
1948	Invention of disc oxygenator.
1950	First modern bubble oxygenator, first application of effective defoaming (Clark and coworkers).
1951	Successful organ bypass in man: direct bubble oxygenation device (Dogliotti and Costantini).
1951	First attempt of total bypass in man (Dennis and coworkers).
1953	Success in open heart surgery using only hypothermia (Lewis and Mansar).
1955	Successful bypass, using human lung in cross circulation (Lillehei and coworkers).
1956	Invention of disposable bubble design (DeWall and coworkers).
1956	First clinical use of membrane oxygenator (Clowes and coworkers).

coronary artery diseases, valve lesions and aneurysms of the left ventricle. Each of these heart abnormalities can be repaired with a low intraoperative mortality risk and a good longterm survival. In combination with the advanced diagnostic techniques, this has lead towards an increasing demand for open-heart surgery. For instance, in the Netherlands the number of open-heart operations nearly doubled within 3 years from a total of 3945 in 1979 to a total of 7112 in 1982. This data mainly refers to coronary bypass surgery (5036), valve replacements (1195) and congenital heart surgery (648) (data from Begeleidingscommissie Hartchirurgie Nederland 1982). It is evident that the rapid development of open-heart surgery in different clinical centres around the world has introduced a variety of techniques and protocols for the performance of the operation. This is especially true for the techniques to protect the heart during the critical ischemic period, when the heart is deprived from blood for a longer period of time (see section 1.2). The need for protection, the protective techniques applied to minimize ischemic damage of heart tissue and the evaluation of their adequacy are discussed in detail in the consecutive sections, at least when relevant to aorto-coronary bypass surgery and to the present study.

Protection includes reduction of the patient's blood temperature to 25°C (systemic temperature) and selective cooling of the heart to 15°C with a cardioplegic solution. A different protection technique is intermittent cross-clamping of the aorta and thus introducing repetitive, short periods of ischemia, each followed by a reperfusion (recovery) period. Development of protective techniques requires reliable evaluation of the clinical outcome. Beside the use of perioperative and postoperative mortality rates, for instance analysis of changes in ECG, hemodynamic function and specific enzyme release during the postoperative period have shown to be valuable tools. By the development of accurate biochemical and ultrastructural indices, a variety of parameters has become available to more specifically evaluate the clinical outcome. These parameters are discussed briefly in section 1.4 and in more detail in section 2.2. Despite the fact that reliable variables to evaluate aorto-coronary bypass surgery are at hand, contradictory results of clinical and experimental research are still being reported (see section 1.5).

The final part of the Introduction (see section 1.6) reports on the set-up of the clinical and experimental parts of this study, which were

Fig 1.1 Schematic design of a heart-lung machine, including an oxygenator, a heat exchanger and a pump, to sustain the blood circulation during open-heart surgery.

Fig 1.2 Site of occlusion of the ascending aorta above the inflow opening of the left and right coronary arteries (AC = aortic cross-clamping).

primarily designed to:

- analyse the clinical and experimental course of three techniques routinely used for myocardial protection during aorto-coronary bypass surgery.
- provide hemodynamic and biochemical parameters to evaluate the behavior of the heart during and immediately after open-heart surgery.

1.2 The need for myocardial protection

When replacing heart and lung function by means of a heart-lung machine the heart becomes unloaded. To perform surgical repair, it is necessary that coronary blood flow is diminished. In particular during aorto-coronary bypass operations a quiet and bloodless operating field is required to perform the bypasses between the ascending aorta and the affected coronary artery. With sustained coronary perfusion incision of the coronary arteries would cause excessive bleeding. A standstill of coronary flow is routinely obtained by occlusion of the ascending aorta, just above the inflow opening of the coronary vessels (fig 1.2).

The duration of aortic cross-clamping during aorto-coronary bypass surgery is in general determined by the total number of aorta to coronary artery grafts. Routinely the performance of one bypass takes 6-15 min. During the period of aortic cross-clamping, when coronary flow is zero (global ischemia), oxygen and substrates will not be delivered to the myocardium and breakdown products will accumulate in this tissue. Because oxygen supply has ceased, and the oxygen reserve is limited within the myocardial cells, the cells immediately change from aerobic to anaerobic metabolism. Endogenous glycogen, which is available in large quantities, will be used for lactate production and the energy delivered should maintain the content of high-energy phosphates at physiological levels. Because meanwhile the removal of breakdown products is hampered, CO_2 and such harmful products as H^+ ions and lactate will accumulate. When ischemia lasts for longer periods of time, the amount of high energy phosphates finally diminishes and cell metabolism deteriorates. Consequently membrane integrity changes and intracellular macromolecules enter the intercellular (interstitial) space.

In order to reduce the metabolic activity of the heart during the critical period of ischemia the myocardium is usually cooled to 15-25°C. This can

be achieved by infusion of cardioplegic solutions or through topical cooling with ice-water or a combination of these techniques. These procedures may reduce the oxygen consumption to 50% at temperatures of 26°C (Badeer, 1956) and to 0.33 ml O₂ per min per 100 g left ventricular tissue at 15°C (Chitwood et al, 1979). This amounts to 6% of the values observed in the empty beating heart at 37°C.

Animal experiments have shown that upto 30 min, global ischemia has no irreversible influence on myocardial ultrastructural appearance (Nohara, 1983; Taylor et al, 1984), while after 60 min ischemia at 37°C the myocardium becomes irreversibly injured (Flameng et al, 1981a).

In between these extremes there is a transition from reversible to irreversible changes of the myocardial cells. The technique of myocardial protection should particularly aim at the extension of the onset of irreversible tissue damage.

1.3 Techniques for myocardial protection

The development of techniques for myocardial protection during open-heart surgery, was initially related to congenital heart abnormalities and valve lesions. Therefore, these techniques had to protect the heart during relatively long, uninterrupted periods of ischemia due to aortic cross-clamping. Since the introduction of aorto-coronary bypass surgery in 1967 (Favaloro), the earlier developed techniques were adapted to this procedure. In myocardial protection two important aims have to be addressed. Firstly, the duration of anaerobic metabolism should be limited and secondly, the intensity of anaerobic metabolism should be reduced. Most of the techniques used nowadays are dealing with one or both of these aspects. In aorto-coronary bypass surgery reduction of the duration of anaerobic metabolism can be achieved by intermittently cross-clamping the aorta (Delva et al, 1978). During each ischemic period the distal anastomosis (coronary artery connection) of the graft can be performed, while during the succeeding reperfusion phase the proximal anastomosis (aortic connection) will be made. The number of cross-clampings mostly equals the number of distal anastomoses unless the "snake" method is applied (multiple distal anastomosis to one graft). The duration of one ischemic period is commonly reduced to 6-15 min and metabolic recovery

during the subsequent reperfusion period (10-20 min) precedes the next ischemic phase. Initially this technique was carried out at a systemic temperature of 34°C (normothermia), but was abandoned because of postoperative complications. Good clinical results were obtained by lowering systemic temperature to 25°C (hypothermia) and combining this method with hypothermia became the technique of choice (Koster et al, 1977; Baur et al, 1979).

Nowadays a different approach to myocardial preservation, known as cardioplegia, is applied in nearly all centres for open-heart surgery (Weisel et al, 1983). Cardioplegia, or cardiac arrest due to selective perfusion of the coronary arteries during aortic cross-clamping with a variety of artificial solutions, primarily aims at reducing the intensity of anaerobic metabolism, and has recently been reviewed by Hearse and his colleagues (1981). In general, cessation of electromechanical activity is achieved by low temperature and a relatively high potassium content of the solution. The composition of the various cardioplegic solutions as described in literature differs enormously and even today the concept, on which the composition is based, varies. Experimental results are not uniform in respect to the optimum combination of constituents. In search of this optimum composition several criteria can be mentioned. The cardioplegic solution should:

- stop heart activity
- minimize anaerobic metabolic activity
- preserve membrane integrity
- have an optimal oxygen capacity
- act as a buffer
- prevent tissue edema
- not contain toxic substances or particles.

Beside the composition of the solution, such factors as temperature, pH, volume infused and intervals between the infusions can be manipulated. Improvement of the technique of cardioplegia is still being reported from many laboratories and it seems to take still some time before the final and most ideal cardioplegic solution has been developed and accepted.

1.4 Evaluation of the clinical outcome

The evaluation of a patient after an open-heart operation can be based upon two different approaches. Firstly, during the operation intensive monitoring in the operating room provides information about the systemic circulation, the blood gas values, the pump function of the heart and the ECG. Correct anesthetic policy sustains the patient at the onset of weaning from cardiopulmonary bypass and determines whether prolonged support of the heart is needed. During the first hours after CPB heart rate, ECG, blood pressure and cardiac output are routinely monitored. Complications as for instance an unacceptable low cardiac output or evolved myocardial infarction can be detected immediately. Within the first days after the operation, repetitive cardiac output and ejection fraction determinations can be performed. This policy takes care of the optimum condition of the patient and the parameters mentioned are in general applied to determine the patient's clinical outcome and recovery in the intensive care unit.

Secondly, comparison of the different surgical and/or protective techniques should involve examination of more specific variables beside the hemodynamic and clinical recovery. Especially the assessment of heart function (i.e. left ventricular pressure and its maximum positive first derivative) is useful when values as obtained following CPB, are compared to the values before CPB. However, these parameters are influenced by multiple interventions and are therefore not solely indicative for damage due to an ischemic insult. Different from the hemodynamic measurements immediately after CPB is the analysis of enzyme release, which is essentially initiated during the operation, but effectuated only after hours or days following the operation. The reliability of the measurement of the activity of one or a set of heart specific enzymes for the detection of myocardial tissue damage has improved during the past years. This is caused by the development of separation techniques to determine isoenzymes and by the application of mathematical models which allow multienzyme analysis.

Detailed information about the myocardium can be derived from myocardial biopsies and coronary sinus blood samples during the period of CPB. To evaluate the effects of ischemia biopsies for studying heart metabolism and ultrastructural examinations can be taken in the immediate postischemic phase.

1.5 Unsolved clinical problems

In interpreting the results from clinical studies in which different groups of patients are compared, patient selection and history, and overall treatment during the preoperative period have to be considered. Great diversity of these factors may influence the final clinical outcome and even hamper adequate interpretation of the results. Especially the hearts of patients submitted to aorto-coronary bypass surgery may be subject to previous myocardial infarction or less well perfused areas. Also during surgery, (even when the operation protocols are meant to be standardized) individual anesthetic policies, to maintain adequate hemodynamic function, may differ between patients.

Another aspect of clinical studies deserves full attention. When the protective technique has been replaced in time, the groups are treated serially and not in a randomized parallel way. The validity of serial observations is debatable because other factors beside myocardial protection may change in time and affect the clinical outcome (Davids, 1982).

Although a variety of studies have been performed on myocardial protection during global ischemia, several aspects need to be clarified. Firstly, the question whether hypothermia should be preferred above normothermia. Deleterious influences of hypothermic ventricular fibrillation as compared to the normothermic empty beating heart, have been reported (Grover et al, 1977; Vinas et al, 1979). On the other hand hypothermia has shown to reduce metabolic demands and hence may be beneficial to the ischemic myocardium. Selective cooling of the heart to 15°C is routinely used and may result in cold damage to cells or more specifically membranes. Whether this type of damage occurs in heart tissue is still subject of discussion.

Secondly, as was mentioned in section 1.3 myocardial protection has evolved to a number of cardioplegic solutions all of which are advocated as being excellent. However, comparison of the cardioplegic solutions used, has only been rarely reported in a clinical trial.

Thirdly, beside the deleterious influence of global ischemia during aorto-coronary bypass operations, other interventions which may be harmful to the myocardial tissue have been recognized. Especially reperfusion damage occurring after ischemia has been hypothesized to influence the state of the myocardial tissue. In this respect the application of different reperfusion

techniques is worthy to note (Engelman et al, 1977).

Fourthly, most of the aorto-coronary bypass operations are performed routinely today and the incidence of myocardial necrosis due to perioperative infarction is low, because of myocardial protection. To detect myocardial necrosis, if any, very sensitive parameters are required. At the same time it will be desirable to select parameters which will discriminate between reversible and irreversible injury as well.

1.6 Aim of the present study

- Clinical set-up

Before this study was started cardiac surgeons from the St. Raphael Clinics in Leuven performed aorto-coronary bypass operations routinely by combining intermittent aortic cross-clamping and normothermia (34° C) or hypothermia (25°C) with good clinical results. Since comparable good results were obtained in patients undergoing extensive aorto-coronary bypass surgery combined with hypothermic St. Thomas Hospital cardioplegia and continuous aortic cross-clamping, this study was designed to evaluate these three techniques in a randomized way. The main question of the study was whether these surgical techniques were different as far as the myocardial changes induced by global ischemia during aortic cross-clamping are concerned. It was observed in the literature that several surgeons operate with the use of one or more of these techniques (Koster et al, 1977; Baur et al, 1979), while evaluation of these techniques within a clinical trial was not reported so far.

Table 1.2 shows the three patient groups including the type of cardio-protective protocol employed (see section 3.1 for detailed information). All patients included in the study were randomly allotted to one of the three groups and they were all subjected to assessment of the parameters listed in table 1.3.

The values of the hemodynamic variables after weaning from CPB were compared with those measured prior to CPB, to obtain insight into the effects of the surgical procedure on systemic circulation. Tissue metabolites were determined to assess the preservation of high-energy phosphates and glycogen stores, and the release of inorganic phosphate into the coronary sinus effluent as an index of high-energy phosphate breakdown. Simultaneously, in

Table 1.2 Three groups of patients with their respective technique of myocardial protection.

Group	Number of patients	Technique of myocardial protection
Normothermia	24	Normothermic intermittent aortic cross-clamping (34°C)
Hypothermia	23	Hypothermic intermittent aortic cross-clamping (25°C)
Cardioplegia	25	Continuous aortic cross-clamping combined with St. Thomas Hospital Cardioplegia

Table 1.3 Parameters and variables used to evaluate the efficacy of the three protective techniques under investigation.

Hemodynamic variables	Cardiac output, arterial pressure, left ventricular pressure (and its maximum positive first derivative), heart rate, pulmonary wedge pressure.
Metabolic substances	Lactate, inorganic phosphate, potassium and oxygen in arterial and coronary sinus blood.
Tissue metabolites	High energy phosphates and glycogen in myocardial biopsies.
Tissue ultrastructure	Mitochondrial appearance in myocardial biopsies.
Catecholamines	Epinephrine, norepinephrine and dopamine in systemic venous blood.
Plasma enzyme activity	AST, creatine kinase, CK-MB and HBDH in systemic venous blood upto 36 hrs after cardiopulmonary bypass.

coronary sinus effluent we determined lactate as a measure of anaerobic glycolysis during myocardial ischemia, potassium as a marker of transsarcolemmal ion transport, and the arterial-coronary sinus difference of the oxygen content to assess tissue oxygen consumption.

Subendo and subepicardial tissue specimens were obtained from the left ventricular free wall to determine ultrastructural changes in mitochondria, as a measure of reversible and irreversible tissue injury. The serum content of catecholamines was assayed to estimate the possible correlation with changes in the hemodynamic performance. Up to 36 hours after CPB serial measurements of plasma enzyme activity of aspartate aminotransferase (AST), creatine kinase, its isoenzyme CK-MB and alpha-hydroxybutyrate dehydrogenase (HBDH) were used to quantitate irreversibly damaged cardiac tissue.

The combination of parameters and variables (Table 1.3) was selected to distinguish between changes from reversible (for instance moderate metabolic disturbances occurring after a short ischemic period) and irreversible cell damage (for instance massive enzyme release in the circulation associated with cell necrosis due to long lasting ischemia).

- Animal experiments

Although a lot of information about the efficacy of protective measures could be obtained from the clinical study, additional animal experiments were performed to investigate aspects, which could not be studied in patients. Therefore, an animal model, resembling the clinical situation, was developed.

The animal model allows the investigation of

- quantitative myocardial metabolism enabled by the measurement of myocardial blood flow with radioactive microspheres and an electromagnetic flow probe.
- transmural distribution of myocardial blood flow to assess whether sub-endocardial regions are preferentially underperfused after cardiopulmonary bypass.
- myocardial metabolism in more detail because of the larger number of biopsies that can be taken. Biopsies just prior to ischemia and at the end of ischemic periods were obtained to differentiate between changes induced by ischemia and by reperfusion.
- the effect of cardiopulmonary bypass per se on the variables and para-

meters measured, because aortic cross-clamping could be deleted.

- the effect of anesthesia and instrumentation on the parameters measured.

Besides, in the animal model we investigated the rapid changes in hematocrit using plasma volume measurements before and after cardiopulmonary bypass.

2 SURVEY OF THE LITERATURE

2.1. Myocardial protection

2.1.1. Introduction

In the early days the clinical outcome after open-heart surgery was not always adequate and in particular perioperative infarction was frequently observed in association with postoperative low cardiac output (see for reviews Bedard et al, 1977; Davids, 1982). Recognition of these complications has been a strong impetus for investigations aiming at the development of techniques to protect the heart during the surgical procedure.

To position the methods of protection presently available in perspective of their development, some important changes in attitude towards myocardial protection will be discussed. Since the first 25 years of myocardial protection have already extensively been reviewed by Hearse and coworkers (1981), and Conahan (1982), three selected topics will be discussed in this chapter.

The first part includes the specific conditions of the heart during the procedure of aorto-coronary bypass surgery. These conditions are the empty beating, fibrillating and flaccid heart as well as the global ischemia of the myocardium due to aortic cross-clamping. Secondly, the effect of the aforementioned conditions of the heart on the tolerance to ischemia as a function of temperature will be discussed. Finally, protective techniques which have been developed from experimental and clinical investigations will be considered, in particular the techniques of intermittent aortic crossclamping and cardioplegia.

The choice of these topics closely relates to the type of surgery used in the present study. Aorto-coronary bypass surgery differs from other openheart surgical procedures, mainly because of the duration of aortic crossclamping and the necessity to operate upon coronary arteries. These differences have resulted in adaptations of the myocardial protection policy. In general, valve replacement and correction of left ventricular aneurysms or congenital heart abnormalities require a continuous period of relatively long-lasting aortic cross-clamping (Flameng et al, 1980). The concomitant oxygen deprivation of the heart is tolerable if profound myocardial cooling, commonly in

combination with cardioplegic solutions, will be applied. In contrast, during aorto-coronary bypass surgery the instalment of each single graft can be succeeded by a reperfusion period allowing the myocardium to recover from a relatively short ischemic period, commonly lasting from 6 to 15 min.

2.1.2. Aorto-coronary bypass surgery

An essential requirement during aorto-coronary bypass surgery is a quiet and bloodless operation field. After instalment of cardiopulmonary bypass the heart is empty beating. This can be changed into a flaccid heart (cardioplegia) or a fibrillating heart (induced electrically or by hypothermia). Next, cessation of coronary artery flow is achieved by cross-clamping the ascending aorta permitting incision of coronary arteries for the performance of distal anastomoses without excessive loss of blood (for details see Conahan, 1982).

The initial employment of a fibrillating state during open-heart surgery offered many practical advantages. However, it has been subject of continuous debate whether fibrillation during this type of surgery is detrimental to the heart in itself. In an experimental model Senning (1952) observed no change in the rate of oxygen consumption during fibrillation as compared to the empty beating heart. In contrast decreased oxygen consumption in the fibrillating heart, as compared with the working heart, was reported by Paul and coworkers (1954). An increased oxygen consumption as compared to the non-working heart, was found by other investigators (Jardetzky, 1956). The functional recovery after prolonged periods of induced fibrillation was reported to be adequate in both dogs and patients (Race et al, 1964).

More recently fibrillation during cardiopulmonary bypass was found to cause an increase in myocardial oxygen consumption and redistribution of coronary blood flow within the left ventricular wall, preferentially to the subepicardial layers (Ghidoni et al, 1969; Hottenrot et al, 1974; Buckberg and Hottenrot, 1975; Brazier et al, 1975; Vinas et al, 1979). These changes indicate that the fibrillating state may cause an imbalance between myocardial metabolic demand and supply resulting in a shortage of oxygen in the subendocardial layers. Under mild hypothermia the supposed deleterious effects of fibrillation were found to be reduced (Vinas et al, 1979). Other expe-

rimental studies did not confirm the negative effects of fibrillation. For instance, McDonagh and coworkers (1982) reported that spontaneous fibrillation during 20 min did not increase myocardial oxygen consumption. Besides, Isom and colleagues (1973) were unable to detect redistribution of flow from the subendocardium towards the epicardium. Nowadays continuous, electrically induced ventricular fibrillation is used less frequently in the clinical setting.

The ultimate effect of fibrillation on myocardial tissue is dependent on the model of investigation (Utley et al, 1981). Vinas and coworkers (1979) and Hottenrot and collaborators (1974) employed continuous, electrically induced fibrillation under normothermic conditions in dogs on an extracorporeal circuit. They observed that during fibrillation coronary flow was lower when electrical stimulation was applied continuously than when the heart was fibrillating without such a stimulus. This difference was especially pronounced after 60 min of fibrillation. Ventricular fibrillation will automatically occur when hypothermia is applied as part of the technique of myocardial protection unless cardioplegia is used (Molina et al, 1977). All reports mentioned so far were dealing with non-ischemic conditions of the heart.

Beside the necessity of a quiet heart during aorto-coronary bypass surgery, cessation of coronary blood flow is also required which can be obtained by cross-clamping the aorta. This situation is referred to as global ischemia and its consequences for the myocardial tissue have been studied extensively. In clinical conditions ischemia affects the myocardial tissue depending on the efficacy of the technique for myocardial protection employed (Reul et al, 1975). The technique of protection should not only reduce the reversible changes induced by ischemia, but should especially prevent irreversible damage to occur.

2.1.3. Hypothermia

One of the first papers on systemic moderate hypothermia in open-heart surgery in infants reports on the successful preservation of the myocardium and other organs after circulatory arrest of 8.5 min without the use of cardiopulmonary bypass (CPB; Swan et al, 1953). Earlier dog experiments had

shown the reversal of changes in cardiodynamics and systemic circulation after cooling to 20°C for a short period of time and subsequent rewarming (Bigelow et al, 1950). The advantage of reduced metabolic demands at reduced body temperature has been applied during the first CPB procedures. Cooling was initially performed by placing the body in a tub of crushed ice and water, which improved survival rate (King et al, 1958). Cooling of the core of the body became possible with the development of CPB equipment, and this technique subsequently replaced peripheral cooling (Galetti and Brechner, 1962). During systemic cooling by means of CPB the temperature of the heart will equal that of the body. An additional advantage of this technique is the fibrillating state of the heart offering a quiet operating field for the surgeon.

It is not practical to decrease body (systemic) temperature below 25°C, but profound cooling of the heart has shown to improve protection during ischemia. External cooling of the heart using ice-cold saline solutions inside the pericardial cradle by addition of crushed ice, has initially been employed (Shumway et al, 1959; Hurley et al, 1964). From then on cold injury to the myocardium became subject of discussion (Speicher et al, 1962; Marco et al, 1977; Shragge et al, 1981). At the time coronary artery perfusion through separate lines was introduced, more uniform cooling of the myocardium could be achieved (Bernhard et al, 1960; Van der Schaar et al, 1966), although inhomogenities due to pre-existing occlusions or stenoses of the coronary arteries have to be considered (see final part of this section).

The possibility to adjust myocardial temperature made it urgent to consider the optimum temperature. In 1979 Chitwood and coworkers reported a study in dogs, subjected to separate, uninterrupted perfusion during CPB, in which they varied myocardial temperature from 37°C to 15°C with the use of potassium enriched cold blood. At this decrease in temperature they noticed a reduction of arterial-coronary sinus difference of oxygen content from 3.92 to 0.42 vol %, and an increase in myocardial blood flow of 43 %. This may be beneficial to patients, especially when underperfused myocardial areas already exist due to atherosclerotic lesions of the coronary arteries.

Profound levels of hypothermia (6-18°C) were investigated by Balderman and coworkers (1983) in dogs on CPB. They found myocardial preservation during 120 min of global ischemia to be satisfactory at a myocardial temperature of 14°C. Cooling to lower degrees impaired postoperative myocardial

function. Kao and coworkers (1982) studied the preservation of high-energy phosphates in the isolated working rat heart during and after one hour of ischemic potassium arrest at temperatures varying from 4 to 37°C with increments of 4 degrees. They observed optimum protection at temperatures of 12°C or lower, without the occurrence of cold injury to the heart. Despite some lack of agreement (Gays, 1980), the experimental and clinical findings indicate that during ischemia the optimum temperature is in the range of 12 to 16°C (Ellis et al, 1979; Manners and Nielsen, 1981).

Since hypothermia decreases oxygen demand of the tissue by slowing down metabolic activity, this aspect has been studied in detail in relation to its potential protective effect. Besides, other biochemical and biophysical aspects should be considered as well. They include the increased solubility of oxygen in plasma and the reduced release of oxygen from hemoglobin for a given oxygen partial pressure. The reduced overall oxygen requirement and the increase in viscosity of blood with decreasing temperature makes hemodilution preferable. Hypothermia and hemodilution are routinely combined in open-heart surgery.

2.1.4. Techniques of myocardial protection

Initially progress in open-heart surgery was dependent on the surgical skill and the adequacy of extracorporeal circulation (i.e. oxygenation, priming solution etc.). The application of aortic cross-clamping resulted in long periods of coronary blood flow interruption and myocardial ischemia, associated with perioperative infarction and a high mortality rate (Favaloro, 1979). In the early years surgical repair mainly involved valve operations and congenital heart abnormalities. These surgical procedures were combined with several types of protective measures. The application of aortic cross-clamping combined with selective coronary artery perfusion permits the uninterrupted delivery of blood to the myocardium. Aortic cross-clamping without coronary artery perfusion at normothermia or with moderate systemic hypothermia, implies no or only a small reduction in metabolic rate, but has the advantage of a short duration of operation and no complications from intracoronary catheters. Besides, local cooling of the heart could be used, resulting in diminished myocardial metabolic demands at the time of ischemia

(Bernhard et al, 1960; Hurley et al, 1964).

Initially these techniques of myocardial protection were adapted to aortocoronary bypass surgery (Favaloro, 1967). Selective coronary artery perfusion was rarely used, because of coronary blood obfuscating the surgical field when the distal anastomoses (on the coronary artery) had to be made. However, aortic cross-clamping at normothermia, hypothermia, or with the use of profound local cooling of the myocardium were assumed to be appropriate in aorto-coronary bypass surgery (Baur et al, 1979).

A completely different technique is the local occlusion of the distal coronary artery. This technique, however, has shown to be inferior to ischemic arrest as induced by aortic cross-clamping (Ellis et al, 1975), although this approach may be preferred under certain circumstances when aortic cross-clamping is less desirable (Akins, 1984).

Especially after Levitsky and Feinberg (1975) reported the depletion of tissue high energy phosphate and glycogen stores after 60 min of continuous normothermic ischemic arrest, surgeons limited the duration of coronary flow obstruction by intermittently cross-clamping the aorta. Myocardial necrosis after prolonged normothermic ischemic cardiac arrest was established by other investigators (Buja et al, 1970; Merchant et al, 1974). Intermittent reperfusion did not improve this situation (Levitsky et al, 1977). These experimental findings caused a shift from moderate hypothermia (30-34°C) towards hypothermic (25°C) intermittent aortic cross-clamping in combination with moderate hemodilution in the clinical setting (Delva et al, 1978). However, intermittent cross-clamping of the aorta was found to have a similar protective efficacy as continuous cross-clamping under profound local cardiac hypothermia (Koster et al, 1977).

As an alternative to intermittent aortic cross-clamping, Melrose and colleagues (1955) and Berglund and coworkers (1957) introduced the so called "cardioplegia" technique. During continuous cross-clamping of the aorta the coronary arteries were perfused with cold solutions introduced via the clamped aortic root. Much research has been performed to find the optimum composition and temperature of the cardioplegic solution. Bretschneider and associates (1975), for instance, advocate solutions, the composition of which is essentially based on the concentration of crystalloids inside the myocardial cells. The most striking feature of their cardioplegic solution is the absence of calcium ions.

In contrast, other investigators proposed solutions related to the extra-cellular composition of the heart, supplemented with a relatively high concentration of potassium and magnesium (McFarland et al, 1960; Tyers et al, 1977; Hearse et al, 1981). In our study we applied the St. Thomas hospital cardioplegic solution as proposed by Hearse and coworkers (1981). This solution has a high sodium chloride content (110 mmol.l^{-1}), and further includes potassium chloride (16 mmol.l^{-1}), magnesium chloride (16 mmol.l^{-1}), calcium chloride (1.2 mmol.l^{-1}) and sodium bicarbonate (10 mmol.l^{-1}). This solution has been studied during recent years with respect to the optimum potassium concentration (Hearse et al, 1976; Jellinek et al, 1981) and the addition of procaine (Hearse et al, 1981) and high-energy phosphates (Hearse et al, 1976; Robinson et al, 1984). Both animal experiments (Sink et al, 1979; Nugent et al, 1982) and clinical studies (Fisk et al, 1977; Tyers et al, 1977) have shown the beneficial effects of potassium cardioplegia, and its superiority as compared to Bretschneider's cardioplegic solution (Jynge et al, 1978).

A different policy in cardioplegic composition was developed in the United States, where the group of Buckberg and Follette concluded that blood cardioplegia was more effective to protect the myocardium from ischemic damage than crystalloid cardioplegia (Follette et al, 1978). The rationale behind blood cardioplegia is an enhanced delivery of oxygen to the myocardial tissue during cross-clamping of the aorta (Hendriks et al, 1983) and an improved colloid osmolality of the cardioplegic solution. The observation of Singh and coworkers (1982) that blood cardioplegia is superior to crystalloid cardioplegia in preserving myocardial ultrastructure and reducing the release of intracellular enzymes, such as CK-MB, however, is at variance with the findings of, for instance, Engelman and coworkers (1981). They did not find differences in myocardial oxygen consumption, when crystalloid or blood cardioplegia was evaluated. The latter results were in keeping with the findings of Shapira and coworkers (1980). An explanation for this discrepancy can not be offered at this moment.

2.2 The myocardium during aorto-coronary bypass surgery

In this section aspects of hemodynamic function, coronary blood flow, local mechanical function, cellular metabolism and ultrastructural appearance, and the loss of intracellular enzymes will be discussed as far as they are relevant to the clinical and experimental study in this thesis. Basic information on the myocardium can readily be obtained from text books (Mc Alpine, 1975; Guyton, 1976; Berne, 1979; Drake-Holland and Noble, 1983).

2.2.1 Hemodynamic function

The early clinical reports on the status of the patient during and after aorto-coronary bypass surgery did not routinely include data on left ventricular function or hemodynamic recovery. More recently, functional parameters such as heart rate, central venous pressure, mean pulmonary artery pressure, mean left atrial pressure, mean radial artery pressure, cardiac index, stroke volume index, stroke work index, systemic vascular resistance and left ventricular minute work index were used by Grieppe and colleagues (1975). On the basis of these parameters, no substantial differences between surgical techniques could be observed, although postoperative enzyme release was significantly different in these techniques. It may therefore be concluded that the assessment of these hemodynamic variables is inadequate to discriminate between different techniques, which is in agreement with the findings of Sapsford and coworkers (1974).

Later studies, performed by Cunningham and coworkers (1979), Fennel and colleagues (1979) and Rosenkranz and Buckberg (1983), indicate that post-operative cardiac output and left ventricular end-diastolic pressure were useful parameters to evaluate the adequacy of myocardial protection during the surgical procedure and that these variables correlated well with peri-operative complications. Jalonen (1980) showed that the end-diastolic volume, rather than the end-diastolic pressure, was more reliable for the examination of individual patients than for groups of patients.

Although the first derivative of left ventricular pressure has shown to be a sensitive marker of myocardial contractility (Mason et al, 1964; 1969; Schaper et al, 1965), in particular to study the effect of regional (Szekeres

and Udvary, 1983) and global ischemia (Standeven et al, 1984), this parameter has been scarcely used in the surgical setting. Using hemodynamic parameters to evaluate the patient outcome after aortocoronary bypass surgery, one should be aware of a variety of factors complicating the correct interpretation of these indices. Changes in hemodynamic function cannot solely be ascribed to inadequate measures against ischemia, but the responses to anesthesia and extracorporeal circulation should also be considered. Differences in the plasma concentration of catecholamines, substances known to influence the hemodynamic function, might occur (Regan et al, 1970; Sebel et al, 1981). Apart from the systemic effects of circulating catecholamines, local cardiac release has been described (Brown et al, 1981). Under circumstances, which are comparable to those observed in cardioplegia, a reduced myocardial epinephrine response may be expected from experimental findings using high potassium solutions (Namm et al, 1968). Besides, severe depression of myocardial sympathetic nerve activity, resulting in a decreased tissue content of norepinephrine, has been reported during hypothermia (Jones, 1981), which might result from a reduction in catecholamine uptake (Reves et al, 1984). For obvious reasons the policy of the anesthesiologist is aiming at a good hemodynamic condition of the patient throughout the operation. Deliberate patient-to-patient differences in the administration of anesthetics, and positive and negative inotropic agents as well as changes in blood volume and artificial adjustment of the filling pressure will influence the values of the hemodynamic parameters. Hence, the effect of, for instance, inadequate protection against perioperative ischemia on these variables could be masked.

2.2.2 Coronary artery blood flow

Blood flow through the coronary arteries during aorto-coronary bypass surgery has been subject of interest for two distinct reasons. Firstly, restoration of flow through the area distal to an occluded or stenosed artery is the ultimate goal of this type of operation. Measurement of the flow through the aorto-coronary graft offers a reliable parameter to evaluate the success of the operation (Reneman and Spencer, 1972; 1975; Glock et al, 1981). Secondly, sufficient nutritional flow should be maintained throughout the operation (Ghidoni et al, 1969; Hottenrot et al, 1974).

In the initial phase of cardiopulmonary bypass, when the heart is empty beating in near normothermic conditions, no signs of impaired subendocardial blood flow could be found (Magrassi et al, 1981). Moreover, total blood flow to the animal heart increased considerably as the result of CPB instalment (Lees et al, 1971; Vinas et al, 1979; Schuette et al, 1982). Adequate subendocardial flow was also observed at reduced perfusion pressures of 6.7 kPa (Chitwood et al, 1981). In contrast, when hypothermia was employed, leading to ventricular fibrillation, myocardial blood flow reached normal values at perfusion pressures of 13.3 kPa, but significantly decreased at lower perfusion pressures particularly in the subendocardial layers (McConnell et al, 1977). The significance of these dog experiments might be important since decreased perfusion pressures are commonly present during the initial phase of aorto-coronary bypass surgery.

The effect of several interventions on myocardial blood flow during aorto-coronary bypass surgery in patients was discussed by Gray and coworkers (1976). They measured coronary sinus flow during CPB, using mild hypothermia (30-32°C) and hemodilution. Their findings indicate that during the initial phase of CPB reduced coronary blood flow may well be associated with a drop in perfusion pressure; increased coronary artery flow was observed after opening of the aorto-coronary bypasses

2.2.3 Mechanical function

In general, most of the variables mentioned in section 2.2.1 provide indirect information about the mechanical function of the heart. For technical reasons, direct measurement of the local mechanical activity of the different myocardial layers is not applied in the clinical setting. In animal experiments, pairs of ultrasonic crystals have been implanted within the myocardial tissue to measure changes in fiber length and in left ventricular shape (Theroux et al, 1974; Weintraub et al, 1981). This technique provides information about local myocardial shortening (usually at the endocardial site) but is dependent on the orientation of the crystals in relation to the local fiber direction. Besides, this approach may influence fiber contractility through local tissue damage (Hattori et al, 1982).

In the animal study of the present thesis a different technique was

applied for the measurement of epicardial deformation using three electromagnetic inductive coils attached to the epicardial surface resulting in little damage of the myocardium (Arts and Reneman, 1980). With this technique epicardial deformation can be described accurately (Arts et al, 1982). From this deformation subepi and subendocardial fiber shortening can be estimated (Prinzen et al, 1984).

Direct measurement of local shortening in the human heart has been described by Glock and coworkers (1981), using angiographic techniques. Their study, performed several months after the operation, showed that local myocardial function had been improved after aorto-coronary bypass surgery when the graft(s) were still patent. Brower and coworkers (1978; 1982) have studied the efficacy of St Thomas Hospital cardioplegia versus topical hypothermia with intermittent aortic cross-clamping with the use of radiopaque markers sutured to the epicardium. Improved regional shortening in the cardioplegia group was found to correlate well with lower values of creatine kinase leakage.

2.2.4 Tissue metabolism

Under normoxic conditions, myocardial cells utilize exogenous substrates for energy production. Non-esterified fatty acids, lactate and, to a lesser extent, glucose are stepwise degraded to carbon dioxide and water in oxygen-depending processes to provide ATP for proper functioning of the cell. Endogenous substrate stores such as glycogen and triacylglycerol are present, but are not used in steady-state conditions (Van der Vusse and Reneman, 1982). However, when the cellular requirements for energy exceed the supply by oxidative processes, anaerobic glycolysis will be accelerated (Opie, 1976). Via this escape mechanism only two molecules of ATP can be generated when one molecule of glucose is converted to lactate. Under circumstances of oxygen deprivation by complete cessation of coronary artery blood flow, as is the case during aortic cross-clamping, breakdown of glycogen will readily occur and lactate will be formed, providing 3 molecules of ATP per glucose moiety. When the amount of glycolytically generated high-energy phosphates is insufficient to meet cellular requirements, net consumption of ATP and creatine phosphate will occur. Beside the formation of creatine and inorganic

phosphate, degradation products of ATP will accumulate. Moreover, accumulation of potassium will take place in the extracellular space probably caused by disturbances at the level of the membrane $\text{Na}^+ - \text{K}^+$ ATPase during energy shortage (Owen et al, 1970) or by a higher potassium conductance during ischemia (Rau and Langer, 1978). The above described changes in myocardial metabolism do not necessarily reflect irreversible damage, but most likely reveal a physiological adaptation to a period of unbalance between energy requirements and supply.

Prolonged duration of this unbalance will ultimately lead to cell death. The mechanisms responsible for the transition of reversible to irreversible injury are not completely understood. Degradation of phospholipid moieties in myocardial membranes by enhanced phospholipase A_2 activity, resulting in impaired cellular ion and fluid homeostasis, and loss of macromolecules likely enzymes, has been suggested to be involved (Katz and Messineo, 1981).

In aorto-coronary bypass surgery measures should be taken to minimize the number of cells irreversibly damaged due to periods of ischemia throughout the operation. Ischemia readily occurs during periods of low perfusion pressure (commonly seen during the initial phase of CPB), due to the low oxygen carrying capacity of the coronary artery perfusate (e.g. hemodilution) and during the period(s) of aortic cross-clamping. The safest approach is to minimize the unbalance between energy demand and supply during ischemia by reduction of the energy requirement. This can be achieved by reducing the workload of the heart. In this respect Kawachi and coworkers (1982) reported that myocardial oxygen consumption reduces by 60% when the heart is empty beating instead of working, whereas Badeer (1956) found an additional reduction of 26% when the heart is cooled to 28°C. These measures appeared to be independent of perfusion pressure in the range from 7 to 13 kPa (McConnell et al, 1977). Despite these precautions, Gray and coworkers (1976) found a significant change from uptake to release of lactate during the initial period of CPB (prior to the first aortic cross-clamping) in their study. This change was closely related to the occurrence of perioperative myocardial infarction.

During aortic cross-clamping oxygen delivery has almost completely ceased. Levitsky and Feinberg (1975) reported a severe reduction of high-energy phosphates (62%) and glycogen (63%) associated with an increase in lactate content to 350% in the dog heart after one hour of normothermic

ischemic arrest. The sustained metabolic activity over a period of 60 min of ischemia was clearly evident and confirmed by others (Merchant et al, 1974; Opie, 1976; Jones et al, 1981; Reimer et al, 1981).

Employment of hypothermia has proved to reduce the rate of substrate breakdown (see section 2.1.3). In particular the use of ice-cold potassium cardioplegia reduces the rate of pH decrease during ischemia (Wilson et al, 1980), while in a clinical study depletion of ATP could completely be prevented when aortic cross-clamping lasted less than 90 min under these circumstances (Balderman et al, 1981). During one hour of ischemic arrest in the isolated working rat heart, the degradation rate of ATP, creatine phosphate and glycogen was greatly reduced when the temperature was lowered to 20°C or less (Tyers et al, 1977).

At reperfusion following unclamping of the aorta, accumulated metabolites will be released into the blood. A rapid regeneration of creatine phosphate occurred, whereas restoration of ATP content and glycogen stores required a prolonged period of recovery (Tyers et al, 1977; Salerno and Chiong, 1980; Balderman et al, 1981). Clinical studies reported lactate release after unclamping of the aorta (Goldschlager et al, 1972; Olin et al, 1981). In particular St Thomas Hospital cardioplegia proved to reduce the washout of lactate as compared to intermittent aortic cross-clamping with topical cooling (Jalonen et al, 1981d).

Myocardial metabolism after weaning from CPB has been examined in a few studies and the results indicate rapid normalization of oxygen and lactate extraction by the heart from the arterial blood (Wallace et al, 1961; Jalonen et al, 1981c; Edlund et al, 1982).

2.2.5 Ultrastructure

Analysis by means of transmission electron microscopy offers the opportunity to study in detail typical cellular components and organelles, such as glycogen granules, mitochondria, nuclei, sarcomeres and sarcolemma in very small myocardial tissue specimen (Jennings et al, 1969; 1978; Buja et al, 1970; Schaper et al, 1977).

On the base of ultrastructural changes several grading systems have been developed to quantitate the severity of ischemic injury (Schaper, 1979;

Apstein et al, 1983; Lindal et al, 1983). In particular the ultrastructural appearance of the mitochondria has been characterized in detail to distinguish between reversible and irreversible ischemic damage. In 1980, Flameng and coworkers proposed a classification system which solely examines the mitochondrial appearance and is basically comparable to the one described by Schaper (1979). We adapted this approach for our patient study and the details are presented in section 3.1.6.

Within minutes after the onset of normothermic ischemia, the specific, small intramitochondrial granules will disappear. After approximately 30 minutes of ischemia fragmentation of cristae and large amounts of gray flocculent material can be observed. After 60 minutes of ischemia severe injury is found and numerous cristae are broken. The ultimate irreversible state of mitochondrial damage is characterized by the presence of large amorphous densities (Jennings et al, 1969). It is generally believed that reduction of the temperature of the ischemic tissue will substantially delay these ultrastructural changes.

It should be emphasized that reperfusion of previously ischemic tissue will lead to restoration of the ultrastructural features when the duration of ischemia remains within limits, but that exaggeration of the morphological signs of injury most likely occur during restoration of flow after a prolonged period of ischemia. This notion is important to consider, because in most clinical studies biopsies for ultrastructural analysis were obtained after variable periods of reperfusion. For instance Balderman and coworkers (1981) allowed 3 min of reperfusion before taking the postischemic biopsies, whereas Flameng and coworkers (1980) allowed the heart to recover during 30 min. Lindal and coworkers (1983) reported that ultrastructural changes observed after more than one hour of aortic cross-clamping (i.e. fragmentation of mitochondrial cristae) had completely normalized after 60 min of reperfusion, using ice-cold St Thomas Hospital cardioplegic solution for myocardial protection.

2.2.6 Enzyme content and enzyme release

Enzyme release after tissue damage can be established with the use of serial plasma enzyme activity measurements. Quantification of tissue injury

can be done by assessing the peak plasma value and the time-delay between the damaging event and the appearance of the peak value in plasma, but more accurately the area under the release curve can be used to calculate cumulative release. Because of the catabolic removal of enzymes from the plasma, and the extravascular return of enzymes during the time intervals of sampling, corrections should be made using known circulatory parameters (Shell et al, 1973; Willems et al, 1979).

Already in 1960 Baer and Blount correlated the AST plasma activity and postoperative mortality. Also in myocardial revascularization studies peak values of plasma AST, ALT and LDH have initially been used to detect myocardial injury, and a positive correlation with the occurrence of ECG disturbances has been reported (Dietrich et al, 1968). These results were confirmed by Hultgren and coworkers (1971) who measured the plasma peak activity of creatine kinase, AST and LDH.

The introduction of isoenzymes of both creatine kinase and LDH rapidly led to numerous studies aiming at determination of the validity of cardiac specific enzyme measurements as a marker of cardiac tissue injury. Differences in the enzyme content of various tissues may well be used to discriminate between several sources of enzyme release (table 2.1). The assessment of the plasma enzyme activity of CK-MB after aorto-coronary bypass surgery is preferred above that of AST, creatine kinase and LDH, since the specific activity of CK-MB is highest in cardiac tissue as was shown by Baur and coworkers (1979). They observed that the detection of myocardial necrosis from changes in the ECG or by scintigraphy correlated best with CK-MB peak values. Similar findings showing the advantage of creatine kinase isoenzyme determinations as compared to total creatine kinase determination, were reported by Roe and coworkers (1979) and Sylven and colleagues (1982). The latter investigators applied the method developed by Shell and coworkers (1973) in a study on uncomplicated aorto-coronary bypass surgery and calculated that an equivalence of about 5 g of myocardial tissue became damaged. Such factors as the duration of aortic cross-clamping, the number of bypasses and the pump time correlated highly with the peak values of plasma CK-MB (Oldham et al, 1973; Strom et al, 1979).

However, the complex nature of enzyme release after aorto-coronary bypass surgery being the resultant of cardiopulmonary bypass, surgical interventions and anesthesia may easily lead to difficulties in interpretation of the

results (Moore et al, 1977; Lell et al, 1977; Davids, 1982). Thoracotomy will damage skeletal muscle. Moreover, surgical dissection of saphenous veins will cause muscle and vessel wall injury, and insertion of cannulas, including those for CPB, is associated with heart and blood vessel wall injury. For example, release of CK-isoenzymes from saphenous veins as a consequence of surgical manipulation was reported by Landaas and coworkers (1981). Hence overestimation of ischemia induced cardiac injury will occur when cardiac damage through surgical manipulation contributes considerably. Besides, hemolysis due to extracorporeal circulation is frequently observed and will result in increased plasma LDH activity. The application of CPB combined with hemodilution results in a decrease of plasma enzyme activity.

Although release of enzymes has generally been considered as a phenomenon related to loss of membrane integrity and hence cell death, Piper and coworkers (1984) have recently proposed that the extrusion of intracellular enzymes may occur in a reversible fashion. Since no data are available to conclude that loss of enzymes during open-heart surgery can occur through a reversible process, we consider the appearance of cellular enzymes in the extravascular space as a sign of irreversible damage (Hermens et al, 1982).

Table 2.1 Enzyme activity in human myocardial, liver and skeletal muscle tissue, and in erythrocytes ($\text{U} \cdot \text{g}^{-1}$; assay temperature of 25°C).
References: (a) - Siegenthaler, 1982; (b) - Hollaar and Van der Laarse, 1979; (c) - Van der Laarse et al, 1980 ; (d) Willems et al, 1982b.

	Heart	Liver	Skeletal muscle	Erythrocytes
AST	145(c)	96(a)	36(a)	0.8(a)
ALT	3(a)	69(a)	3(a)	0.1(a)
Creatine kinase	865(d)	0.7(a)	2800(c)	<0.01(a)
CK-MB	132(b)	-	22(b)	-
LDH	166(c)	156(a)	147(a)	36(a)

3. METHODS AND TECHNIQUES

3.1 Patient study

3.1.1 Patients, anesthesia and instrumentation

Seventy two patients undergoing aorto-coronary bypass surgery were assigned at random to three groups with a different cardioprotective technique: Group N (normothermia), intermittent aortic cross-clamping at 34°C, Group H (hypothermia), intermittent aortic cross-clamping at 25°C, and Group C (cardioplegia) continuous aortic cross-clamping in combination with multidose St. Thomas Hospital cardioplegia. All operations were performed in the St. Rafael Clinics, Leuven, Belgium, between November 1980 and October 1981. The two surgeons involved, Dr. W. Flameng and Prof R. Suy, were already familiar with the three protective techniques before the start of the study. The anesthetic policy and the surgical procedures were standardized in the patients as described before (Flameng et al, 1984).

Selection of the patients to enter the study was based on the diagnosis of three vessel disease requiring at least four distal anastomoses. Patients with associated cardiac diseases, signs of acute myocardial infarction or an ejection fraction of less than 30% were not included. The profile of the patients is presented in table 3.1.

The treatment of patients with nitrates and beta-blocking agents was discontinued on the evening before the operation. As night sedation lorazepam (3 mg orally) was given. Premedication, administered 60 min before induction, consisted of scopolamine (0.5 mg), fentanyl (75 µg) and droperidol (3.75 mg) intramuscularly. Anesthesia was induced with pancuronium (0.1 mg.kg⁻¹), fentanyl (15 µg.kg⁻¹) and sodium thiopentone (3 mg.kg⁻¹) while the patients inspired 100% oxygen by mask. Pancuronium was given before fentanyl administration to prevent the onset of truncal rigidity.

After endotracheal intubation, the lungs were ventilated with nitrous oxide and oxygen (FIO₂ 0.5). The ventilation was adjusted to maintain an arterial PCO₂ of 4.7-5.3 kPa corresponding to an endtidal CO₂ of 4.5-5% (capnogram). A 7F Edwards balloon guided thermistor catheter was inserted through the right internal jugular vein into the pulmonary artery for cardiac

Table 3.1. Profile of the patients in the three groups. Mean values and standard deviations are presented.

	Group		
	normothermia	hypothermia	cardioplegia
number of patients	24	23	25
age (years)	52 ± 9	54 ± 6	53 ± 7
sex	22M , 2F	19M , 4F	22M , 3F
body weight (kg)	73 ± 9	69 ± 8	71 ± 9
preoperative			
ejection fraction (%)	60 ±17	59 ±16	64 ±15
NYHA class	2.3± 0.7	2.3± 0.7	2.5 ± 0.8

Operation scheme :

Normothermia (34 °C) ; Hypothermia (25 °C) :

St Thomas hospital cardioplegia :

H - hemodynamics

E - enzymes

B - biopsies

C - multidose cardioplegia

AC - aortic cross-clamping

Fig 3.1 Protocol as followed in the three groups. AC = aortic cross-clamping, B = biopsies, C = multidose cardioplegia, E = enzymes, H = hemodynamics. (Note that both hemodynamics and enzymes were measured at more sample times; see for details section 3.1.3 and 3.1.5).

output determinations and to measure pulmonary artery wedge pressure (PWP). The thorax was opened by a median sternotomy, after the patients had received an extra dose of fentanyl ($60 \mu\text{g.kg}^{-1}$). During the preoperative period an isotonic solution of Normosol^R (composition see technical note in section 3.3) was administered aiming at a PWP value of at least 1.1 kPa. Initial heparinization (300 IU.kg^{-1}) before cardiopulmonary bypass (CPB) was followed by supplementary doses to obtain a three fold increase in actual clotting time, which was maintained until 15 min after CPB. Through the right atrium a 7F catheter was inserted into the coronary sinus to collect blood samples. A 7F Millar or Philips catheter-tip micromanometer was placed in the left ventricle via the right pulmonary vein for monitoring left ventricular pressure and its maximum positive first derivative. The left ascending aorta was cannulated for the return of oxygenated blood from the oxygenator and usually two cannulas were placed in the superior and inferior caval veins for the drainage of blood to the oxygenator by gravity. Just prior to the start of CPB an additional dose of pancuronium (0.1 mg.kg^{-1}) was given. During CPB a mean arterial pressure of 11-13 kPa was maintained, using droperidol or nitroglycerine. During CPB the lungs were not ventilated and were allowed to remain collapsed with a static inflation pressure of 0.7 kPa. Cardiopulmonary bypass was conducted using a Harvey H-1500, a Bentley Spiraflo BOS-10 or a Shiley 100A bubble oxygenator with heat exchanger. The oxygenator was primed with Normosol pH 7.4, (25 ml.kg^{-1}), human albumin (80-100 g), fresh donor blood (500 ml) and heparin (500 IU). This produced a hemodilution with an average minimum hematocrit of 25% during the bypass period. Using Sarns roller pumps, a non-pulsatile flow rate of $60\text{-}80 \text{ ml.kg}^{-1}.\text{min}^{-1}$ was maintained. During CPB the left ventricle was vented through the left atrial appendage. Fresh blood and Normosol were supplemented when needed. Before weaning from CPB ventilation was started with oxygen in air ($\text{FI}_{\text{O}_2} 0.5$). At the same time fresh blood or the remaining content of the oxygenator was slowly transfused to the patients to reach a final PWP of 1.1-1.3 kPa. After the pumps were stopped and the heart performed adequately, the cannulas were removed and the lungs were ventilated with nitrous oxide in oxygen ($\text{FI}_{\text{O}_2} 0.5$). Between 15 and 20 min after CPB, protamine hydrochloride was given to counteract heparin.

3.1.2 Surgical technique

The patients were randomized and allotted to the three groups N, H and C. The operation conditions were similar for all groups, with the exception of the myocardial protective technique used to minimize harmful influences of aortic cross-clamping during CPB. The protocols as followed in the groups are schematically shown in fig 3.1.

- Group N (normothermia with intermittent aortic cross-clamping, n=24). The rectal temperature of the patients was maintained at 34°C throughout the CPB period. The aorta was cross-clamped intermittently to allow performance of the distal anastomosis. Meanwhile, the left ventricle was fibrillating. After each clamp the hearts were defibrillated, if necessary, and reperfused for 6-18 min. The reperfusion period was used to perform the proximal anastomosis consisting of a vein graft connection to the ascending aorta. After the last cross-clamp the patients were rewarmed to at least 36°C rectal temperature, before weaning from CPB.

- Group H (hypothermia with intermittent aortic cross-clamping, n=23). The patients were cooled to 25°C (rectal temperature) immediately after starting the extracorporeal circulation. Analogously to group N the aorta was intermittently cross-clamped for performance of the distal anastomosis. In contrast to group N the hearts remained fibrillating during the periods of reperfusion due to the low temperature. The patients were rewarmed immediately after the last aortic cross-clamping and the hearts were defibrillated when rectal temperature had reached a value of 34°C.

- Group C (hypothermic cardioplegia with continuous aortic cross-clamping, n=25). The patients were cooled to 25°C of rectal temperature after the onset of CPB. Unlike group N and group H the aorta remained cross-clamped throughout the period needed to perform all distal anastomoses. During this period an ice-cold St. Thomas hospital cardioplegic solution (composition see technical note section 3.3) was infused into the aortic root with a roller pump. Infusion pressure as measured in the aortic root was kept at about 7 kPa. Initially 700 ml of the cardioplegic solution were infused, which was suffi-

cient to lower septal temperature to 8-15°C. Infusion was repeated (with approximately 400 ml of St. Thomas solution) every 20 min to keep septal temperature below 18°C. The patients were rewarmed after release of the aortic clamp and defibrillated at a rectal temperature of 34°C.

Individual variations were mainly caused by the number of aorto-coronary bypass grafts and, hence, the number of aortic cross-clampings in group N and group H and the duration of the single aortic cross-clamp period in group C.

3.1.3 Hemodynamic assessment

After all catheters and cannulas were installed, reference data was collected during a stable hemodynamic period preceding the start of CPB (pre CPB values). Hemodynamic variables included in this study were heart rate, systolic and diastolic arterial blood pressure, left ventricular pressure, pulmonary artery wedge pressure and cardiac output. Derived hemodynamic indices were calculated using standard formulas for the cardiac index ($CI = \text{cardiac output divided by body surface area; } l \cdot \text{min}^{-1} \cdot \text{m}^{-2}$) and left ventricular stroke work index ($LVS\text{WI} = \text{cardiac index multiplied by mean arterial blood pressure minus pulmonary artery wedge pressure and divided by heart rate; } J \cdot \text{m}^{-2} \cdot \text{beat}^{-1}$). The variables were also measured 5, 10 and 15 minutes after CPB. During CPB mean arterial perfusion pressure and pump flow were recorded at regular intervals.

Rectal and systemic blood temperature were measured routinely during the whole surgical procedure, and in group C septal temperature was recorded during aortic cross-clamping.

3.1.4 Sampling of tissue

In each patient two transmural biopsies were taken from the anterior free wall of the left ventricle about 2 cm above the apex using a small biopsy needle (Tru-cut Travenol Laboratories, Inc. Deerfield, Ill, USA) as described by Flameng and coworkers (1980). Two preischemic biopsies were taken within 5 min after the start of CPB, and a second pair of postischemic biopsies was obtained approximately 20 min after release of the (last) aortic cross-clamp, or when rectal temperature was at least 32°C. One of each pair of biopsies

was used for electronmicroscopic evaluation, while the second one was analysed for glycogen, ATP and creatine phosphate.

The biopsies for ultrastructural evaluation were immediately immersed in a cold fixative solution. The biopsies were divided into a subepi and a subendocardial part.

The biopsies for biochemical evaluation were immediately (within 3 seconds) frozen in liquid nitrogen and stored at -80°C until analysis. Biopsies were subsequently freeze dried and any adherent blood was carefully removed. The dry weight of these biopsies amounted to 3.0 mg (2.4-3.5 mg) (median value and 95% confidence limits) for all 72 patients.

3.1.5 Sampling of blood

Several chemical substances were measured in blood or plasma obtained before, during and after CPB.

- Lactate, inorganic phosphate, potassium and oxygen were determined in arterial (radial artery; during CPB directly from the oxygenator content) and coronary sinus blood to evaluate myocardial metabolic activity. Blood was sampled at the following intervals: 1) prior to CPB, immediately after insertion of the coronary sinus catheter, 2) after onset of CPB when the heart was empty beating, 3) just before cross-clamping of the aorta when the heart was fibrillating, 4) during reperfusion after each period of aortic cross-clamping within 30 sec and at 1, 2, 3, 4, 5, 6, 7.5 and 10 min (shown as wash-out curves), 5) at 20, 30 and 40 min after release of the last aortic clamp and 6) during the post CPB period at 5, 10 and 15 min after weaning from CPB.

- Plasma catecholamines were measured to evaluate distinct anesthetic and surgical interventions during the course of the operation. Epinephrine, norepinephrine and dopamine were determined in venous blood samples at the following sample times: 1) before induction of the patient, 2) at the time of endotracheal intubation, 3) immediately following sternotomy, 4) just prior to the start of CPB, but after cannulation of the aorta, 5) at the end of CPB when controlled ventilation of the lungs was started, 6) 5 min after cessation of CPB, and 7) 15 min after cessation of CPB when the aorta was already

decannulated.

- Plasma was assayed for creatine kinase, its isoenzyme CK-MB, AST and HBDH, enzymes known to mark myocardial muscle damage or hemolysis. The hematocrit and free hemoglobin values were determined simultaneously. For these purposes systematic venous blood samples (10 ml) were collected prior to sternotomy and 15 min, 4, 8, 12, 24 as well as 36 hours after cessation of CPB.

3.1.6 Ultrastructural analysis

Initial fixation of biopsies for electronmicroscopic analysis lasted for at least 24 hrs, using a cold 3% glutaraldehyde solution containing 90 mmol.l⁻¹ oxalate and adjusted to pH 7.4 with KOH. Next the tissue was rinsed in potassium oxalate buffer, containing 0.22 mol.l⁻¹ sucrose, and subsequently postfixation was achieved in 2% OsO₄, buffered with 0.05 mol.l⁻¹ veronal acetate to pH 7.4. The tissue was embedded in epoxy resin and ultrathin sections were cut. These sections were contrasted with uranyl acetate, followed by lead citrate, and pictures were taken on a Philips EM 300 electron microscope.

From the ultrathin sections of each subepi and subendocardial tissue specimen five photographs were made for semiquantitative evaluation of mitochondria. The procedure of evaluation as described by Flameng and coworkers (1980), involved grading of twenty randomly chosen mitochondria per photograph, using a scale from 0 to 4 (see fig 3.2):

- 0 - normal ultrastructure of the mitochondrion.
- 1 - normal appearance of the crests and matrix, but absence of granular deposits (small electron dense granules).
- 2 - loss of matrix granules and clarification of the matrix without breaking of crests.
- 3 - loss of matrix granules, uniform clarification of the matrix and disruption of crests (focal condensation may be seen: inclusions having at least twice the diameter of normal mitochondrial granules).
- 4 - loss of matrix granules, uniform disruption of crests and loss of integrity of mitochondrial inner and outer membranes.

Fig 3.2 Five stages of ultrastructural appearance of mitochondria as used for the classification of tissue injury. Meaning of the five distinct classes of mitochondrial appearance. 0 = normal ultrastructure, 1 = absence of granular deposits, 2 = clarification of the matrix, 3 = disruption of crests, 4 = loss of integrity of mitochondrial inner and outer membranes.

The average score of 100 mitochondria per tissue sample was calculated. All micrographs were examined in a blind fashion.

3.1.7 Biochemical analysis of tissue

Analysis of ATP, creatine phosphate and glycogen was performed as described in detail by Van der Vusse and coworkers (1984). The dried tissue was crushed with a glass rod in a 0.2 ml water-acetone (1:1, v/v) mixture, containing 2 mmol.l^{-1} EDTA and 0.4 mol.l^{-1} perchloric acid at a temperature of -15°C . This mixture was subsequently neutralized with 0.1 ml ice-cold Tris-KOH and centrifuged for 10 min at 4°C with a relative centrifugal force of 1100 g. The clear supernatant was carefully removed and aliquots were used for the determination of ATP and creatine phosphate. The residue, containing fragments of tissue and insoluble salts was used for the determination of glycogen.

ATP and creatine phosphate were analyzed with a fluorometric technique (Lamprecht and Trautschold, 1974; Lamprecht et al, 1974), modified by Drake and coworkers (1980). The composition of the reaction mixture per liter was: Tris buffer (55 mmol, $\text{pH}=8.1$), NADP^{+} (1 mmol), glucose (1 mmol), MgCl_2 (1 mmol) and glucose 6-phosphate dehydrogenase (160 U). After addition of 10 μl tissue extract to 1.0 ml of the reaction mixture, the reaction was started with hexokinase (690 mU). The amount of ATP was calculated by comparison of the change in fluorescence in the test cuvet with the standard cuvet (standardized with $5 \times 10^{-9} \text{ mol NADPH}$ in 1.0 ml buffer). From a subsequent addition of ADP (final concentration 0.05 mmol.l^{-1}) and creatine kinase (980 mU) the amount of creatine phosphate was determined in the same cuvet.

For glycogen analysis the residue mentioned before was incubated at 37°C for 3 hours, after addition of 1.0 ml of 1.0 mol.l^{-1} NaOH. Subsequently, 1.0 ml 95% ethanol was added and the whole mixture was heated for 10 min at 85°C . After cooling to 4°C , the mixture was stored at this temperature for 24 hours. Then the mixture was centrifuged at 4°C with 1100 g for 20 min. The clear supernatant was carefully removed with a pipette, and the residue was washed twice with 0.5 ml 60% ethanol. After centrifugation at 4°C with 1100 g for 30 min, the glycogen residue was hydrolyzed at 85°C for 3 hours, after the addition of 1.0 ml of 1.0 mol.l^{-1} HCl to the residue. The mixture was

subsequently neutralized with 0.5 ml Tris-KOH and aliquots were used for the fluorometric assay of the glucose moieties. Instead of glucose, ATP (1 mmol.l^{-1}) was added to the afore mentioned reaction mixture. The reaction was started with hexokinase (690 mU), using 10 μl of tissue extract. The determinations were performed with a Zeiss ZFM4 Fluorometer at 25°C.

3.1.8 Biochemical analysis of blood

Lactate, inorganic phosphate and potassium were measured in plasma derived from arterial and coronary sinus blood. Lactate and inorganic phosphate were analyzed with a Technicon-autoanalyzer as described before (Van der Vusse et al, 1979). Potassium was determined with a Technicon Flame Photometer IV. The blood samples were also used to determine total hemoglobin content and oxygen saturation immediately after collection, using a Radiometer OSM 2 hemoximeter. Oxygen content was estimated by multiplying venous hemoglobin content and the arterial-coronary sinus (A-CS) differences in oxygen saturation.

The concentration of norepinephrine, epinephrine and dopamine was determined in venous blood plasma, using the method of simultaneous single isotope radioenzyme assay as described by Peuler and Johnson (1977).

In pre and postoperative plasma samples the enzymatic activity of creatine kinase, its isoenzyme CK-MB, AST, HBDH and free hemoglobin were determined. Creatine kinase (EC 2.7.3.2) was measured with creatine phosphate as a substrate (Merck testkit 14111). The isoenzymes of creatine kinase (EC 2.7.3.2) were separated by ion exchange chromatography on DEAE-sephadex A50 (Pharmacia) into 3 isoenzyme fractions: CK-MM, CK-MB and CK-BB according to Mercer and Varat (1975). HBDH (EC 1.1.1.27) was measured with α -ketobutyrate as a substrate, as described by Rosalki and Wilkinson (1960), using a testkit (Boehringer 124818). AST (EC 2.6.1.1) was measured with α -ketoglutarate as a substrate according to Bergmeyer and Bernt (1974) (Boehringer testkit 124397). All enzyme activities were assayed at 25°C using an Eppendorf Photometer 1101 M in combination with a CKE calculator. Enzyme activities were expressed as Units (IU), in which 1 IU of enzyme catalyses the conversion of 1 μmol of substrate per min at 25°C. Free hemoglobin in plasma was measured spectrophotometrically at 540 nm after addition of cyanide and ferricyanide

according to Van Kampen and Zijlstra (1961), with the use of a testkit (Boehringer 124729).

3.1.9 Mathematical analysis of enzyme activity data

Recently Willems and coworkers (1982) described a method to calculate enzyme release due to cardiac injury, with the elimination of the contribution of non-cardiac enzyme sources. The mathematical method allows multi-enzyme analysis of plasma enzyme activity data. Using this method the plasma enzyme activity for creatine kinase, CK-MB, AST and HBDH was measured before and at various intervals after CPB upto and including 36 hrs. Postoperative data for enzyme activity was corrected for the mean value of the standard activity, found in control samples. These control values (C_s) were determined for creatine kinase (32.5 U.l^{-1}), CK-MB (5.6 U.l^{-1}), AST (8.8 U.l^{-1}) and HBDH (80 U.l^{-1} ; Willems et al, 1982). Corrected values of enzyme activity ($C_t - C_s$) are presented in this study, and subsequently used for the calculation of cumulative enzyme release $Q(t)$ (release as a function of time, expressed per liter of plasma). Analyses of $Q(t)$ values were based on a two compartment model (Willems et al, 1979). This two compartment distribution model consists of one intravascular (plasma pool) and one extravascular space as is illustrated in fig 3.3. Enzymes entering the circulation are subject to three processes:

- degradation from the plasma pool $P(t)$ at a rate $\text{FCR} \times P(t)$, where FCR is the fractional catabolic rate constant.
- escape from $P(t)$ to the extravascular pool $E(t)$ at a rate $\text{TER} \times P(t)$, where TER is the transcapillary escape rate constant.
- return from $E(t)$ to $P(t)$ at a rate $\text{ERR} \times E(t)$, where ERR is the extravascular return rate constant.

Quantification of total plasma enzyme release follows from three equations:

$$A(t) = P(t) + E(t) + \text{FCR} \int_0^t P(\tau) d\tau$$

Fig 3.3 Two compartment model as reported by Willems and coworkers (1982).
TER = transcapillary escape rate constant, ERR = extravascular return rate constant, FCR = fractional catabolic rate constant, $P(t)$ = plasma pool, $E(t)$ = extravascular pool, $F(t)$ = rate of input of enzyme into plasma, $V_p(t)$ = plasma volume, $C_p(t)$ = plasma enzyme activity.

$$E(t) = \text{TER} \cdot \int_0^t P(\tau) e^{-\text{ERR}(t-\tau)} d\tau$$

$$P(t) = C(t) \times V_p$$

where: $C(t)$ = plasma enzyme activity (U.l^{-1})

V_p = plasma volume

Since the actual plasma volume was not determined in the patients, the equations mentioned before were transformed to calculate the cumulative release expressed in units per liter of plasma:

$$Q(t) = P(t) + E'(t) + \text{FCR} \int_0^t P(\tau) d\tau$$

$$E'(t) = \text{TER} \cdot \int_0^t P(\tau) e^{-\text{ERR}(t-\tau)} d\tau$$

These processes determine the relation between (total) enzyme release and the plasma enzyme activity measured. The mathematical model mentioned before quantitates these processes, using parameter values from standard conditions for the enzymes involved, and finally results in cumulative values for enzyme release $Q(t)$.

It is obvious that the two compartment model and the derived $Q(t)$ values are useful in case of enzymes leaking from one specific tissue or organ. Under conditions of open heart surgery, calculations based on the measurement of AST and creatine kinase will overestimate the amount of damaged myocardial tissue because AST, creatine kinase and HBDH may also be released from non-cardiac sources. To correct for hemolysis, the amount of HBDH released during CPB was subtracted from the $Q(t)$ values calculated over the total postoperative period of 24 to 36 hrs. Cumulative values of CK-MB were not corrected for release from non-cardiac sources, such as arterial and venous vascular structure and skeletal muscle, since their contribution has been assumed to be small. The two remaining marker enzymes, AST and creatine kinase, can be released from skeletal muscle (SM) as well as from cardiac muscle (H).

Recently, a mathematical procedure was developed to discriminate between these two sources using the following equations:

$$Q_{AST} = I(AST;H) \cdot Q_H + I(AST;SM) \cdot Q_{SM} \quad (\text{eq 1})$$

$$Q_{CK} = I(CK;H) \cdot Q_H + I(CK;SM) \cdot Q_{SM} \quad (\text{eq 2})$$

In equation 1, Q_{AST} refers to the amount of AST released during the postoperative phase, assuming that only cytoplasmatic AST was released. $I(AST;H)$ and $I(AST;SM)$ refers to the content of cytoplasmatic AST in heart (H) and skeletal muscle (SM), respectively. These enzyme contents were reported to be 54 U.g^{-1} for heart tissue (Van der Laarse et al, 1980) and 32 U.g^{-1} for skeletal muscle tissue (Willems et al, 1982b). Q_{CK} , from equation 2, refers to the amount of creatine kinase released during the postoperative period. $I(CK;H)$ and $I(CK;SM)$ refers to the content of creatine kinase in heart muscle (798 U.g^{-1} according to Willems et al, 1979) and in skeletal muscle tissue (2800 U.g^{-1} according to Van der Laarse et al, 1980), respectively. Finally cumulative enzyme release from cardiac tissue (Q_H) and from skeletal muscle (Q_{SM}) after cessation of CPB can be calculated from the equations 1 and 2 by conversion to:

$$Q_{SM} = \frac{Q_{AST} \cdot I(CK;H) - Q_{CK} \cdot I(AST;H)}{I(AST;SM) \cdot I(CK;H) - I(CK;SM) \cdot I(AST;H)}$$

$$Q_H = \frac{Q_{AST} \cdot I(CK;SM) - Q_{CK} \cdot I(AST;SM)}{I(AST;H) \cdot I(CK;SM) - I(CK;H) \cdot I(AST;SM)}$$

Substitution of the known tissue enzyme contents and of the calculated cumulative release values of these enzymes results in Q_{SM} and Q_H values, reflecting gramequivalents of tissue irreversibly damaged.

3.2 Animal experiments

3.2.1 Animals and anesthesia

Investigations on hearts in situ implicate that one has to choose between pigs, calves and dogs, depending on the aim of the study. Dogs have generally been used in cardiopulmonary bypass (CPB) research during the past 30 years (Senning, 1952). Well documented information about anesthesia, CPB equipment and blood transfusion further stressed the preference of using the dog model in this specific study (De Vries, 1976; Van der Vusse et al, 1979; Jalonen, 1980; Prinzen, 1982).

Additional advantages of the use of dogs in this type of experiments, as compared to other species, are their seize and weight, making them suitable for handling. More specifically their vessels are strong and their heart is not very susceptible to surgical and anesthetic interventions. It should be emphasized that dogs allow the use of most of the clinical instrumentations, catheters and CPB devices, including oxygenators.

Comparing experimental and clinical models implies the consideration of differences in, for instance, anesthetic policy and blood constituents which may affect the hemodynamic and metabolic state. Another important difference is that in patients with obstructions of the coronary arteries myocardial areas with inadequate perfusion have to be considered. On the other hand various fairly similar conditions are observed in dogs and man, which validates most of the comparisons made in this study. The myocardial metabolism, the coronary vasculature (James, 1961), in particular the presence of collaterals (Schaper, 1971), myocardial and systemic blood flow, hemodynamic function and gross anatomy are comparable, which allows the extrapolation of data from animal experiments to the clinical situation.

Mongrel dogs of either sex and unknown age, ranging in weight from 18-36 kg were used in this study. Prior to the experiments the animals were housed individually for at least one week. The dogs were allowed to take water ad libitum and were fed with Doko dog food (Trouw, Putten; composition see technical notes 3.3). Data on body weight and heart weight is shown in appendix 1.

The animals were premedicated with Hypnorm (1 ml.kg^{-1}), containing 10 mg fluanisone and 0.3 mg fentanyl citrate per ml, as described by Marsboom and coworkers (1964). Anesthesia was induced intravenously with at least 10 mg per kg body weight sodium pentobarbital (Narcovet) and, after endotracheal intubation, was maintained with oxygen/nitrous oxide and a continuous infusion of sodium pentobarbital ($2 \text{ mg.kg}^{-1}.\text{hr}^{-1}$). The lungs were ventilated with a positive pressure respirator (Pulmomat). The inflation pressure was kept at 0.05-0.15 kPa and the respiratory volume at 10 ml per kg body weight. The respiratory rate was maintained at 18 inflations per min. If necessary, ventilation was adjusted on the basis of the blood gas values, which were kept within physiological ranges, i.e. PCO_2 : 4.7-6.0 kPa; pH: 7.35-7.45.

Prior to sternotomy, succinylcholine (2 mg.kg^{-1} body weight i.m.; Curatest) was injected to prevent muscle movement induced by electrocoagulation. The sternum was split over its total length and the pericardium was opened completely. Throughout the experiments two heating mattresses were placed underneath the animal to regulate body temperature. During hypothermic perfusions, fentanyl was administered at regular intervals (total amount of fentanyl not exceeding 0.5 mg). Just before cessation of CPB, the animals routinely received 500 mg calcium gluconate. There was no support with inotropic drugs in the animal experiments. Anesthesia was standardized as much as possible to minimize influences of different anesthetic protocols on the parameters measured.

3.2.2 Instrumentation

The experimental protocol and the sample scheme for the assessment of most parameters and variables of the 10 groups in the experimental study is given in section 3.2.3. The devices were installed in the following order:

- A Braunnulle was placed in the right brachial vein for compound administration.
- ECG cables were connected to the animals (limb leads).
- A polyethylene catheter was inserted into the left femoral artery and connected to an external pressure transducer to measure arterial pressure. This catheter was also used for sampling arterial blood.
- Right arterial pressure was measured with a polyethylene catheter connected

to an external pressure transducer. The catheter was inserted into the right atrium via the left femoral vein.

- A polyethylene catheter was inserted into the left brachial artery for sampling arterial blood as a reference for the calculation of regional myocardial blood flow, when using the microsphere technique (see section 3.2.10).
- After heparinization and sternotomy, a Swann-Ganz balloon guided thermistor catheter (7F, KMA) was inserted through the left jugular vein for cardiac output measurements, using the thermodilution technique.
- A stainless steel cannula, used for the arterial return from the oxygenator, was inserted into the right femoral artery.
- A thermistor (Ellab, type A-R7) was placed for the recording of rectal temperature. At the same time a urinary catheter was inserted if possible.
- The pericardium was opened and the aorta and the left anterior interventricular coronary artery (LAICA) were dissected. Electromagnetic flowprobes were mounted on the ascending aorta and the LAICA to measure volume blood flow. The flow probes (Skalar) were connected to an electromagnetic flow meter (Transflow 600).
- A 5F or 7F catheter was inserted into the left jugular vein and moved into the right atrium. Approximately 2 cm of the catheter ending was carefully placed in the coronary sinus for collection of blood.
- In all dogs of the cardioplegia group one thermistor (type A-K3, Ellab) was placed deep in the septum. In a few experiments, thermistors were placed in the anterior and posterior left ventricular free wall (thermistor, type A-KC1, Ellab).
- A silastic catheter was placed in the left atrium, allowing the injection of radioactive microspheres for the measurement of regional and total myocardial blood flow.
- Left ventricular pressure was measured with a catheter-tip micrometer (Millar, 7F micromanometer), inserted through the left atrium. The left ventricular end-diastolic pressure was separately recorded (amplification 0-3 kPa) and the first derivative was determined as described by Schaper and coworkers (1965).
- Epicardial deformation of the left ventricular free wall was measured using three coils situated on the epicardium as described in section 3.2.11. The field generating coil was attached by suction (underpressure not exceeding -25 kPa) and the two sensor coils were attached to the epicardium with a

suture string.

- Just prior to instalment of CPB, a tube was inserted in the right atrium for venous drainage by gravity. Immediately after the start of CPB the left ventricular vent was inserted through the apex.
- In the animals of the cardioplegia group a double lumen metal needle was inserted into the aortic root through the ascending aorta, for the infusion of the cardioplegic solution as well as for measurement of aortic perfusion pressure.

All hemodynamic variables and the epicardial deformation parameters were recorded continuously on a 10-channel Schwarzer recorder at a speed of 1.25 mm.s^{-1} and intermittently at speeds of 50 and 200 mm.s^{-1} . All thermistors were connected to an Ellab temperature display (Type DU-3).

3.2.3 Design of the experiments

- Introduction

Eighty four animals were included in this study and allotted to ten groups as shown in table 3.2. In this section all groups will be described in detail.

In the control group (CON), the animals were not subjected to CPB, and the influence of anesthesia and complete instrumentation on the hemodynamic variables, mechanical performance, myocardial substrate uptake and plasma enzyme activity was examined. These data served as reference values. Two groups were designed to evaluate the influence of extracorporeal circulation on the above mentioned parameters. For this purpose dogs were subjected to CPB without periods of ischemia. In one group systemic temperature during CPB was maintained at 34°C (Normothermic control; NC) and in the other group this temperature was kept at 25°C (Hypothermic control; HC). In the latter group the heart fibrillated continuously and in the NC group the heart was fibrillated four times during 10 min.

Three other groups were designed to simulate the patient situation. In two of them the animals were subjected to CPB and intermittent aortic cross-clamping, either normothermic (N) or hypothermic (H). In the third group the animals were subjected to CPB and the aorta was cross-clamped continuously. In this group (C) cardioplegic solution was infused into the aorta during

Table 3.2 Survey of the animal groups. + = present, - = absent, o = not relevant, CPB = cardiopulmonary bypass, AC = aortic cross-clamping.

Group nr	Group name	Abbreviation	Number of animals	CPB	AC	post CPB recovery
1	Normothermia	N	14	+	+	+
2	Hypothermia	H	10	+	+	+
3	Cardioplegia	C	8	+	+	+
4	Normothermia/Biopsies	NB	6	+	+	-
5	Hypothermia/Biopsies	HB	6	+	+	-
6	Normothermia/Control	NC	11	+	-	+
7	Hypothermia/Control	HC	11	+	-	+
8	Enzyme release/Control	EC	5	-	-	o
9	Hypothermia/Plasma volume	HPLV	5	+	+	+
10	Control	CON	8	-	-	o

Table 3.3. Variables and parameters measured in the groups N, H, C, NC, HC and CON.

hemodynamics	Heart rate, Pao,syst, Pao,dia, Plv, Plv,ed, dPlv/dtmax,pos, cardiac output, coronary flow.
plasma enzyme activity	AST, ALT, creatine kinase, MDH, HBDH.
biochemical variables in blood	PO ₂ , PCO ₂ , pH, Hb, O ₂ SAT, lactate, inorganic phosphate, potassium and glucose.
biochemical variables in tissue	ATP, creatine phosphate, inorganic phosphate, glycogen and lactate.
mechanical function	Epicardial deformation.
regional coronary artery flow	Left ventricular wall (subendo and subepicardium).

cross-clamping. In the groups N and H ischemia was induced four times during 10 min by aortic cross-clamping. Intermittent reperfusion lasted 15 min, analogous to the clinical situation. In group C the aortic cross-clamp period lasted for 60 min. In these three groups protection was evaluated by assessing hemodynamic and mechanical recovery, myocardial substrate uptake, plasma enzyme activity and myocardial temperature. In addition myocardial biopsies were taken in group C.

Two other groups were designed to study more extensively myocardial tissue metabolism during the CPB period. In one group normothermia (34°C) was applied (NB) and in the other one hypothermia (25°C; HB). Multiple transmural myocardial biopsies were collected at 12 sample points, mainly at the end of the ischemic and reperfusion periods, as shown in fig 3.4. Tissue samples were analyzed for glycogen, ATP, creatine phosphate, inorganic phosphate and lactate, and these data were related to the release and uptake of lactate and inorganic phosphate during reperfusion as measured in coronary sinus blood. The groups NB and HB were similar to the groups N and H, respectively, except for the multiple biopsies taken. Because the multiple biopsy holes can be expected to influence post CPB myocardial function, the experiments were terminated at the end of CPB.

Finally two groups were designed to analyse more precisely the enzyme release (EC) as a result of tissue damage and plasma volume changes (HPLV) after CPB. In group EC the animals were anesthetized and the thorax was opened through sternotomy. No other interventions were performed. Hemodynamic measurements such as heart rate and arterial pressure were recorded at regular intervals, and blood samples were taken to analyze plasma enzyme activities. Using this procedure enzyme release resulting from anesthesia and sternotomy, could be determined. These values were used to estimate enzyme release as the result of tissue damage for all other groups mentioned. The group HPLV (hypothermia with six plasma volume determinations) was designed to analyse plasma volume changes under circumstances similar to those in group H. CPB and intermittent aortic cross-clamping were included in the protocol, but the number of variables measured was kept to a minimum. Only hemodynamic variables, mechanical performance and plasma enzyme activity were determined during a post CPB recovery period of 2 hours instead of the routine 1 hour recovery period following CPB.

- Protocol of subgroups

Group N. Normothermia with intermittent aortic cross-clamping (n=14).

After complete instrumentation of the animals control measurements were performed (table 3.3). Subsequently CPB was started and the rectal temperature was maintained at 34°C. Within five minutes after the start of CPB, when the hearts were empty beating, a second set of measurements was performed. Thereafter ventricular fibrillation was induced and the next set of measurements was taken. The time course of CPB is shown in fig 3.4. The aorta was cross-clamped four times during 10 min and reperfused in between for 15 min. During cross-clamping the hearts were fibrillating and immediately after release of each clamp the hearts were defibrillated. During the first 10 min of reperfusion arterial and coronary sinus blood was sampled for biochemical analysis. This data will be presented as wash-out curves. Myocardial release and uptake of the substrates, mentioned in table 3.3, were calculated by multiplying arterial-coronary sinus differences and coronary artery blood flow. After the last cross-clamping the dogs were rewarmed and a reperfusion period of 45 min allowed the hearts to recover before cessation of CPB. After weaning from CPB the recovery of the dogs was studied during 60 min. Measurements were performed at two time intervals, one within 15 min and one at the end of the 60 min recovery period. Finally the dogs were killed with an overdose of thiopental. In some dogs biopsies of myocardial tissue were taken at the end of the experiment for biochemical determinations.

Group H. Hypothermia with intermittent aortic cross-clamping (n=10)

After the start of CPB the rectal temperature was lowered to 25°C using the heat exchanger in the oxygenator. Throughout the hypothermic period the hearts fibrillated. Cross-clamping was performed as described for group N. After the last clamp the dogs were rewarmed and defibrillated when rectal temperature exceeded 34°C. The protocol of the experiments is shown in fig 3.4 and the variables measured are summarized in table 3.3.

Group C. Hypothermic potassium cardioplegia with continuous aortic cross-clamping (n=8).

The protocol of the experiments is shown in fig 3.4. In addition to the variables assessed in the groups N and H, myocardial biopsies were taken

Fig 3.4 Protocols of the animal groups. AC = Aortic cross-clamping. CPB = Cardiopulmonary bypass. M = measurement of plasma enzyme activity, biochemical variables, regional coronary flow and mechanical activity. B = biopsies. (note that both hemodynamics and enzymes were measured at more sample times; see for details sections 3.2.5 and 3.2.7).

before and at the end of the period of global ischemia, at the end of CPB and after a 60 min recovery period. Rectal temperature was decreased to 25°C after the start of CPB. Unlike group N and group H the aorta remained cross-clamped during a period of 60 min. After cross-clamping the aorta an ice-cold St. Thomas hospital cardioplegia solution (for composition see technical note section 3.3) was infused into the aortic root with a roller pump. Infusion pressure as measured within the aortic root, did not exceed 5.3 kPa. Initially 300 ml of the cardioplegic solution was infused, sufficient to decrease septal temperature below 15°C. Infusion of the St. Thomas cardioplegic solution was repeated every 20 min (with approximately 250 ml) to maintain septal temperature at a level below 18°C. Data on septal temperature is shown in appendix 3. The dogs were rewarmed after release of the aortic clamp and defibrillated when rectal temperature reached 34°C.

Group NB. Normothermia with intermittent aortic cross-clamping and multiple left ventricular biopsies (n=6)

The protocol followed in these experiments was similar to that followed in group N, with the exception that during CPB multiple left ventricular biopsies for biochemical analysis were taken just before and at the end of each aortic cross-clamping (fig 3.4). The experiments were terminated at the end of the CPB period. The variables measured are listed in table 3.4.

Group HB. Hypothermia with intermittent aortic cross-clamping and multiple left ventricular biopsies (n=6)

The protocol of these experiments is shown in fig 3.4. The only difference between this group and group NB is the level of rectal temperature during CPB, which was maintained at 25°C in group HB. The variables and parameters measured are shown in table 3.4.

Group NC. Normothermia without intermittent aortic cross-clamping (n=11)

The protocol followed in this group is similar to that of group N, but during CPB aortic cross-clamping was deleted. The hearts remained empty beating during the CPB period, except for four periods of 10 min each, when they were fibrillating. The protocol of these experiments is shown in fig 3.4. During the CPB period rectal temperature was kept at 34°C. The variables and parameters measured for routine evaluation are listed in table 3.3.

Table 3.4. List of variables measured in group NB and group HB.

hemodynamics	Heart rate, Pao,sys, Pao,dia, coronary flow.
biochemical variables in blood	PO ₂ , PCO ₂ , pH, Hb, O ₂ SAT, lactate, inorganic phosphate, potassium.
biochemical variables in tissue	ATP, creatine phosphate, inorganic phosphate, glycogen and lactate.
regional coronary artery flow	Left ventricular wall (subendo and subepicardium).

Table 3.5. Variables measured in group HPLV.

hemodynamics	Heart rate, Pao,sys, Pao,dia.
mechanical function	Epicardial deformation.
plasma enzyme activity	AST, ALT, MDH, HBDH, creatine kinase.

Group HC. Hypothermia without intermittent aortic cross-clamping (n=9)

The protocol of these experiments was analogous to the protocol of group NC, except that the rectal temperature was decreased to 25°C (fig 3.4). The hearts remained fibrillating during the hypothermic period and were defibrillated after rewarming to 34°C as described for group H. The variables and parameters measured during the experiments are shown in table 3.3.

Group CON. Control group with complete instrumentation but without CPB (n=8)

The dogs were instrumented as in the groups N, H and C. Subsequently the dogs were studied during a period of 205 min without further intervention (fig 3.4). The variables and parameters measured during the experiments are shown in table 3.3.

Group EC. Enzyme release during control conditions (n=5)

After induction of anesthesia and sternotomy, enzyme release was studied for a period, comparable to the duration of the experiments in the groups N, H and C. All cardiac interventions were avoided (fig 3.4).

Group HPLV. Hypothermia with intermittent aortic cross-clamping and multiple plasma volume determinations (n=5)

The protocol of these experiments was similar to that in group H, but before and after CPB in total six plasma volume determinations were performed in controlled fluid infusion conditions. The total post CPB period lasted for 120 min. The protocol is shown in fig 3.4. and the variables and parameters measured during these experiments are summarized in table 3.5.

3.2.4 Cardiopulmonary bypass

In the extracorporeal circuit blood was drained by gravity from the animal to the oxygenator through a tube in the right atrium. The blood was returned from the oxygenator via a canula in the femoral artery. The cardiopulmonary bypass set-up is shown schematically in fig 3.5.

The blood was oxygenated with a Harvey (H200) or a Polystan (VT 5000)

Fig 3.5 Schematic representation of cardiopulmonary bypass. AC = aortic clamp, AD = apex drain; AV = aortic valve; HE = heat exchanger; L = lung; LV = left ventricle; OX = oxygenator; P1, P2, P3 = roller pumps; PC = peripheral circulation; RA = right atrium; RFA = right femoral artery; RV = right ventricle; S = suction; VC = venous clamp; VT = venous tube. CI = site of infusion of cardioplegic solution. The dotted area represents the extracorporeal circulation (After Van Renterghem, 1983).

bubble oxygenator, including a heat exchanger. A blood level detector (light sensitive photo cell) and a thermistor (Ellab, type A-F6) were installed. Polystan tubing was used in this circuit except for the roller pumps in which silicone tubing was employed. Arterial inflow was controlled with a Polystan pump supplied with a precalibrated flow scale. A Bentley and a Sarns roller pump were used to drain the left ventricular cavity and to collect intrathoracic blood, respectively. Intrathoracic blood and donor blood were stored in a Harvey Cardiomy Reservoir (H-700F) and filtered (5 micron filter) before return to the extracorporeal circuit.

The oxygenator was primed with 1 l of fresh heparinized donor blood, 1000 IU of heparin, 2 l of Normosol and 80 ml of sodium bicarbonate (4.2%). Since plasma measurements showed an initial drop in ionized calcium content in the first phase of CPB of $0.29 \pm 0.03 \text{ mmol.l}^{-1}$, 1 g of calciumgluconate (in solution) was added to the priming solution (Van der Veen et al, 1983). Heparinized donor blood was collected on the day of the experiments, or ultimately 2 days before and stored at 5°C if necessary. The priming solution was equilibrated with 3 l oxygen and 2 l carbogen (95% O₂, 5% CO₂) per minute at 37°C. During the CPB period the CO₂ percentage in the sweep gas of the oxygenator was measured using a Mijndhardt infrared gas analyzer. Routinely this CO₂ percentage was kept at 4-5%, corresponding to an arterial PCO₂ of 4.7-6.0 kPa. At the same time arterial PO₂ was kept above 21 kPa. Only during hypothermic perfusions (25°C) the PCO₂ of arterial blood was lowered to 1.3-2.7 kPa, resulting in an increase of pH to 7.6-7.8, according to Becker and coworkers (1981). Changing blood temperature resulted in different blood gas and pH values which could be corrected in the ABL-3 after introducing the actual value of the blood temperature. Heparinization was started at the time of sternotomy with a bolus of 250 IU heparin.kg⁻¹ body weight intravenously, resulting in an increase of the actual clotting time (ACT), as measured on a Hemochron 400 (Bull et al, 1975). In the normothermia, hypothermia and cardioplegia groups the ACT increased from 108 to 257, 100 to 333 and 101 to 202 seconds prior to CPB, respectively. No further heparin was administered unless ACT became less than 400 seconds during CPB.

When all connections were made, the arterial pump was started at a rate of 0.3-0.5 l per min. The venous return was partially opened to maintain the initial oxygenator content. After at least five minutes, this exchange phase was terminated and bypass was started. Initially the arterial inflow was

adjusted according to the pre CPB cardiac output values and the venous out-flow tract was opened completely. Meanwhile blood within the extracorporeal circuit was cooled to 25°C in the groups H, HC, HB and C, and to 34°C in the groups N, NC and NB as is shown in appendix 2.

During the CPB period additional fluid for the oxygenator was selected on the basis of the hematocrit value. Donorblood was given when the hematocrit had decreased below 25%. In all other instances Normosol or Haemacel was added to the oxygenator. Mean arterial blood pressure did not exceed 10.7 kPa by adjusting the pump flow rate. The data on perfusion pressure and pump flow during CPB is shown in appendix 5, and indicates that under normothermic circumstances adequate perfusion was generally associated with lower perfusion pressures. Systemic venous pressure was generally within the physiological range (0.4-0.8 kPa). In groups with normothermic perfusion conditions, induction of fibrillation was performed by touching the heart with battery electrodes. In all experiments the hearts were defibrillated with a DC defibrillator (B-D Electrodyne) delivering 40 Joule totally.

3.2.5 Hemodynamic measurements

- Blood pressure

Systolic and diastolic arterial blood pressure were assessed from the instantaneous tracings and mean pressure was calculated by adding systolic and twice the diastolic value, and dividing this sum by three. The venous catheter, inserted into the right atrium, through the left femoral vein, was used for the registration of central venous blood pressure. All catheters were connected to external pressure transducers (Ailtech). Prior to each experiment the pressure transducers were calibrated, using a mercury column. The Millar catheter-tip micromanometer was calibrated before the experiments. During the experiment, the zero drift, if any, was checked by connecting the catheter lumen to an external pressure transducer (Ailtech).

- Blood flow

From aortic blood flow tracings the start and the end of the ejection phase was estimated. This information is required for the assessment of left ventricular mechanical function (see section 3.2.11).

Changes in coronary blood flow were continuously recorded with the electromagnetic flow probe, mounted on the LAICA, just distal to the site of branching from the main left coronary artery. These flow probes were calibrated in vivo on a dog hind leg artery with the use of a Harvard withdrawal pump.

- Cardiac output

Cardiac output was measured by thermodilution, using a Swann-Ganz balloon guided thermistor catheter (KMA). This triple lumen catheter (7F) was connected to a KMA Cardiac Output Computer (Model 3500 E) combined with a KMA Mini-Corder (Model 3501). Before each experiment, the cardiac output computer was checked by internal calibration simultaneously shown on the recorder. During the experiment cardiac output measurements were performed in triplicate. Just prior to the injection of 5 ml ice cold saline and after the saline was injected manually, the content of the catheter was withdrawn to prevent cold dissipation from the catheter according to Snoeckx and coworkers (1976).

3.2.6 Sampling of tissue

Transmural myocardial biopsies were taken from the left ventricular free wall in the perfusion area of the LAICA as described in section 3.1.4. Biochemical evaluation of the tissue included the determination of ATP, creatine phosphate, glycogen, lactate and inorganic phosphate.

Myocardial biopsies were routinely collected at the end of each experiment (the groups N, H, C, NC, HC, CON). An additional series of biopsies was taken during the experiments in the groups C, NB and HB. Biopsies in these three groups were taken within 5 min after the start of CPB (preischemic biopsies, similar to the clinical study) and at the end of each ischemic and reperfusion phase. All biopsies were immediately (within 3 s) frozen in liquid nitrogen and stored at -80°C until further analysis. The biopsies were subsequently freeze-dried and adherent blood was carefully removed.

3.2.7 Sampling of blood

Chemical substances were determined in either coronary sinus blood, or arterial blood. During the period of CPB arterial blood was collected from the oxygenator.

For the determination of lactate, inorganic phosphate, potassium and oxygen 5 ml of blood were used at the following sample times: 1) before CPB, during a period of stable hemodynamic function, 2) after onset of CPB when the heart was empty beating and the left ventricle was vented, 3) just before cross-clamping of the aorta when the heart was fibrillating in cases of hypothermic perfusions, 4) in the immediate reperfusion phase after each period of aortic cross-clamping within 30 seconds and at 1, 2, 3, 4, 5, 6, 7.5 and 10 min and 5) during the post CPB period at 5, 10, 15 and 60 min after cessation of CPB. During CPB experiments without aortic cross-clamping, the sample times were reduced during the period of CPB and blood samples were collected at 15, 45, 75 and 105 min after the start of CPB. All blood samples were immediately centrifuged and plasma was kept at -80°C until analysis. The arterial glucose concentration was measured before CPB, 60 and 135 min after the start of CPB, and 15 min after weaning from CPB.

Plasma enzyme activity (AST, ALT, creatine kinase, MDH, HBDH), free hemoglobin content and hematocrit were determined routinely using arterial blood samples of 8 ml. These samples were collected prior to CPB, at 10, 45, 75 and 105 min after the start of CPB as well as 15 and 60 min after cessation of CPB. Blood samples were centrifuged at 1000 g during 15 min and subsequently stored at -80°C . The hematocrit was measured immediately.

Arterial blood gases were measured routinely as an indication of adequate anesthesia and ventilation. Arterial and coronary sinus blood (40 μl) were separately analyzed for oxygen saturation and hemoglobin content at all sample times mentioned for blood analysis.

During the instrumentation of the dog, the actual clotting time was measured prior to and after heparin administration as well as 5, 35 and 105 min after the start of CPB. At 15 and 60 min after CPB blood samples were analyzed as well.

3.2.8 Biochemical analysis of tissue and blood

- Blood

The analysis of chemical substances in blood samples was similar to that reported in the patient study (section 3.1.8). Glucose was determined in arterial blood samples by spectrophotometry on a Technicon AAI autoanalyzer with hexokinase and glucose-6-phosphate dehydrogenase, as reported by Bergmeyer and coworkers (1974).

- Chemical substrates in myocardial tissue

The tissue specimens were prepared as described for ATP, creatine phosphate and glycogen analysis (section 3.1.7). Shortly, the dried biopsy was crushed in a 3 mol.l^{-1} perchloric acid solution at -15°C . This mixture was neutralized with KHCO_3 and after centrifugation aliquots were obtained from the supernatant for the determination of ATP, creatine phosphate, inorganic phosphate and lactate. The residue was used for glycogen analysis. The biochemical analyses for ATP, creatine phosphate and glycogen were reported in section 3.1.7.

Inorganic phosphate was analyzed by spectrophotometry. The reaction mixture for inorganic phosphate contained malachite green-HCl (0.06% solution) and sodium molybdate (0.3% solution). Measurements were performed at 625 nm (according to Van Belle, 1970). The reaction mixture for lactate (analyzed by fluorometry at 25°C) contained per 100 ml tridest water: 4 ml Hydrazine solution (1 M), 0.8 ml LDH suspension (3400 U.ml^{-1}), 0.5 ml NAD^{+} solution (0.1 M), and was adjusted to pH 9.8 according to Passonneau (1974).

- Plasma enzymes

In the animal experiments plasma samples were analyzed for creatine kinase, AST, ALT, HBDH and MDH. The analysis techniques for AST, creatine kinase, HBDH and free hemoglobin have been described in section 3.1.8. ALT (EC 2.6.1.2) was measured using α -ketoglutarate as a substrate according to Bergmeyer and Bernt (1974a). MDH (EC 1.1.1.37) was determined using α -ketoglutarate as a substrate according to Bergmeyer and Bernt (1974b) using a testkit (Boehringer 124940). Hematocrit values were determined immediately after collection of the blood samples by the microcapillary method of McGovern and coworkers (1955). Values are expressed as the percentage of

total blood volume.

- Blood gases and blood pH

Determination of blood gases and blood pH was performed at 37°C using a blood gas analyzer (Radiometer ABL 3). Blood pH was measured potentiometrically using a pH glass-electrode and a calomel reference electrode. The CO₂ partial pressure (PCO₂) was determined using a PCO₂ electrode, and O₂ partial pressure (PO₂) was determined with an amperometrical method. When during CPB body temperature had decreased, temperature corrections were made.

3.2.9 Plasma volume determination and cumulative enzyme release

Plasma volume was measured in a selected number of experiments before and after CPB. The technique of plasma volume determination has been described in detail by Visser and coworkers (1982) and involves the intravascular injection of a known activity of enzyme. The subsequent increase in plasma enzyme activity quantitates the contribution of plasma to the intravascular volume.

Prior to injection of the enzyme bolus one reference blood sample was collected. Then a concentrated amount of AST (Sigma; purified from porcine heart) or ALT (dog liver extract) was introduced quantitatively into the left atrium. Approximately 250 U of AST or 500 U of ALT were administered during each plasma volume determination. Exactly 5 min after injection of the enzyme bolus one blood sample was withdrawn for measurement of enzyme activity.

Cumulative release of enzymes was calculated during the last 45 min of the experiments starting at 15 min after weaning from CPB and 60 min after CPB. Both creatine kinase and AST were appropriate for this purpose since the characteristics of the rate of enzyme breakdown in dogs were available for these enzymes. In the groups without extracorporeal circulation (group CON and group EC) the data on plasma volume, transport of enzyme from the plasma to the extravascular pool (TER) and the intravascular degradation of enzyme (FCR) was assumed to follow the normal values and rates. In the remaining groups (N, H, C, NC and HC) changes in plasma volume and TER, as observed in group HPLV, were employed. Because known values of the plasma volume could be employed in the animal experiments, the cumulative release is expressed in units of enzyme activity. In the patient study plasma volume was not measured

and therefore cumulative release was calculated as units per liter of plasma (see section 3.1.9).

Calculation of the cumulative release from two values of plasma activity was performed using the formula:

$$\Delta A = P1 - P2 + k_d \times T \frac{P1+P2}{2} \quad (\text{eq. 1})$$

where: ΔA = cumulative enzyme release (U)

$P1$ = plasma enzyme content at $t1$ (U)

$P2$ = plasma enzyme content at $t2$ (U)

k_d = apparent enzyme disappearance constant

T = total time interval between $t1$ and $t2$ (hr)

and $P1 = V1 \times C1$

$P2 = V2 \times C2$

where $V1$ = plasma volume at $t1$ (l)

$V2$ = plasma volume at $t2$ (l)

$C1$ = plasma enzyme activity at $t1$ (u.l^{-1})

$C2$ = plasma enzyme activity at $t2$ (u.l^{-1})

Therefore eq. 1 transforms to:

$$\Delta A = V1 \times C1 - V2 \times C2 + k_d \times T \frac{V2 \times C2 + V1 \times C1}{2} \quad (\text{eq. 2})$$

The apparent enzyme disappearance constant (k_d), as introduced in a two compartment model for enzymes, is determined by both TER and FCR ($k_d = \text{TER} + \text{FCR}$) (Hermens et al, 1982). These authors measured the transcapillary escape rate ($\text{TER}=0.031$) and the fractional catabolic rate for AST ($\text{FCR}=0.21$) and for creatine kinase ($\text{FCR}=0.36$) in dogs. The control values of plasma volume as measured in the present study were 1.19 ± 0.36 l (mean $\pm$ SD; $N=17$).

Subsequently calculations were performed in the groups without cardiopulmonary bypass with the use of normal circulatory parameters, whereas in the groups with cardiopulmonary bypass the data as obtained in group HPLV

were employed.

Group CON and group EC:

In these two groups without CPB the cumulative release of CK and AST was calculated using equation 2 and known values of plasma volume (1.2 l), k_d of AST (0.24), k_d of creatine kinase (0.39) and T (0.75 hrs). Using these values, equation 2 can be written as follows:

$$\text{AST: } \Delta A = 1.26 C_2 - 1.13 C_1$$

$$\text{CK: } \Delta A = 1.31 C_2 - 1.09 C_1.$$

Groups with CPB:

From the observations in group HPLV we can conclude that PLV and TER changed after the period of CPB. Plasma volume was 140% at 15 min after CPB (t_1) as compared to 60 min (100%) after CPB (t_2). The transport of enzyme from plasma to the extravascular pool (TER) was increased from 0.031 to 0.48 throughout the period after CPB. These data changes equation 2 into:

$$\text{AST: } \Delta A = 1.38 C_2 - 1.38 C_1.$$

$$\text{CK: } \Delta A = 1.42 C_2 - 1.01 C_1.$$

3.2.10 Microsphere technique

- Introduction

The microsphere method was used to measure regional blood flow (Rudolph and Heymann, 1967). In several studies (Domenech et al, 1969; Buckberg et al, 1971; Yipintsoi et al, 1973; Utley et al, 1974) it was shown that the fraction of injected radio-activity retrieved in various organs is similar to the fraction of cardiac output, reaching these organs during the transit of the microspheres. If the flow to one organ (for example a reference organ) and its microsphere content is known, the flow to any other organ with a known microsphere content can be calculated.

Since both Heymann and coworkers (1977) and Schamhardt (1980) reviewed the microsphere technique extensively, and Prinzen (1982) has reported on the accuracy of the method used in our laboratory, the microsphere method will be described only briefly.

- Choice of the type of microspheres

In the present study microspheres with a diameter of 15 μm were used because they seem to be the best compromise between the particle size induced deviation from normal transmural blood flow and the entrapment of microspheres in the microvessels (Andersen et al, 1983; Meesmann et al, 1983). Moreover, microspheres of 15 μm are less expensive as compared to the smaller sized ones. The specific activity of the microspheres used in this study was lower than that normally used. The reduced specificity was applicable because at a specific activity of 3 mCi.g^{-1} the number of microspheres per sample rather than the gamma counting determines the accuracy of the method. Additional advantages are the lower radiation load for the investigator and the more favourable price per number of microspheres.

- Checks before experimental use

The microspheres were purchased from New England Nuclear (Boston, USA). Microspheres were delivered in injection vials, suspended in 10% Dextran with 0.05% Tween 80 as surfactant to reduce aggregation of the microspheres. Before use, each batch was checked for: a) purity of the nuclide, b) specific activity of the microspheres, c) integrity of the spheres, d) leakage of the nuclides from the spheres and e) diameter distribution of the beads.

Ad a):

Purity of the nuclide is required for accurate gamma-spectrometry. The purity was checked by analysis of the spectrum of a small sample of each batch on the monitor of the Multi-Channel Analyzer. In each batch the right nuclide was delivered and no contamination with other nuclides was found.

Ad b):

In each batch of microspheres the specific activity was measured to check whether the materials delivered were according to the manufacturer's specifications. Furthermore, for accurate determination of the number of microspheres in each blood or tissue sample, the specific activity of the beads has to be known precisely. A droplet of a diluted suspension of microspheres was spread on a piece of graph paper and the number of microspheres (usually 2000-4000) was counted microscopically. After this procedure the radioactivity of this piece of paper was counted in a gamma counter. Subsequently, it served as an isotope reference sample during counting of each series of samples from an experiment (see Experimental use of microspheres).

Ad c):

Damaged microspheres may have a different flow behavior, may fail to be entrapped and may cause leakage of the nuclide from the spheres. These factors negatively influence the accuracy of the method. Therefore, microsphere integrity was checked microscopically in a small sample at an enlargement of 100x. A few hundred beads were checked per sample. Routinely, less than 1% damaged spheres was observed.

Ad d):

Substantial leakage of nuclides from the spheres will invalidate the method because in this case injected radioactivity is not only trapped in the microcirculation, but will also circulate as soluble radioactivity. This leakage was checked as follows. A sample of microspheres was taken from the batch, sedimentated and once washed to mimic the treatment of microspheres under experimental conditions. After one ml 10% Dextran and 0.05% Tween 80 was added, and the tube was thoroughly shaken on a Vortex agitator during 1 min and sonificated in an ultrasound bath during 5 min, the content of the tube was counted. Thereafter, the suspension was mixed again on the Vortex and subsequently filtered through a 0.22 μm millipore filter (Cathivex). The supernatant was counted and the percentage leakage was calculated. This acute leakage was maximally 0.15%.

The leakage at 37°C over longer periods of time was determined in all batches of microspheres. Samples of these batches were suspended in 10% Dextran solution after vigorous shaking and were stored in a 37°C water bath for 1 week. Intermittently the suspensions were shaken and after this period leakage was determined in the same way as in the acute leakage experiments. The values of leakage were found to be about twice as high as the acute leakage values, which is in agreement with the manufacturer's specifications.

Ad e):

Diameter distribution of the microspheres. The importance of a narrow diameter distribution of each batch of microspheres has been emphasized by Reneman and coworkers (1975). Because beads of different size have different rheological properties, a large variation in microsphere size will also introduce larger coefficients of variation. For this reason and also to check whether the right size of spheres was delivered, in each batch the diameter distribution was determined in a sample of 50 microspheres. The diameter of the spheres was measured using an image splitting eye piece (Vickers A.E.I.) mounted on a light microscope. The observed diameters and their distributions were comparable with the data provided by the manufacturer.

- Experimental use of microspheres

The number of spheres in each batch was calculated using the data on total activity (mCi) and specific activity (mCi.g^{-1}) as well as the number of spheres per gram as provided by the manufacturer. From each batch a stock suspension in fresh 10% Dextran and 0.05% Tween 80 was made containing about 5×10^6 microspheres per ml. Prior to each experiment, the required number of microspheres was taken from this stock solution and diluted twice in 10% Dextran in a 5 ml syringe. The syringe was closed by a stopcock and thoroughly shaken on a Vortex agitator to suspend all microspheres prior to injection. Then the syringe was put in a beaker in an ultrasonic bath. The beads were sonicated for 5-10 min to break up clumps of beads, occasionally present (Reneman and Verheyen, 1977). Shortly before injection, the syringe was taken out of the bath and again shaken on the Vortex mixer for 1 min. Within 1 min after the last procedure, the microspheres were injected into the left atrium via a silastic catheter (inner diameter 2 mm, length about 25 cm). The syringe was washed once with about 3 ml physiological saline and subsequently the catheter was flushed with another 7 ml of physiological

saline to remove residual microspheres. In case of microsphere injection during CPB, the left atrium could not be used as the site of injection. Considering the retrograde aortic perfusion during CPB, the microspheres were injected into the arterial line, in between the oxygenator and the main pump.

An arterial reference sample was taken from the brachial artery at a rate of about $20.7 \text{ ml} \cdot \text{min}^{-1}$, using a Harvard withdrawal pump. Suction of blood started 5 s before the injection of the microspheres and was continued for at least 1 min. The arterial blood was put into test tubes and the sample syringe was flushed once with a few ml of water. After the experiment the heart was excised, rinsed and stored in formaldehyde 5%. The free wall of the left ventricle was cut into 5 slices (parallel to the base of the heart). Each slice was cut into 5 pieces of approximately 3 gram, and each piece was divided into a subendocardial, a middle and a subepicardial layer. Only the subendocardial and subepicardial data is presented (see fig 3.6). Total flow and transmural differences were calculated from a selected number of tissue pieces. These five pieces correspond to B2, D2, C3, B4 and D4 in fig 3.6. Overall myocardial blood flow was calculated from these pieces assuming that the flow was uniformly distributed. All tissue samples were put into 10 ml PVC test tubes. These tubes were weighed empty and with the sample. The weighing results were typed in on a data-entry system with a portable memory (3.84 kbytes). In the Packard Multi-Channel Analyzer the following samples were counted according to the MIC II program input (Schosser et al, 1979): background samples, isotope reference samples, subtract samples (meant for empty syringes), arterial and venous reference samples, and tissue samples. To be able to distinguish between the radioactivity of different nuclides within one sample as many counting windows were programmed as isotopes were present. Each window was located around the photopeak(s) of an isotope, in such a way that the overlap of other nuclides in that window was as small as possible. The spectrum of each isotope could be visualized on a display, facilitating the positioning of the windows.

- Calculation of regional myocardial blood flow

Data obtained from the gammacounter, e.g. counting time and the gross counts in each window, were printed on paper and on papertape. The papertape data were fed into the MINC II computer, using a Facit papertape reader, and stored on file on a floppy disc. The weighing data in the portable memory

Fig 3.6 Sectioning of left ventricular free wall for transmurality blood flow measurements. (Reproduced with permission of Prinzen et al, 1981).

were read into the computer by a data entry system (manufactured at the University of Limburg) in octal code and subsequently converted into alpha numeric figures. By using a screening program these data were checked on errors in the input and the data were ranked in negative numbers (for the empty tubes) and positive numbers (for tubes with samples) for direct use in the MICRO program.

The MICRO program is based on the MIC II program of Schosser and coworkers (1979). Shortly, the analysis of the energy spectra is performed according to the matrix method. Isotope reference samples (containing a certain amount of one pure isotope each) are measured to obtain the overlap matrix. The unknown amounts of the nuclides in the samples are calculated by solving a set of linear equations. The original MIC II program has been changed for convenience with respect to calculation of the ratio count per min (cpm) per microsphere. This ratio was originally determined by dividing activity of the whole injectate by the estimated number of injected microspheres. Direct gamma counting of the injectate, however, is not feasible, because a high dose of radioactivity is required when a large experimental animal is used. Therefore, from each nuclide 2000-4000 microspheres were counted microscopically on a piece of graph paper. This piece of paper was carefully brought into a counting tube to serve as a reference. Manual input into the MICRO program of this number of microspheres, together with the paper tape input of the counting data, results in the cpm per microsphere. Subsequently the counts per gram of tissue for each isotope in the tissue were calculated, and then the local myocardial flow (expressed in $\text{ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$) was obtained from the equation:

$$\text{myocardial blood flow} = \frac{\text{CPM (tissue)} \times \text{reference flow}}{\text{CPM (reference sample of arterial blood)}}$$

3.2.11 Myocardial mechanical function

- Introduction

Beside the routine determination of global myocardial mechanical function by means of the measurement of left ventricular pressure and its maximal positive first derivative ($dP_{lv}/dt_{max,pos}$), local mechanical performance was

Fig 3.7 Rectangle OABC deforms to parallelogram OA'B'C'. Deformation is defined by natural strain e_c along AO, natural strain e_z along OC and shear angle e_{zc} .

Fig 3.8 Measurement of the deformation parameters. A magnetic field rotates around the magnetic field generating coil (MFGC) and induces voltages V_c and V_z in sensor coils SC_c and SC_z , respectively. The deformation parameters are determined from the amplitudes of and the phase difference between V_c and V_z

assessed, using an electromagnetic induction technique (Arts and Reneman, 1980). This technique enables the measurement of epicardial deformation during the ejection phase, and allows the estimation of fiber shortening in subepi and subendocardial layers as well as the assessment of torsion of the left ventricular free wall (Prinzen 1982, Prinzen et al, 1984).

- Epicardial deformation

The principle of measuring epicardial deformation is based on assessing the distance between three coils attached to the epicardial surface in a predetermined position. Theoretically the three coils are part of the rectangle OABC as shown in fig 3.7. During contraction this rectangle deforms to a parallelogram OA'B'C'. O is assumed to be the origin, OA and OA' the direction along the c-axis (circumferential direction) and OC and OC' the direction along the z-axis (base to apex direction, perpendicular to the c-axis). The deformation of the rectangle can be described by the natural tangential strain (e_c), the natural axial strain (e_z), and the shear angle e_{zc} as defined in fig 3.7. The variables e_c , e_z and e_{zc} determine the dynamic change in the position of the three coils with the addition of torsion.

In the practical situation, one magnetic field generating coil (MFGC) and two sensor coils (SC) are situated within the perfusion area of the LAICA at the outer surface of the left ventricular free wall. The MFGC is attached at the origin O and the two sensor coils at the position A and C as indicated in fig 3.7. The MFGC is supplied by a three-phase alternating-current system, thus generating a rotating magnetic field (fig 3.8). This magnetic field induces a voltage in each sensor coil, proportional to the time derivative of the magnetic field strength. Changes in the induced voltages are subsequently used to calculate strains between the MFGC and sensor coils. The angle $90^\circ - e_{zc}$ equals the phase difference between the voltages induced in the sensor coils (fig 3.8).

The changes in the induced voltages and the phase difference were registered with a recorder (Schwarzer). The actual changes of the strains were recorded as 0.2, 0.4 or 0.8% per mm and the phase differences as 0.005 or 0.01 rad per mm.

- Subepi and subendocardial fiber shortening

From the epicardial deformation parameters e_c , e_z and e_{zc} , the direction

and magnitude of minimal and maximal shortening can be calculated. The direction of maximal shortening approximately coincides with the fiber direction in the outer layers (Arts et al, 1982; Prinzen et al, 1984). Consequently, the magnitude of maximal shortening at the epicardium ($e_{\max}$) is almost equal to shortening along the fibers in the outer layers. The direction of minimal shortening at the epicardium is approximately along the fiber direction in the inner layers (Prinzen et al, 1984). A mathematical model of left ventricular mechanics predicted that the magnitude of fiber shortening in the inner layers can be estimated ($e_{\text{endo,est}}$) using:

$$e_{\text{endo,est}} = 1.42 e_{\min} + 0.78 e_{\max}$$

where:

$$e_{\min} = 0.5 (e_c + e_z) - 0.5 \sqrt{(e_c - e_z)^2 + e_{zc}^2}$$

$$e_{\max} = 0.5 (e_c + e_z) + 0.5 \sqrt{(e_c - e_z)^2 + e_{zc}^2}$$

The calculated values of $e_{\text{endo,est}}$ were found to correlate well with shortening in the inner layers, as measured approximately along the fiber direction (Prinzen et al, 1984). Therefore, in the present study $e_{\text{endo,est}}$ was used as estimate of fiber shortening in the inner layers. This approach to assess fiber shortening across the myocardial wall is advantageous because the magnitude of $e_{\max}$ and $e_{\min}$ is essentially independent of the orientation of the inductive coils with respect to the fiber direction.

3.3 Technical notes

Chemical composition of solutions and dog food:

<u>Normosol (R)</u>	NaCl	90	mmol.l ⁻¹
	Na-acetate	43	mmol.l ⁻¹
	Na-glutamate	23	mmol.l ⁻¹
	KCl	5	mmol.l ⁻¹
	MgCl ₂	0.7	mmol.l ⁻¹

St Thomas hospital cardioplegia

NaCl	92	mmol.l ⁻¹
NaHCO ₃	25	mmol.l ⁻¹
KCl	15	mmol.l ⁻¹
MgSO ₄ .7H ₂ O	1.2	mmol.l ⁻¹
MgCl ₂ .6H ₂ O	15	mmol.l ⁻¹
KH ₂ PO ₄	1.1	mmol.l ⁻¹
CaCl ₂ .2H ₂ O	1.2	mmol.l ⁻¹

Haemacel (R)

NaCl	145	mmol.l ⁻¹
K ⁺	5.1	mmol.l ⁻¹
Ca ²⁺	6.25	mmol.l ⁻¹
polypeptides	3.5	%

Doko Food

Protein	28	%
Fat	9	%
Linoleic acid	1.7	%
Water	10	%
Carbohydrates	48	%
Minerals and vitamins	trace amounts	

3.4 Statistical analysis

The data from the patient study are presented as median values and 95% confidence limits or as mean values and standard deviations. Differences in the values of the various variables were evaluated for statistical significance in two manners. Intraindividual differences (within one group) were analyzed by applying Wilcoxon's matched pairs signed-rank test (two-tailed probability) using the program BMDP 3S. Intergroup differences were evaluated for statistical significance with Kruskal-Wallis non-parametric one-way analysis of variance with the BMDP 3S program. The changes in arterial-coronary sinus differences of biochemical substances in blood, as determined immediately after the periods of aortic cross-clamping, were evaluated for statistical significance with the randomization analysis of univariate responses according to Zerbe (1979).

The data from the animal experiments are presented as mean values and standard deviation, or as mean values and standard error of the mean. Intraindividual differences within one group were analyzed using Wilcoxon's matched pairs signed-rank test (two-tailed probability) with the BMDP 3S program, or with the use of the two sample t-test (BMDP 3D program) when the number of observations was less than five. Intergroup differences were evaluated for statistical significance by applying the one sample t-test with the BMDP 3D program.

4. RESULTS

4.1. Patient study

4.1.1. Clinical outcome

Patients in the clinical study were treated according to a routine anesthetic policy before, during and immediately after the surgical procedure. Only when mean arterial blood pressure had decreased to 70% or less of the control value, postoperative inotropic support was applied. For this purpose dopamine was administered to two patients in each group within one hour after cessation of CPB.

One patient in each group died within 3 weeks after the operation. One patient died because of bacterial sepsis in combination with respiratory complications (hypothermia group), one because of bacterial sepsis alone (cardioplegia group), and one because of heart failure (normothermia group) due to an occluded graft on the right descending coronary artery, as was observed at autopsy.

The intraoperative data of the patients of group N, H and C are shown in table 4.1. The number of distal grafts was not significantly different between the three groups. In groups N and H the mean duration of aortic cross-clamping varied from 9 to 10 min for the first through fourth cross-clamping and the intermediate reperfusion periods lasted between 12 and 14 min. The differences between groups N and H were not statistically significant. The total duration of aortic cross-clamping was significantly longer in group C (63 ± 13 min) than in group N (42 ± 11 min) and group H (45 ± 11 min). The total CPB time was significantly shorter in group N.

Table 4.1 Intraoperative data of group N (normothermia), group H (hypothermia) and group C (cardioplegia). AC = aortic cross-clamping, CPB = cardiopulmonary bypass. Mean values and S.D. are presented.

	GROUP		
	N	H	C
number of distal grafts	4.2±0.5	4.7±1.1	4.5±0.9
duration of aortic cross-clamping (min):			
first AC	9±3	10±3	63±13
second AC	10±3	10±4	
third AC	10±4	10±3	
fourth AC	10±2	9±2	
duration of intermediate reperfusion periods (min):			
first reperfusion	13±6	14±6	
second reperfusion	14±6	13±4	
third reperfusion	14±6	12±7	
total duration of aortic cross-clamping (min)	42±11	45±11	63±13*
total CPB time (min)	128±21*	151±26	144±23

*= significantly different from the other two groups ($p<0.05$).

4.1.2. Hemodynamics

The values of the hemodynamic variables as measured before and after CPB are summarized in table 4.2. Heart rate significantly increased in all three groups, whereas both systolic ($P_{ao,sys}$) and diastolic ($P_{ao,dia}$) arterial blood pressures significantly decreased as compared to their corresponding pre CPB values.

PWP was significantly increased in group H and group C, but was unchanged in group N. After CPB the maximum positive first derivative of left ventricular pressure ($dP_{lv}/dt_{max,pos}$) was significantly decreased, whereas cardiac index (CI) was significantly increased in group N and group C. A relatively small but significant decrease in left ventricular stroke work index (LVSWI) was noticed in all three groups 15 min after weaning from CPB.

Perfusion pressure (mean arterial blood pressure) and arterial pump flow during the period of CPB is shown in fig 4.1 and fig 4.2, respectively. Both during the first 15 min and the final period of CPB perfusion pressure was considerably depressed in all groups, as compared to the mean arterial blood pressure prior to CPB. In comparison with groups H and C, patients in group N had higher perfusion pressures immediately after the start of CPB, preceding the first aortic cross-clamping, while pump flow was similar in all three groups. This indicates a higher systemic vascular resistance in group N. Around 45 to 75 min after the start of CPB pump flow in group N exceeded pump flow in the groups H and C.

Data on rectal and systemic blood temperatures, which were registrated continuously during the surgical procedure, are shown in fig 4.3 and fig 4.4, respectively. The rectal temperature was set at 25°C in the groups C and H, and at 34°C in group N by means of the heat exchanger in the oxygenator. A small delay in the decrease of rectal temperature was noticed in comparison to systemic blood in both hypothermically perfused groups. A similar phenomenon was observed during rewarming after the last aortic cross-clamp.

Septal temperature in group C was kept below 18°C throughout the period of cardioplegic infusion. The latter temperature was registrated intermittently with a needle probe pierced into the septum after performance of each distal graft.

Table 4.2. Hemodynamic variables in the three groups before and 15 min after CPB. Median values and 95% confidence limits are presented.

	GROUP					
	Normothermia (n=24)		Hypothermia (n=23)		Cardioplegia (n=25)	
	before CPB	after CPB	before CPB	after CPB	before CPB	after CPB
heart rate (beat. min ⁻¹)	79 65-90	97* 90-101	74 64-80	94* 83-98	72 63-84	89* 79-93
Pao,sys (kPa)	15.9 14.3-16.8	13.7* 12.3-14.8	15.2 13.6-16.4	12.1* 10.8-13.1	14.8 14.0-16.1	12.5* 11.1-13.7
Pao,dia (kPa)	9.6 8.3-10.4	7.5* 6.9-8.1	8.1 7.7-9.2	6.5* 6.0-7.6	8.0 7.5-9.2	6.3* 6.1-7.5
PWP (kPa)	1.1 0.9-1.2	1.1 1.1-1.2	0.9 0.8-1.1	1.4* 1.1-1.5	0.9 0.8-1.1	1.1* 0.9-1.5
dPlv/dtmax,pos (kPa. s ⁻¹)	140 120-170	115* 100-140	108 97-125	97 90-127	112 103-140	107* 87-143
CI (l.min ⁻¹ .m ⁻²)	2.21 1.84-2.47	2.60* 2.46-3.08	2.25 1.88-2.53	2.56 2.29-2.85	2.17 1.91-2.78	2.66* 2.20-3.16
LVSWI (J.m ⁻² .beat ⁻¹)	0.32 0.28-0.38	0.28* 0.24-0.32	0.34 0.27-0.41	0.24* 0.22-0.26	0.32 0.26-0.37	0.28* 0.22-0.34

* significantly different from pre CPB values (p<0.05).

Fig 4.1 Mean aortic blood pressure before, during and 5, 10 and 15 min after cardiopulmonary bypass. Median values and 95% confidence limits are presented. ■ = normothermia, ● = hypothermia, ▲ = cardioplegia, E = empty beating, F = fibrillating. * = significantly different from the value in the groups H and C, ** = significantly different from the value in the group C, *** = significantly different from the value in the group H.

Fig 4.2 Cardiac output prior to and after CPB, and arterial pump flow during cardiopulmonary bypass. Median values and 95% confidence limits are presented. ■ = normothermia, ● = hypothermia, ▲ = cardioplegia, E = empty beating, F = fibrillating. For further explanation see legend to fig 4.1.

Fig 4.3 Rectal temperature of patients on CPB. Median values and 95% confidence limits are presented. ■ = normothermia, ● = hypothermia, ▲ = cardioplegia, E = empty beating, F = fibrillating. For further explanation see legend to fig 4.1.

Fig 4.4 Arterial systemic blood temperature during CPB. Median values and 95% confidence limits are presented. ■ = normothermia, ● = hypothermia, ▲ = cardioplegia, E = empty beating, F = fibrillating. For further explanation see legend to fig 4.1.

4.1.3. Biochemical analysis

4.1.3.1. Plasma catecholamine concentration

Norepinephrine, epinephrine and dopamine, were selected to evaluate the effect of anesthetic or surgical interventions on the sympathetic nervous system during the surgical procedure.

The plasma concentration of norepinephrine measured prior to intubation was about 0.7 ng.ml^{-1} in all patients, and decreased after intubation and sternotomy (fig 4.5). Before the start of CPB norepinephrine had increased to preinduction values. In all groups plasma levels of norepinephrine further increased during CPB and remained elevated in group N (1.85 ng.ml^{-1}), group H (1.23 ng.ml^{-1}) and group C (1.49 ng.ml^{-1}) until 15 min after weaning from CPB. During the first 15 min postoperatively the norepinephrine concentrations were significantly higher in group N than in groups H and C.

Before the start of CPB the epinephrine concentration decreased significantly in all three groups (fig 4.5). At the end of CPB the plasma level had increased significantly as compared to the immediate pre CPB values and remained elevated during the first 15 min after CPB. There were no significant differences between the groups throughout the period of investigation.

In contrast to norepinephrine and epinephrine, no change in the dopamine plasma concentration was observed during the pre CPB phase. The patients of group H had significantly higher plasma levels as compared to the other two groups immediately before the onset of CPB (fig 4.5). During the first 15 min and at the end of CPB, only the patients in group N and group C had elevated plasma dopamine concentrations as compared with the pre CPB values. The differences between the three groups did not reach the level of significance.

Fig 4.5 Plasma concentration of norepinephrine, epinephrine and dopamine. Median values and 95% confidence limits are presented. ■ = normothermia (n=16); ● = hypothermia (n=15); ▲ = cardioplegia (n=18). $p < 0.05$ was considered as statistically significant.

- a, different from preinduction values by paired analysis
- b, different from pre CPB values by paired analysis
- c, different from corresponding values in the group N by non-paired analysis
- d, different from the corresponding value in group H by non-paired analysis.

4.1.3.2. Postoperative enzyme release

The plasma activity of AST, creatine kinase, CK-MB and HBDH was not significantly different in the three groups before the period of CPB. The following median values and 95% confidence limits were found: AST= 11 (10-12) U.l^{-1} ; creatine kinase= 30 (28-33) U.l^{-1} ; CK-MB= 4.4 (3.6-6.6) U.l^{-1} ; HBDH= 117 (92-212) U.l^{-1} (n=72). These values were comparable to the control values as found in healthy subjects, except for an increased value of HBDH, which might be due to hemolysis (Hermens et al, 1982). The plasma enzyme activity in the postoperative period is presented after correction for control plasma values (see section 3.1.9) in fig 4.6. Creatine kinase levels significantly increased after the operation and reached a maximum value at 24 hrs after weaning from CPB. In contrast, CK-MB reached a maximum value between 4 and 8 hrs and levelled thereafter. The activity of AST reached a maximum 24 hrs after weaning from CPB. HBDH plasma activity was already increased immediately after cessation of CPB and remained elevated thereafter. No differences between the three groups could be observed with respect to the plasma activity of these four enzymes during the investigated postoperative period.

All plasma enzyme activities were subsequently used to calculate the cumulative enzyme release, in order to estimate the amount of tissue damaged, if any, during the surgical procedure (see section 3.1.9). The 24 and 36 hour values for cumulative release of creatine kinase, AST, HBDH and CK-MB (expressed as gram-equivalents of tissue per liter plasma) are summarized in table 4.3. In each group the values measured at 36 hrs after CPB are generally higher than those measured at 24 hrs. The plasma values observed during the last 12 hrs of plasma enzyme analyses were not significantly different between the three groups.

Fig 4.6 Plasma activities of creatine kinase, its isoenzyme CK-MB, AST and HBDH during 36 hours after cessation of CPB. Values given in this figure were corrected for normal pre CPB values (Cs, see section 3.1.9). Median values and 95% confidence limits are presented. ■ = normothermia, ● = hypothermia, ▲ = cardioplegia.

Table 4.3 Cumulative release of enzymes during the first 24 and 36 hours after CPB. Data refer to median values and 95% confidence limits, a, cumulative release expressed as gram-equivalents of myocardial tissue per liter plasma. In this case, the heart was considered as the sole source of postoperative enzyme release into the blood. b, cumulative release of HBDH after subtraction of the amount of enzyme released from erythrocytes damaged during CPB (see section 3.1.9). c, cumulative release of enzymes from heart and skeletal muscle, respectively. Calculations were based on the release of creatine kinase and AST after weaning from CPB (see section 3.1.9).

Enzyme	Time	Group		
		Normothermia	Hypothermia	Cardioplegia
creatinine kinase ^a (g-eq.l ⁻¹)	24 h	6.1 5.3-8.8	6.0 5.0-6.7	5.2 4.8-6.2
	36 h	10.3 7.9-13.4	9.2 7.1-11.0	8.1 7.4-11.1
AST ^a (g-eq.l ⁻¹)	24 h	1.8 1.4-2.9	2.1 1.8-2.4	1.8 1.7-2.3
	36 h	2.5 2.1-4.1	2.8 1.9-3.5	2.5 2.3-3.0
HBDH ^b (g-eq.l ⁻¹)	24 h	0.6 0.4-0.9	0.6 0.6-1.0	0.7 0.4-0.9
	36 h	0.8 0.5-1.3	0.9 0.6-1.4	0.9 0.7-1.3
CK-MB ^a (g-eq.l ⁻¹)	24 h	1.2 0.7-1.8	0.7 0.3-1.7	0.8 0.5-1.4
	36 h	1.6 1.0-2.4	1.2 0.4-2.3	0.9 0.5-2.0
Q _{Heart} ^c (g-eq.l ⁻¹)	24 h	0.7 0.4-1.2	1.2 0.7-1.4	1.1 0.6-1.5
	36 h	0.8 0.5-1.2	1.2 0.6-2.0	1.5 0.7-2.0
Q _{skeletal muscle} ^c (g-eq.l ⁻¹)	24 h	1.7 1.4-2.3	1.4 1.1-1.7	1.3 1.1-1.6
	36 h	2.9 1.5-3.9	2.4 1.5-2.9	2.2 1.7-3.0

Calculation of the amount of injured tissue revealed an equivalent for heart tissue in the order of 1 gram and for skeletal muscle of about 2.5 gram per liter plasma during 36 hrs. The amount of heart enzyme (Q_H) released is in good agreement with the direct measured and calculated cumulative values based on HBDH and CK-MB.

Assuming a mean heart weight of about 400 g and a circulating plasma volume of 3 l in these patients, an equivalent for myocardial tissue of 1 to 1.5 g per liter plasma suggests that about 1% of the myocardial cells became injured due to the surgical procedure. The equivalents of 2.5 gram per liter plasma for skeletal muscle indicates that 0.02% of all skeletal muscle cells were damaged, when assuming that skeletal muscle represents about 50 % of total body weight.

4.1.3.3. Tissue content of ATP, creatine phosphate and glycogen

Changes in the tissue content of ATP, creatine phosphate and glycogen during the period of CPB were investigated in pre and postischemic transmural myocardial biopsies (figs 4.7, 4.8 and 4.9).

After the reperfusion period of about 20 min, following the release of the (last) aortic cross-clamp, the content of ATP was found to be 83, 74 and 100% of the control values in group N, group H and group C, respectively. In both the groups N and H the decrease was significant.

No significant difference in the content of creatine phosphate could be observed between the pre and postischemic samples in all three groups (fig 4.8).

In the groups N and H the glycogen content was significantly reduced to 54% and 57% of the preischemic values, respectively. No significant change in the carbohydrate store could be observed in the group C.

Fig 4.7 Content of ATP in pre and postischemic myocardial biopsies of all individual patients. —■— refer to median values and 95% confidence limits. Values are expressed as $\mu\text{mol.g}^{-1}$ dry weight of tissue. p indicates the level of significance of the difference between the pre and postischemic values.

Fig 4.8 Content of creatine phosphate in pre and postischemic myocardial biopsies of all patients (for explanation see legend to fig 4.7).

Fig 4.9 Content of glycogen (expressed as μmol glucose moieties per gram dry weight of tissue) in pre and postischemic myocardial biopsies of all patients (for explanation see legend to fig 4.7).

Fig 4.10 Histograms representing the interindividual variation of the tissue contents of ATP, creatine phosphate and glycogen in preischemic myocardial biopsies, taken during cardiopulmonary bypass (n=70 patients). Tissue values were grouped in cohorts of 2, 5 and 20 $\mu\text{mol.g}^{-1}$ dry weight in case of ATP, creatine phosphate and glycogen, respectively. n = the number of values belonging to one specific cohort. (reproduced by permission of Van der Vusse et al, 1982)

Fig 4.11 Intraindividual variation of the contents of ATP, creatine phosphate and glycogen in the human left ventricle. Biopsy 1 and 2 were taken from adjacent areas within 2 min, in the preischemic period during cardiopulmonary bypass ($n=14$). —■— = refers to median values and 95% confidence limits. (reproduced by permission of Van der Vusse et al, 1982)

The content of ATP, creatine phosphate and glycogen varied substantially in the preischemic myocardial biopsies of the individual hearts as is shown in the figs 4.7, 4.8 and 4.9. The preischemic data, when transformed to histograms (fig 4.10), depicts a near normal distribution, but does not eliminate the possibility of spatial inhomogenieties within the heart. Inter-individual variation amounted to 35% for ATP, 37% for creatine phosphate and 34% for glycogen. Whether inhomogenieties rather than interindividual variation prevail, was analyzed in a subset of the three patient groups. To this purpose two preischemic biopsies of adjacent left ventricular free wall areas were collected in a limited number of patients (n=14). There was no significant difference in the mean ATP, creatine phosphate or glycogen content between these two biopsies (fig 4.11). However, in some hearts pronounced differences exist between the two sampling sites, which resulted in a mean intraindividual variation of 11% for ATP, 11% for creatine phosphate and 20% for glycogen. Since these patients had 3-vessel disease these differences may be explained by spatial inhomogeneities due to partly underperfused areas of the left ventricular free wall (Jones et al, 1981).

4.1.3.4. Plasma levels of lactate, inorganic phosphate, potassium, and oxygen content

Insight into the metabolic functioning of the myocardium during the surgical procedure was obtained by determining the plasma concentrations of lactate, inorganic phosphate and potassium and the blood oxygen content in coronary sinus and arterial blood samples and assessing the arterial-coronary sinus (A-CS) differences. Besides, the arterial concentrations of these substances do give some information about systemic metabolism.

- Arterial plasma concentrations of lactate, inorganic phosphate, potassium and oxygen.

The arterial concentrations of lactate are shown in fig 4.12 for the three groups of patients. In all groups arterial lactate tended to increase during the surgical procedure, and about 10 min after the start of CPB all values were significantly higher as compared to the pre CPB lactate levels. These levels remained increased after CPB. Statistically significant diffe-

Fig 4.12 Arterial plasma concentrations of lactate, inorganic phosphate and potassium in the three patient groups. Horizontal bars refer to the period of CPB, and the inserted dashed areas represents the duration of aortic cross-clamping. Median values and 95% confidence limits are shown for group N (■), group H (●) and group C (▲). E = empty beating heart, F = fibrillating heart. Post CPB samples were collected 3 and 10 min after weaning from CPB.

rences between the groups were not observed.

The arterial concentration of inorganic phosphate fell significantly after the start of CPB in all three groups (fig 4.12). The arterial inorganic phosphate levels in group N tended to remain low during the periods of aortic cross-clamping, but in group H the arterial concentration increased in this period, reaching values not significantly different anymore from the pre CPB concentration. After aortic cross-clamping arterial inorganic phosphate levels increased gradually in the three groups, resulting in significantly higher levels after CPB, as compared to the pre CPB values.

In the early phase of CPB the arterial concentration of potassium increased significantly in all groups (fig 4.12). In the groups N and H the arterial potassium concentration initially remained at pre CPB levels, but increased significantly during the second half of the CPB period. In group C the arterial potassium concentration increased immediately during the reperfusion phase, probably as a result of the contribution of the cardioplegic solution to the circulating blood volume. Within the first 10 min after cessation of CPB the arterial potassium concentration tended to decrease to pre CPB values in all groups.

The arterial oxygen saturation remained within a narrow range, close to 100% (data not shown) throughout the operation.

- Arterial-coronary sinus differences of substances

The A-CS differences of lactate, inorganic phosphate, potassium and O_2 are presented in the figs 4.13, 4.14, 4.15 and 4.16, respectively.

The A-CS differences of lactate show that in most hearts hardly no lactate was extracted before CPB. During empty beating (E) and especially ventricular fibrillation (F), the A-CS differences of lactate were significantly changed into release as compared to their corresponding pre CPB values in all groups. The negative values indicate release of lactate during the initial period of CPB.

The A-CS differences of inorganic phosphate (fig 4.14) were hardly different from zero in all groups during the periods before and after aortic cross-clamping.

Prior to CPB the A-CS differences of potassium (fig 4.15) were slightly negative in most patients, indicating some release of this ion. During empty beating and fibrillation of the hearts the negative values appeared to de-

Fig 4.13 Arterial-coronary sinus difference of lactate in group N (normothermia, ■), group H (hypothermia, ●) and group C (cardioplegia, ▲). Median values and 95% confidence limits are presented in mmol.l⁻¹. E = empty beating heart; F = fibrillating heart (in groups H and C). Horizontal bars refer to the period of CPB, and inserted dashed areas represent duration of aortic cross-clamping. Negative values of A-CS differences correspond to washout of lactate into coronary sinus blood.

- q - initial peak value (0-30 s) of group N differs significantly from the corresponding value measured in group H.
- r - initial peak value (0-30 s) of the reperfusion period following the first aortic cross-clamping differs significantly from corresponding values of the subsequent reperfusion periods within the same group of patients.
- s - release curve of group N differs significantly from the corresponding curve in group H during a given reperfusion period.
- t - release curve of the first reperfusion period differs significantly from the curves of subsequent reperfusion periods within the same group of patients.
- u - value obtained after cardiopulmonary bypass differs significantly from the value prior to CPB.

Statistical analysis of differences between group C and the other two groups during the reperfusion periods was not performed.

Fig 4.14 A-CS difference of inorganic phosphate in the three patient groups.
For explanation see legend to fig 4.13.

Fig 4.15 A-CS difference of potassium in the three patient groups. For explanation see legend to fig 4.13.

Fig 4.16 A-CS difference of oxygen content in the three patient groups. For explanation see legend to fig 4.13.

crease further in both group H and group C, as compared to group N. The pre and post bypass A-CS differences were not significantly different from each other in all three groups.

The A-CS differences of oxygen were expressed as oxygen content. The venous oxygen saturation was subtracted from 100% (arterial oxygen saturation) and subsequently multiplied by the venous hemoglobin content. The data as presented in fig 4.16, indicates that there are no differences between the three groups before CPB. Within a few min after the start of CPB, venous oxygen content increased significantly in all groups and a further increase was observed in group C and group H at the time of fibrillation, possibly due to cooling of the heart. After cessation of CPB, A-CS differences of oxygen remained significantly decreased in all groups, as compared to the pre CPB values.

- Arterial-coronary sinus differences of substances during the immediate reperfusion period following aortic cross-clamping.

Release of the aortic clamp resulted in instantaneous reperfusion of the global ischemic myocardium. During this reperfusion phase release of substances, accumulated during the preceding period of ischemia, readily occurred. Examples of these substances are lactate, potassium and inorganic phosphate known as reliable markers of metabolic changes induced by ischemia (Owen et al, 1970; Opie et al, 1972; Van der Vusse et al, 1979, 1980).

The presentation of the values of A-CS differences is restricted to 0.5, 2, 6 and 10 min after release of the aortic clamp for the sake of clarity, whereas the intermediate values were also used for statistical analysis.

The release of lactate (fig 4.13) during the first 10 min following each aortic cross-clamp period was highest in the samples collected immediately after release of the clamp in both group N and group H (sample period from 0 to 30 s). During 10 min of reperfusion the A-CS differences gradually decreased.

In both group N and group H the A-CS differences during the very first reperfusion period were higher than the corresponding differences during the following reperfusion periods at each sample time. In the four reperfusion periods at all first sample times (0-30 s) the A-CS differences of lactate were significantly higher in group N than in group H. The A-CS differences of lactate in the cardioplegia group were hardly different from zero, indicating

that virtually no lactate was released.

In group N and group H the release of inorganic phosphate at the first sample time (0-30 s) was significantly higher in the first reperfusion period than in the three following reperfusion periods (fig 4.14). The A-CS differences of inorganic phosphate rapidly fell to zero. In the group N even a measurable amount of inorganic phosphate was taken up by the heart during the second, third and fourth reperfusion period at sample times 6 and 10 min. This phenomenon was also observed in the group H after release of the fourth cross-clamping. The A-CS differences of inorganic phosphate in the cardioplegia group were highest at the first sample time (0-30 s) and gradually decreased during the succeeding 6 min of reperfusion.

In both group N and group H, the A-CS differences of potassium (fig 4.15) during reperfusion were initially high, but fell rapidly to low levels. The A-CS differences as measured in the first samples (0-30 s), were significantly higher in group N than in group H during each reperfusion period. In group N potassium was extracted by the heart at sample time 2 min during the first reperfusion period, and at sample times 2 through 10 min during the second, third and fourth reperfusion periods. In contrast, sustained negative A-CS differences of this ion were measured during the first 10 min of reperfusion in group C.

The patterns of the A-CS differences of oxygen (fig 4.16) show that during reperfusion the initially high values rapidly fell to low levels in group N and group H. The initial A-CS differences of oxygen were significantly higher in group N than in group H. During reperfusion A-CS differences of oxygen rapidly fell to very low values in both groups. In group N these values reached a minimum at 2 min levelling off at a value of about $1.0-1.6 \text{ mmol O}_2 \cdot \text{l}^{-1}$. In contrast the A-CS differences remained significantly decreased (median value ranging from $0.2-0.6 \text{ mmol O}_2 \cdot \text{l}^{-1}$) in group H. In the cardioplegia group the A-CS differences of oxygen during reperfusion were initially small, but gradually increased to values of $1.2-1.5 \text{ mmol O}_2 \cdot \text{l}^{-1}$, which was not significantly different from the values as measured in group N.

4.1.4. Mitochondrial ultrastructural findings

Mitochondrial ultrastructure was evaluated before and after the periods of ischemia using semiquantitative parameters (table 4.7). This scoring system has been discussed in detail in section 3.1.6.

In the three groups the preischemic tissue specimens were well preserved and over 95% of the mitochondria belong to the grading score 0 or 1, which indicates an excellent membrane ultrastructure with or without typical small intramitochondrial particles. Hardly no heavily damaged mitochondria were observed.

After ischemia (and reperfusion) no significant changes in the value of score 0 and 1 were found in all myocardial layers of the three groups, except for the endocardial layers of group C, where significantly more mitochondria with score 0 were found in the postischemic biopsy. Simultaneously, a decrease of the percentage of mitochondria with score 1 was observed ($p < 0.05$). In both intermittently cross-clamping groups the mitochondria showed an increased tendency to clarification of the matrix (score 2) in the postischemic specimens as compared to preischemic specimens. This increase was noticed in both the subendo and the subepicardial layer, but did not reach the level of significance most likely due to a substantial patient to patient variation.

Table 4.7 Mitochondrial ultrastructural appearance. Data refer to mean values and standard deviations. Meaning of score (see also section 3.1.6): 0 = normal ultrastructure of the mitochondrion, 1 = normal appearance of the crests and matrix, but absence of granular deposits (small electron dense granules), 2 = loss of matrix granules and clarification of the matrix without breaking of crests, 3 = loss of matrix granules, uniform clarification of the matrix and disruption of crests (focal condensation may be seen: inclusions having at least twice the diameter of normal mitochondrial granules), 4 = loss of matrix granules, uniform disruption of crests and loss of integrity of mitochondrial inner and outer membranes.

Group		Score				
		0	1	2	3	4
<u>Normothermia</u>						
Epicard:	preischemic	61±29	36±26	3.3±7.3	0.1±0.4	0 ±0
	postischemic	53±28	40±22	6.2±11	0.5±1.4	0.1±0.4
Endocard:	preischemic	50±23	43±21	5.4±13	1.0±2.8	0.4±1.6
	postischemic	50±28	39±23	10 ±18	0.6±1.3	0 ±0
<u>Hypothermia</u>						
Epicard:	preischemic	55±26	44±25	1.1± 2.3	0.1±0.3	0 ±0
	postischemic	62±24	32±15	5.9±15	0.2±0.7	0 ±0.1
Endocard:	preischemic	62±25	38±25	0.7± 1.8	0 ±0	0.1±0.4
	postischemic	66±19	32±17	1.5± 4.4	0.2±0.5	0 ±0.1
<u>Cardioplegia</u>						
Epicard:	preischemic	49±29	48±22	2.3± 4.3	0.6±2.5	0.1±0.5
	postischemic	58±30	39±27	2.8± 5.8	0.1±5.8	0.3±0.5
Endocard:	preischemic	44±29	53±29	2.7± 4.7	0.2±0.4	0.2±0.5
	postischemic	64±27*	33±24*	2.4± 8.1	0.3±0.8	0.1±0.3

*= significantly different from corresponding values in preischemic tissue specimens (p<0.05).

4.2 Animal experiments

4.2.1. Introduction

The procedures followed in the animal experiments were aimed at being comparable to those in the clinical study, with respect to duration of CPB, ischemia, and reperfusion, and to the techniques of myocardial protection. The experiments were terminated one hour after cessation of CPB, when myocardial biopsies were taken. Within this post CPB period the hemodynamic and metabolic variables could be determined for a longer period than in the clinical study, in which the last measurement was performed at 15 min after termination of CPB.

In this section the results of the animal experiments are presented as far as used for further evaluation and comparison to clinical measurements. Additionally, data already briefly discussed in the method section, are summarized in the appendix. Animal data (body weight and left ventricular weight) is presented in appendix 1. Data on cardiopulmonary bypass (rectal and septal temperature, perfusion pressure, pump flow) are shown in appendix 2, 3 and 5, respectively and indicates adequate perfusion circumstances.

4.2.2. Hemodynamics

In fig 4.17 the hemodynamic variables as measured before and 15 and 60 min after CPB in the groups N, H and C are shown. In all groups heart rate was relatively high before CPB and did not change significantly during the postoperative period. After CPB cardiac output was increased in the groups H and C, but not in group N. As compared to the pre CPB values, Pao_{sys} and Pao_{dia} were significantly decreased in group H, and Pao_{dia} in the groups N and C after CPB. Although Plv did not change significantly in either group a tendency to decrease was observed in groups N and H. The mean values of Plv_{ed} tended to be increased after CPB, but only reached the level of significance in group C. In group H mean $dPlv/dt_{max,pos}$ showed a transient decrease from 220 to 160 $kPa.s^{-1}$ 15 min after CPB. The hemodynamic variables as measured before CPB in the groups NB, HB, NC, HC and HPLV, were comparable

Fig 4.17 Time course of hemodynamic variables before and 15 and 60 min after cardiopulmonary bypass in the groups N, H and C. The variables presented are heart rate, cardiac output (CO), systolic (Pao,sys) and diastolic aortic pressure (Pao,dia), left ventricular pressure (Plv), left ventricular end-diastolic pressure (Plv,ed) and the positive first derivative of Plv (dPlv/dtmax,pos). Mean values and SD are presented. * = significantly different from the pre CPB values (p < 0.05).

to those as measured in the groups mentioned above (appendix 4). The significant decrease in $P_{ao,sys}$ in group HPLV (mean $P_{ao,sys}$ decreased from 10.8 kPa before CPB to 7.1 kPa 120 min after CPB) was mainly caused by the different policy in fluid administration after cessation of CPB, necessary to determine the apparent disappearance constant rate and the plasma volume. In group CON, in which all instrumentation was employed but CPB was excluded, Plv significantly decreased which may indicate the influence of instrumentation and experimental procedure on the hemodynamic stability of the preparation, although the other variables did not change significantly.

4.2.3. Myocardial blood flow

Coronary artery blood flow was measured continuously during the experiments, using an electromagnetic flow probe placed around the LAICA. Information about transmural distribution of blood flow was obtained from radioactive microspheres.

The myocardial blood flow values were similar in the groups N, H and C during the pre CPB period (fig 4.18). After weaning from CPB significantly higher flows were measured in the groups N, H and C as compared to the pre CPB values for a substantial period of time.

In all groups coronary artery blood flow increased to the same extent after the start of CPB, both under empty beating and fibrillating conditions. Release of the aortic cross-clamp resulted in a reactive hyperemia response in all three groups. In the groups N and H this response tended to be less pronounced after the release of succeeding aortic cross-clampings as compared to the first release. This was indicated by the diminished peak reactive hyperemia (group N), and the more rapid fall to the control values (groups N and H). A pronounced reactive hyperemia response was observed after release of the cross-clamping in group C, reaching higher peak values ($1.90 \pm 0.40 \text{ ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$), and lasting longer than in the groups N and H.

The subendo to subepicardial blood flow ratios in the left ventricle are summarized in table 4.8. This ratio did not change in group N and group C, but decreased significantly in group H after reperfusion, indicating relative underperfusion of the subendocardial layers at that moment. The subendo to subepicardial blood flow ratios in the groups NC, HC and CON did not change

Fig 4.18 Coronary artery blood flow of the groups N, H and C. Mean values and SEM ($\text{ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$) are presented. AC = aortic cross-clamping, CPB = cardiopulmonary bypass, E = empty beating, F = fibrillating. * = significantly different from pre CPB values ($p < 0.05$).

Table 4.8 Subendo to subepicardial coronary blood flow ratios in the left ventricular free wall in the groups N, H, C, NC, HC and CON. Mean values and SD are presented.

Group						
Time	N	H	C	NC	HC	CON
pre CPB	1.13±0.18	1.11±0.17	1.05±0.15	1.27±0.63	1.10±0.21	1.04±0.23
15 min post CPB	1.03±0.25	0.82±0.26*	1.04±0.37	1.09±0.25	0.98±0.45	0.95±0.12
60 min post CPB	1.12±0.25	0.89±0.29	0.98±0.16*	1.06±0.35	0.98±0.24	0.88±0.28

* = significantly different from pre CPB values ($p < 0.05$).

Table 4.9 Epicardial ($e_{\max}$) and endocardial ($e_{\text{endo,est}}$) fiber shortening before and 15 and 60 min after CPB. Mean values and SD are presented.

Group	Time	$e_{\max}$ (%)	$e_{\text{endo, est}}$ (%)
N (n=7)	pre CPB	8.8± 4.2	9.3± 4.9
	post CPB		
	15 min	9.4± 6.5	6.2± 6.4
	60 min	10.5± 7.0	9.6±12.4
H (n=4)	pre CPB	7.3±10.6	5.5±19.8
	post CPB		
	15 min	7.2± 3.8	4.6± 7.7
	60 min	7.8± 2.8	7.0± 4.7
C (n=8)	pre CPB	8.9± 3.2	5.2± 4.5
	post CPB		
	15 min	10.3± 3.6	7.5± 5.7
	60 min	11.8± 3.9*	9.5± 5.9

* = significantly different from the pre CPB value ($p < 0.05$).

significantly during the experimental procedure.

More details about the myocardial blood flow measurements in all groups are presented in appendix 6.

4.2.4 Myocardial mechanical performance

The measured values of subepicardial fiber shortening ($e_{\max}$) and the estimated values of subendocardial fiber shortening ($e_{\text{endo,est}}$) for the groups N, H and C are presented in table 4.9.

After CPB the mean values of $e_{\max}$ and $e_{\text{endo,est}}$ were not reduced as compared to the pre CPB values in the groups N, H and C, indicating that regional fiber shortening was not impaired in these groups. In group C, $e_{\max}$ was even significantly increased 60 min after CPB.

However, the large variation within each group might implicate regionally impaired fiber shortening, both before and after CPB. Depressed values were noticed in some dogs in all three groups, especially with respect to $e_{\text{endo,est}}$. The abnormal positioning of the dog on his back, may have affected the technique of measurement and certainly limits the area of the coils attached at the epicardium to a small part of the left ventricular free wall just above one of the papillary muscles.

4.2.5. Biochemical analysis

4.2.5.1. Release and uptake of lactate, inorganic phosphate and potassium, and uptake of oxygen

- Lactate

Fig 4.19 shows the A-CS differences of lactate for the groups N, H and C. Before and after CPB, no major differences could be detected between the three groups. Before CPB lactate was taken up by the heart in all groups, but after the start of CPB lactate was released in the groups N and C, and to a lesser extent in group H. In the groups N and H during the reperfusion periods following the release of aortic cross-clamping lactate was initially

released, but lactate was taken up again within 10 min. The peak release values at 0.5 min after the start of the first reperfusion period were comparable in group N and group H, but this peak value significantly decreased in group H during the succeeding reperfusion periods. In group C only minor A-CS differences of lactate were found immediately after reperfusion. The calculated values of lactate uptake on release (fig 4.20) show that changes in coronary artery blood flow did not substantially affect the qualitative patterns of A-CS differences before and after CPB. However, during CPB the quantity of lactate released was more prominent in group N. Yet during the initial phase of reperfusion the peak values in group H remained constant during the four reperfusion periods, which is at variance with the A-CS differences.

- Inorganic phosphate

The A-CS differences of inorganic phosphate are shown in fig 4.21. At the sample times before and 60 min after CPB these differences were not significantly different from zero, but immediately after the start of CPB the venous concentration tended to increase. Five min later the values were normalized. Release of inorganic phosphate was observed within the first min after release of the aortic cross-clamp in all groups. In group N the mean peak value was about $-1.00 \text{ mmol.l}^{-1}$, whereas the mean peak value in group H and group C was on the average -0.40 and $-0.06 \text{ mmol.l}^{-1}$, respectively. Only in group H the peak value during the first reperfusion period was significantly higher than the peak values in the subsequent reperfusion periods. Positive A-CS differences were measured within 4 min after the start of reperfusion in all instances, indicating extraction of inorganic phosphate from the blood.

Calculated values of net inorganic phosphate uptake and release (fig 4.22) show that during the initial phase of reperfusion the differences between group N and group H were comparable to the differences observed on the basis of the A-CS differences alone. The average peak value within 0.5 min after release of aortic cross-clamping was $-1.30 \text{ } \mu\text{mol.g}^{-1}.\text{min}^{-1}$ in group N, $-0.50 \text{ } \mu\text{mol.g}^{-1}.\text{min}^{-1}$ in group H, but only $-0.12 \text{ } \mu\text{mol.g}^{-1}.\text{min}^{-1}$ in group C. After 5 to 10 min of reperfusion the uptake of inorganic phosphate was quantitatively higher in group N. In all groups considerable amounts of inorganic phosphate were taken up by the heart during the prolonged reperfusion phase

Fig 4.19 Arterial-coronary sinus difference of lactate in the three dog groups with aortic cross-clamping (N, H and C). Mean values and SD are presented in mmol.l^{-1} . Negative values of A-CS differences correspond to washout of lactate into coronary sinus blood. CPB = cardiopulmonary bypass, AC = aortic cross-clamping, E = empty beating, F = fibrillating heart. a = peak value different from the first peak value ($p < 0.05$), b = peak value different from the preceding peak value ($p < 0.05$), c = peak value different from the second peak value ($p < 0.05$), d = value different from the pre CPB value ($p < 0.05$).

Fig 4.20 Uptake/release values of lactate ($\mu\text{mol.g}^{-1}\text{min}^{-1}$). For explanation see legend to fig 4.19.

Arterial-coronary sinus difference
Inorganic phosphate

Fig 4.21 Arterial-coronary sinus difference of inorganic phosphate (mmol.l⁻¹). For explanation see legend to fig 4.19.

Fig 4.22 Uptake/release values of inorganic phosphate ($\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$). For explanation see legend to fig 4.19.

Fig 4.23 Arterial-coronary sinus difference of potassium (mmol.l⁻¹). For explanation see legend to fig 4.19.

Fig 4.24 Uptake/release values of potassium ($\mu\text{mol.g}^{-1}\text{min}^{-1}$). For explanation see legend to fig 4.19.

Fig 4.26 Uptake values of oxygen ($\mu\text{mol.g}^{-1}\text{min}^{-1}$). For explanation see legend to fig 4.19.

after the last aortic cross-clamp (2.2 to $5.0 \mu\text{mol.g}^{-1}$ wet weight). Values at 60 min after CPB were not different from zero, as was the case in the pre CPB period.

- Potassium

At most instances before and after CPB only minor A-CS differences were found for potassium, but after the start of CPB at all instances potassium was extracted from the blood (fig 4.23). During reperfusion after release of aortic cross-clamping considerable A-CS differences were observed in all three groups. In group N the mean peak A-CS difference of potassium varied from -2.35 to $-3.51 \text{ mmol.l}^{-1}$, indicating release of this ion. Within 2 min this release was followed by uptake of potassium. In group H potassium was also released initially as indicated by the mean peak value A-CS difference, ranging from -1.52 to $-2.12 \text{ mmol.l}^{-1}$. This release was rapidly followed by uptake during the reperfusion period. In contrast, in group C negative A-CS differences of potassium, with a mean peak value of $-2.01 \text{ mmol.l}^{-1}$, was continued for a longer period of time, indicating sustained washout of this ion. Calculation of the quantitative release and uptake of potassium confirmed the observations on A-CS differences (fig 4.24). The peak release values during the repetitive reperfusion periods were higher in group N than in group H. The initial values during each reperfusion period were similar in group N and in group H, respectively. The uptake of potassium from 2 to 10 min of reperfusion was usually higher in group N than in group H. In group C the sustained release of potassium most likely results from the preceding infusion with the potassium containing cardioplegic solution (i.e. 16 mmol.l^{-1}).

- Oxygen content

The mean A-CS differences of oxygen content varied between 4 and 5 mmol.l^{-1} before CPB and were found to vary around 3 mmol.l^{-1} after CPB in the groups N, H and C (fig 4.25). Immediately after the start of CPB, the A-CS differences of oxygen were about 2 mmol.l^{-1} with a tendency to further decrease during ventricular fibrillation in group H and group C. During the immediate reperfusion phase, the peak values of A-CS differences increased significantly in the groups N and H. In contrast, this initial high value of

A-CS difference of oxygen was not observed in the cardioplegia group (fig 4.25 lower panel). In group N the initial peak in A-CS difference was followed by transient low A-CS differences, which increased again later during reperfusion, reaching values between 1 and 2 mmol.l^{-1} after 10 min. In contrast, in group H the A-CS differences of oxygen content remained low following the initial peak (below 0.5 mmol.l^{-1}). However, following rewarming after the last aortic cross-clamping, a rapid increase in the A-CS difference was observed in this group. This increase was found to be delayed in group C (fig 4.25 lower panel).

Calculated values of oxygen uptake are presented in fig 4.26. Although the pre CPB values were higher in group N than in group C, the mean values of oxygen uptake after CPB were not significantly different in all three groups (ranging from 2 to 3 $\mu\text{mol.g}^{-1}.\text{min}^{-1}$). After the start of extracorporeal circulation the mean values of oxygen uptake decreased in group N and group H, but only during fibrillation in group C. Within the first 30 seconds of all reperfusion periods high values of oxygen uptake were measured in group N and group H. Thereafter oxygen uptake remained at a low level in group H, but after a transient fall increased again in group N. Upon rewarming in group H (after 100 min of CPB) and in group C (after 75 min of CPB) comparable patterns were observed as far as increase in oxygen uptake is concerned.

4.2.5.2. Tissue content of metabolic substances

Myocardial transmural tissue biopsies were routinely obtained in groups N, H and C at 60 min after CPB and in group C also at three intervals during CPB. In two different groups of animals (NB and HB), comparable with the normothermic and hypothermic intermittent cross-clamp groups N and H, respectively, multiple biopsies before the first cross-clamping, and at the end of each cross-clamping and each reperfusion period were obtained. For statistical analysis the results as obtained in comparable groups (groups N and NB as normothermia group, and groups H and HB as hypothermia group) were combined. The data on ATP, creatine phosphate, inorganic phosphate, glycogen and lactate is shown in the figs 4.27, 4.28, 4.29, 4.30 and 4.31, respectively. The first biopsy, taken when the heart was empty beating, was considered as the control or preischemic value.

Fig 4.27 Content of ATP in myocardial biopsies obtained during and 60 min after CPB. Mean values and SD are presented ($\mu\text{mol.g}^{-1}$ dry weight). The single observations in the Normothermia and Hypothermia groups after CPB refer to the groups N and H.

AC = aortic cross-clamping, CPB = cardiopulmonary bypass, E = empty beating, F = fibrillating. a = significantly different from the corresponding value in the other two groups ($p < 0.05$), b = significantly different from the preceding value within the same group ($p < 0.05$), c = significantly different from the control value obtained during empty beating (E) ($p < 0.05$).

Fig 4.28 Content of creatine phosphate in myocardial biopsies. Mean values and SD are presented ($\mu\text{mol.g}^{-1}$ dry weight). For explanation see legend to fig 4.27.

Fig 4.29 Content of inorganic phosphate in myocardial biopsies. Mean values and SD are presented. ($\mu\text{mol.g}^{-1}$ dry weight). For explanation see legend to fig 4.27.

Fig 4.30 Content of glycogen in myocardial biopsies. Mean values and SD are presented ($\mu\text{mol glucose moieties.g}^{-1}$ dry weight). For explanation see legend to fig 4.27.

Fig 4.31 Content of lactate in myocardial biopsies. Mean values and SD are presented ($\mu\text{mol.g}^{-1}$ dry weight). For explanation see legend to fig 4.27. Each value in the hypothermia group refers to two measurements.

- ATP

In group NB, the ATP content at the end of the first and third ischemic period was significantly lower than the value measured at the end of the preceding normoxic period, but the changes observed in the groups HB and C were not significant (fig 4.27). The ATP content of the biopsies obtained at the end of the experiment, following a 60 min recovery period, was not significantly different between the three groups.

- Creatine phosphate

The creatine phosphate content of biopsies decreased significantly in the normothermia group and the hypothermia group after each period of ischemia, and returned to or exceeded the control values after 15 min of reperfusion (fig 4.28). The pattern of changes in creatine phosphate content was uniform during the succeeding periods of reperfusion. In group C lower values were observed at the end of ischemia, but this decrease did not reach the level of significance. In this group significantly increased levels of creatine phosphate were found within 15 min of reperfusion.

The creatine phosphate content in the final biopsies, taken 60 min after CPB, were comparable to the control values in the hypothermia groups and cardioplegia group, but higher values were found in the normothermia groups amounting to 170 % of the control value. Differences in creatine phosphate content between preischemic (before aortic cross-clamping) and postischemic (just prior to the end of cardiopulmonary bypass) biopsies were found in the groups NB and C, but no significant differences were observed in group HB.

- Inorganic phosphate

The inorganic phosphate content of myocardial biopsies is shown in fig 4.29 for the groups N, H, C, NB and HB. The content of inorganic phosphate was significantly increased after each ischemic period of 10 min in group NB and HB, and after 60 min of ischemia in group C. The increase was most outspoken in the group HB (ranging from 61 to 81 $\mu\text{mol.g}^{-1}$ d.w., after the various cross-clamp periods) and group NB (ranging from 37 to 51 $\mu\text{mol.g}^{-1}$ d.w.) but much less in group C (maximal increase 20 $\mu\text{mol.g}^{-1}$ d.w.). Restoration to the preischemic (control) levels of inorganic phosphate readily occurred in all three groups within 15 min of reperfusion.

- Glycogen

The glycogen content in the myocardial biopsies was determined as glucose moieties and the results are shown in fig 4.30. The data shows that the maximum decrease was observed after the fourth aortic cross-clamping in the group NB, where the glycogen content had decreased to 74% of the initial preischemic values. In group HB the maximum decrease to 63% was observed after the third reperfusion period. Otherwise inconsistent changes were seen during the periods of ischemia and reperfusion. The analysis of the values at the end of CPB as compared to the preischemic glycogen content did not show a significant decrease.

- Lactate

Data on myocardial lactate content in the normothermia, hypothermia and cardioplegia groups is shown in fig 4.31. This substance was measured in a limited number of biopsies of the group HB for technical reasons, and as a result no statistical analysis were performed. Before ischemia the myocardial lactate content was low (mean values varying from 25 to 37 $\mu\text{mol.g}^{-1}$ d.w.) in all groups, but remarkably increased after each period of aortic cross-clamping in group NB and group HB (mean increase ranging from 65 to 117 $\mu\text{mol.g}^{-1}$ d.w.). After 15 min of reperfusion the lactate content decreased to low values in both groups with intermittent aortic cross-clamping. The myocardial lactate content at the end of CPB was not different from the preischemic levels in both the group NB and group HB. In the group C no significant differences from the control values were observed throughout the experiment.

4.2.6. Hemodynamic and biochemical changes during the initial period of cardiopulmonary bypass

Since several investigators (Hottenrott et al, 1974; Vinas et al, 1979) have suggested that the heart is very susceptible to damage during the initial extracorporeal perfusion phase, especially during ventricular fibrillation, we have investigated this particular phase in more detail. During this period, lasting in the average 15 min, CPB had completely replaced the normal circulation.

About 5 min after the start of CPB, when the heart was vented through the apex, the first set of measurements was performed, referred to as "during the empty beating state" ('E1'). Approximately 5 min later, when systemic cooling in the hypothermia and cardioplegia groups had caused the heart to fibrillate, a second set of measurements during this "fibrillating state" ('F') was performed. In the normothermia group the heart was still empty beating after CPB had lasted approximately 10 min and the sample interval was designated as 'E2'.

Table 4.10 summarizes the functional and biochemical parameters of the heart during the empty beating and fibrillating conditions. For the sake of comparison the values obtained before CPB are shown as well. The normothermia group refers to the combination of the animal groups N, NC and NB, and the hypothermia group refers to the animal groups H, HC, HB and C.

The rectal temperature was slightly decreased in both groups at the time of 'E1', which confirms that cooling has started only recently. The rectal temperature at the time of 'F' indicates progression of cooling in the hypothermia group.

Mean aortic pressure decreased considerably during 'E1' and 'F' in both groups as compared to the values before CPB. However, differences between the values during 'E1', 'E2' and 'FIB' could not be detected. Similar falls in aortic pressures were observed in the patients of groups H and C (section 4.1.2), but no differences between the values obtained during the initial phase of CPB could be detected.

Coronary artery blood flow increased considerably in all animal groups after the start of CPB. Whether this increase in total blood flow was directed preferentially to the subendo or subepicardial layers of the left ventricular free wall was investigated in group H with the use of radioactively labelled microspheres. The subendo to subepicardial ratio decreased from 1.11 ± 0.17 to 0.48 ± 0.16 during 'E1', indicating redistribution of flow towards the epicardial layers under these circumstances (mean $\pm$ SD; $n=5$). During fibrillation this ratio increased to 1.21 ± 0.69 , suggesting that in this condition blood flow is shifted towards the endocardial layers.

The influence of empty beating and fibrillating conditions on myocardial flow resistance appeared to be marked in all groups (table 4.10). In group H myocardial resistance fell from 0.17 ± 0.06 kPa.min.g.ml⁻¹ before CPB to 0.10 ± 0.07 kPa.min.g.ml⁻¹ during 'E1' and 0.10 ± 0.11 kPa.min.g.ml⁻¹ during 'F'.

Table 4.10 Functional and biochemical variables in dogs during the initial period of cardiopulmonary bypass in dogs. Mean values and SD are

Group	Normothermia		
	pre CPB	'E1'	'E2'
Time interval			
Parameter		5 min on CPB	10 min on CPB
Rectal temperature (°C)	36±2	36±1	35±1
Perfusion pressure, mean aortic pressure (kPa)	8.7±1.7	6.3±2.0 ^q	6.7±2.0 ^q
Coronary flow (ml.min ⁻¹ .g ⁻¹)	0.66±0.40	1.12±0.77 ^q	1.07±0.87 ^q
Myocardial flow resistance(kPa.min.ml ⁻¹)	0.17±0.07	0.08±0.05 ^q	0.09±0.04 ^q
<u>Uptake/Release:</u>			
(μmol.g ⁻¹ .min ⁻¹)			
Lactate	0.16±0.76	-0.18±0.43	0.49±0.86 ^r
Inorganic phosphate	-0.01±0.06	-0.14±0.21	0.02±0.06 ^r
Potassium	-0.13±0.38	0.17±0.34	0.12±0.33
Oxygen	3.26±2.09	2.31±1.97 ^q	2.28±1.92 ^q
<u>Myocardial content:</u>			
(μmol.g ⁻¹ dry weight)			
ATP	-	22± 6	24± 5
Creatine phosphate	-	46±12	52±11
Inorganic phosphate	-	19± 5	18± 7
Glycogen	-	160±56	143±40
Lactate	-	25± 9	28±12

q = significantly different from the pre CPB value (p<0.05), r = significantly different from the value obtained after 5 min on CPB (p<0.05), s = significantly different from the corresponding value in the hypothermia groups (p<0.05).

presented. * = the mean value of two dogs is presented. 'E1' and 'E2' = empty beating, 'F' = fibrillating.

Group	Hypothermia		
	pre CPB	'E1'	'F'
Time interval			
Parameter		5 min on CPB	10 min on CPB
Rectal temperature (°C)	37±1	35±3	31±3
Perfusion pressure, mean aortic pressure	8.5±1.5	6.4±2.1 ^q	6.1±1.6 ^q
Coronary flow	0.52±0.18	0.85±0.45 ^q	0.92±0.53 ^q
Myocardial flow resistance	0.17±0.06	0.10±0.07 ^q	0.10±0.11 ^q
<u>Uptake/Release:</u>			
Lactate	0.29±0.62	-0.01±0.36	0.09±0.20
Inorganic phosphate	0.00±0.03	-0.02±0.05	0.01±0.04
Potassium	0.02±0.13	0.11±0.18	0.05±0.26
Oxygen	2.43±1.07	1.38±0.78 ^q	1.04±0.59 ^q
<u>Myocardial content:</u>			
ATP	-	23±11	19± 2
Creatine phosphate	-	52±13	57±12
Inorganic phosphate	-	29±22	14± 4
Glycogen	-	158±78	136±38
Lactate	-	37*	19*

These values were not different from those measured in the group N.

The uptake of lactate as observed before CPB decreased considerably in both groups, resulting in an average net release of lactate in group N. Five min later this release was reversed again significantly in uptake of lactate. The uptake/release of inorganic phosphate did not alter significantly after instalment of CPB, but in the normothermia group an initial release was restored to control values within 5 min ('E2').

Potassium was not significantly extracted from or released by the heart, neither before CPB nor during the initial period of CPB.

As a consequence of reduced work load and decreased temperature, the oxygen uptake was significantly reduced in group H. Evaluation of differences in oxygen consumption, if any, due to the shift from empty beating to fibrillation could not be performed because blood temperature of the heart changed as well. In group N, prolonged empty beating did not significantly affect oxygen uptake.

Biochemical analysis of myocardial biopsies revealed only minor differences between 'E1', 'E2' and 'F'.

4.2.7 Enzyme release

In the dog experiments plasma samples for enzyme activity were routinely obtained before, during, upto and including one hour after cessation of CPB. To illustrate the course of changes during the experiments in the groups N, H and C the results of AST measurements are shown in fig 4.32. Immediately after the start of CPB the mean values were decreased in all groups as a result of dilution of the blood with the priming solution. During CPB the AST activity gradually increased in all groups. This trend was accentuated after CPB. It should be noted, however, that after CPB the total circulating plasma volume was considerably increased, so that enzyme release may be underestimated.

The slope of the curves indicate that even 60 min after CPB sustained release of AST into the plasma can be observed and the maximum values have not been reached. At the last sample point no significant differences in total AST plasma activity could be detected between the three groups.

The data of AST, creatine kinase, MDH, ALT, HBDH, free hemoglobin and

Fig 4.32 Plasma enzyme activity of AST as measured in group N (■), group H (●) and group C (▲). Mean values and SD (U.l⁻¹) are presented. CPB = cardiopulmonary bypass.

Table 4.11 Calculated enzyme release from 15 to 60 min after weaning from CPB. Mean values and SEM are presented, and expressed as units released per hour.

Enzyme	Group						
	N (n=8)	H (n=9)	C (n=8)	NC (n=9)	HC (n=8)	CON (n=8)	EC (n=5)
AST	30±11	12±2	31±5 ¹	24±8	22±7	11±7	20±15
CK	503±119 ^{2,3}	541±110 ^{2,3}	476±95 ^{2,3}	682±239 ³	722±116 ^{2,3}	184±86	80±7

¹ = different from group H and CON ($p < 0.05$), ² = different from group CON ($p < 0.05$), ³ = different from group EC ($p < 0.05$).

hematocrit are shown in appendix 13 for all groups of animals. Comparison of the groups N, H and C shows that the free hemoglobin content was higher in group C than in the other two groups. Beside the initial decrease in plasma AST activity (see above), no marked changes were observed during the first 35 min of CPB. Therefore plasma enzyme activity increased at the end of CPB except for ALT. During that period the total volume of blood within the intravascular space and the oxygenator should be considered when plasma activity is used for quantitative calculations. The gradual increase of enzyme activity is also observed for creatine kinase and MDH at 15 and 60 min after CPB. The plasma enzyme activity for HBDH and the free hemoglobin content increase to a lesser extent after CPB, which is in favor of the idea that the release mainly results from pump-induced damage of red blood cells during CPB.

The data as presented in appendix 13 indicates that the enzyme release in group NC and group HC was not different from the groups with aortic cross-clamping (i.e. the groups N, H and C). In contrast, in group CON (without CPB) the release of creatine kinase, MDH, HBDH and the formation of free hemoglobin was reduced which indicates the detrimental influence of CPB. In group EC (without cardiac manipulation) reduced release of AST, ALT and creatine kinase was observed, as compared to the groups with CPB (MDH and HBDH activity and free hemoglobin content were not measured in group EC). Comparison of the results of group EC and group CON indicates that the increased plasma activity of AST and ALT in group CON is most likely caused by cardiac manipulation and instrumentation.

From fig 4.32, it is evident that quantification of enzyme release in the present series of dog experiments can only be performed on a small intercept of the total enzyme release curve, i.e. during 60 min after termination of CPB. From the data obtained at the last two time intervals (15 and 60 min after CPB) the cumulative release can be assessed after correction for enzyme degradation and plasma volume changes (see section 3.2.9 for full details). This calculation was applied to AST and creatine kinase with the use of formerly obtained specific circulatory parameters. The cumulative release of these enzymes was also calculated in the groups NC, HC, CON and EC, to analyse the influences, if any, of aortic cross-clamping, CPB and cardiac instrumentation (table 4.11). The data on plasma enzyme activity of these groups are summarized in appendix 12. The release of AST was significantly

higher in the groups N and C, as compared to group H. The differences between the groups N, H and C were not statistically significant for creatine kinase. The effect of aortic cross-clamping on enzyme release was evaluated in groups NC and HC. The cumulative release of AST in both groups was not significantly different from that in the corresponding groups with aortic cross-clamping, N and H, respectively. However, the creatine kinase release was less in group H than in group HC.

For group CON and group EC, in which the animals were not subjected to CPB, the cumulative release of creatine kinase was decreased as compared to the groups with CPB, but this difference was not significant when comparison was made between group CON and group NC.

4.2.8. Plasma volume

In group HPLV serial plasma volume measurements were performed before and after CPB. The experimental protocol in this group was comparable to that in group H, including hypothermic CPB and four periods of aortic cross-clamping. In group HPLV the total post CPB recovery period was extended to 120 min. Data on plasma volume was obtained 5 min after the administration of a bolus of AST into the circulation (fig 4.33). The plasma values calculated are shown in fig 4.34 and indicate that changes in plasma volume after anesthesia and sternotomy were insignificant. Fifteen min after CPB, however, significantly increased plasma volumes, amounting to 150% of the values before induction of anesthesia, were measured. During the recovery period plasma volume rapidly normalized within 90 min to 110% of the plasma volumes before anesthesia.

Beside plasma volume the disappearance rate constant (k_d) of ALT was determined in the same group of animals (HPLV) after CPB, using an ALT bolus injection (fig 4.35). Analysis of the k_d value for ALT as a measure of the enzyme leakage from the intravascular space, revealed that k_d had increased to $50 \pm 19\%$ per hour (mean value $\pm$ SD; $n=5$). This indicates a markedly increased value of TER, from 0.03 to 0.46, assuming a constant value of FCR equal to 0.04 for ALT. Both the plasma volume changes and the k_d value after CPB were used to calculate cumulative release of AST and creatine kinase as reported in section 4.2.7.

Fig 4.33 Plasma levels of AST used for plasma volume calculation with the use of an AST bolus injection (indicated by an arrow). Mean values are presented (U.l⁻¹). The value of PLV1 was obtained after induction, PLV2 after sternotomy and PLV3 just prior to CPB. PLV4, PLV5 and PLV6 was determined 15, 90 and 120 min after weaning from CPB, respectively. AC = aortic cross-clamping, CPB = cardiopulmonary bypass.

Fig 4.34 Plasma volume (PLV) in dogs before and after cardiopulmonary bypass (CPB) as calculated from the data shown in fig 4.33. Mean values and SEM (ml.kg^{-1} body weight) are presented ($n=5$).

Fig 4.35 Plasma levels of ALT used for k_d calculation after an ALT bolus injection. Mean values and SD (U.l^{-1}) are presented. AC = aortic cross-clamping, CPB = cardiopulmonary bypass.

See legend of fig 4.33.

5. DISCUSSION

In this chapter the results of the clinical and experimental studies will be discussed. In the clinical study the efficacy of the protective measures against ischemia during three different operation techniques, i.e. intermittent aortic cross-clamping at 34°C and at 25°C, and continuous aortic cross-clamping in combination with St Thomas hospital cardioplegia, were evaluated.

From the experimental study more detailed information about the significance of some of the variables measured could be obtained. These findings will be discussed in relation to the observations in the clinical study. Besides, topics such as the hemodynamic and biochemical function of the heart during the initial phase of cardiopulmonary bypass (CPB), and the effect of CPB on coronary artery blood flow will be discussed separately.

5.1 General topics

5.1.1 Clinical outcome and hemodynamic recovery

The myocardial protection offered by the three techniques under investigation resulted in adequate postoperative recovery, a low incidence of inotropic support and an hospital mortality of about 4% (one patient in each group).

The hemodynamic function as assessed during the 15 minute time interval after cessation of CPB, showed that both left ventricular dP/dt max, pos and stroke work index were depressed in all three groups. The postoperative increase in pulmonary artery wedge pressure observed in the hypothermia and cardioplegia groups should be interpreted with care, because the pulmonary artery wedge pressure was deliberately set at a value between 1.1 and 1.4 kPa by the anesthesiologist at the end of the CPB phase. This level of pulmonary artery wedge pressure was achieved by infusing the content of the heart-lung machine and donor blood into the patient. The increase of cardiac index in all three groups after the operation most likely results from a decreased systemic vascular resistance.

It should be emphasised that the changes in hemodynamic function do not

solely reflect the influences of the periods of ischemia during aortic cross-clamping, but are also caused by the anesthetic policy and the CPB procedure. This is illustrated by the results of the animal study, because hemodynamic recovery was not different in the groups with and without aortic cross-clamping (Appendix 4), indicating that the application of global ischemia did not adversely influence systemic hemodynamics after CPB. Yet no significant difference could be detected in subendo and subepicardial fiber shortening in these two experimental groups.

In addition, hemodynamic function during and after CPB can also be influenced by humoral factors, such as circulating and locally released catecholamines. The higher perfusion pressure during CPB observed in the normothermia group may be related to an increase in the plasma level of norepinephrine (Kim et al, 1981), as measured immediately after CPB in this group, as suggested by Kim and coworkers (1981). Whether the significantly higher norepinephrine levels in the normothermia group exert a positive effect on left ventricular hemodynamics and thereby masking a more pronounced deleterious effect of the intermittent aortic cross-clamp technique at 34°C on post CPB hemodynamic function, remains to be elucidated.

The origin of the postoperative increase in plasma catecholamine levels is not exactly known. The adrenal glands might be involved, although the involvement of other organs can not be excluded. Mori and coworkers (1983), for instance, proposed that myocardial ischemia itself caused an increase in plasma norepinephrine content, while Reves and colleagues (1984) concluded that increased catecholamine levels resulted from reperfusion of the heart following ischemia. In our clinical study the highest correlation between the maximum plasma norepinephrine level and the total duration of aortic cross-clamping was found in the normothermia group ($r=0.77$). No such correlation was observed in the other two groups. The latter observation is in agreement with Engelman and coworkers (1983), who did not find a correlation between duration of aortic cross-clamping on the one hand and plasma levels of catecholamines on the other in patients submitted to aortocoronary bypass surgery or valve replacement in hypothermic conditions.

In the clinical study hemodynamic function was determined during a relatively short postoperative period of 15 minutes after CPB. The rationale was to assess early hemodynamic recovery and to exclude the influence of various interventions routinely applied postoperatively at the intensive care unit,

because in principal no medication was given during the initial period after CPB. This does not imply that the condition immediately after the surgical intervention is representative of the situation after one or two days. Several studies report a changed hemodynamic function immediately after the operation, and a return to preoperative conditions within two days (Levinsky et al, 1979; Roberts et al, 1981; Jalonon et al, 1981c).

In conclusion, hemodynamic function immediately after CPB was changed in all patient groups, but no major differences between the groups could be observed. It is likely that myocardial ischemia as induced during the surgical procedure, is not the sole cause of the reduced values of parameters related to cardiac contractility (left ventricular dp/dt max, pos and stroke work index), but that the anesthetic policy, CPB procedures and humoral factors should be considered as well.

5.1.2 Biochemical variables in arterial blood

- Lactate

The gradual increase of serum lactate concentration in the three patient groups under investigation indicates that the patients were not stable from a metabolic point of view. This confirms the findings of Olin and coworkers (1981). Since no differences were observed between the normothermia group and the two other groups, we might conclude that the metabolic instability is not directly related to body temperature during CPB. Theoretically, an increased production of this metabolite, most likely in skeletal muscle, or a reduced degradation, for instance, in the liver and the heart, might occur.

A similar phenomenon was observed in dogs subjected to CPB. Since the increase of serum lactate was less prominent in the dogs not subjected to CPB, the imbalance between lactate production and degradation is partly induced by extracorporeal circulation.

- Inorganic phosphate

With respect to inorganic phosphate the arterial serum concentration did not substantially change, neither in patients nor in dogs, although a small but significant transient decrease was observed after the start of CPB in all three patient groups and in the animal groups. This is most likely due to

hemodilution by the priming solution of the oxygenator.

- Potassium

The concentration of potassium showed a rapid, but transient increase in the last period of CPB in the cardioplegia group. Part of these ions originates from the cardioplegic solution. Since the arterial potassium concentration also increased in the patients of the hypothermia group, an endogenous source of potassium should be considered as well. In both groups mentioned before, the enhanced mean potassium levels did not exceed a value of 5.5 mmol.l^{-1} , so that serious disturbances caused by high circulating levels of this ion should not readily be anticipated (Weber and Yarnoz, 1982).

5.1.3 Arterial-coronary sinus differences of biochemical variables in blood

- Lactate

In the clinical study the arterial-coronary sinus (A-CS) difference of lactate during the hemodynamically stable phase prior to CPB, did not differ from zero. This indicates no net uptake of this substrate by the heart. In contrast, a substantial amount of lactate was extracted from the blood in the pre CPB phase by the dog heart. Net lactate uptake by animal hearts has been considered as a sign of an adequate aerobic condition (Opie et al, 1973; Apstein et al, 1979; Drake et al, 1980a; Van der Vusse et al, 1982). The discrepancy between human and dog hearts most likely results from spatial inhomogeneities in the former hearts. Lactate release from diseased parts of the patient hearts, might mask the ability to extract net amounts of lactate (Apstein et al, 1979). In this respect it is noteworthy that healthy human hearts are able to extract significant amounts of lactate (Kaijser et al, 1979). A similar discrepancy was observed in the post CPB phase. In both the human and experimental study the A-CS differences returned to their pre CPB levels. No net uptake of lactate was found in the human situation despite an assumed adequate O_2 supply of the formerly potentially underperfused areas through the aortocoronary bypass grafts. The significance of this observation is open to speculation.

The release of lactate during the reperfusion phase, following aortic cross-clamping in the normothermia and the hypothermia groups indicates that anaerobic glycolysis was activated during the periods of global ischemia. In contrast, the finding that hardly no release of lactate was observed after unclamping of the aorta in the cardioplegia group most likely reflects the absence of glycolytic activity in the cardioplegia treated hearts, although washout of lactate by the intermittently administered cardioplegic solution cannot be excluded. The observation that the myocardial glycogen content was not significantly altered in this group (see section 4.1.3.3) makes the latter explanation less feasible.

The higher peak values of the A-CS differences of lactate in the normothermia group than in the hypothermia group suggest that the glycolytic activity during the preceding period of ischemia is more enhanced at normothermia. This conclusion is supported by the calculation of the net release of lactate in the dog experiments. The loss of lactate during reperfusion in the normothermia and hypothermia patient groups gradually decreased, reaching zero values 10 minutes after unclamping of the aorta. In contrast, in the dog experiments within 5 minutes of reperfusion release generally changed into net uptake of lactate. This difference between dogs and humans may also result from the partially diseased state of the human hearts under investigation.

This data shows that washout of lactate accumulated during ischemia, generally occurs in the first two minutes of reperfusion. In other studies a delayed washout of lactate was observed (Gray et al, 1976; Olin et al, 1981; Jalonen, 1981d; Edlund et al, 1982). These investigators, however, only took blood samples 3 to 10 minutes after release of the aortic clamp. At the present state of the art it is impossible to decide whether this is a different pattern of lactate washout, because in these studies the main lactate release during the first two minutes of reperfusion might have been missed. For proper judgement of the occurrence of ischemic episodes during aortic crossclamping, early sampling is required.

- Inorganic phosphate

With respect to the A-CS differences of inorganic phosphate, basically no disturbances in the cardiac homeostasis of this ion could be observed during the pre and post CPB phase, in both the human and dog study.

In the patient groups washout of inorganic phosphate was prominent in the first minutes after release of the aortic cross-clamping, and the duration of this imbalance was shorter than observed for lactate. A significantly higher A-CS difference after the first aortic cross-clamping as compared to the succeeding peak values of A-CS differences, was prominent in the normothermia and hypothermia patient groups. This phenomenon was not observed in the dogs of the normothermia group, and only to a minor degree in the dogs of the hypothermia group. No explanation is at hand for the differences in release pattern between man and dog. The peak values of the A-CS differences of inorganic phosphate after unclamping the aorta were less pronounced in the patient cardioplegia group, as compared to the other two groups. A similar observation was made in the dog study.

In contrast to the patient study, in the animal experiments hypothermic conditions during aortic cross-clamping resulted in reduced inorganic phosphate washout at reperfusion, as compared to the normothermic situation. The significance of this finding will be discussed in section 5.1.4.

In dogs uptake of inorganic phosphate from the blood occurred after approximately 4 minutes of reperfusion in all groups, but in patients a positive A-CS difference of inorganic phosphate in the normothermia and hypothermia groups was only observed during reperfusion after the fourth aortic cross-clamping. Similar to the findings of lactate, this observation most likely reflects differences in the state of healthiness between human and dog hearts.

Since the findings in the experiments with dogs on CPB without aortic cross-clamping revealed that A-CS differences were not different from zero under both normothermic and hypothermic circumstances (Appendix 11), we may conclude that the extraction of inorganic phosphate from the blood does reflect replenishment of intracellular inorganic phosphate stores, which are reduced during the preceding reperfusion phase. This observation suggests the reversible nature of the repetitive ischemic insults.

- Potassium

Since the A-CS differences of potassium did not substantially differ from zero in the pre and post CPB period in both patients and dogs, disturbances in the potassium homeostasis of the heart can be excluded. The rapid release of potassium after unclamping of the aorta in the normothermia and hypother-

mia groups reflects accumulation of this ion in the extracellular space during ischemia. A variety of authors has shown that washout of potassium from myocardial tissue occurs within the first minutes of ischemia, when only reversible ischemic changes are involved (Opie et al, 1973; Van der Vusse et al, 1980; Kleber et al, 1984). This phenomenon may be caused by disturbances at the level of the membrane $\text{Na}^+\text{-K}^+\text{ATPase}$ or by a higher potassium conductance induced by ischemia (section 2.2.4). The observation that the initial high washout of this ion is followed by a significant uptake indicates the ability of the heart to replenish the reduced intracellular potassium stores. This supports the notion that the insult of the preceding ischemic period is reversible in nature. It is noteworthy that the time interval of potassium release, following unclamping of the aorta, is much shorter in dogs than in patients which might reflect a less healthier state of the myocardial tissue in the latter.

The higher peak values and more sustained release of potassium in the cardioplegia group in comparison with the other two groups, in both patients and dogs, reflects the washout of potassium ions. These ions are most likely derived from the vascular space filled with cardioplegia solution containing a relatively high concentration of potassium (16 mmol.l^{-1}). From the dog experiments we were able to estimate the quantitative contribution of the potassium derived from the cardioplegic solution, trapped in the vascular space during ischemia, to the amount of potassium released during reperfusion. On the average $8.6 \text{ } \mu\text{mol potassium.g}^{-1}$ wet weight of heart tissue were released. This amount corresponds with about 1.3 mmol per dog heart. Assuming a left ventricular weight of 150 g and a vascular volume of 15 ml filled with the cardioplegic solution, about 0.24 mmol can be expected to originate from this solution. This difference can only be explained when potassium ions originating from the intracellular space are released as well. It is not likely that potassium release due to the period of ischemia will contribute to the release calculated above, since only minor metabolic disturbances on the base of the tissue lactate and high-energy phosphate contents were observed.

- Oxygen

The A-CS differences in the oxygen content were significantly decreased during the post CPB period as compared to the A-CS differences before CPB, in

both the animal and the patient groups. This observation was also reported in patients by Olin and coworkers (1981) and by Jalonen (1981d). This change in A-CS differences most likely results from a simultaneously increased myocardial blood flow as was observed after CPB in the animal groups. The latter observation is in agreement with the finding of Gray and coworkers (1976).

After the release of aortic cross-clamping initially high values of A-CS differences in the oxygen content were observed in patients of the normothermia and hypothermia groups, which suggests that the blood sampled from the coronary sinus during the first 30 seconds is low in oxygen due to repayment of oxygen debt.

The animal experiments show that oxygen consumption was lower during CPB under hypothermic than under normothermic conditions. This clearly indicates a reduction of energy demand of the heart due to a lower temperature of the heart (Appendix 11).

In general, the findings in the patient and animal studies were comparable and indicate that the effect of aortic cross-clamping on oxygen consumption was reversible; a stable condition was reached again within 5 min of reperfusion.

5.1.4 Biochemical variables in tissue

5.1.4.1 Tissue high-energy phosphates and glycogen in patients

The reduction in the ATP content and the instantaneous release of inorganic phosphate during the periods of reperfusion in the normothermia and hypothermia patient groups, clearly indicate that the myocardial energy requirements exceeded the oxygen and substrate supply during ischemia. Although the tissue content of creatine phosphate was not changed after ischemia, a decrease during ischemia might have been restored to control values during the short reperfusion period prior to taking biopsies. In the cardioplegia group, the energy demand has obviously been reduced to low levels since no change in myocardial ATP was observed. Besides, only minor differences in the content of inorganic phosphate between arterial and coronary sinus blood could be detected upon reperfusion. The apparent abolishment of energy consuming processes such as basal metabolism and electro-mechanical

activity, likely results from the low temperature of the heart ($<18^{\circ}\text{C}$ during the ischemic period) and the high levels of potassium and magnesium present in the infusate.

This conclusion is supported by the observations on tissue glycogen. The reduced content of myocardial tissue glycogen and the release of lactate after restoration of coronary artery blood flow in the normothermia and hypothermia patient groups indicates that anaerobic glycolysis is activated during the periods of global ischemia in these groups. In contrast, hardly any sign of accelerated glycolytic breakdown of glycogen stores could be detected in the cardioplegia group.

Reports on the determination of high-energy phosphates in myocardial tissue are limited. During valve replacements Olin and coworkers (1981) observed that ATP and creatine phosphate levels were diminished at the end of one hour of aortic cross-clamping, using one single dose of blood cardioplegia. In contrast, these levels did not change when continuous blood cardioplegia was employed. In patients operated upon for aorto-coronary bypass surgery, protection with the use of multidose blood cardioplegia plus topical cooling of the heart during an ischemic insult of less than 80 minutes was evaluated by Cunningham and coworkers (1979). They could differentiate between "adequate" and "inadequate" myocardial protection on the basis of high-energy phosphate levels.

Lowering the temperature of the heart in the patient groups from 34°C to 25°C did apparently not result in a decrease of the energy needs of the heart, since the content of glycogen and ATP was lowered to a comparable extent in both groups. One should conclude on the basis of these findings that the difference between energy demand and supply was equal in both groups. This conclusion is not supported by the finding, that the A-CS differences in oxygen content was reduced at 25°C as compared to 34°C , which was confirmed quantitatively in the dog study. It is likely that the metabolic activity during ischemia, and the subsequent restoration during reperfusion, is more prominent under normothermic than under hypothermic circumstances and that these two processes will ultimately result in a similar reduction of energy stores at the moment of measurement.

The consequences of the reduced ATP and glycogen contents in human myocardial tissue is open to speculation. In dogs a relationship between low ATP levels and lethal injury in myocardial ischemia was found by Jennings and

colleagues (1978). Loss of 65% of the tissue ATP was associated with minimal ultrastructural changes. Depletion of more than 90% of the ATP was associated with significant morphological disturbances and markedly altered cell volume regulation. The decrease in tissue ATP content in patients of the normothermia and hypothermia groups was found to be 28 and 29%, respectively. Although extrapolations from animal data have to be done with care, it may be concluded that the levels of ATP as observed in the human hearts after global ischemia and reperfusion are not likely to be associated with severe myocardial damage.

It should be realized that the tissue content of high-energy phosphates and glycogen were measured in biopsies obtained at least 20 minutes after unclamping of the aorta. Therefore, a combined effect of ischemia and reperfusion on the tissue content of these substances should be considered. In the dog experiments we have investigated the separate effects of ischemia and reperfusion in more detail.

5.1.4.2 Tissue content of ATP and glycogen in dog hearts. Comparison with the human situation

In the course of the experimental study we became aware of an interesting observation. The significantly decreased levels of ATP and glycogen as observed in the postischemic biopsies of the patients of the normothermia and hypothermia groups, was not seen in the comparable dog groups. To understand this discrepancy between the clinical and experimental results two factors should be considered. Firstly, the last postischemic biopsy in dogs was obtained 45 minutes after the last aortic cross-clamping, while in patients these biopsies were taken about 20 minutes after release of the last cross-clamp. This difference in recovery time might allow the resynthesis of tissue ATP. Secondly, differences in the functional state of the heart (i.e. healthy dog hearts versus regionally diseased human hearts) or species differences might play a role. With respect to ATP it is noteworthy that in the dog study after each individual reperfusion interval of 15 minutes the ATP levels were not different from the control values. In contrast, the values as measured at the end of the ischemic period, were significantly decreased after the first and third aortic cross-clamping in the normothermia group. This clearly

indicates that dog hearts can easily restore their ATP content, as reduced during a period of 10 minutes of ischemia.

With respect to glycogen in dog hearts, no significant change in the content of this carbohydrate store could be observed neither at the end of the ischemic, nor after the reperfusion period. These findings most likely reflect that the enzymatic system involved in the glycogen degradation is less sensitive to ischemia in dog than in human hearts.

5.1.4.3 Tissue content of creatine phosphate in dog hearts

Similar to the observations in the three patient groups, no significant decrease in the tissue content of creatine phosphate could be observed in the last reperfusion biopsy as compared to preischemic values. Since Jones and colleagues (1976) in regional ischemic hearts, and Kao and coworkers (1982) in global ischemic dog hearts have clearly demonstrated that the myocardial creatine phosphate content rapidly decreased during ischemia, a process of alternating dephosphorylation during ischemia and rephosphorylation during reperfusion is most likely responsible for the virtually unchanged levels in the postischemic biopsies. The findings in the normothermia and hypothermia dog groups demonstrate the feasibility of this notion, and clearly indicate the reversibility of the degradation of creatine phosphate in creatine and inorganic phosphate during the ischemic insult. The conclusion of Engelman and coworkers (1977) that multiple short periods of ischemia are deleterious to the heart is therefore only justified in case of short periods of intermediate reperfusion (5 min).

Despite the decrease in myocardial temperature from approximately 34°C (normothermia) to 25°C (hypothermia) in the animal groups, the decrease in tissue content of creatine phosphate during ischemia was not different between these two animal groups (table 5.1). This finding does not necessarily imply that the energy demands during hypothermic and normothermic ischemia are similar. The reduction in tissue creatine phosphate content has shown to be a very rapid process at 37°C (Hearse, 1979; Dunn, 1984; Prinzen et al, submitted for publication), ultimately levelling off in the dog heart at about 10-20 $\mu\text{mol.g}^{-1}$ wet weight of tissue after 5 minutes of oxygen deprivation. Reduction of temperature may delay the rapidity of the degradation of creati-

Table 5.1 Relation between the content of ATP, creatine phosphate and inorganic phosphate in myocardial biopsies at the end of ischemia, and inorganic phosphate release during reperfusion in dogs. Mean values and SEM are presented ($\mu\text{mol.g}^{-1}$ d.w.). Each period of aortic cross-clamping was separately used in the normothermia and hypothermia groups.

	Group		
	normothermia (n=24)	hypothermia (n=24)	cardioplegia (n=8)
Duration of ischemia (min)	10	10	60
Decrease in tissue content:			
ATP	4 $\pm$ 2	2 $\pm$ 2	5 $\pm$ 3
creatine phosphate	41 $\pm$ 4	38 $\pm$ 4	10 $\pm$ 4
Increase in tissue content:			
inorganic phosphate	44 $\pm$ 3	70 $\pm$ 7	20 $\pm$ 3
Washout of inorganic phosphate into venous blood	4.9 $\pm$ 1.3	1.1 $\pm$ 0.2	0.3 $\pm$ 0.2

ne phosphate but not prevent it.

5.1.4.4 Tissue inorganic phosphate content in relation to high-energy phosphate content and inorganic phosphate release.

The findings in the animal study show that the content of inorganic phosphate increased rapidly during ischemia and decreased to the same extent upon reperfusion. An important difference was found between the two dog groups with intermittent aortic cross-clamping, with respect to the quantitative rise in tissue inorganic phosphate content at the end of the ischemic period. In the normothermia group the increase of inorganic phosphate at the end of ischemia could be explained by the tissue ATP and creatine phosphate reduction, but in the hypothermia group, this increase exceeded the simultaneous reduction in high-energy phosphates (table 5.1). From these findings it might be concluded that another intracellular source of inorganic phosphate, for instance phosphorylated proteins, is responsible for the rise in tissue content of this ion.

Despite the higher tissue levels of inorganic phosphate at the end of ischemia in the hypothermia group, the total amount released was less than in the normothermia group (table 5.1). This implicates that the release during reperfusion was not quantitatively related to the high tissue levels as the result of ischemia. A possible explanation for this finding is that the rate of passage through cell membranes at lower temperatures is reduced (Pine et al, 1981). The significance of this finding might be that the simultaneously higher total tissue level of inorganic phosphate may represent a potential danger for the ischemic tissue, since a positive correlation between the height of the inorganic phosphate content and disturbances in the calcium homeostasis has been described (Ponce-Hornos and Langer, 1982; Ponce-Hornos et al, 1982).

5.1.4.5 Tissue lactate in relation to glycogen and release of lactate in the coronary sinus blood

The myocardial lactate content rapidly changed during ischemia and reper-

Table 5.2 Relation between the content of glycogen and lactate in myocardial tissue at the end of ischemia, and the lactate release during reperfusion in dogs. Mean values and SEM are presented ($\mu\text{mol.g}^{-1}$ d.w.). Each period of aortic cross-clamping was separately used in the normothermia and hypothermia groups. * = tissue lactate was determined in 2 dogs of the hypothermia group.

	Group		
	normothermia	hypothermia	cardioplegia
	(n=24)	(n=24)	(n=8)
Change in tissue glycogen content	-15± 8	13±14	16±15
Lactate increase in tissue *	68± 6	55	6± 4
Washout of lactate into venous blood	48±11	16± 2	7± 4

fusion in the animal groups with intermittent aortic cross-clamping. The increase of the lactate content during ischemia to two to four times the initial preischemic value and the return to the initial low levels during the subsequent reperfusion phase confirms the findings of Levitsky and Feinberg (1975), Imai and colleagues (1979) and Guilbeau and coworkers (1984).

The quantitative relation between the tissue content of glycogen and of lactate at the end of ischemia, and the release of lactate during the following reperfusion period is presented in table 5.2. This data shows that the increase in tissue lactate as measured at the end of ischemia, could not be explained by a simultaneous decrease in the glycogen content. An alternative source of tissue lactate may be the glucose, present in the cells at the start of ischemia. To calculate the possible contribution of tissue glucose to the lactate increase during ischemia, we assumed that the tissue glucose content was in equilibrium with the arterial glucose concentration. The latter concentration was found to be about 5 mmol.l^{-1} in the dogs on CPB (Appendix 9). This amount of glucose can be converted to $50 \text{ } \mu\text{mol}$ lactate per gram dry weight, which is in the range of the total increase in tissue lactate as observed in the dog groups. The assumption that during the initial phase of ischemia lactate is most probably originating from glucose breakdown, is supported by the findings of Prinzen and colleagues (submitted for publication). In a model of regional myocardial ischemia they observed that a significant venous washout of lactate was associated with an increased glucose uptake during the first minutes of ischemia. Within this time interval net glycogen degradation was not measurable.

5.1.4.6 Variations in the high-energy phosphate and glycogen contents in preischemic myocardial biopsies

In the myocardial biopsies obtained from the patient study in the pre-ischemic period, relatively large variations in ATP, creatine phosphate and glycogen content were measured. To evaluate whether these variations were the result of intracardial inhomogenieties in the patients with three vessel disease or of interindividual differences, the concomitant coefficients of variation were calculated (table 5.3). Besides, the coefficient of variation calculated for the preischemic biopsies obtained in the dog groups is shown,

Table 5.3 Coefficients of variation (%SD/mean value) of the ATP, creatine phosphate and glycogen contents in human and dog myocardial biopsies obtained shortly after instalment of CPB, but before induction of ischemia. Inter-individual values refer to the biopsies as obtained from different hearts, while intraindividual values refer to two biopsies obtained in the same heart.

	HUMAN		DOG
	interindividual (n=70)	intraindividual (n=14)	interindividual (n=20)
ATP	35	11	29
Creatine phosphate	37	11	29
Glycogen	34	20	36

to elucidate whether differences between species may be involved. It is evident from table 5.3 that the large variation as observed in preischemic biopsies, occurs in both animals and man and that the occurrence of myocardial inhomogenieties does not contribute considerably to the total variation. From this finding it may be concluded that evaluation of the glycogen and high-energy phosphate contents in small postischemic myocardial biopsies implies the occurrence of large interindividual variations. These findings indicate that the use of changes in tissue content of these biochemical substances as markers for myocardial ischemia, is only allowed when using the patient or the animal as its own control. Therefore, pre and postischemic biopsies are required.

5.1.4.7 Summary of biochemical findings in tissue

The reduction in ATP and glycogen content, and the release of inorganic phosphate and lactate during reperfusion in the normothermia and hypothermia patient groups, indicate sustained metabolic requirements during ischemia in these patient groups. The rapid process of alternating dephosphorylation and rephosphorylation of creatine phosphate as observed in the dog study, is most likely responsible for the unchanged levels of creatine phosphate in the postischemic biopsies as was observed in patients. The animal study demonstrates that the temperature difference between the normothermia and hypothermia groups is not reflected in the changes in the high-energy phosphate tissue levels at the end of ischemia. However, the washout of inorganic phosphate was more prominent in the normothermia than in the hypothermia group. Similarly, the decrease in carbohydrate content does not differentiate between the latter two groups, except for the lactate washout during reperfusion. In general, the metabolic activity in the cardioplegia group was reduced to low levels as can be concluded from the absence of changes in tissue content of high-energy phosphates and glycogen, and the small release of metabolic markers of ischemia during reperfusion.

5.1.5 Changes in mitochondrial ultrastructure

Preservation of mitochondrial ultrastructure has been accepted as a

reliable parameter to determine the efficacy of myocardial protection during cardiac surgery (Cunningham et al, 1979; Flameng et al, 1980; Balderman et al, 1981).

Deterioration of mitochondrial ultrastructure during the operation was absent in the three patient groups under investigation. Both before and after aortic cross-clamping less than one percent of all mitochondria examined, exhibited signs of irreversible injury. Since five photographs were examined for mitochondrial evaluation, maximally 15 cells were included for evaluation. Therefore, a low incidence of cell damage could not accurately be detected.

It should be noted that the postischemic biopsy was obtained after ischemia and reperfusion for approximately 20 min. Therefore, possible changes at the end of ischemia may have been restored during the following reperfusion phase. Because this recovery of reversible changes is known to occur, our findings allow the conclusion that changes at the end of ischemia, if any, were not irreversible.

The observations in the present study show that the majority of the mitochondria in preischemic tissue specimens from subepi and subendocardial layers had normal cristae. Within approximately half of the mitochondria specific small granules were observed and in some cases irreversibly injured mitochondria were present. This observation is in agreement with the findings reported by Levinsky and coworkers (1979) and most likely reflects the morphologically inhomogeneous state of cardiac tissue of patients with three vessel disease (Laguens et al, 1979).

Ultrastructural evaluation was not employed in the animal study because the findings in patients had shown that no changes could be expected in the three techniques of myocardial protection.

In summary, ultrastructural evaluation of mitochondria revealed adequate protection in the three patient groups.

5.1.6 Enzyme release

In the patient study the amount of irreversibly damaged myocardial tissue due to the surgical procedure was estimated from serial determinations of plasma enzymes after CPB.

The curves of the plasma enzyme activity reveal a comparable pattern in the three patient groups. The plasma peak values occurred within 24 hours postoperatively for the enzymes under investigation, and these values were not significantly different in the three groups.

Although plasma enzyme activity can be applied to quantitate myocardial injury, this is more difficult in patients after cardiac surgery due to additional interventions which may cause enzyme release from non-cardiac sources (Hermens et al, 1982). Similar to quantification of enzyme release after acute myocardial infarction (Shell et al, 1973; Willems et al, 1979), we calculated the cumulative release of heart specific enzymes. Corrections were made for the occurrence of hemolysis, changes in circulatory parameters and the release of enzymes from non-cardiac sources (see section 2.2.6). The cumulative enzyme release was transformed into the equivalence of myocardial tissue injured and revealed comparable values of irreversibly damaged cardiac tissue in the three patient groups.

Assuming that the elevated levels of CK-MB are specifically originating from myocardial tissue, we may conclude that release of cardiac enzymes mainly occurs within the first 12 hours after CPB. Calculation of the cumulative release of CK-MB indicates that the amount of irreversibly damaged tissue is in the order of 1% of the total heart of the patients of the three groups. Comparable findings were reported by Sylven and coworkers (1982). They calculated that enzyme release equivalent to 5 g of myocardial tissue was present after uncomplicated aorto-coronary bypass surgery. In contrast, Roe and coworkers (1979) found that upto 16 g of heart tissue was irreversibly injured in patients operated under comparable circumstances.

Cumulative release of HBDH, corrected for HBDH released from red blood cells due to hemolysis, was in keeping with the findings on the basis of the CK-MB release. However, when the heart was considered as the sole source of creatine kinase released into the circulation, erroneously high values of damaged myocardial tissue were estimated. This was also observed for AST, although to a lesser extent. Beside the heart, prominent sources of these two enzymes will be the intercostal, pectoral and diaphragmatic muscles which are apparently at risk during open-heart surgery (Hermens et al, 1982). It should be realized that cardiac enzyme release can be caused by other interventions than global ischemia of the heart due to aortic cross-clamping. Such procedures as application of thermoprobes and biopsy needles, and insertion of

tubes for venous return to the heart-lung machine and for venting of the left ventricle should be considered. In addition, defibrillation may cause tissue injury (Dahl et al, 1974). Finally, reperfusion of chronically ischemic tissue through the installed aorto-coronary bypasses might provoke the release of enzymes as well. The quantitative contribution of these factors to cardiac enzyme release is difficult to estimate, but we assumed these factors contributed similarly in the three patient groups.

The findings in the animal experiments support the above mentioned assumptions. The increase of plasma HBDH was observed in the groups with CPB, independent of aortic cross-clamping, and occurred parallel to the increase of plasma free hemoglobin (Appendix tables 12e and 12f). This indicates that hemolysis is induced by the arterial pump in combination with extracorporeal oxygenation, which is supported by the finding that hemolysis did not occur when the employment of CPB was omitted.

The cumulative release of creatine kinase was calculated in the dogs during the first hour after cessation of CPB, and revealed that release of this enzyme did not allow to discriminate between the three techniques of myocardial protection. In groups without CPB reduced release was observed, even when thoracotomy and insertion of cardiac cannulas was performed (table 4.11). This indicates that the CPB procedure of 145 minutes in itself caused a significant release of creatine kinase. The cumulative release of AST in the dogs did not discriminate between groups because of the small release during this phase of the operation.

Decreased hematocrit values were commonly observed during and after CPB as a consequence of both hemodilution and increased plasma volume during the immediate postoperative phase. It should be noted that in the patient groups the hematocrit returned to normal values within 4 hours (Willems et al, accepted for publication). Cumulative enzyme release is basically determined by the release between 0 and 36 hours postoperatively. Because during the first four hours a significant contribution to the totally measured release can be expected (creatinine kinase 10%; AST 20%; CK-MB 30%; HBDH 40%), changes in hematocrit will have some effect. It was assumed that this effect was rather small as compared with the total amount of enzymes released.

In one specific dog group we also measured a considerable increase of k_d after CPB, which was used as a measure of enzymes disappearing from the intravascular space. It is not known, whether the transcapillary exchange

rate (responsible for the change in k_d value) remains increased thereafter. Most likely the changes in colloid osmotic capacitance of the circulating blood plasma are important determinants of the transcapillary exchange rate of proteins. Since the colloid osmotic properties have been reported to return to normal values within 2 hours postoperatively (Ghadiali and Cox, 1981), we assume that changes in k_d will be less pronounced during the time interval of enzyme release.

In summary, the enzyme release during the first 36 hours after the operation was similar in the three patient groups under investigation. With the use of multi-enzyme analysis, we were able to calculate the contribution of non-cardiac sources of enzyme release (i.e. hemolysis, skeletal muscle). After correction for changes in circulatory parameters like plasma volume and transcapillary exchange rate, we calculated that in the three patient groups less than one percent of the heart was irreversibly damaged. From the animal study we can conclude that the cardiac enzyme release due to the periods of ischemia could not be separated from other causes of enzyme release. Thoracotomy, anesthesia and extracorporeal circulation should be considered as important causes of increased enzyme activity in postoperative plasma.

5.2 Selected topics

5.2.1 Hemodynamic and biochemical changes in the initial period of cardiopulmonary bypass

The initial extracorporeal perfusion phase was examined in more detail, because several investigators have suggested that this particular period may be harmful to the heart (Hottenrot et al, 1974; Vinas et al, 1979). Hemodilution, hypotension (Gray et al, 1976) and fibrillation (Hottenrot et al, 1974), or a combination of these conditions have been suggested to cause a shortage of myocardial oxygen leading to a considerable ischemic insult.

In the animal study we did observe hemodilution and hypotension, but were unable to demonstrate impaired coronary artery blood flow (section 4.2.3). The oxygen consumption reduced significantly after instalment of CPB in normothermic (from 3.26 to 2.31 $\mu\text{mol.g}^{-1}.\text{min}^{-1}$) and hypothermic conditions

(from 2.43 to 1.38 $\mu\text{mol.g}^{-1}.\text{min}^{-1}$). This can be ascribed to the lower metabolic activity of the empty beating heart especially because the delivery of oxygen was not diminished. Despite these findings net lactate uptake simultaneously disappeared in the dogs and turned into lactate release in the patients, indicating that anaerobic glycolysis was activated despite adequate oxygen delivery.

In contrast to the patients, in dog hearts lactate uptake had returned to pre CPB values within 10 min after the start of CPB. In all three patient groups release of lactate remained. This difference in metabolic response between dogs and patients probably results from regional ischemic areas in the patient hearts. From these findings we may conclude, that the instalment of CPB, hemodilution and hypotension caused a metabolic change in both dogs and patients. This emphasises that the early phase of CPB may affect the heart, although this insult was shown to be reversible in the dog hearts.

From the hemodynamic and biochemical variables as measured in the hypothermic animal group, we could not confirm the harmful influences of fibrillation. Because these influences were reported to occur during fibrillation under continuous electrical stimulation (Hottenrott et al, 1974; Vinas et al, 1979), we conclude that spontaneous fibrillation or fibrillation without continuous stimulation, does not exert harmful effects on the heart.

5.2.2 Coronary artery blood flow

In the normothermia, hypothermia and cardioplegia animal groups an increase in coronary artery blood flow in combination with a decrease in A-CS difference of the oxygen content was observed after the period of CPB. The net oxygen consumption was calculated to be comparable during the pre and post CPB phase. Most likely the reduced hematocrit value after CPB, resulting in a lower oxygen carrying capacity, was compensated by an increased myocardial blood flow.

An increase in coronary artery blood flow was also reported in patients after aorto-coronary bypass surgery (Albert et al, 1970; Gray et al, 1976), although these reports do not reveal whether the enhanced blood flow is caused by hemodilution or improved distal run-off through the grafts (Reneman and Spencer, 1972; 1975).

Marked changes in coronary artery flow were observed during CPB, follow-

ing unclamping of the aorta. This hyperemic response appeared to be delayed in the normothermia group. In contrast, this response occurred immediately in the other two groups and was followed by a rapid (within 5 minutes) reduction of coronary artery blood flow. The difference in hyperemic myocardial flow pattern between the normothermia and hypothermia groups might be explained by a combined effect of ischemia, reduced temperature and different state of the myocardium (i.e. fibrillating in the hypothermia group versus empty beating in the normothermia group). Although it was not the aim of the present study, some attention has been paid to the differences in reactive hyperemia between the two groups with intermittent aortic cross-clamping. It should be noted, that such differences might influence the interpretation of the release of substances from the heart observed during reperfusion. In the animal study, however, the patterns of A-CS differences were basically comparable with the curves related to net uptake or release, (i.e. A-CS differences multiplied by coronary flow). Although extrapolation of animal data on the reactive hyperemic response to the human situation should be done with care, we may assume that the A-CS differences as assessed in the human study, globally reflect the changes in metabolic condition of the heart.

5.3 General considerations on normothermic, hypothermic and cardioplegic techniques in open-heart surgery

Continuous aortic cross-clamping to perform the distal anastomosis in combination with multidose cardioplegic solutions is commonly employed in aorto-coronary bypass surgery. The technique of myocardial protection through several short lasting periods of aortic cross-clamping, in both hypothermic (25°C) and normothermic (34°C) conditions is less commonly used, although good clinical results have been reported (Flameng et al, 1981b; Pepper et al, 1982). In the present study these three techniques were compared in a randomized clinical trial, and evaluated by hemodynamic, biochemical and ultra-structural variables.

The hemodynamic recovery of the patients after weaning from CPB was adequate. No major differences between the three groups could be observed. The reduced left ventricular stroke work index and left ventricular dP/dt max, pos and the increase in cardiac index are generally observed as a transient response to anesthetics used and to the CPB procedure per se (this study, Roberts et al, 1981; Jalonen et al, 1981c).

The increase of plasma catecholamine levels during CPB, which was also reported by other investigators, most likely result from the non-pulsatile flow, as generated by the heart-lung machine, and hemodilution (Sebel et al, 1981; Estaphanous et al, 1984). The higher plasma levels of norepinephrine immediately after CPB in the normothermia patient group suggests a more prominent release as the result of normothermic perfusion. As a consequence cardiac function can be stimulated in this group as was suggested by Regan and coworkers (1970).

The amount of irreversibly damaged myocardial tissue was very small as assessed with the use of postoperative enzyme release. Besides, no significant differences between the three operative techniques could be observed. These findings confirm the results of earlier reports in which cardioplegia was compared with intermittent aortic cross-clamping combined with moderate (32°C; Roberts et al, 1981; Pepper et al, 1982), or more profound hypothermia (Conti et al, 1978; Lolley et al, 1980). In contrast, other investigators found significantly higher CK-MB plasma levels in patients after intermittent aortic cross-clamping at 28 to 32°C as compared to cardioplegia (Adappa et

al, 1978; Weisel et al, 1978; Follette et al, 1978; Brower et al, 1982). The conclusion from the data on enzyme release, that less than one percent of myocardial tissue was irreversibly damaged, is in agreement with the finding that postischemic ultrastructure was very well preserved.

The decrease of glycogen content in postischemic biopsies and the instantaneous release of lactate during the intermittent reperfusion periods revealed that glycolysis was accelerated in the normothermia and hypothermia groups. Although this process is stimulated by the imbalance in energy demand and supply, this metabolic change failed to maintain the ATP contents at their preischemic values. Obviously, the decreased ATP levels remained within safety limits. In the cardioplegia group the ATP and glycogen tissue contents did not decrease, and the washout of metabolites was clearly reduced. In conclusion cardioplegia is superior to the other techniques as far as the biochemical data is concerned.

6. Appendix: animal data

Appendix 1. Body weight and left ventricular weight (including the free wall and septum) of the 10 animal groups. Mean values and SD are presented.

Group	Body Weight (kg)	Left ventricular weight (g)
N	26± 3	152±31
H	26± 6	147±60
C	23± 3	129±21
NC	28± 5	150±45
HC	27± 5	150±27
NB	24± 2	129±27
HB	28± 6	155±29
EC	34±10	-
HPLV	22± 2	-
CON	28± 6	141±28

Appendix 2. Rectal temperature of the animals in group N (■), group H (●) and group C (▲). Mean values and SD are presented. E = empty beating, F = fibrillating.

Appendix 3. Septal temperature during cardioplegic infusions in group C. Mean values and SEM are presented (n=8). The cardioplegic solution was infused each 20 min as indicated by the arrows. Mean volume of 450, 475 and 325 ml was infused, respectively.

Appendix 4. Hemodynamic variables determined immediately before (time 0 min) and 15 and 60 min after cardiopulmonary bypass. Mean values and SD are presented.

Variable	Time (min)	Group					
		N	H	C	NC	HC	CON
Heart rate (beats. min ⁻¹)	0	142±38	143±46	117±16	112±38	138±35	124±44
	15	149±30	161±24	122±10	133±26	132±25	122±25
	60	132±31	138±29	119±15	125±25	130±25	128±35
Pao,sys (kPa)	0	9.6±1.7	10.5±1.5	10.0±1.2	10.0±1.9	10.7±2.5	10.9±2.4
	15	7.7±2.0*	8.5±2.7*	9.5±1.5	10.1±2.9	10.1±2.5	10.8±3.1
	60	8.8±1.5	8.5±2.1	9.3±2.7	9.1±2.1	9.5±2.1	9.9±2.8
Pao,dia (kPa)	0	6.8±1.7	7.6±1.5	7.2±1.2	7.3±1.9	7.2±1.3	7.3±2.0
	15	4.8±1.5*	5.6±1.6*	6.1±0.9	6.9±2.4	6.0±1.7	7.6±2.7
	60	5.6±1.6	5.7±1.6	5.7±1.3	6.5±2.1	6.4±2.0	6.5±2.3
CO (l.min ⁻¹)	0	2.5±0.7	2.4±0.4	2.0±0.5	2.1±0.6	2.3±0.9	2.4±0.7
	15	2.8±0.7*	3.3±0.7*	2.5±0.4*	3.1±1.4*	3.8±1.3*	2.0±0.4
	60	2.7±0.7*	3.1±0.7*	2.9±0.8*	2.8±1.1*	2.9±1.2*	1.8±0.3
Plv (kPa)	0	10.7±1.6	11.3±1.7	11.1±1.5	10.7±2.1	11.6±3.5	12.1±2.4
	15	10.0±1.9	10.4±3.2	11.5±1.5	10.7±3.3	11.7±3.6	9.9±3.6*
	60	10.9±2.9	10.1±1.6	11.7±3.5	10.4±1.6	10.1±1.7	9.2±2.9*
Plv,ed (kPa)	0	0.5±0.4	0.4±0.3	0.3±0.3	0.5±0.5	0.5±0.5	0.5±0.5
	15	0.5±0.3	0.5±0.5	0.8±0.3	0.9±0.7	0.9±0.4	0.7±0.1
	60	0.8±0.7	0.8±0.7	0.7±0.4	0.8±0.7	0.7±0.4	0.7±0.3
dP/dt max (kPa.s ⁻¹)	0	180±40	220±67	193±60	173±40	173±53	167±60
	15	193±80	160±53*	173±40	187±47	187±107	140±27
	60	193±73	193±80	180±60	160±47	173±56	147±47

* - different from the value before cardiopulmonary bypass (p<0.05).

Appendix 5. Mean aortic pressure and pump flow during cardiopulmonary bypass.

The data of the mean aortic pressure are supplemented with the mean aortic blood pressure measured prior to and after cardiopulmonary bypass. Values of the pump flow and the results of cardiac output measurements obtained prior to and 15 min after cardiopulmonary bypass are presented. The results of the groups N and NB, and of the groups H and HB were combined. Mean values and SD are presented. CPB = cardiopulmonary bypass, EB = empty beating heart, F = fibrillating heart. Time indication in min refers to the duration of cardiopulmonary bypass.

Mean aortic pressure (kPa).

Time	Group				
	N+NB	H+HB	C	NC	HC
pre CPB	8.7±1.9	8.5±1.3	8.1±1.2	8.8±1.7	8.8±1.7
during CPB					
5 min(EB)	5.9±1.6	5.9±2.0	5.9±2.7	6.5±2.3	7.3±2.0
10 min(F)	6.1±1.5	5.6±1.3	5.9±1.3	7.3±2.3	6.8±1.9
45 min	7.7±2.3	9.3±2.0	9.5±1.2	7.9±1.5	8.8±2.3
75 min	6.4±1.5	9.3±2.3	10.9±2.3	7.9±1.6	9.3±3.5
105 min	6.1±1.7	9.5±1.7	7.2±1.2	8.4±2.4	8.5±2.1
135 min	6.0±2.1	8.4±2.4	7.7±1.6	8.0±1.6	6.4±1.1
post CPB	6.7±1.5	6.7±1.7	7.1±1.9	7.5±2.0	8.6±2.5

Pump flow and cardiac output ($\text{l}\cdot\text{min}^{-1}$).

Time	Group				
	N+NB	H+HB	C	NC	HC
pre CPB	2.5±0.7	2.4±0.4	2.0±0.5	2.1±0.6	2.3±0.9
during CPB					
5 min(EB)	2.3±0.7	2.6±0.5	1.7±0.3	1.8±0.7	2.3±0.8
10 min(F)	2.2±0.7	2.5±0.5	1.6±0.3	1.8±0.7	2.2±1.0
45 min	2.1±0.7	2.4±0.6	1.4±0.3	1.8±0.7	1.8±1.0
75 min	2.1±0.7	2.3±0.6	1.3±0.2	1.5±0.4	1.5±0.8
105 min	2.1±0.8	2.1±0.5	1.4±0.3	1.7±0.6	1.5±0.9
135 min	2.1±0.8	2.1±0.6	1.5±0.2	1.5±0.4	1.6±0.8
post CPB	2.8±0.7	3.3±0.7	2.5±0.4	3.1±1.4	3.8±1.3

Appendix 6. Coronary artery blood flow. The overall left ventricular blood flow data are obtained from the combined measurements with radioactive microspheres and magnetic flow probes situated around the LAICA. Mean values and SD are presented ($\text{ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ of heart tissue).

Time	Group		
	NC	HC	CON
pre CPB	0.57±0.21	0.57±0.15	0.54±0.16
during CPB			
5 min	0.88±0.80	0.78±0.23	-
10 min	0.56±0.18	0.63±0.19	-
45 min	0.68±0.19	0.50±0.27	-
75 min	0.54±0.20	0.43±0.21	0.57±0.23
105 min	0.71±0.42	0.68±0.45	-
135 min	0.58±0.17	0.72±0.24	-
post CPB			
15 min	0.64±0.28	1.38±0.76	0.65±0.30
30 min	0.91±0.71	1.31±0.55	-
60 min	0.68±0.22	0.81±0.20	0.57±0.25

Appendix 7. Epicardial deformation parameters and derived parameters before (time 0 min) and 15 and 60 min after cardiopulmonary bypass. Mean values and SD are presented. e_c = natural tangential strain, e_z = natural axial strain, e_{zc} = shear angle, e_{max} = maximum shortening on the epicardium, $e_{endo,est}$ = estimated subendocardial fiber shortening.

Variable	Time (min)	Group					
		N (n=7)	H (n=4)	C (n=8)	NC (n=5)	HC (n=6)	CON (n=5)
e_c (%)	0	5.2±3.7	2.7±6.6	3.6±2.8	2.7±1.9	2.6±4.1	6.8±4.2
	15	2.8±4.0	2.6±1.9	5.9±3.7	10.3±6.5	6.2±5.6	5.6±2.5
	60	5.9±5.4	3.3±3.1	6.9±3.0	6.8±6.0	4.1±6.0	4.4±1.3
e_z (%)	0	4.8±4.5	4.9±10.2	4.0±3.8	2.0±1.1	1.8±3.4	0.2±5.6
	15	5.8±5.6	4.0±5.9	4.0±5.9	3.1±1.7	3.9±5.1	-1.3±3.6
	60	5.7±6.9	2.8±3.2	5.0±5.9	4.2±5.5	1.2±3.3	1.6±4.2
e_{zc} (rad)	0	-0.01±0.06	0.01±0.07	0.09±0.06	0.07±0.06	0.00±0.07	0.04±0.09
	15	0.03±0.10	0.02±0.07	0.04±0.07	0.08±0.01	0.04±0.08	0.00±0.04
	60	0.04±0.11	0.03±0.08	0.06±0.10	0.05±0.07	0.04±0.07	0.01±0.06
e_{max} (%)	0	8.8±4.2	7.3±10.6	8.9±3.2	5.8±3.4	6.1±3.0	10.0±3.0
	15	9.4±6.5	7.2± 3.8	10.3±3.6	12.4±5.3	10.7±4.1	6.4±2.3
	60	10.5±7.0	7.8± 2.8	11.8±3.9	10.4±6.3	6.9±6.1	6.5±1.6
$e_{endo,est}$ (%)	0	9.3± 4.9	5.5±19.8	5.2±4.5	3.0±3.3	2.5±4.6	3.6±3.1
	15	6.2± 6.4	4.6± 7.6	7.5±5.7	9.4±9.5	7.5±5.5	2.7±3.8
	60	9.6±12.4	7.0± 4.7	9.5±5.9	8.9±4.8	3.1±5.6	4.3±5.5

Appendix 8. Blood gas values. Mean values and SD are presented.

pH values of arterial blood.

Time	Group		
	N	H	C
pre CPB	7.389±0.071	7.392±0.080	7.393±0.054
during CPB			
5 min	7.483±0.134	7.450±0.104	7.498±0.127
10 min	7.488±0.154	7.444±0.102	7.594±0.102
45 min	7.485±0.089	7.528±0.169	7.658±0.101
75 min	7.449±0.075	7.549±0.144	7.676±0.095
105 min	7.437±0.084	7.550±0.121	7.436±0.049
135 min	7.407±0.080	7.469±0.067	7.415±0.033
post CPB			
15 min	7.364±0.088	7.353±0.087	7.341±0.062
30 min	7.351±0.104	7.363±0.087	7.340±0.078
60 min	7.387±0.074	7.357±0.120	7.350±0.085

Time	Group			
	NC	HC	CON	EC
pre CPB	7.387±0.034	7.407±0.051	7.418±0.087	7.410±0.089
during CPB				
5 min	7.407±0.075	7.488±0.114	-	-
10 min	7.380±0.086	7.583±0.147	7.415±0.076	-
45 min	7.441±0.072	7.558±0.138	7.393±0.081	7.422±0.064
75 min	7.398±0.090	7.573±0.141	7.402±0.062	-
105 min	7.409±0.066	7.597±0.165	7.384±0.058	7.407±0.036
135 min	7.426±0.062	7.509±0.057	7.394±0.079	-
post CPB				
15 min	7.336±0.095	7.346±0.100	-	7.434±0.027
30 min	7.328±0.131	7.348±0.093	7.416±0.049	-
60 min	7.415±0.048	7.402±0.065	7.425±0.073	7.412±0.028

PCO₂ values of arterial blood (kPa).

Time	Group		
	N	H	C
pre CPB	5.4±1.2	5.4±1.3	5.2±0.5
during CPB			
5 min	4.7±1.1	5.1±1.6	4.5±1.0
10 min	4.8±1.1	5.1±1.9	3.3±0.8
45 min	4.3±0.5	4.0±2.5	2.5±0.5
75 min	4.5±0.5	3.6±2.0	2.3±0.4
105 min	4.6±0.6	3.4±1.5	4.4±0.7
135 min	4.9±0.8	3.9±1.1	4.6±0.5
post CPB			
15 min	5.8±0.8	6.5±1.5	6.1±1.2
30 min	5.8±1.2	6.3±1.5	5.8±1.5
60 min	5.5±1.2	6.1±1.9	6.1±1.3

Time	Group			
	NC	HC	CON	EC
pre CPB	5.5±0.6	5.3±0.7	5.1±1.1	5.0±0.8
during CPB				
5 min	5.5±1.2	4.8±1.6	-	-
10 min	5.4±1.1	4.0±1.6	5.1±1.4	-
45 min	4.5±0.6	3.6±1.8	4.5±0.8	5.8±1.4
75 min	4.7±0.9	3.3±1.8	5.0±1.1	-
105 min	4.9±0.8	3.2±1.8	4.9±1.2	5.7±1.1
135 min	4.7±1.0	3.9±0.8	4.8±1.1	-
post CPB				
15 min	5.7±1.3	6.1±1.7	-	5.2±0.8
30 min	5.9±1.6	5.9±1.3	4.7±1.0	-
60 min	5.3±0.5	5.5±0.8	4.7±1.4	5.2±0.4

PO₂ values of arterial blood (kPa).

Time	Group		
	N	H	C
pre CPB	24.8± 6.7	28.4± 7.1	29.2± 5.5
during CPB			
5 min	48.3±20.7	41.9±27.1	50.1±20.8
10 min	56.3±18.4	41.1±22.8	52.4±22.8
45 min	58.8±16.3	58.3±26.4	65.6±16.0
75 min	60.0±18.1	61.7±25.6	51.7±17.1
105 min	64.8±17.5	52.9±28.8	50.9±19.2
135 min	58.1±20.4	50.0±24.3	54.0±15.7
post CPB			
15 min	40.1±21.3	21.9±19.9	24.8±16.3
30 min	37.7±24.3	24.7±17.7	22.8±15.3
60 min	45.3±24.4	17.5± 7.9	21.9±16.4

Time	Group			
	NC	HC	CON	EC
pre CPB	24.5± 6.5	27.7± 5.6	28.4±6.5	20.9±7.7
during CPB				
5 min	32.2±20.4	52.7±28.7	-	-
10 min	40.0±19.2	57.1±13.9	27.7±5.5	-
45 min	36.7±20.1	63.1±14.3	28.3±5.3	22.1±6.1
75 min	39.3±10.8	63.1±18.9	27.5±4.5	-
105 min	38.9±20.7	49.5±24.3	28.4±5.9	26.5±9.7
135 min	31.1±13.3	45.1±20.5	30.1±8.1	-
post CPB				
15 min	28.0±24.7	21.3± 9.7	-	27.1±8.7
30 min	20.8±13.7	24.5±18.7	28.5±8.3	-
60 min	22.4±14.0	26.1±16.8	28.7±8.7	28.3±8.3

Appendix 9. Arterial concentration of lactate, inorganic phosphate, potassium and glucose. Mean values and SD are presented (mmol.l^{-1}).

Arterial lactate concentration (mmol.l^{-1}).

Time	Group		
	N	H	C
pre CPB	2.37±1.30	3.02±2.20	2.24±1.32
during CPB			
5 min	3.92±2.28	4.86±2.43	3.31±1.95
10 min	4.37±1.98	6.34±3.45	4.75±2.16
45 min	6.77±2.94	8.14±2.48	-
75 min	7.83±2.54	8.19±2.65	6.27±2.16
105 min	7.54±1.92	8.24±2.55	6.31±2.51
135 min	8.13±2.64	8.62±2.54	6.25±1.89
post CPB			
15 min	7.17±1.46	8.87±2.42	6.26±2.61
30 min	6.99±1.97	8.31±2.70	5.83±2.35
60 min	6.85±2.61	8.11±3.53	6.02±2.84

Time	Group		
	NC	HC	CON
pre CPB	2.33±1.20	2.46±1.92	2.68±1.15
during CPB			
5 min	4.10±2.69	4.22±2.32	2.61±1.07
10 min	4.87±2.63	4.02±1.64	-
45 min	6.10±1.93	6.26±2.01	2.70±1.11
75 min	6.20±1.96	6.40±2.06	2.44±0.97
105 min	6.58±1.68	6.27±1.99	3.02±1.41
135 min	6.36±2.26	6.50±2.09	3.34±1.77
post CPB			
15 min	5.39±2.40	7.04±2.66	3.74±2.44
30 min	5.49±2.52	6.37±2.45	-
60 min	5.15±2.63	5.72±2.47	4.43±3.97

Arterial concentration of inorganic phosphate (mmol.l-1).

Time	Group		
	N	H	C
pre CPB	1.98±0.54	1.58±0.72	1.97±0.33
during CPB			
5 min	1.39±0.47	1.20±0.53	1.40±0.13
10 min	1.44±0.43	1.08±0.59	1.59±0.27
45 min	1.36±0.42	1.07±0.50	-
75 min	1.27±0.48	0.93±0.54	1.15±0.22
105 min	1.17±0.32	0.75±0.43	1.19±0.24
135 min	1.16±0.34	0.78±0.41	1.27±0.25
post CPB			
15 min	1.20±0.14	0.99±0.47	1.48±0.24
30 min	1.25±0.03	1.16±0.48	1.60±0.25
60 min	1.52±0.15	1.47±0.53	1.83±0.31

Time	Group		
	NC	HC	CON
pre CPB	1.84±0.49	1.66±0.55	1.76±0.33
during CPB			
5 min	1.35±0.30	1.28±0.32	1.77±0.33
10 min	1.52±0.19	1.39±0.24	-
45 min	1.13±0.32	1.13±0.30	1.67±0.22
75 min	1.15±0.32	1.00±0.28	1.82±0.28
105 min	1.10±0.32	0.88±0.24	1.86±0.43
135 min	1.18±0.35	0.89±0.26	1.86±0.35
post CPB			
15 min	1.46±0.30	1.08±0.31	1.92±0.35
30 min	1.54±0.28	1.20±0.36	-
60 min	1.75±0.28	1.50±0.49	2.07±0.41

Arterial potassium concentration (mmol.l-1).

Time	Group		
	N	H	C
pre CPB	3.49±0.26	3.54±0.70	3.45±0.57
during CPB			
5 min	3.78±0.46	3.78±0.61	3.90±0.44
10 min	3.63±0.43	3.08±0.44	3.83±0.74
45 min	3.22±0.62	2.94±0.84	-
75 min	3.08±0.51	2.80±1.11	3.76±0.61
105 min	2.89±0.23	2.93±0.88	3.49±0.31
135 min	3.11±0.55	2.96±0.69	3.47±0.26
post CPB			
15 min	2.94±0.20	2.60±0.43	3.23±0.18
30 min	2.90±0.19	2.49±0.31	3.23±0.30
60 min	2.95±0.24	2.76±0.38	3.29±0.31

Time	Group		
	NC	HC	CON
pre CPB	3.75±0.37	3.60±0.69	3.48±0.68
during CPB			
5 min	4.15±0.38	3.87±0.69	3.53±0.67
10 min	4.01±0.19	3.97±0.81	-
45 min	3.52±0.39	3.08±0.76	3.25±0.47
75 min	3.45±0.38	3.14±0.73	3.64±0.46
105 min	3.25±0.30	3.19±0.65	3.71±0.63
135 min	3.48±0.52	3.44±0.68	3.74±0.52
post CPB			
15 min	3.40±0.68	2.78±0.45	-
30 min	3.15±0.40	2.71±0.44	3.69±0.52
60 min	3.55±0.75	2.86±0.42	4.06±0.81

Arterial glucose concentration (mmol.l^{-1}).

Time	Group		
	N	H	C
pre CPB	6.3±0.9	7.2±0.9	7.9±2.3
during CPB			
60 min	3.6±1.4	4.9±1.2	-
135 min	2.4±1.7	5.0±1.5	5.0±2.1
post CPB			
15 min	2.2±1.9	4.7±2.1	4.6±2.4

Time	Group		
	NC	HC	CON
pre CPB	7.1±0.8	7.5±1.8	6.6±1.7
during CPB			
60 min	5.3±1.6	4.8±1.6	6.1±1.5
135 min	4.3±2.3	4.8±1.6	-
post CPB			
15 min	3.8±2.3	4.8±2.2	5.8±1.4

Appendix 10. Arterial-coronary sinus differences of lactate, inorganic phosphate, potassium and oxygen content. Mean values and SD are presented (mmol.l⁻¹).

Lactate (mmol.l⁻¹).

Time	Group		
	NC	HC	CON
pre CPB	0.40±0.41	-0.07±0.84	0.29±0.56
during CPB			
5 min	0.07±0.73	-0.02±0.93	0.30±0.25
45 min	0.40±0.33	0.33±0.58	0.04±0.61
75 min	0.58±0.31	0.33±0.59	0.22±0.52
105 min	0.57±0.46	0.27±0.42	0.19±0.60
135 min	0.72±0.37	0.05±0.64	-0.06±0.81
post CPB			
15 min	0.66±0.46	0.38±0.24	0.09±0.71
30 min	0.86±0.23	0.44±0.33	-
60 min	0.81±0.20	0.24±0.24	0.20±0.61

Inorganic phosphate (mmol.l⁻¹).

Time	Group		
	NC	HC	CON
pre CPB	-0.02±0.09	-0.05±0.12	-0.03±0.05
during CPB			
5 min	-0.11±0.19	0.00±0.11	-0.08±0.09
45 min	0.02±0.04	0.04±0.05	-0.13±0.25
75 min	0.05±0.05	0.05±0.05	-0.07±0.13
105 min	0.05±0.04	0.04±0.05	-0.04±0.12
135 min	0.03±0.06	-0.02±0.07	-0.11±0.25
post CPB			
15 min	-0.04±0.07	-0.01±0.04	-0.08±0.20
30 min	-0.04±0.09	0.03±0.04	-
60 min	0.02±0.08	0.02±0.05	-0.01±0.11

Potassium (mmol.l^{-1}).

Time	Group		
	NC	HC	CON
pre CPB	-0.04±0.20	-0.14±0.33	-0.04±0.06
during CPB			
5 min	0.03±0.27	0.24±0.36	-0.16±0.18
45 min	0.18±0.08	0.14±0.14	-0.30±0.49
75 min	0.16±0.15	0.17±0.17	-0.21±0.29
105 min	0.10±0.15	0.05±0.24	-0.11±0.19
135 min	0.08±0.16	-0.04±0.19	-0.31±0.57
post CPB			
15 min	-0.01±0.11	0.03±0.13	-0.11±0.34
30 min	-0.08±0.18	-0.01±0.20	-
60 min	0.09±0.28	-0.02±0.08	-0.20±0.33

Oxygen (mmol.l^{-1}).

Time	Group		
	NC	HC	CON
pre CPB	4.51±1.43	3.73±1.56	4.23±1.53
during CPB			
5 min	1.90±0.82	2.08±0.85	4.43±1.08
45 min	2.11±0.60	1.09±0.46	4.50±1.18
75 min	2.25±1.11	1.02±0.63	3.99±0.73
105 min	1.88±1.00	1.43±0.51	4.09±1.07
135 min	2.32±1.07	2.12±0.61	4.18±0.70
post CPB			
15 min	3.06±0.79	2.36±0.53	4.08±0.77
30 min	3.18±0.84	2.54±0.57	-
60 min	3.92±0.50	2.86±0.69	4.53±1.37

Appendix 11. Uptake and release of lactate, inorganic phosphate and potassium, and oxygen consumption as calculated from the arterial-coronary sinus differences and coronary flow values. Mean values and SD are presented ($\mu\text{mol.g}^{-1}.\text{min}^{-1}$).

Lactate ($\mu\text{mol.g}^{-1}.\text{min}^{-1}$)

Time	Group		
	NC	HC	CON
pre CPB	0.23±0.31	-0.07±0.46	0.27±0.40
during CPB			
5 min	0.26±1.02	-0.32±0.44	0.29±0.16
45 min	0.25±0.17	0.10±0.16	0.20±0.55
75 min	0.22±0.15	0.10±0.11	0.17±0.37
105 min	0.33±0.45	0.05±0.20	0.42±0.18
135 min	0.41±0.24	-0.07±0.36	0.35±0.17
post CPB			
15 min	0.49±0.27	0.57±0.48	0.18±0.53
30 min	0.82±0.85	0.60±0.47	-
60 min	0.53±0.23	0.19±0.21	0.13±0.28

Inorganic phosphate ($\mu\text{mol.g}^{-1}.\text{min}^{-1}$)

Time	Group		
	NC	HC	CON
pre CPB	-0.01±0.04	-0.03±0.07	-0.01±0.03
during CPB			
5 min	-0.09±0.08	-0.06±0.13	-0.07±0.08
45 min	0.00±0.03	0.02±0.02	-0.17±0.30
75 min	0.01±0.02	0.02±0.02	-0.05±0.08
105 min	0.03±0.06	0.01±0.03	-0.01±0.10
135 min	0.00±0.03	-0.03±0.05	-0.07±0.13
post CPB			
15 min	-0.03±0.03	-0.02±0.10	0.01±0.13
30 min	-0.05±0.07	0.02±0.05	-
60 min	0.02±0.05	0.02±0.05	0.00±0.06

Potassium ($\mu\text{mol.g}^{-1}.\text{min}^{-1}$)

Time	Group		
	NC	HC	CON
pre CPB	0.01±0.10	-0.08±0.19	-0.02±0.04
during CPB			
5 min	0.07±0.30	0.07±0.23	-0.09±0.15
45 min	0.12±0.06	0.07±0.09	-0.39±0.53
75 min	0.08±0.08	0.03±0.05	-0.12±0.18
105 min	0.10±0.19	-0.04±0.13	-0.06±0.20
135 min	0.01±0.08	-0.10±0.07	-0.19±0.17
post CPB			
15 min	-0.03±0.07	0.07±0.18	-0.03±0.31
30 min	-0.10±0.15	0.01±0.11	-
60 min	0.04±0.18	-0.01±0.08	-0.10±0.15

Oxygen ($\mu\text{mol.g}^{-1}.\text{min}^{-1}$)

Time	Group		
	NC	HC	CON
pre CPB	2.97±1.58	2.03±1.00	2.43±1.24
during CPB			
5 min	1.58±1.19	1.89±0.91	3.43±0.54
45 min	1.48±0.86	0.53±0.22	4.01±0.49
75 min	1.09±0.50	0.44±0.28	2.39±1.30
105 min	1.20±0.85	0.84±0.43	3.29±1.25
135 min	1.29±0.76	1.45±0.41	3.21±0.66
post CPB			
15 min	1.79±0.84	3.83±2.62	2.80±1.70
30 min	2.76±2.14	3.03±1.13	-
60 min	2.30±0.87	2.53±0.85	2.54±1.11

Appendix 12. Plasma enzyme activity, hematocrit and free hemoglobin.

Mean values and SD are presented. Enzyme activity is expressed in units per liter of plasma.

Table a. Plasma enzyme activity of AST (U.l⁻¹).

Time	Group		
	N	H	C
pre CPB	32±19	29±27	28±12
during CPB			
10 min	26±14	25±16	21± 9
35 min	40±19	34±37	25±10
110 min	47± 8	55±42	40±17
post CPB			
15 min	65± 8	72±54	64±18
60 min	86±23	80±54	86±14

Time	Group			
	CON	NC	HC	EC
pre CPB	36±18	25±21	33±15	16± 3
during CPB				
10 min	-	18±10	22± 8	21± 3
35 min	44±15	22±12	31±13	23± 4
110 min	53±21	48±31	41±10	25± 3
post CPB				
15 min	-	60±41	59±13	26± 4
60 min	56±23	86±67	70±25	39±27

Table b. Plasma enzyme activity of ALT (U.l^{-1}).

Time	Group		
	N	H	C
pre CPB	13± 7	19±11	21± 9
during CPB			
10 min	14± 8	14± 5	14± 2
35 min	19±10	14± 8	15± 3
110 min	21± 9	25±21	22±15
post CPB			
15 min	20± 3	31±21	35±26
60 min	- *	32±21	53±34

* - no measurements due to the determination of plasma volume (injection of liverenzymes).

Time	Group			
	CON	NC	HC	EC
pre CPB	24±21	26±22	26±17	19± 6
during CPB				
10 min	-	16±11	30±45	18± 5
35 min	24±13	16±10	33±51	19± 7
110 min	26±15	23± 9	33±43	18± 6
post CPB				
15 min	-	28± 8	35±39	19± 6
60 min	29±21	32± 8	48±52	18± 6

Table c. Plasma enzyme activity of creatine kinase (U.l⁻¹).

Time	Group		
	N	H	C
pre CPB	327±240	461±408	421±492
during CPB			
10 min	231±115	310±207	240±161
35 min	333±222	452±464	284±184
110 min	405±166	787±563	490±313
post CPB			
15 min	636±247	975±661	720±405
60 min	797±380	1080±697	851±465

Time	Group			
	CON	NC	HC	EC
pre CPB	335±146	335±433	470±327	94±38
during CPB				
10 min	-	214±184	254±131	145±96
35 min	444±201	264±134	362±192	187±75
110 min	535±223	653±578	543±224	229±77
post CPB				
15 min	-	939±834	752±299	237±67
60 min	586±258	1165±1213	988±450	259±52

Table d. Plasma enzyme activity of MDH (U.l^{-1}).

Time	Group		
	N	H	C
pre CPB	262±271	278±301	191±109
during CPB			
10 min	220±192	219±103	194±100
35 min	287±193	271±210	260±141
110 min	388±140	463±218	431±223
post CPB			
15 min	540±207	615±241	653±337
60 min	825±354	727±294	824±324

Time	Group		
	CON	NC	HC
pre CPB	206± 78	154±133	227± 90
during CPB			
10 min	-	162±126	172± 61
35 min	307± 58	212±150	266±110
110 min	384± 61	460±197	391±117
post CPB			
15 min	-	614±272	586±184
60 min	489±148	709±380	797±288

Table e. Plasma enzyme activity of HBDH (U.l^{-1}).

Time	Group		
	N	H	C
pre CPB	65± 82	33±29	33± 5
during CPB			
10 min	42± 33	51±37	44±31
35 min	78± 56	55±41	48±39
110 min	127± 58	108±38	118±74
post CPB			
15 min	166± 78	158±13	175±88
60 min	192±114	162±51	203±99

Time	Group		
	CON	NC	HC
pre CPB	32± 8	21± 7	26± 9
during CPB			
10 min	-	26±25	35±19
35 min	52± 7	49±32	62±33
110 min	50±14	122±40	105±41
post CPB			
15 min	-	149±35	160±55
60 min	51±23	133±37	168±71

Table f. Plasma content of free hemoglobin (nmol.l⁻¹).

Time	Group		
	N	H	C
pre CPB	0.044±0.007	0.043±0.023	0.043±0.017
during CPB			
10 min	0.041±0.025	0.050±0.051	0.066±0.058
35 min	0.061±0.031	0.062±0.051	0.087±0.076
110 min	0.094±0.046	0.094±0.052	0.141±0.112
post CPB			
15 min	0.118±0.036	0.134±0.066	0.189±0.151
60 min	0.118±0.043	0.137±0.083	0.205±0.175

Time	Group		
	CON	NC	HC
pre CPB	0.028±0.010	0.034±0.015	0.038±0.029
during CPB			
10 min	-	0.049±0.056	0.040±0.023
35 min	0.037±0.025	0.047±0.050	0.068±0.036
110 min	0.036±0.023	0.087±0.058	0.098±0.056
post CPB			
15 min	-	0.087±0.060	0.139±0.084
60 min	0.036±0.029	0.082±0.049	0.140±0.102

Table g. Hematocrit values (%).

Time	Group			
	N	H	C	NC
pre CPB	28±9	30±8	27±5	31±9
during CPB				
45 min	23±6	21±3	22±4	23±5
125 min	21±5	23±3	21±3	26±7
post CPB				
60 min	24±3	24±5	24±7	29±8

Time	Group			
	HC	CON	HPLV	EC
pre CPB	29±7	33±6	26±7	31±5
during CPB				
45 min	21±3	33±5	23±2	26±3
125 min	22±5	31±7	25±2	28±3
post CPB				
60 min	25±7	30±8	32±3	26±4

7. LITERATURE

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8 SUMMARY

In this thesis three techniques of myocardial protection, which are routinely employed during aorto-coronary bypass surgery, were evaluated in a randomized clinical trial and in an experimental dog study. The three techniques of investigation were continuous aortic cross-clamping in combination with multidose ice-cold St. Thomas Hospital cardioplegia, and intermittent aortic cross-clamping at 34°C (normothermia) and at 25°C (hypothermia). On the average, the period of ischemia lasted 63 minutes in the cardioplegia group (heart temperature less than 18°C and body temperature 25°C), and the single periods of aortic cross-clamping in the other two groups lasted 10 minutes and were followed by reperfusion periods of approximately 15 minutes. Since 4 to 7 anastomoses were performed, the mean total duration of ischemia was 42 and 45 minutes in the normothermia and hypothermia groups, respectively. The dog study was performed to obtain more detailed information about specific questions raised in the clinical study.

The clinical study revealed adequate hemodynamic recovery immediately after weaning from extracorporeal circulation. These findings were confirmed in the dog study. Besides, the results in this part of the investigation also showed good recovery of subendo and subepicardial fiber shortening.

The cumulative release of heart-specific enzymes was very small and no marked ultrastructural changes in mitochondria of both the subendo and subepicardial layers of the left ventricular free wall could be detected in the patients. Both findings indicated that no substantial amount of cardiac tissue became damaged. Besides, no significant differences could be observed between the three operation techniques. The evaluation of circulatory parameters in the dog experiments revealed a transient increase in plasma volume and apparent disappearance rate constant for circulating enzymes. Because these parameters had returned to control values within 4 hours and peak values of plasma enzyme activity were observed thereafter, the changes in circulatory parameters will not influence the calculation of cumulative enzyme release to a great extent. Cumulative creatine kinase release was found to be a sensitive parameter for the assessment of damage by the extracorporeal circulation procedure, rather than the ischemic insult.

Biochemical analysis of transmural left ventricular biopsies revealed that myocardial ATP and glycogen contents were decreased in postischemic tis-

sue in the intermittent aortic cross-clamp patient groups. This decrease was associated with release of lactate and inorganic phosphate, during the repetitive periods of reperfusion. In contrast no change in myocardial ATP and glycogen content could be observed in the cardioplegia group. The sustained metabolic activity during the periods of intermittent aortic cross-clamping was also observed in the dogs, irrespective of a reduction in myocardial temperature from 34°C to 25°C. Pronounced changes of the tissue contents of creatine phosphate, inorganic phosphate and lactate could be observed during the periods of ischemia and reperfusion, but these ischemia induced changes appeared to be completely reversible. Although in both groups with intermittent aortic cross-clamping comparable metabolic disturbances were measured in the myocardial tissue, the release of lactate and inorganic phosphate as markers of the ischemic insult, was considerably reduced under hypothermic circumstances.

In general we conclude from both the clinical and the experimental study that

- hardly any sign of irreversible myocardial damage could be observed in the three groups under investigation.
- cardioplegia offered a better protection against the ischemic insult than intermittent aortic cross-clamping, both on the basis of metabolic disturbances in the myocardial tissue and the release of metabolites during reperfusion.
- a decrease in myocardial metabolic activity during ischemia as the result of a reduced temperature in conditions of intermittent aortic cross-clamping did not result in a better preservation of high-energy phosphates and glycogen stores in patients. However, the release of lactate and inorganic phosphate during reperfusion was reduced in hypothermic conditions.

9. SAMENVATTING

Eén van de methoden om een vernauwing van de kransslagaders te behandelen is het uitvoeren van een aorta-coronaire bypass operatie. Hierbij worden beenaders vrij geprepareerd en aangebracht tussen de aorta en de kransslagader voorbij de vernauwing. Op deze manier ontstaat een overbrugging om het bedreigde gedeelte van het hart dat door de betrokken kransslagader bevoeid wordt weer van bloed te voorzien. Tijdens het uitvoeren van deze operatie is het noodzakelijk dat het hart wordt stilgelegd en de doorbloeding van de hartspeer gestopt wordt. Dit laatste gebeurt door het plaatsen van een klem op de aorta of grote lichaamsslagader. Stilleggen van het hart is mogelijk door de hart- en longfunctie over te laten nemen door een hart-longmachine (extracorporale circulatie).

Het stoppen van de hartspeerdoorbloeding is een potentieel gevaar, omdat er geen toevoer van zuurstof en voedingsstoffen meer plaatsvindt en afbraakprodukten niet meer worden afgevoerd. Deze omstandigheid wordt ischemie genoemd. Na verloop van tijd kan de ischemie de hartspeerzellen onherstelbaar beschadigen. Aangezien het regelmatig voorkomt dat meerdere overbruggingen aangebracht worden per patiënt, dienen maatregelen genomen te worden om de ischemieduur binnen aanvaardbare grenzen te houden. Hiertoe kan men de aorta voor elke overbrugging apart afklemmen, waarbij tussen de afklemmingen het hartweefsel opnieuw doorbloed wordt om het weefsel te laten herstellen van de aangebrachte ischemie. De lichaams- en harttemperatuur kan ingesteld zijn op waarden variërend van 25°C tot 34°C. Anderzijds wordt de techniek gehanteerd om de aorta continu af te klemmen tijdens het plaatsen van de overbruggingen. In dit geval dienen aanvullende beschermende maatregelen, bijvoorbeeld in de vorm van cardioplegie, getroffen te worden. Hiertoe worden in het algemeen de kransslagaders doorstroomd met een ijskoude vloeistof met een hoog kaliumgehalte waardoor de hartactiviteit stopt. Nadat alle overbruggingen zijn aangebracht wordt de temperatuur van de patiënt weer teruggebracht tot de fysiologisch normale temperatuur en de hart-longmachine stilgezet zodra het hart zelf weer voldoende pompcapaciteit kan opbrengen.

In dit proefschrift is een onderzoek beschreven waarin drie operatietechnieken met elkaar worden vergeleken. De drie technieken zijn: continue

afklemming van de aorta en gelijktijdige behandeling met de zogenaamde St. Thomas Hospital cardioplegische vloeistof en intermitterende afklemming van de aorta bij een lichaamstemperatuur van 34°C (normothermie) of bij 25°C (hypothermie). In het onderzoek is enerzijds een klinische studie uitgevoerd, waarbij aan de hand van de bloedsomloop, de hartspierstofwisseling en de structuur van de mitochondriën in de cel, drie groepen patiënten met elkaar zijn vergeleken gedurende een korte periode voor en na de operatie en tijdens de fase van de extracorporale circulatie. Anderzijds zijn de drie operatietechnieken bestudeerd in een proefdiermodel bij de hond. Van het proefdiermodel is gebruik gemaakt om enkele onopgeloste vragen uit de klinische studie in meer detail te kunnen onderzoeken.

In de klinische studie zijn 72 patiënten willekeurig verdeeld over drie groepen, die behandeld zijn volgens de genoemde drie operatietechnieken: cardioplegie en intermitterende aorta-afklemming met normothermie of hypothermie. In de cardioplegie groep bleef de aorta gemiddeld 63 minuten afgeklemd, bij een harttemperatuur van minder dan 18°C en een lichaamstemperatuur van 25°C. De ijskoude cardioplegische vloeistof met een relatief hoog kaliumgehalte (16 mmol.l^{-1}) en een normaal calciumgehalte (1.2 mmol.l^{-1}) werd iedere 20 minuten door de kransslagaders gespoeld. In de normothermie en de hypothermie groepen werd de aorta voor het aanbrengen van iedere overbrugging gemiddeld ongeveer 10 minuten afgeklemd. Tussen deze perioden van afklemming werd de hartspier gedurende gemiddeld ongeveer 15 minuten doorbloed. De totale afklemtijd bedroeg in deze twee groepen respectievelijk 42 en 45 minuten. Per patiënt werden 4 tot 7 overbruggingen tussen de aorta en de kransslagaders aangebracht. Dit aantal was niet verschillend tussen de drie groepen.

In de proefdierstudie werden meerdere groepen honden bestudeerd volgens de protocollen die overeenkomen met die van de drie patiënten groepen. Tijdens de extracorporale circulatie (145 minuten) is in de normothermie en de hypothermie groepen de aorta intermitterend afgeklemd (4 maal 10 minuten) en de afklemmingen zijn afgewisseld door perioden van doorbloeding die 15 minuten duren. In de cardioplegie groep is de aorta 60 minuten afgeklemd en is de St. Thomas Hospital cardioplegische vloeistof tijdens de afklemming drie maal door de kransslagaders gespoeld.

Na de operatie was bij de patiënten het herstel van de hartfunctie en de bloedsomloop bevredigend. Er konden geen duidelijke verschillen tussen de drie groepen worden aangetoond. Het verhoogde hartritme, de daling in arteriële bloeddruk en de stijging in cardiac index (de hoeveelheid bloed die per minuut door het hart wordt uitgedrukt gedeeld door het lichaamsoppervlak) zijn wellicht volledig toe te schrijven aan de toegediende narcosemiddelen en de bloedverdunding die plaats vindt door het mengen van het bloed van de patiënt met de vloeistofinhoud van de hart-longmachine. Ook de daling van de kracht waarmee de linker hartkamer samentrekt was vergelijkbaar in de drie groepen. Het staat ter discussie of deze daling kan worden toegeschreven aan een direct effect van de toegediende narcosemiddelen op de nabelasting van het hart, of op de hartspeer zelf. In het proefdiermodel is behalve een adequaat herstel van de bloedsomloop en de hartfunctie ook waargenomen dat de hartspeerfunctie in het hart, na extracorporale circulatie niet verschillend is tussen de groepen. Hieruit kan worden geconcludeerd dat de periode(n) van ischemie geen aantoonbare blijvende invloed heeft gehad op de werking van de hartspeer. De hogere plasma adrenaliniespiegel die in de normothermie groep is gemeten na extracorporale circulatie, kan een positieve invloed hebben op het functioneren van het hart van deze patiënten en hierdoor een mogelijk negatief effect van het operatieprotocol maskeren.

Eén in de kliniek veel gehanteerde parameter voor het vaststellen van onherstelbare hartweefselbeschadiging na ischemie is het verlies van enzymen uit het hart, hetgeen gemeten wordt als een stijging in de plasma-enzym activiteit na afloop van de operatie. In de klinische studie bleek in de drie groepen de plasma-activiteit van CK-MB en HBDH piekwaarden te bereiken van 4 tot 8 uur na de operatie, terwijl piekwaarden voor AST en creatine kinase pas na 12 tot 24 uur werden gevonden. Uit deze plasma waarden is de totale uitstorting berekend door gebruik te maken van bekende waarden voor de afbraaksnelheid van de enzymen. Vervolgens is deze hoeveelheid uitgedrukt in gram equivalenten hartweefsel door van een bekende weefselenzyminhoud uit te gaan. Hierbij is rekening gehouden met het vrijkomen van enzymen uit skeletspieren en uit de tijdens de extracorporale circulatie beschadigde rode bloedcellen. Op deze wijze kon worden berekend dat minder dan 1% van de totale enzyminhoud van de hartspeer in het bloed komt, hetgeen wijst op een geringe beschadiging van de hartspeer.

Uit de proefdierstudie is naar voren gekomen dat bij het meten van het

vrijkomen van enzymen als maat voor weefselschade na extracorporale circulatie, rekening moet worden gehouden met veranderde waarden van de parameters waarmee de totale hoeveelheid vrijgekomen enzymen berekend wordt. Het plasma-volume bleek tot 150% te stijgen en de verdwijningssnelheid van de enzymen uit de bloedsomloop was aanmerkelijk groter dan onder controle omstandigheden. Aangezien het plasmavolume en de verdwijningssnelheid van de enzymen binnen 4 uur na de operatie weer vergelijkbaar waren met de omstandigheden voor de extracorporale circulatie en het vrijkomen van de enzymen voornamelijk na 4 uur plaatsvond, zal de invloed van deze parameters op de berekening van de totale uitstorting gering zijn.

Morfologische veranderingen in de hartspiercel werden bestudeerd in bipten die vóór en ongeveer 20 minuten na de periode(n) van aorta afklemming werden genomen. In deze bipten werd de structuur van mitochondriën onderzocht met behulp van een electronenmicroscop. Het bleek dat vrijwel alle mitochondriën in de stukjes hartweefsel, die voor het afklemmen van de aorta waren verkregen, een normale structuur vertoonden en dat deze structuur na de perioden(n) van afklemming in alle drie de groepen behouden bleef.

De stofwisseling van de hartspier werd bestudeerd in stukjes hartweefsel, die in de klinische studie verkregen werden op dezelfde tijdstippen als de weefselstukjes voor electronenmicroscopisch onderzoek. In de hartspier bleek het gehalte aan ATP en glycogeen te zijn gedaald na de perioden van ischemie in de normothermie en hypothermie groepen, terwijl het gehalte aan creatine fosfaat niet veranderd bleek te zijn. In de cardioplegie groep werden geen veranderingen waargenomen. Bovendien werd het melkzuur, anorganisch fosfaat, kalium en zuurstofgehalte bepaald in het aderlijke bloed uit het hart, zowel voor als na de perioden van aorta afklemming. De concentratie van deze substanties in het aderlijke bloed werd vergeleken met die in het slagaderlijke bloed en het concentratie verschil werd gebruikt als maat voor de ernst van de ischemische veranderingen ten gevolge van de afklemming. Deze concentratie verschillen werden in de proefdierstudie omgerekend tot opname en afgifte van deze chemische substanties per gram hartweefsel door gelijktijdige meting van de hartspier doorbloeding. Het bleek dat direct na het losmaken van de aortaklem aanzienlijke hoeveelheden melkzuur en anorganisch fosfaat uit het hart stroomden in de normotherme groep en de hypotherme groep, wat respectievelijk wijst op anaerobe glycolyse en de afbraak van energierijke fosfaatverbindingen. In de met cardioplegie behandelde harten bleken deze veranderingen

vrijwel niet op te treden, waaruit kan worden geconcludeerd dat de energie-behoefte in voldoende mate was verlaagd zodat de stofwisseling tijdens de periode van ischemie vrijwel geheel geremd was.

Berekening van de hoeveelheid anorganisch fosfaat en melkzuur in de proefdierstudie geeft aan dat onder normotherme omstandigheden aanzienlijk meer van deze substanties het hart verlaten. Daar tegenover staat dat de weefselconcentraties van deze stoffen aan het einde van de ischemie niet verschillend zijn tussen de normotherme en de hypotherme groepen. Dit geldt globaal ook voor de weefselconcentraties van stoffen met energierijke verbin-dingen zoals ATP, creatine fosfaat en glycogeen. Hieruit kan worden geconclu-deerd dat verlaging van de temperatuur van 34°C naar 25°C leidt tot een verlaging van de zuurstofconsumptie tijdens normale doorbloeding van het hart. Daartegenover staat dat de afname van energierijke fosfaten en de stijging van melkzuur in het weefsel na ischemie niet minder was bij de lagere temperatuur van 25°C. De bevinding dat bij een lagere temperatuur veel minder melkzuur en anorganisch fosfaat het hart verlaat mag daarom niet worden gezien als een gevolg van een verschil in stofwisseling, maar als een gevolg van een verminderde uitstroom van deze stoffen bij een lagere tempera-tuur.

In het algemeen kan worden geconcludeerd dat bescherming van het hart door cardioplegie de voorkeur verdient boven de toepassing van intermitteren-de afklemming van de aorta. Dit is gebaseerd op de geringe veranderingen in de stofwisseling van de hartspier tijdens ischemie onder cardioplegische omstandigheden. Het is van belang om hier op te merken dat relatief korte perioden van ischemie zijn onderzocht. Of tijdens de langere ischemieduur die bij andere soorten van openhartoperaties voorkomen dezelfde mate van bescher-ming door middel van cardioplegie gevonden wordt moet nog worden bevestigd.

Nawoord

Het onderzoek dat in dit proefschrift is beschreven werd uitgevoerd met steun van de Nederlandse Hartstichting (projectleider G.J. van der Vusse, projectnr. 79.098). Het klinische deel van dit onderzoek is verricht in samenwerking met Prof. R. Suy, Prof. dr. W. Flameng en Dr. R. De Meyere op de afdeling Cardiovasculaire Chirurgie van de Katholieke Universiteit te Leuven in België. Het dierexperimentele gedeelte van deze studie, alsook de biochemische analyses en de dataverwerking zijn uitgevoerd op de Rijksuniversiteit Limburg te Maastricht.

Het electronenmicroscopisch deel van dit onderzoek werd door Marcel Borgers en Fred Xhonneux verricht bij Janssen Pharmaceutica (Beerse, België). De calciumanalysator werd ter beschikking gesteld door Radiometer, Nederland.

Aan de computerverwerking hebben met name Lex Volovics, Henk Jas en Vic Dreessen meegewerkt. Peter Willemsen en Will Coumans hebben een grote bijdrage geleverd tijdens de uitvoering van respectievelijk de experimentele en de klinische studies, en daarnaast de meeste biochemische analyses verricht. De analyse van plasma enzymactiviteiten is uitgevoerd door Marij Krill en Marie-Louise Boumans, en de mathematische uitwerking van deze data door George Willems. De dierproeven zijn uitgevoerd in samenwerking met Ruud Kruger, Theo van der Nagel, Inger Simons-Achtenberg en Jet Beekman. Huub Simons en Frans Slangen hebben zorg gedragen voor de proefdieren. Aan de verwerking van het manuscript hebben Mariel Coenen, Emmy van Roosmalen en Lucienne de Boer meegewerkt. Vele discussies over de inhoud van dit proefschrift zijn gevoerd met Wim Hermens en Theo Arts.

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Curriculum vitae

Erik van der Veen werd op 3 februari 1950 geboren te 's-Gravenhage. Na het behalen van het HBS-b diploma aan het Erasmus Lyceum te Almelo (1969) is hij in dat zelfde jaar aan de scheikunde studie begonnen te Groningen. Na een verplichte studie onderbreking op vliegbasis Twente (1970-1971) is hij in Wageningen begonnen met de studie Humane Voeding. Het kandidaatsexamen met als verzwaarde vakken celbiologie, dierfysiologie en levensmiddelenchemie werd in 1979 behaald. De 6-maands praktijktijd heeft hij gewerkt in het Central Food Technology and Research Institute in Mysore, Zuid-India. Het ingenieurs examen haalde hij met lof in september 1980, met de hoofdvakken humane voeding (Prof. dr. J.G.A.J. Hautvast) en celbiologie (Prof. dr. L.P.M. Timmermans).

Na 3 maanden studentassistentschappen te Wageningen is hij per 1 januari 1981 in dienst getreden als wetenschappelijk ambtenaar bij de capaciteitsgroep Fysiologie van de Rijksuniversiteit Limburg te Maastricht. Tot 1 december 1983 heeft hij gewerkt in het project "Myocard protectie tijdens aorta-coronaire bypass chirurgie" (project van de Nederlandse Hartstichting, nr. 79.098). De resultaten van dat onderzoek zijn in dit proefschrift weergegeven. Sindsdien werkt hij in de capaciteitsgroep Fysiologie voor de duur van drie jaar in het project "Schatting van hartspierschade en andere toepassingen van kwantificeringsmethoden voor circulerende eiwitten" (dr. W.Th. Hermens).