From the
Department of Surgical Sciences, Karolinska Institutet,
Section of Thoracic Surgery, Karolinska Hospital,
Stockholm, Sweden

Cardioplegia and Cardiac Function

Evaluated by

Left Ventricular Pressure-Volume Relations

by

Anders B. Ericsson, M.D.

Stockholm 2000

Cardioplegia and Cardiac Function
Evaluated by
Left Ventricular Pressure-Volume Relations

By: Anders B. Ericsson

Cover picture: Left, cardiac pressure-volume loops during preload reduction by occlusion of inferior vena cava;
Right, a conductance catheter and a pressure catheter, for acquisition of pressure-volume loops,
inserted through the apex of the left ventricle.

Printed at Elanders Gotab, Stockholm, 2000
ISBN 91-628-4138-6

To

my parents

Table of Contents

Abstract

Purpose: This thesis is focused on studies of blood cardioplegia in order to evaluate regimens of myocardial protection during cardiac surgery. To further bring down operative mortality and morbidity, in patients with acutely ischaemic hearts, an optimal peroperative myocardial management is essential. During the early 90ties several new regimens of cardioplegia emerged, but their place in clinical practice has been debated. By using an animal model, subjected to severe myocardial ischaemia, different regimens of cardioplegia were investigated. By using load independent indices of cardiac function our intention was to gain clinical relevant information about the appropriateness of these cardioplegic methods.

We specifically investigated the impact of cardioplegic temperature and mode of administration on postoperative cardiac function. In study I: warm vs. cold cardioplegia; in study II: antegrade vs. simultaneous antegrade-retrograde cardioplegia; and in study III: warm intermittent vs. continuous cardioplegia. A study was also performed to further study the conductance catheter method for cardiac volume measurements (study IV), and to calibrate indices of cardiac function for changes in heart rate (study V).

Methods: In study I - III pigs were put on cardiopulmonary bypass (CPB) and subjected to global ischaemia followed by cardioplegic resuscitation. Following reperfusion and weaning from CPB, cardiac function was measured using the conductance catheter technique with on-line acquisitions of left ventricular (LV) pressure-volume loops. Among measured indices were the preload recruitable stroke work relation (PRSW; global cardiac function) and the end-diastolic pressure-volume relation (EDPVR; diastolic chamber stiffness). In study IV volume measurements were used to compare (by an algorithm) the wave form of the volume curve from a mid-cardiac volume segment and the volume curve of the global LV volume. In study V the influence of heart rate on indices of cardiac function was investigated and the influence of heart rate on pressure-volume loops by atrial pacing in increments up to 160 beats per minute (bpm).

Results and Implications: Blood cardioplegia given continuously showed to have a potential to resuscitate the acute severely damaged myocardium. The temperature of continuously delivered cardioplegia was of minor importance for acute post-bypass cardiac function. Warm cardioplegia caused an increasing coronary vascular resistance during the time of delivery which might have implications for the cardioplegic distribution. Since warm continuous cardioplegia is not proved better than cold cardioplegia, the cold technique is advocated due to the added safety of hypothermia.Warm intermittent blood cardioplegia showed to be detrimental for post-bypass cardiac function when used in the ischaemically insulted heart and should be avoided in these situations. Simultaneous warm antegrade-retrograde continuos blood cardioplegia did not, in comparison with antegrade perfusion, impair post-cardioplegic cardiac function despite a small, but significant, increase in myocardial water content. Simultaneous cardioplegia has thus the potential of being an optimal technique for cardioplegic delivery.

The LV mid-segmental and global volume curves showed a good agreement. This suggests that the conductance catheter might be simplified with potential for clinical use.

PRSW was rate independent up to 140 bpm. At 160 bpm EDPVR increased significantly and probably resulted in a decline of PRSW at 160 bpm. ESPVR was rate dependent up to 100 bpm and should be used with caution when heart rate is different from baseline. From 140 bpm LV filling started at an increased LV pressure. LV pressure then declined during the whole filling. This might be the result of a suction mechanism facilitating LV filling during tachycardia at rest.

Keywords: *Cardioplegia; simultaneous cardioplegia, intermittent warm cardioplegia; cardiac function; pressure-volume loops; conductance catheter; heart rate.*

List of original articles

The thesis is based on the following original articles, which will be referred to by their Roman numerals:

I. Ericsson AB, Takeshima S, Vaage J. **Warm or cold blood cardioplegia provide similar myocardial protection.** Ann Thorac Surg 1999;68:454-9.

II. Ericsson AB, Takeshima S, Vaage J. **Simultaneous antegrade and retrograde delivery of continuous warm blood cardioplegia after global ischemia.** J Thorac Cardiovasc Surg 1998;115:716-22.

III. Ericsson AB, Kawakami T, Vaage J. **Intermittent warm blood cardioplegia does not provide adequate myocardial resuscitation after global ischaemia.** Eur J Cardio-Thorac Surg 1999;16:233-9.

IV. Ericsson AB, Kronander H, Söderqvist E, Vaage J, Brodin LÅ. **The correlation of a mid-ventricular volume segment to global left ventricular volume measured by the conductance catheter.** Scand Cardiovasc J, Accepted

V. Ericsson AB, Vaage J**. The influence of heart rate on left ventricular function and diastolic filling.** (Manuscript)

Abbreviations

α	slope factor, CO (conductance catheter) /CO (thermodilution).
β	beta, the stiffness constant (unit less), diastolic chamber stiffness; EDP=a *x* $e^{EDV\,x\,\beta}$
bpm	beats per minute
CBP	cardiopulmonary bypass
CO	cardiac output (litre/minute)
DFT	diastolic filling time (ms)
dP/dt	first derivative of left ventricular pressure (mmHg/s)
E_a	arterial elastance (mmHg/ml)
EDP	end-diastolic left ventricular pressure (mmHg)
EDPVR	end-diastolic pressure-volume relation (mmHg/ml), diastolic chamber stiffness
EDV	end-diastolic left ventricular volume (ml)
E_{ed}	regression coefficient of EDPVR (mmHg/ml)
E_{es}	regression coefficient of ESPVR (mmHg/ml)
EF	ejection fraction (%)
ESP	end-systolic left ventricular pressure (mmHg)
ESPVR	end-systolic pressure-volume relation (mmHg/ml), systolic LV function
ESV	end-systolic left ventricular volume (ml)
HR	heart rate (beats per minute)
LV	left ventricular
MAP	mean arterial pressure (mmHg)
M_w	regression coefficient of PRSW (mmHg or erg/ml; 1 mmHg= 1.33 $x10^3$ erg/ml)
PAP_s	systolic pulmonary artery pressure (mmHg)
PFR	peak filling rate (ml/s)
PRSW	preload recruitable stroke work relation (mmHg or erg/ml), global LV function
PRSWA	preload recruitable stroke work area (erg*ml), global LV function
SV	stroke volume (ml)
SW	stroke work (mmHg*ml or erg)
ι	tau, the time constant of left ventricular isovolumic pressure relaxation (ms)
V_0	the volume intercept (ml) of PRSW (study I-III) or ESPVR (study V)
V_c	parallel conductance (ml)
V_{ed}	the volume intercept (ml) of EDPVR
V_w	the volume intercept (ml) of PRSW (study V)

Introduction

Myocardial protection

Historical overview – the 50ties

Protection of organs from ischaemic damage was early identified as a prerequisite for open heart surgery. In 1950 Bigelow introduced systemic hypothermia for cerebral and myocardial protection [Bigelow 1950]. Without hypothermia open cardiac procedures had been limited to 2 minutes of caval inflow occlusion. In 1955 Lam et al [Lam 1955] reported about intraventricular injections of potassium chloride to induce cardiac arrest to facilitate operations during hypothermia. With the introduction of the heart-lung machine in the mid 50ties by Gibbon [Gibbon 1954] it was possible to operate on the beating or fibrillating [Senning 1952] and continuously blood perfused heart (with the second successful human operation with a heart-lung machine1954, performed in Stockholm by Clarence Crafoord [Crafoord 1957]). During aortic surgery with crossclamping of the aorta retrograde blood perfusion through the coronary sinus was used by Lillehei already in 1956 [Lillie 1956]. In order to protect the brain from air embolism during open beating heart procedures, Melrose 1955 described a method for chemical cardiac arrest, using potassium citrate [Melrose 1955]. Gott and Lillehei used Melrose solution to arrest the hearts in combination with warm antegrade or retrograde continuos warm blood perfusion [Gott 1957]. However, this technique was soon abandoned due to disappointing clinical results with myocardial necrosis, persistent ventricular fibrillation and poor ventricular function [Allen 1957, Waldhausen 1960, Björk 1961]. Initially the citrate was thought to be the damaging agent [Hölcher 1961, 1967], but later the high potassium concentration (200 mM) was shown to be the deleterious factor [Tyers 1975]. By using potassium in a toxic dose, due to inadequate preclinical testing, the use of chemical myocardial protection (cardioplegia) was delayed for twenty years until the mid 70ties!

Topical cooling was introduced by Shumway 1959 [Shumway 1959], using cold saline to irrigate the heart, and later Hufnagel used ice-slush [Hufnagel 1961]. By cooling the heart myocardial oxygen demand decreased thus increasing the tolerance for ischaemia. To protect the heart from ischaemic damage the prevailing maxim among cardiac surgeons for many years then became: "to operate as fast and to operate as cold as possible" [Buckberg 1991].

The 60ties and 70ties

Many non-cardioplegic strategies for myocardial protection were used during the sixties to mid seventies and beginning of the eighties. With the aid of cardiopulmonary bypass using systemic hypothermia or normothermia, with or without topical cooling of the heart, operations were performed on the fibrillating or beating heart with or without intermittent crossclamping of the aorta. For aortic surgery continuous antegrade blood perfusion with or without hypothermia was used. These techniques were gradually abandoned when more safer and more convenient cardioplegic techniques emerged. A risk for myocardal infarction was shown with antegrade blood perfusion at operations both on the beating [Sapsford 1974] and fibrillating [Hottentrott 1974] heart,

possibly due to abnormal distributions of coronary blood flow [Miayamoto 1978, Hottentrott 1974].

Myocardial infarction was not identified as a complication of cardiac surgery until late 60ties [Kirklin 1993] when myocardial necrosis was described as a cause of death after cardiac surgery [Morales1967]. It was later suggested that it had to do with the peroperative protection of the myocardium [Najafi 1969, Hultgren 1973]. With the development of enzymatic and ECG diagnosis also surviving patients with perioperative myocardial infarction were identified [Breweret 1973, Roberts 1973]. As late as 1972 Cooley described the "stone heart" as a fatal complication of cardiac surgery when normothermic ischaemia was prolonged over 45 minutes [Cooley 1972]! Also post-ischaemic myocardial dysfunction or "myocardial stunning" was identified as a separate entity [Vatner 1975]. Contrary to myocardial infarction this was defined as a contractile dysfunction with fully reversible cell damage without any cellular necrosis[Piper and Preusse 1993]. It was also described that re-oxygenation after a prolonged period of ischaemia could further damage the myocardium [Dabforth 1960, Hearse 1973, McCord 1985] and "reperfusion injury" as a separate entity was defined [Hearse 1977]. All these findings further initiated interest in improving perioperative myocardial management.

Cardioplegia

The word cardioplegia, originally meaning only "cardiac arrest", was introduced by Lam et al [Lam 1957] when experimenting with acetylcholine for induction of reversible cardiac arrest. However, the word "cardioplegia" has also become to be the name of the solution given to induce the cardioplegia. Thus, "cardioplegic arrest" means: cardiac arrest (or cardioplegia) induced by cardioplegia (or cardioplegia induced by a cardioplegic solution) (which chemically arrests the heart).

Development of cardioplegia

During the 60ties Bretschneider in Germany continued to investigate cardioplegic solutions and their potential to also protect the myocardium from ischaemic damage by preserving the pool of energy-rich phosphates. He developed the so-called "intracellular" type of cardioplegic solution being a crystalloid solution, calcium-free, sodium-poor and with moderate potassium (9mM). Later histidine, as a buffer, was added to the solution [Bretschneider 1964, 1975]. Another type of "intracellular solution" later developed was the University of Wisconsin solution, used for preservation of donor hearts at cardiac transplantations, also being sodium-poor, but potassium-rich [Stein 1991]. In 1973 Gay and Ebert reported of a cardioplegic solution with an "extracellular " composition: high potassium and isotonic sodium concentration [Gay 1973]. This led to the subsequent development of the St. Thomas´ Hospital cardioplegic solution (144 mM sodium, and 20 mM potassium chloride) by Hearse and Braimbridge in London 1976 [Hearse 1976] with their first reported clinical series in 1977 [Braimbridge 1977]. Crystalloid solutions for cardioplegic arrest were soon widely adopted [Nelson 1976] an crystalloid cardioplegia was used at the Karolinska Hospital from 1977.

Development of blood cardioplegia

During the 70ties Buckberg and Barner reinvestigated the solution by Melrose which used blood as a vehicle. Now with a non-toxic potassium dose, they (re)introduced blood cardioplegia [Folette 1977, Barner 1979, Buckberg 1979]. Compared with crystalloid solutions one advantage of blood cardioplegia is the larger oxygen carrying capacity. Other advantages with blood as the cardioplegic vehicle include the buffering capacity of blood proteins with histidine-imidazole groups [Reeves 1972], improved microvascular flow due to rheologic effects [Heitmiller 1985, Bing 1982], improved cardioplegic distribution beyond stenoses [Robertson 1983], less myocardial oedema [Foglia 1979], less hemodilution, and endogenous scavengers for oxygen free radicals [Brown 1989]. The use of blood cardio-

plegia was extended by Bomfim and colleagues [Bomfim 1981] at the Karolinska Hospital by their first report 1981 of continuous cold blood cardioplegia to reduce myocardial ischaemia and to preserve ATP during aortic valve surgery. However, this method was abandoned due to the inconvenience with blood in the operating field.

Numerous modifications has then been done in the compositions and regimens of cardioplegic solutions. Besides using depolarising agents such as potassium, magnesium or procaine for inducing cardiac arrest, hyperpolarising agents such as adenosine, potassium-channel openers, and sodium-channel blockers have been and are investigated as possible arresting agents in cardioplegic solutions [Chambers 1999], as well as short-acting β-blockers [Mehlhorn 1999]. A further description about the different constituencies and additives of cardioplegic solutions and the objectives for their use is beyond the scope of this reviews.

However, Buckberg later formulated a concept of blood cardioplegia where the energy-depleted heart was arrested with potassium chloride and ”resuscitated” with a continuous infusion of a warm, aspartate and glutamate enriched, blood cardioplegic solution, improving the ischaemic tolerance of the myocardium [Buckberg 1987]. This was followed by an intermittent infusions of cold blood cardioplegia during surgery. After surgery before flooding the heart with the ordinary blood, the heart was continuously reperfused with a warm substrate enriched cardioplegic solution to optimise metabolic repair and restoring the pool of energy-rich phosphates. Many well-conducted experimental and clinical studies have documented the excellent performance of this cardioplegic regimen [Rosenkranz 1982, 1983, Follette 1977, 1981, Theo 1986, Buckberg 1995, Schlensak 1999].

Still today the debate is going on if blood cardioplegia have benefits compared to crystalloid solutions. Although a plethora of experimental studies have documented the advantages of blood cardioplegia, there is no large prospective, randomised clinical study showing a superiority of blood cardioplegia, because no such study is ever conducted. There are several reasons why such a study is missing, Such a study is difficult to do, it might be that the difference is of minor significance in routine cardiac surgery, but superior as myocardial protection during operations with prolonged cross-clamp time or in the preoperative ischaemically deranged or metabolically exhausted hearts [Fremes 1984, Conti 1978, Daggett 1987, Engelman 1980, Rosenkranz 1986, Krukenkamp 1987, Takamoto 1980, Ibramin 1999, Schlensak 1998]. Consequently, unless the patient population studied is at high risk, and then not so numerous, a large number of patients would be necessary. Additionally, the proponents of blood cardioplegia claim that the data from experimental studies and in some high-risk patient groups, like patients with evolving myocardial infarction [Beyersdorf 1993, 1995, Schlensak 1999], are so good that these surgeons would not be willing to use crystalloid cardioplegia. To date there is no experimental or clinical study indicating an advantage of crystalloid cardioplegia, most studies find an advantage of blood cardioplegia or no difference between them.

However, today in the era of cardioplegia, intermittent cross-clamping with ischaemic fibrillatory arrest is still used in some centres, with its advocates showing excellent results [Boncheck 1992]. At Karolinska Hospital crystalloid cardioplegia was with some exceptions, routinely used for all cardiac procedures until the mid nineties, but today blood cardioplegia is used in more than 90% of the cases.

The aim of modern perioperative myocardial protection is to enable safe cardiac operations, also in damaged hearts, for prolonged time without resultant structural, metabolic or functional deterioration. This is accomplished by decreasing the cardiac metabolism and optimising the energy supply/demand ratio to avoid or decrease ischaemia and reperfusion injury. In the severely deranged heart the aim is also to resuscitate the myocardium before further ischaemic injury is initiated by interrupted coronary circulation during the cardiac surgery.

Myocardial oxygen demand

The *normally working heart* requires at 37ºC approximately 9 (SD3) ml O_2/100g heart weight/ minute [Gertz 1988]. The oxygen demand per minute is mainly depending on heart rate and stroke work including oxygen expenditure for the degree of wall tension and contractility. In the *empty beating heart* the oxygen consumption is lowered to approximately 5.6 (SD2.0) ml O_2/100g/min [Buckberg 1977], but oxygen consumption is still heavily dependant and linearly related to heart rate (0.033 [Buckberg 1977] to 0.045 [Preusse 1984] ml O2/100g/*beat*). The fibrillating, vented non-hypertrophied heart at 37ºC has approximately the same oxygen demand [Buckberg 1977, Krukenkamp 1991]. In the *arrested heart* at 37ºC the oxygen demand declines to 1 - 2 ml O_2/minute /100g [Buckberg 1977, Bernard 1961], i.e. only 10% to 20 % of the oxygen consumption in the normally working heart. When hypothermia, at 22ºC and 5ºC, is added to the arrested heart there is only a small further reduction in oxygen consumption (0.31 and 0.13 ml O_2/ 100g/minute, respectively) [Buckberg 1977, Bretschneider 1975]. Hypothermia below 22ºC might be unnecessary since not much more oxygen lowering is gained [Buckberg 1979]. However, to avoid increased oxygen demand due to rewarming by collateral blood flow, the temperature of cold intermittent blood cardioplegia has generally been lowered to 6-10 ºC.

Oxygen delivery to myocardial tissue

There have been great concern about the ability for blood to deliver oxygen to myocardial tissue at low temperatures [Buckberg 1979, Magovern 1982]. An in vitro study showed that only 50% of the oxygen content in blood cardioplegia is available at 20°C and 38 % at 10°C [Digerness 1981], with oxygenated crystalloid solutions actually delivering more oxygen. The oxyhemoglobin dissociation curve shifts to the left by hypothermia [Astrup 1965] impairing the release of oxygen to the tissue. However, a low tissue PO_2 , a greater affinity of tissue for O_2 with hypothermia [Longimuir 1962], and an opposing rightward shift of the dissociation curve (Bohr effect) induced by tissue acidosis and hypercarbia [Astrup 1965, Reeves 1982] may contribute to a significant tissue oxygenation despite the hypothermia [Penrod 1951, Gutierrez 1986]. Although controversial [Illes 1989] it has been shown that the protective effect of hypothermic blood cardioplegia is critical dependent on the oxygen content despite the greater affinity of haemoglobin for O_2 at low temperatures (4-8°C) [Vinten-Johansen 1991].

Warm heart surgery

Since most of the myocardial oxygen demand (80-90%) is due electromechanical work, hypothermia might be unnecessary in the arrested heart, if it is continuously oxygenated. This was the rationale behind the concept of warm aerobic arrest. First used by Lillehei et al. 1957 (se above), warm heart surgery was reintroduced by Lichtenstein et al in 1989, but now using a modern composition of cardioplegia [Lichtenstein 1989, 91]. By continuously perfusing the heart with normothermic blood cardioplegia the heart was arrested and ischaemia was avoided. The addition of hypothermia was no longer considered necessary. Instead of cold anaerobic arrest this was a concept of warm *aerobic* arrest where cross-clamp time had little to do with ischaemic time. Detrimental effects of hypothermia such as membrane destabilisation [Martin 1972], Na^+-K^+ adenosine triphosphatase inhibition with oedema formation [Martin 1972], and calcium sequestration [Sakai 1985] could be avoided. As shown by Kaijser and colleagues [Kaijser 1986] the production of high-energy phosphates during deep hypothermia (10°C vs. 15° C) is reduced more than energy consumption. This was another negative effect now avoided.

With an ongoing metabolism this method had the potential for peroperative preservation of mitochondrial-, membrane-, and enzymatic function. Consequently, possible improvements in postoperative outcome was expected, especially in patients with limited cardiac reserve and following long cross-clamp times [Lichtenstein 1992]. However, despite the theoretical advantages using warm continuous blood car-

dioplegia, results diverge when compared with cold intermittent blood cardioplegic techniques. With warm cardioplegia good [Lichtenstein 1991, Salerno 1991, "Warm heart invest." 1994, Brown 1993, Yau 1993], but also detrimental cardiac effects are shown [Misare 1993, Bufkin 1994, Stahl 1994, Van Camp 1995]. Systemic effects as increased risk for neurologic events and low systemic vascular resistance are reported [Martin 1994, Christakis 1992]

Warm and cold continuous blood cardioplegia - Objectives of study I

Despite the theoretical advantages with warm heart surgery not all reports have been in favour of this method (se above). A warm heart requires 10-20 % more oxygen than a cold heart. This oxygen demand, though small, may not be neglectable even in the perfused cardioplegic heart. If not the continuous cardioplegic perfusion is able to completely and homogeneously perfuse the whole myocardium, small areas of ischaemia in a warm heart may be the net result. Consequently, the warm heart is more susceptible for inhomogeneity in cardioplegic distribution. Cardioplegic delivery to myocardium beyond proximal occlusions or critical stenoses is not optimal, and the distribution may be inhomogeneous [Hilton 1979 Tian 1998]. Retrograde delivery via the coronary sinus is in these situations probably a superior route of administration [Partington 1989-1, Haan 1991, Menasche 1991], but also retrograde cardioplegia is inhomogeneously distributed [Gates 1993-1, Caldarone 1994], with hypoperfusion of the right ventricle. Several studies has concluded that only 25 to 80% of the retrograde delivered cardioplegia is nutritive [Partington 1989 -1, Solorzano 1978, Gates 1993-2, Carrier 1994, Ardehali 1995].

Even in the absence of coronary artery obstructions antegrade perfusion to the cardioplegic heart is not homogeneous [Miyamoto 1978] with subendocardial layers being more vulnerable [Aldea 1990]. Cardioplegic flow and pressures may in the warm heart be of more critical importance, and pump accidents may be fatal due to decreased time margins. Even in the warm, antegradely perfused myocardium without coronary obstructions, post-cardioplegic cardiac function is shown to deteriorate [Mehlhorn 1995]. Ischaemic damage may further result when warm "continuous" cardioplegia is temporarily interrupted, as done by many surgeons to better visualise the operating field [Matsuura 1916, Lichtenstein-Warm Heart investigators 1995].

These remarks may have important implications for the use of warm heart surgery. When reviewing studies on warm continuous cardioplegia we could only find studies where the warm continuous cardioplegia had been compared with *intermittent* cold cardioplegia (see above). Was then the observed differences between the methods due to different cardioplegic temperature and metabolism or a result of a different myocardial perfusion? As a result of this review we hypothesised that parts of the observed advantages with warm continuous cardioplegia might be attributed to the continuous perfusion per se, rather than to favourable metabolic effects of normothermia. There have only been a few studies on continuous cold blood cardioplegia [Bomfim 1981, Khuri 1988, Louagie 1997], but none of them has been in comparison with warm blood cardioplegia. Even with continuous cardioplegia, hypothermia may still be an important adjunct to myocardial protection. Hence the purpose of study I (warm vs. cold blood cardioplegia) was to isolate and investigate the role of cardioplegic temperature on post-bypass functional recovery per se, keeping parameters as cardioplegic flow, pressure and route of administration similar between groups. If cold continuous cardioplegia is not inferior to warm continuous, then the cold may have advantages due to the greater safety margins of hypothermia.

Simultaneous antegrade-retrograde blood cardioplegia - Objectives of study II

As discussed above a homogenous distribution of the cardioplegic solution may be of critical importance particularly in conjunction with warm heart surgery. One solution is to combine antegrade and retrograde cardioplegia. The heart is then arrested with an initial dose of antegrade cardioplegia, with the subsequent doses given through the coronary sinus, or given by alternating between antegrade and retrograde perfusion. [Partington 1989-2, Drinkwater 1992, Hayashida 1995, Aldea 1994]. A further reason for a such regimen is that even in the absence of coronary stenoses, antegrade and retrograde cardioplegia may perfuse complementary vascular beds in the cardioplegic heart [Aldea 1994, Gates 1995, Gates 1996].

To provide an optimal and fast distribution of cardioplegia, even in the presence of obstructed vessels, simultaneous antegrade-retrograde delivery of the cardioplegic solution was proposed by Buckberg's group [Ihnken 1994]. This simultaneous technique is easy to use without any change from antegrade to retrograde or vice versa. With the theoretical potential for homogenous distribution, the simultaneous antegrade-retrograde cardioplegia might be an optimal mode of cardioplegic delivery. However, several questions are raised: Is it a safe method? Will the microvascular hydrostatic pressure increase to such an extent that myocardial oedema will cause deterioration of left ventricular function? [Spotnitz 1994]. This possibility is particularly relevant in situations with acute occlusions and ischaemia, which would be an important indication for simultaneous cardioplegia. Ischaemic injury to the coronary endothelium and myocytes will increase microvascular permeability and cause myocardial oedema [Spotnitz 1994], rendering the heart more susceptible to increased hydrostatic pressure during cardioplegia and reperfusion. Giving large quantities of cardioplegia might then further enhance myocardial oedema. A study by Mehlhorn and co-workers [Mehlhorn 1995] showed oedema formation during continuous antegrade warm blood cardioplegia with subsequent reduction in systolic function, and in a canine study myocardial water content increase during reperfusion after global ischaemia resulting in increased chamber stiffness[Weng 1992]. Hence the purpose of study II of this thesis became trying to answer theses questions about the safety of simultaneous cardioplegia. Antegrade and simultaneous antegrade-retrograde cardioplegia given via the aortic root and the coronary sinus was compared with regard to post-bypass functional recovery, troponin-T release, and myocardial water content.

Intermittent warm blood cardioplegia - Objectives of study III

A problem with continuous infusions of cardioplegia is that the operating field will be flooded with blood. One solution is to use retrograde perfusion which increase visibility due to decreased coronary artery flow [Salerno 1991, Lichtenstein 1992], but the perfusion of the right ventricle will be suboptimal as discussed above. Continuous saline irrigations, suction, or blower devices can be applied in the vicinity of the opened vessel to improve visibility [Teoh 1991, Maddaus 1992], but despite this, conditions will not be optimal. The alternative to shut off blood flow by circumscribing the arteries with ligatures was at this time, in the middle of the nineties before the real beginning of the off-pump surgery, not considered prudent due to possible risks of endothelial damage [Pabst 1989].

Consequently, both cold and warm continuous perfusion was considered cumbersome by most surgeons. Thus in practice the warm blood cardioplegic perfusion, when used, was interrupted during shorter or longer intervals to better visualise the operating field, converting the continuous perfusion to an *intermittent* one. Many studies on "continuous" warm blood cardioplegia have in fact been studies on warm intermittent rather than continuous cardioplegia. In Lichtenstein's original publication on warm heart surgery it was stated that cardioplegic perfusion was occasionally turned off for 10 to 15 minutes [Lichtenstein

1991]. In a later randomised trial a mean of 48 % of the cross-clamp time was off cardioplegia, the highest mean for any surgeon being 64 % [Warm heart invest. 1994]. In that study the longest ischaemic time off cardioplegia was on average 11 minutes, and further analysis suggested that warm blood cardioplegia interrupted for more than 13 minutes is a risk factor for adverse outcome [Liechtenstein 1995].

The practice of interrupting cardioplegic delivery influenced surgeons to specifically address the intermittence of warm cardioplegia. During the mid 90ties there were an increasing number of papers advocating this technique. The majority of studies showed excellent results, concluding that intermittent warm blood cardioplegia in the protocols used was safe and provides a myocardial protection equivalent or superior to the various regimens of myocardial protection used for comparison [Yau 1992, Landymore 1992, Matsuura 1993, Ko 1993, Ali 1994, Pelletier 1994, Landymore 1994, Calafiore 1995, Tian 1995, Mezzetti 1995, Landymore 1996, Tönz 1996, Torracca 1996, Carias de Oliveira 1997,Caputo 1998]. Indeed, Calafiore et al [Calafiore 1995] showed that intermittent warm blood cardioplegia given during only 12 % of the total cross-clamp time and with ischaemic episodes of up to 15 minutes "is a safe, reliable, and effective technique of myocardial protection".

However, in the warm cardioplegic heart an oxygen debt occurs after 3.5 minutes of warm ischaemia [Landymore 1992], and two studies conclude that interruptions of warm blood cardioplegia for 7- or 10-minute episodes cause ischaemic damage with detrimental functional effects and structural defects [Matsuura 1993, Ko 1993]. It is important to emphasise that intermittent warm cardioplegia, is not a defined entity, but represents a wide range of regimens of in the hands of the different investigators. Warm intermittent cardioplegia is also warm intermittent ischemia. With increasing ischaemic time the result will inevitably be an ischaemic injury.

When reviewing the literature we found that most investigators have studied warm intermittent cardioplegia in the *normal heart,* evaluating its safety and trying to establish a longest safe interval off cardioplegia. The purpose of study III was to investigate intermittent warm cardioplegia in *the already ischaemic and compromised heart.* The regimen we chose was similar to, but not identical, that used by Calafiore et al [Calafiore 1995]. As in study I and II antegrade continuous blood cardioplegia was used for the comparison. Variables studied were post-bypass left ventricular function, release of troponin T and myocardial water content.

Measurements of cardiac function

Preload, afterload and contractility

The classic determinants of muscular performance; preload, afterload, heart rate and force of contraction (contractility) were originally defined during studies of isolated muscle strips. Preload was the load put on the muscle before it was stimulated to contract. Practically, it was a weight hanged at the end of the muscle stretching it to a length consistent with its resting length-tension relation. When the muscle was stimulated and shortened during contraction this was called an isotonic contraction. If the muscle was allowed to shorten first after having developed an extra force in addition to the preload this additional force was called the afterload. Practically, this was also a weight hanged at the end of the muscle strip, but with a device made not to lengthen in addition to the length caused by the preload weight. This contraction was then called an afterloaded isotonic contraction and the total load was the sum of the preload and afterload weights. In this setting preload and afterload were completely independent of muscular contractility and the load was constant during the contraction.

In the nineteenth century Fick [Fick 1882] and Blix (in Sweden) [Blix 1885] demon-

strated the existence of a direct relationship between muscle fibre length and contractile force. However, problems have arisen when transferring this concept to the intact heart. As the heart starts to contract and ejection begins both preload (measured as ventricular pressure or volume) and afterload (measured as arterial or ventricular pressure, ventricular wall stress or arterial impedance) will change. Furthermore there is no way to direct measure ventricular force, tension or wall stress. The load will also be different in different parts of the heart due to different wall radius and wall thickness, and due to different orientation of the muscle fibres at different depths of the myocardial wall [Sagawa 1988]. Although preload and afterload, at the beginning of the contraction, are reasonable well defined, the force of contraction or the contractility is even more difficult to quantify and translate to the cardiac system in vivo.

Cardiac function curves, Frank-Starling relationship

Cardiac contractility described in terms of function curves was first done by Otto Frank in 1885 describing a pressure-volume diagram of a frog ventricle plotting the peak pressure in the ventricle during contraction at different volumes. (At that time pressure-volume diagrams had also been used to describe the work of engines). However, the non-linearity of his pressure-volume diagram and its load dependence made it difficult to use practically [Frank 1885]. Later Starling formulated: ”//the larger the volume of the heart, the greater is the energy of its contraction//” [Starling 1918]. Starling and his group [Patterson 1914 x2] have later been associated with three relations:

1. The pressure-volume relation used by Frank;
2. Cardiac output plotted against atrial pressure, a concept further developed by Guyton with his pump function curves 1955 [Guyton 55];
3. Stroke work plotted against end-diastolic pressure, end-diastolic volume or atrial pressure.

The Frank-Starling relations (length-tension relations) were further developed by Sarnoff and Berglund 1955 [Sarnoff and Berglund 1955], investigating the relation between ventricular stroke work and filling pressure, showing the existence of numerous function curves depending on inotropic state, and suggesting that the relation between stroke work and end-diastolic volume might be linear. Later force-velocity relations were investigated by Sonnenblick [Sonnenblick 1962] and the maximal velocity of shortening was used as a preload independent index of contractility [Ross 1966]. Also various indices derived from the first derivative of left ventricular pressure increase, dP/dt [Gleason 1962] was investigated and is still used, but being preload dependent [Little 1985], (afterload independent if occurring before opening of the aortic valve). Among many indices of cardiac function are the load dependent, but clinically much used stroke work index and ejection fraction. The latter is easily obtained non-invasively. In the 70ties Suga and Sagawa developed the end-systolic pressure-volume relation (ESPVR) as an index of contractility, independent of both pre- and afterload [Suga 1971, Sagawa 1977, 78]. Unfortunately, ESPVR has later been shown to be influenced by afterload [Maughan 1984, Baan 1988, Van der Velde 1991], and is in vivo often curvilinear [Burkhoff 1987, Su 1989, Kass 1989]. With the development of better methods for on-line volume measurements (sonomicrometry), Rankin´s group could further investigate the function curves of Sarnoff and Berglund and presented in 1985 the concept of preload recruitable stroke relation (PRSW) [Glower 1985]. This is left ventricular stroke work plotted against the end-diastolic volume and it is claimed to be a preload- and afterload-independent index of cardiac function [Glower 1985].

Interdependence between load and contractility

A problem when defining “contractility” is the intimate interdependence between load and contractility which is seen in the intracellular regulation of the availability and sensitivity of

calcium-ions to the contractile elements. Consequently, on the myocyte level contractility, preload, and afterload cannot be defined independent of each other [Kass 1988, Lakatta 1987]. Changes in load influence the length of muscle fibres (length-tension relation), which again influences the Ca^{2+}-sensitivity of the myofilaments (also as the contraction proceeds) and thus the inotropic state or contractility of the myocardium.

However, in both clinically and in experimental studies the ability to describe changes in pump performance, independent of changes in pre- or afterload, is of great importance. Thus a surrogate for cardiac force, whether or not representing true "contractility" on the basic muscular level, will be a useful tool as long it is operational, does change with inotropic state, and is reasonably independent of "preload" and "afterload". Such indices are the ESPVR and PRSW which today are used mostly for experimental evaluation of systolic and global ventricular function. These indices are relatively insensitive to changes in preload (Suga 1979, Spratt 1987, Glower 1985), but ESPVR, and to a lesser degree PRSW, are not completely insensitive to afterload [Maughan 1984, Baan 1988, Van der Velde 1991].

The force-frequency relation

A positive inotropic effect of increased contraction frequency was described in frog ventricles by Bowditch already in 1871 [Bowditch 1871]. This force-frequency relation also known as the interval-force relation or staircase ("Treppe") [Woodworth 1902], is considered to be one of the fundamental determinants of myocardial contractility [Braunwald 1992, Seed 1992]. It has been seen in isolated cardiac muscles [Koch-Weser 1963] and in anaesthetised animals [Covell 1967] as well as in conscious animals [Mahler 1974, Freeman 1987] and humans [Sonnenblick 1965]. Research by Ross et al have shown that in addition to the regulation of contractility by direct beta-adrenergic stimulation, length-dependant activation (Frank-Starling relation), and heart rate, there is also an indirect beta –adrenergic effect [Ross 1995]. Beta –adrenergic stimulation is shown to amplify the force-frequency relation and thereby influence both systolic and diastolic function possibly a mechanism involved in the pathophysiology of heart failure [Kambayashi 1992, Eising 1994, Hasenfuss 1999].

Measurements of cardiac function using mechanical function indices

In this thesis the most applicable and important variable for investigation was the preload recruitable stroke work relation (PRSW) used to quantify global cardiac function (Fig. 2 and "Materials and methods"). In addition the end-diastolic pressure-volume relation (EDPVR) and the stiffness constant (beta) were used to quantify chamber stiffness (late diastolic function) (Fig. 1). The end-systolic pressure-volume relation (ESPVR) was only used in study V due to its tendency of being curvilinear in vivo making it difficult to use accurately. The ESPVR not only change its steepness, but also its curvilinearity with changing inotropic state [Burkhoff 1987, Su 1989, Kass 1989] (Fig. 1, 3 and 4, also see further discussion in paper V). These indices of cardiac function are called "mechanical function indices" as they are cardiac function relations obtained by preload or afterload interventions. To derive end-systolic and end-diastolic pressure and volume, as well as stroke work for individual heart beats during changing of preload or afterload on-line acquisitions of pressure-volume loops have to be done. For on-line volume measurements we used a conductance catheter and for pressure measurements a transducer-tipped pressure catheter. A pressure-volume loop is a convenient way to describe the heart beat during its various phases. A "guide" to the interpretation of the pressure-volume loop is given in figure 5.

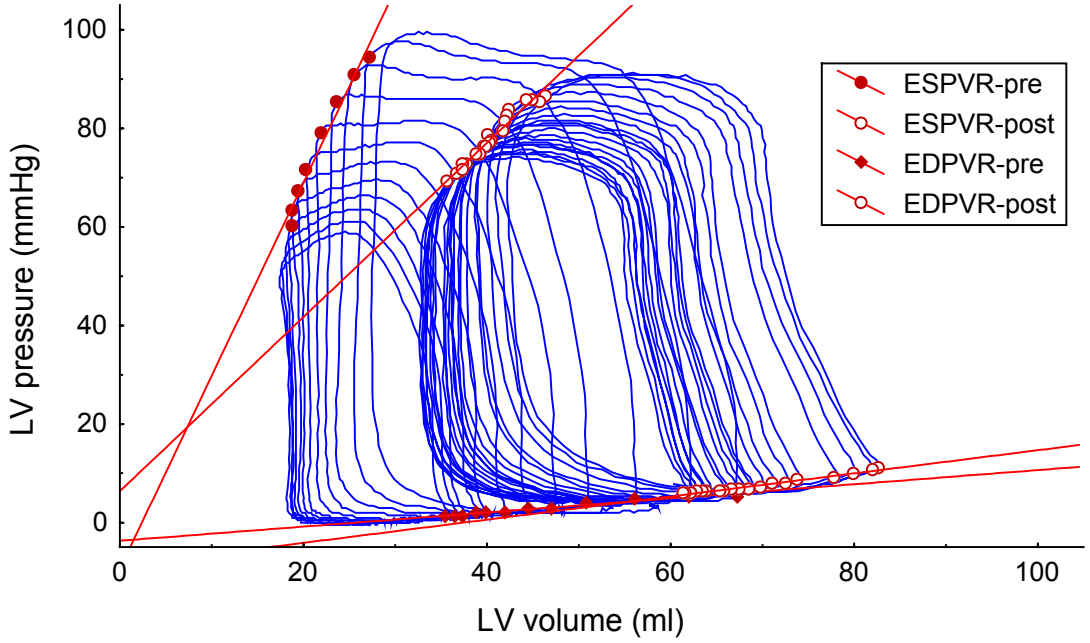

Fig 1. *Pressure-volume loops during vena cava occlusion at baseline (pre; left loops) and after ischaemia (post; right loops) in one animal. After ischaemia the end-systolic pressure-volume relation (ESPVR) has become less steep indicating a deterioration in systolic left ventricular (LV) function. The end-diastolic pressure-volume relation (EDPVR) has become steeper indicating an increased chamber stiffness (deterioration in late diastolic function). After ischaemia the loops have moved rightwards and slightly upwards due to LV failure (increased end-diastolic volume and pressure). After ischaemia the loops are closer to each other due to tachycardia. The heart rate is in average 87 and 122 beats per minute at baseline and after ischaemia. Note the increased blunting of the left lower corner of the pressure-volume loops after ischaemia. The left ventricle starts to fill at a higher LV pressure.*

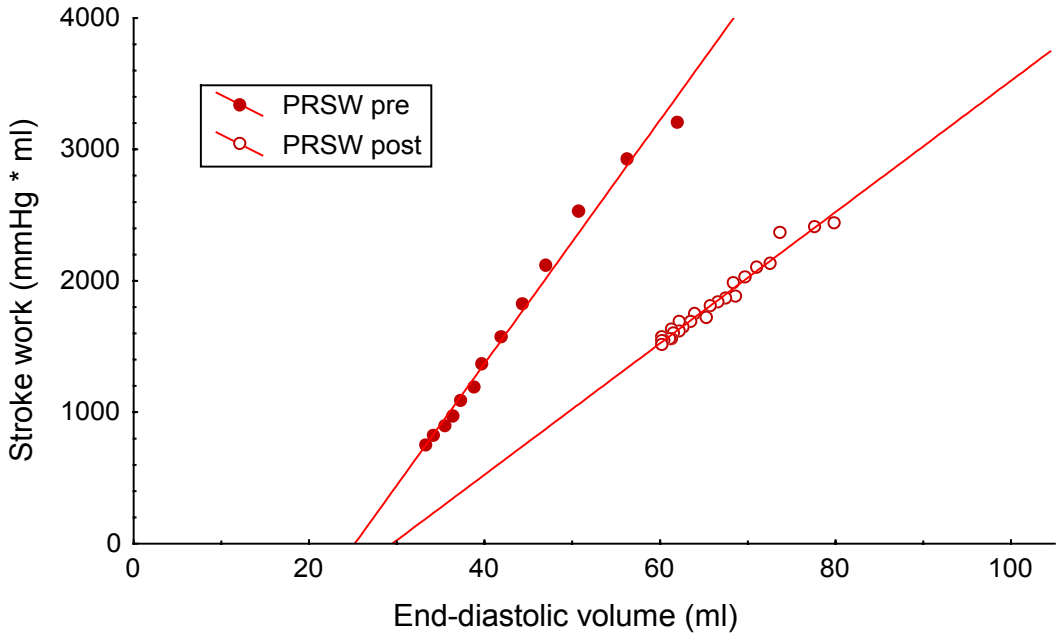

Fig 2. *The preload recruitable stroke work relation (PRSW) before (pre) and after (post) global ischaemia in one study subject. PRSW calculated from the loops in Fig. 1. Each point in this diagram is calculated from the area (stroke work) of a single pressure volume loop and its corresponding end-diastolic volume. The regression coefficient (M_w) of PRSW is 123 and 67 erg x10^3/ml respectively, indicating a severe post-ischaemic deterioration of global left ventricular function.*

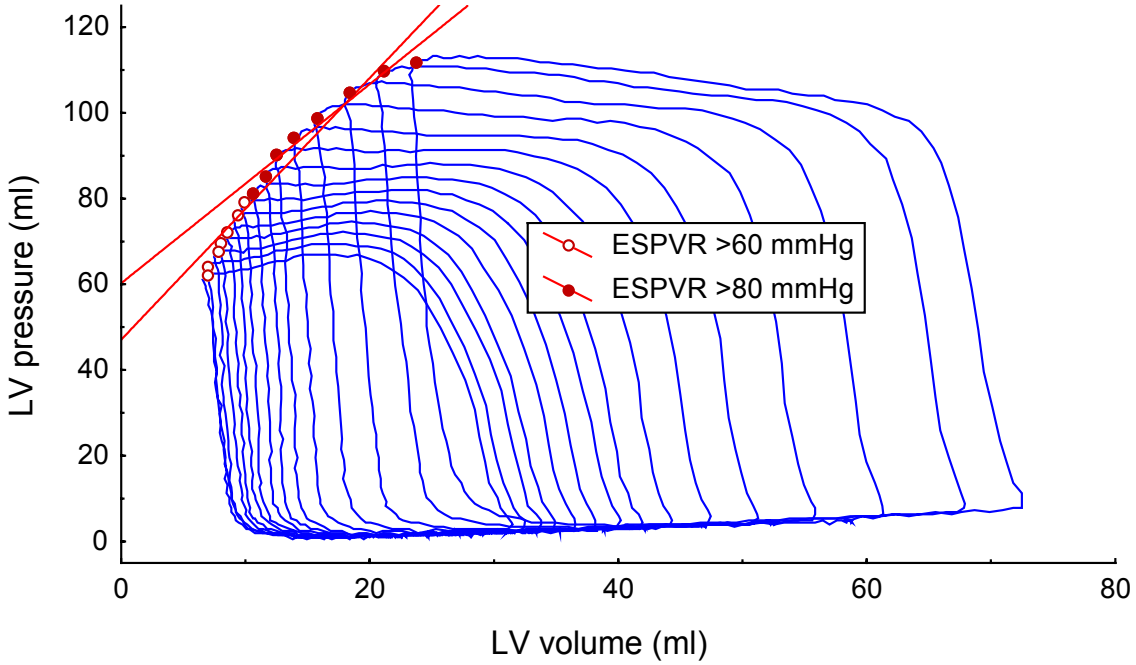

Fig 3. *Pressure-volume loops during an acute occlusion of vena cava inferior. For each new heart beat the end-diastolic volume is reduced and consequently also reduction of end-systolic pressure, end-systolic volume, and stroke work (loop area). The end-systolic points are used for calculation of the end-systolic pressure-volume relation (ESPVR). Since ESPVR often is slightly curvilinear there is a problem when adjusting it to a straight line in order to quantify systolic function as Ees (the regression coefficient of ESPVR). It is of uttermost importance that limits for the calculation are defined, otherwise Ees will easily be a function of the number of loops used for calculation! In this figure Ees increases 35% when all pressure-volume points down to a pressure of 60 mmHg are used instead of eight points down to 80 mmHg (Ees is 2.3 and 3.1 mmHg/ml respectively).*

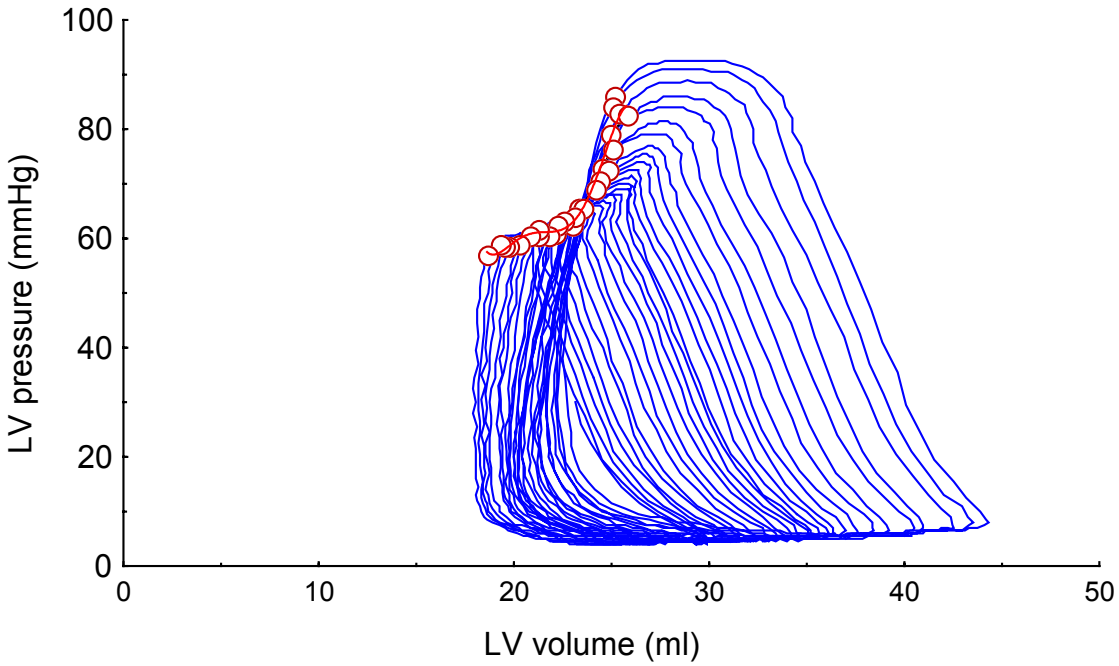

Fig 4. *Pressure-volume loops during a vena cava occlusion in one animal with the ESPVR shown as dots. These loops are from an acutely failing heart due to global ischemia (not from the same animal as in the figure 3). The ESPVR has become concave against the y-axis. This is seen in severely failing hearts [Burkhoff 1987]. The first part of the ESPVR can, as in this figure, even be steeper than in the same heart before the failure. The problem of calculating a linear Ees is obvious. Thus ESPVR was not used in any of the studies I-III, but was investigated in study V (se material and methods for study V).*

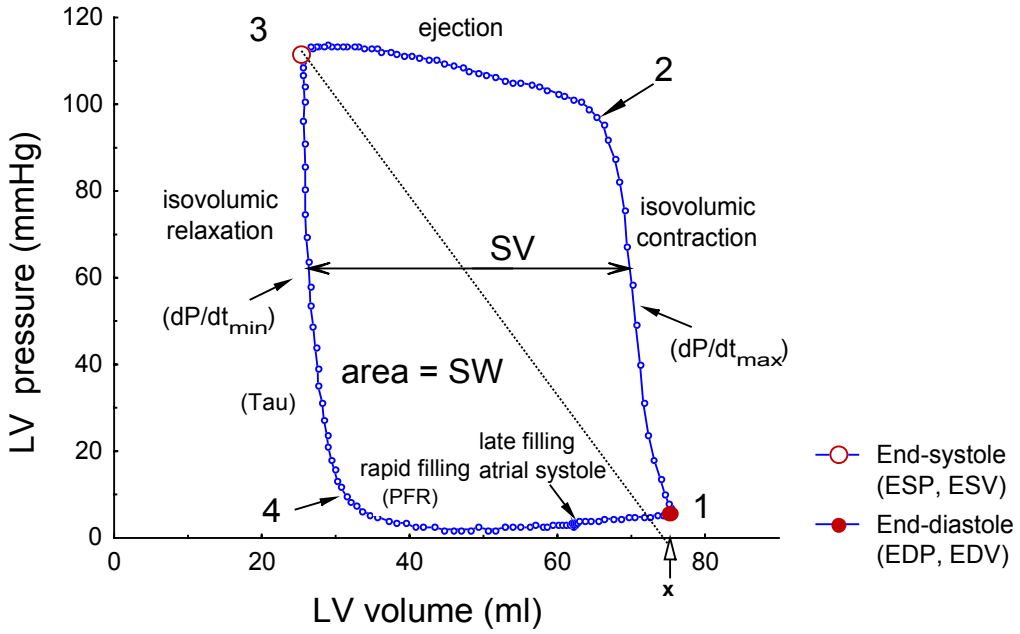

Fig 5. *A single heart beat, from a study animal, is presented as a pressure-volume loop. To follow the heart cycle the loop is followed counter-clockwise, starting at end-diastole (1). Heart rate is 82 beats per minute. It is 4 milliseconds between the small dots in the loop showing its relative speed during different parts of the heart cycle.* **1.** *The beginning of systole and the heart beat. End-diastole with closure of the mitral valve and start of the "isovolumic contraction".* **2.** *Opening of the aortic valve and start of the "ejection" phase. The left ventricle is emptied.* **3.** *End-systole, ejection stops, and the aortic valve closes. Beginning of diastole and the "isovolumic relaxation" with declining left ventricular pressure (relaxation velocity is measured as tau).* **4.** *Opening of the mitral valve and start of left ventricular filling. First the passive "rapid filling", enhanced by active diastolic relaxation (filling velocity is measured as PFR), followed by the "late filling" due to atrial systole (starting at the arrow). Tau is the time constant of isovolumic pressure relaxation (ms) measured from dP/dt$_{min}$, and PFR is the peak filling rate (ml/s) (For definitions of tau and PFR se "materials and methods").(The velocity of the rapid and late filling is measured by echocardiography as the E- and A- wave.) Note the small protuberance in the loop where atrial systole begins. For this heart beat atrial systole contributes 26% to the filling volume. The width of the loop represents stroke volume (SV) and the area stroke work (SW), dp/dt max and min (mmHg/s) is the maximum positive and negative first derivative of the LV pressure increase and decrease, respectively. ESP and ESV are end-systolic pressure and volume. EDP and EDV are end-diastolic pressure and volume. The ejection fraction can be estimated by just looking at the width (SV) of the loop and its location. (If the width of the loop is larger than its distance from zero volume (y-axis), then ejection fraction is more than 50%.) In a single pressure-volume loop the angle of a line drawn from "x" on the volume-axis to the end-systolic point is proportional to the effective arterial elastance (E_a) which is a measure of afterload ($E_a \approx$ ESP/SV). For calculation of mechanical function indices as PRSW (preload recruitable stroke work - global function), ESPVR (end-systolic pressure-volume relation – systolic function), EDPVR (end-diastolic pressure-volume relation - chamber stiffness/late diastolic function) or Beta (stiffness constant- EDPVR described as a curvilinear function), multiple pressure-volume loops have to be obtained by changing preload or afterload.*

Heart rate and cardiac function - Objectives of study V

In study I, II, and III the pigs were tachycardic after ischaemia-cardioplegia and cardiopulmonary bypass, probably as a compensation for failing cardiac pump function. Even though the increase in heart rate was almost the same in the different study groups we were concerned about the validation of the different indices used to measure cardiac function (se materials and methods). The main variable for the evaluation of cardiac function in study I to III was the regression coefficient of the preload recruitable stroke work relation (PRSW), being an index of global cardiac function or "contractility".

By the force-frequency relation, also known as the interval-force relation, heart rate will influence myocardial contractility in anaesthetised animals [Covell 1967; Skinner 1963, Koch-Weser 1963, Mahler 1974, Bowditch 1871]. Consequently cardiac function may be influenced by changes in heart rate, even when investigated by a preload independent function index such the PRSW. When reviewing the literature there were no studies really applicable to our adult pigs with regard to the their range of heart frequencies. There are only a few studies on heart rate and PRSW. Glower et al [Glower 1985] paced six dogs from 110 to 160 beats per minute (bpm) with no change in PRSW. In eight humans [Liu 1993] PRSW increased 20% during pacing from 71 to 120 bpm, and two studies in new-born piglets showed no changes in PRSW with increasing pacing rate, but studied from a baseline heart rate of about 150 bpm [Klautz 97, Davies]. Accordingly the behaviour of PRSW may be different in species having different ranges of heart rates or in the same species at different maturity of the myocardium.

The human study was also the only study where diastolic function indices were concomitantly investigated. Since PRSW as a global index contains diastolic properties, diastolic dysfunction may also influence PRSW. Since much cardiovascular research is done on anaesthetised pigs, we wanted to "calibrate" the adult anaesthetised pig model with increasing heart rate. Beside the PRSW we also wanted to investigate the end-systolic pressure-volume relation (ESPVR) (systolic function), the end-diastolic pressure-volume relation (EDPVR) (chamber stiffness), the stiffness constant (chamber stiffness), and tau (early diastolic relaxation) [Glower 1985, Suga 1973, Mirsky 1984]. In addition standard hemodynamics were recorded and pressure-volume loops were analysed. By designing the study as multiple increments of heart rate from a steady-state level we wanted to reduce possible influences of the preceding heart rate history.

Segmental and global LV volumes

- Objectives of study IV

Measurements of left ventricular (LV) volume are necessary for more exact assessment of cardiac function. In this thesis measurements of LV volume was done with the conductance catheter [Baan 1984] (se material and methods). Other more common methods for volume measurements such as contrast angiography, radioisotope imaging, magnetic resonance or computer tomography have all the disadvantages of not being on-line, or demanding gated sampling over several beats at steady state conditions, or as the conductance catheter, being invasive. Methods for on-line registration of LV volume are radiopaque metallic markers [Vine 1976], sonomicrometry [Ranking 1976] and the conductance catheter method [Baan 1981]. LV measurements by the conductance catheter is the latest developed method, but metallic markers and sonomicrometry have their place in long term animal models also giving the possibility to measure LV wall thickness. The conductance catheter technique has been used in humans [Baan 1984;

Liu 1993], but the invasiveness of this technique has made it unsuitable for most clinical applications.

Echocardiography is today the clinical method of choice for assessment of LV dimensions, having the advantage of being a bedside and non-invasive procedure. By using transesophageal echocardiography in conjunction with automated border detection LV area curves can be displayed on-line [Perez 1992, Vandenberg 1992]. These area-changes are shown to be good estimates of the corresponding changes in LV volume and the technique can be used for on-line monitoring in clinical practice[Gorcsan 1993x2]. However, with increased cardiac dyskinesia, assumptions drawn from area changes in single cross-sections may be violated. One solution is to use multiple area discs by which the LV volume, measured by echocardiography has shown to be linearly related to global volume measured by the conductance catheter [Gorcsan 1996].

A weakness of methods using areas is that geometric assumptions and calculations of global volume have to be done [Wyatt 1980]. This can be avoided by further using the conductance catheter technique, by which the momentaneous volume in multiple intraventricular volume segments can be measured [van der Velde 1992]. With both segmental and global volume measured by the conductance catheter, we hypothesised that changes in five intraventricular volume segments correlate with concomitantly measured changes in global LV. If valid, it would be a further indication that echocardiography, perhaps with thicker areas, could be used for on-line measurements of LV volume. There might also be a possibility that the conductance catheter not necessarily has to cover the whole left ventricular long axis, but only a central segment thus making the technique more easy. For this study, which was done in co-operation with the Division of Medical Engineering at Karolinska Institutet, an algorithm was constructed for purpose of comparing the momentaneous differences between the curve shape of global and segmental volumes. The comparisons were done at baseline, after 60 minutes of apical ischaemia, during changes of preload and changes of afterload.

.

Aims of the study

This thesis is focused on studies of blood cardioplegia in order to evaluate some novel regimens of myocardial protection in the acutely ischaemic heart. The studies were performed in pigs subjected to global ischaemia, and outcome was evaluation by load independent indices of cardiac function derived from pressure-volume relations acquired with the conductance catheter method. Studies were undertaken to further study the conductance catheter method for cardiac volume measurements and to calibrate the technique for changes in heart rate. The objectives were:

- To evaluate the effect of the temperature of blood cardioplegia. Is continuous cold blood cardioplegia similar to continuous warm cardioplegia with respect to post-cardioplegic cardiac function?

- To investigate if simultaneous antegrade and retrograde blood cardioplegia is detrimental to post-cardioplegic cardiac function. Will myocardial oedema develop, and if so, will it influence cardiac function?

- To study if warm intermittent blood cardioplegia is a method to be used in the metabolically deranged heart. Is warm intermittent blood cardioplegia as good as warm continuous cardioplegia with respect to post-cardioplegic cardiac function?

- To correlate, if possible, continuous volume changes in a mid-cardiac volume segment with volume changes in the global left ventricular volume (with volumes measured by conductance catheter). Is volume changes in a mid-cardiac volume segment an estimate of volume changes in global volume?

- To examine, in a comprehensive study, the rate dependency of systolic and mechanical function indices. How do indices of mechanical function and cardiac performance change with changing heart rate in the anaesthetised pig?

Materials and methods

Anaesthesia and surgical procedures

Study population

All animals received humane care in compliance with the European Convention on Animal Care and all studies were approved by the Ethics Committee for Animal Research at Karolinska Institutet, Stockholm, Sweden.

In all 5 studies Swedish farm pigs were used (study **I**: 44 (40-48) kg; study **II**: 42 (40-44) kg; study **III**: 40 (39-42) kg; study **IV**: 41 (40-41) kg; study **V**: 42 (41-43) kg [median (quartile limits)]). A model with pigs on cardiopulmonary bypass (CPB) was principally the same in study **I-III**, differing in the different regimens of cardioplegic administration. All studies were conducted in anaesthetised, open chest pigs, but study **IV** and **V** without CPB.

Anaesthesia and surgical procedures (study I-V)

All pigs were premedicated with intramuscular ketamine hydrochloride (20 mg/kg) and atropine sulphate (0.5 mg). Anaesthesia was induced with intravenous sodium pentobarbital (15 mg/kg) and maintained by a cocktail (0.35 ml/kg per hour) containing 2 mg fentanyl citrate, 25 mg midazolam and 24 mg pancuronium bromide in a volume of 57 ml. The infusion was preceded by a bolus of 0.15 ml/kg. The pigs were intubated and ventilated with a volume-cycled ventilator (Engström 300, Datex-Engström AB, Bromma, Sweden).

Catheters were inserted into the right femoral artery and vein for drug and fluid administration, blood sampling, and pressure monitoring. A catheter and a temperature probe were surgically introduced into the urinary bladder (study **I-III**). An electrocardiogram was recorded by surface electrodes. In study **II-V** a Swan-Ganz catheter (Baxter Healthcare Corp.) was placed in the pulmonary artery through the right external jugular vein for pressure monitoring, cardiac output measurements, and injections of hypertonic saline solution for parallel conductance calibrations. In study **I** an ordinary catheter was placed in the pulmonary artery instead of a Swan-Ganz catheter, hence cardiac output was in this study only measured by the conductance catheter (see below).

The pericardium was opened after median sternotomy. A 5F transducer-tipped pressure catheter (Mikro-Tip, Millar Instruments Inc.) and a 7F, 12-pole conductance catheter with 9 or 7-mm spacing between the electrodes, depending on heart size (Cordis Webster), were introduced into the left ventricle through a stab wound in the apex. The catheters were fixed by a purse string suture and a tourniquet (Fig. 6)

The tip of the conductance catheter was brought through the aortic valve. A proper position of the catheter was confirmed before each set of measurements by inspection of the individual segmental volume signals. In study **I-II** a "single field" and in study **III-V** a "dual filed" conductance catheter was used. The later is an improved model for increased accuracy of volume measurement. The catheters were inserted through the apex, in order not having to remove and reposition them before and after cross-clamping the aorta. In study **IV** two extracardial pacemaker wires were sutured to the right atrium and connected to an external pacemaker generator (Medtronic 5375, Medtronic Inc.). In study **I-III** ventricular or atrial pacing was sometimes used during reperfusion and weaning from CPB.

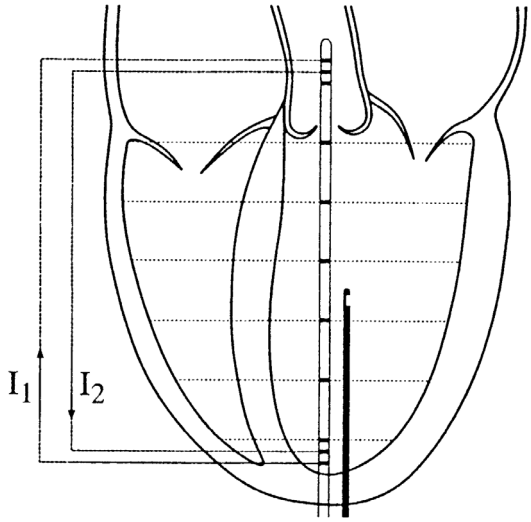

Fig 6. *Catheters for acquisition of pressure-volume loops. A conductance catheter with five segments and a pressure catheter inserted through the heart apex (can also be inserted from aorta). An electrical field is set up between the two most proximal and distal electrodes (here "dual excitation" with two currents, I_1 and I_2 , having opposite polarity). Note the extra segment in aorta. This segment can be recruited depending on heart size. (This segment will then be "number 5" and the mid-cardiac segment will be doubled in thickness). Also note that the electrical field will spread to the right ventricle and to other surrounding structures.*

*Surgical procedure, cardiopulmonary bypass (study **I-III**).*

After heparinisation (activated clotting time > 480 seconds), the ascending aorta was cannulated for cardiopulmonary bypass (CBP) with a 20F arterial cannula. Venous return was through a 32F two-stage cannula in the right atrium. CPB was initiated with a flow of 75 to 90 ml/kg per minute using a roller pump (7400, Sarns Inc./3M Health Care) and a membrane oxygenator (Maxima, Medtronic Blood System) primed with Ringer's acetate solution, in study **III** also with Dextran 70 (Macrodex). During CPB the temperature was allowed to drift to 29°C (study **I**) and 34°C (study **II-III**). The left ventricle was vented by a 16 - 20F catheter inserted through the left atrial appendage, brought through the mitral valve and connected to the venous line for passive drainage. A cardioplegia cannula was inserted into the aortic root with a side branch for pressure monitoring. Cardioplegic flow, in study **I,** was continuously measured by an ultrasound transit-time flowmeter with the flow probe on the cardioplegia tubing (HT207, Transonic Systems Inc.), and in study **III** by the cardioplegia delivery pump (continuous group) and by calculating delivered volume (intermittent group). For study **II** se below (Fig. 7).

Surgical procedure, additional in study **II**.

The hemiazygos vein, which in the pig connects with the great cardiac vein to form the coronary sinus, was ligated. The ostium of the coronary sinus was dissected, and a suture with a tourniquet was placed around. This was done also in the group given only antegrade cardioplegia to provide the same surgical trauma as in the simultaneous antegrade-retrograde cardioplegia group (se below). A retrograde cardioplegia catheter (Research Medical, Inc.), with the balloon removed, was then introduced into the coronary sinus through a stab wound in the right atrium and secured with a purse string suture. In the group given simultaneous cardioplegia, the cardioplegia lines to the aortic root and coronary sinus were connected by a Y-connector. During administration of simultaneous cardioplegia the suture around the coronary sinus ostium was tightened around the retrograde catheter. Pressure was measured both in the coronary sinus and in the aortic root. Cardioplegic flow was continuously measured by the cardioplegia delivery pump and by an ultrasound transit-time flowmeter (CM 1000, CardioMed A/S, Oslo, Norway) with a flow probe on the cardioplegia tubing to the coronary sinus to measure the relative antegrade and retrograde distribution of cardioplegia. Mean cardioplegic delivery pressure and flow were continuously displayed. (Fig.7)

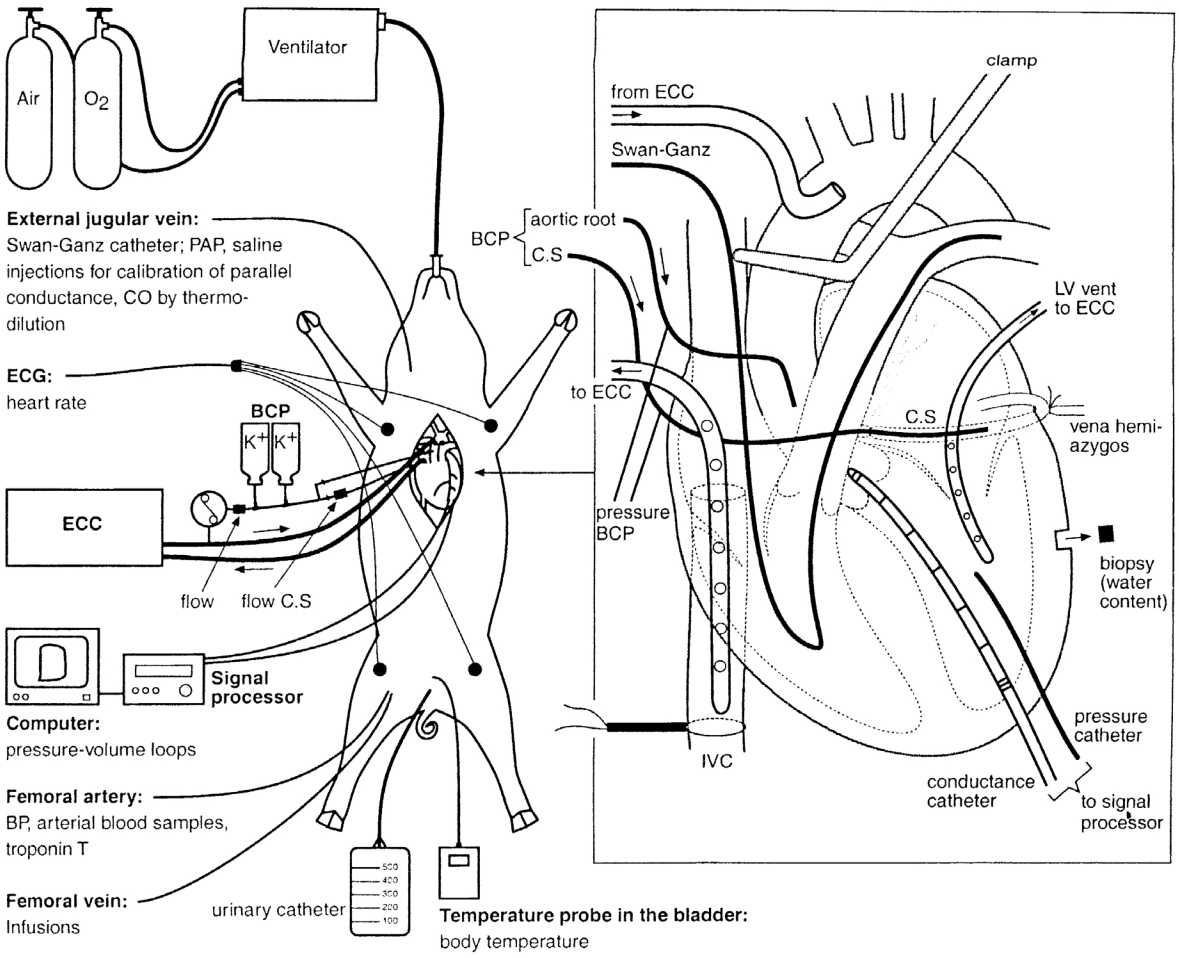

Fig 7. *This figure shows the instrumentation in study* ***II*** *(antegrade vs. simultaneous antegrade-retrograde cardioplegia). The same principal instrumentation was also applied in study* ***I*** *(warm vs. cold antegrade cardioplegia), and in study* ***III*** *(warm intermittent vs. warm continuous antegrade cardioplegia), but without the line to coronary sinus (C.S.) (and with no ligation of vena hemiazygos or snaring of coronary sinus). The pig is cannulated for cardiopulmonary bypass (ECC) with inflow cannula in the ascending aorta ("from ECC") and outflow cannula in the right atrium ("to ECC"), as well as a vent in the left ventricle ("LV vent") draining blood flow from collaterals and Thebesian veins. Blood cardioplegia ("BCP") is given through a separate line, with oxygenated blood from the heart-lung machine (ECC). High or low potassium cardioplegic solutions are given into the coronary circulation via the aortic root (study I and III), and in study II also via the coronary sinus . Cardioplegic flow and pressure are measured. During ischaemia and cardiopulmonary bypass the ascending aorta is clamped ("clamp"). A conductance catheter for volume measurements and a pressure catheter are inserted through the LV apex. These catheters are connected to a signal processor and a computer to display the pressure-volume loops on-line. A snare is put around inferior vena cava (IVC) for preload reductions to obtain pressure-volume relations. A Swan-Ganz catheter is put into the pulmonary artery for (1) measurements op pulmonary artery pressure (PAP), (2) injections of saline for parallel conductance corrections (offset calibration), (3) and for cardiac output (CO) measurements to correct conductance catheter volumes to absolute volumes (gain calibration).*

Data Acquisition (study I-V)

Hemodynamic and mechanical data were acquired during disconnection of the ventilation for 15 to 20 seconds in end-expiration to minimise the effects of intrathoracic pressure variations. The mechanical data were acquired during unloading of the heart by clamping the inferior vena cava for 10 to 15 seconds. In study **IV** there was an additional intervention where afterload was increased by clamping the descending thoracic aorta during a few beats. Every measurement was repeated at least twice with an interval of two minutes for circulatory stabilisation between acquisitions.

The conductance catheter was connected to a Leycom Sigma-5 signal-conditioner processor (CardioDynamics BV). The pressure and volume signals were processed (Conductance-PC software, CardioDynamics BV), and the left ventricular pressure-volume loops were displayed on-line and stored on the computer hard disk for later analysis. The volume signal was calibrated to absolute volume by correcting the signal for the parallel conductance, for the cardiac output measured by thermodilution (not in study **I**) and for the blood resistivity. The principle and technique for volume measurement have previously been presented in detail [Baan 1984, 1989]. Data were acquired with an IBM-compatible computer with an AD converting board (DAS-1601, Keithley Data Acquisition) at a sampling frequency of 200 to 250 Hz. The time-varying conductance G(t) from five intraventricular cylindrical segments, perpendicular to the LV long axis of the conductance catheter, was recorded and added in the Leycom Sigma-5 giving the time-varying volume V(t) by the formula:

$$V(t) = [1/\alpha]\,[L^2/\sigma] / [G(t)-Gp]$$

α is a slope factor giving the relation between the "true" cardiac output and the cardiac output measured by the conductance catheter. In study **I** cardiac output by thermodilution was not measured, but approximated to 0.6 [Steendijk 1993]. In study **II-V** cardiac output was measured by thermodilution (CO) (Cardiac computer, Sat-2, Baxter Healthcare Corp.) 3-5 times after each set of recordings by injecting 10 ml of cold (4°C) glucose 5%. L is the distance between the electrodes on the catheter and σ is the resistivity of the blood, which was measured in a cuvette before each set of volume recordings. Gp, the parallel conductance from structures surrounding the left ventricle (septum, right ventricle, lungs and the great vessels), was calculated by a bolus injection of 4 ml hypertonic (10%) saline solution into the pulmonary artery (Baan et al 1989). This was repeated at least twice after each set of baseline recordings. The transducer tipped pressure catheter was calibrated to zero pressure with the sensor just below the surface of water and the corresponding electrical zero was checked in the computer.

Data analysis and calculations

*Hemodynamics, study **I-V** (with some exceptions)*. End-diastole was defined as the lower right-hand corner of the pressure-volume loop. At this point left ventricular (LV) end-diastolic pressure (EDP) and volume (EDV) were measured. Variables calculated were stroke volume (SV) as maximum minus minimum volume, conductance catheter measured cardiac output as SV *x* heart rate, ejection fraction (EF) as SV/ maximum volume x 100, stroke work (SW) as the area within the pressure-volume loop, mean arterial pressure (MAP) (study **I-III**), pulmonary artery pressure (PAP) (study **I-III**), the time constant of LV isovolumic pressure relaxation (τ) as the time from peak -dP/dt until LV pressure had reached half the value at peak -dP/dt (study **II, III, V**) (Mirsky 1984).

*Additional hemodynamics in study **I***. Coronary vascular resistance was calculated as the cardioplegic perfusion pressure, measured in the aortic root, divided by cardioplegic flow.

*Additional hemodynamics in study **V***. End-systole for steady state beats was defined at the

timepoint of maximum systolic elastance (maximum LV pressure/ LV volume). At this point LV end-systolic pressure (ESP) and volume (ESV) were measured. Variables also calculated were peak filling rate (PFR) as maximum dV/dt (V= LV volume), peak dP/dt (first derivative of LV pressure), diastolic filling time (DFT) as the time from onset of peak -dP/dt to end-diastole, beat-to-beat interval (RR-interval) as 1/heart rate, and systolic time (systole) as RR-interval minus DFT. Arterial load was quantitated as the effective arterial elastance (E_a) being ESP / SV [Kelly et al 1992]. The LV pressure at the opening of the mitral valve (MVOP) was, by us, defined as the point of the pressure-volume loop, where a line parallel to the diagonal connecting the end-diastolic and end-systolic points was tangent to the left lower corner of the pressure-volume loop.

*LV mechanical function (study **I-III, V**)*. All curves were individually analysed and inspected visually beat by beat. Global LV function was quantitated by the slope (M_w) (mmHg or erg*10^3/ml) of PRSW, with V_w (ml)as its x-axis intercept (in study **I-III**: V_0), using the equation (Glower et al 1985):

$$SW = M_w (EDV-V_w)$$

LV diastolic chamber stiffness was quantitated by the stiffness constant (β) (unit less) and by the slope (E_{ed}) (mmHg/ml) of EDPVR with "a" as y-axis (pressure) intercept, and V_{ed} (ml) as x-axis intercept. Since E_{ed} is an adjustment to a curvilinear relation the value is dependent on the amount of pressure-volume points used for the calculation. This differed between, but not within study I-III, giving different baseline values in these studies. The following equations were used:

$$EDP = ae^{(\beta \, x \, EDV)}$$

$$EDP = E_{ed} (EDV-V_{ed})$$

*Additional mechanical function study **II***. Preload recruitable stroke work area was calculated, with V_{0max} as the maximal volume intercept obtained over the entire study (= 68 ml) and V_0 as the volume intercept of PRSW, using the equation (Glower et al 1988):

$$PRSWA = ½ \, M_w (1.2V_{0max}-V_0)^2$$

*Additional mechanical function study **V***. LV systolic function was quantitated by end-systolic elastance E_{es} (mmHg/ml), which is the slope of the ESPVR using the equation (Sagawa 1978):

$$ESP = E_{es} (ESV- V_0)$$

V_0 is the x-axis intercept calculated by the iterative method [Kono 1984]. The reason not using ESPVR in study **I-III** was its tendency to be curvilinear [Burkhoff 1987, Kass 1989]. To adjust the often curvilinear ESPVR to a straight line, according to the above formula, we strictly followed a few rules when analysing ESPVR in study V: (1) the drop in ESP between the first and second beat used for the Ees calculation should be >2.0 mmHg, (2) twelve heart beats were used unless condition 3 and 4 interfered, (3) only beats with ESP >60 mmHg were used, (4) the ESPVR used for analysis should be a straight line or a smooth curve convex against the y-axis, if not, this ESPVR was not used, (5) single heart beats giving an irregularity in the beginning or end of the ESPVR could be taken away, but ESPVR´s where the volume increased for one or more beats were not used (as with extra beats), (6) all analysis was done by one investigator.

Calculations - study IV

A computer algorithm was constructed to compare the curve shape of segmental volumes with the curve shape of global volume. In order to analyse the absolute difference between curve shapes of segmental versus global volume, segmental volumes were normalised to equalise the corresponding global volume 1:1 at global end-diastole and end-systole. For the calculations the mean of ten beats were

used. During vena cava and aortic occlusion the first beat in each acquisition was used as reference. Both mean and peak differences are presented as per cent of global stroke volume.

Myocardial water content
- study II and III

Transmural samples of the left ventricular anterolateral wall were taken at the end of the experiments, weighted, and dried to a constant weight. Water content was determined by the following formula:

Water content (%) =
100 (wet weight - dry weight) / wet weight.

Troponin T
- study II and III

Troponin T concentration in serum was analysed with the second generation of cardiac troponin T enzyme-linked immunosorbent assay (Katus wt al 1992) using the Enzymun-Test® troponin T (Boehringer Mannheim). The serum was stored at -70 °C and all samples were analysed at the same time. Troponin T was analysed in arterial blood at baseline and after 90 minutes of reperfusion, in study **II** also in coronary sinus blood.

Experimental protocols

Study I-III (beginning and end)

After circulatory stabilisation baseline haemodynamic and mechanical data were recorded followed by cannulation and start of totally vented CBP. The aorta was clamped and the hearts fibrillated (study **I, II**) if still beating five minutes after cross-clamping. Following 30 minutes of "unprotected", normothermic global, ischaemia cardioplegia was delivered during 45 minutes according to the protocols in study **I-III** (se below and Fig. 8).

After the 45 minutes of cardioplegic delivery the infusion was stopped, the aorta unclamped and rewarming begun. After 30 minutes of reperfusion the LV vent was discontinued and after additional 15 minutes the pigs were gradually weaned from bypass. Within 60 minutes after declamping all pigs were weaned from CBP and decannulated. No inotropic support was ever used, but during reperfusion and weaning atrial or ventricular pacing was sometimes used but not thereafter. After 90 minutes of reperfusion post-bypass haemodynamic and mechanical data were acquired. In study III arterial blood and in study II arterial and coronary sinus blood was sampled for troponin T. At the end of all experiments, in all studies, the pigs were given a lethal intravenous injection of pentobarbital and potassium.

Study I (Cold vs. warm continuos blood cardioplegia)

Pigs were randomised to cold (n= 12; 6-7° C) or warm (n= 13; 34-35° C) continuous antegrade blood cardioplegia. An initial dose of 500 ml high-potassium cardioplegia was given, followed by a low-potassium solution (Table I). Cardioplegic flow was continuously adjusted to maintain an aortic root pressure of 55 to 60 mm Hg and flow was recorded after 5, 15, 25, 35, and 45 minutes. High potassium cardioplegia was reintroduced if needed to sustain a totally arrested heart. In the cold group body temperature was allowed to drift, but in no animal below 29° C. In the warm group it was maintained at 33-37° C.

Study II (Antegrade vs. simultaneous antegrade-retrograde blood cardioplegia)

Pigs were randomised to antegrade (n=8) or simultaneous antegrade-retrograde (n=9) continuous warm (35-36°C) blood cardioplegia.

An initial dose of 500 ml of high-potassium cardioplegia, followed by low-potassium cardioplegia was given. In the simultaneous group the coronary sinus tourniquet was tightened to prevent backflow of cardioplegia into the right atrium during cardioplegia infusion. Cardiople-

gic flow was continuously adjusted in the antegrade group to maintain an aortic root pressure of 70-80 mmHg. In the simultaneous group flow was adjusted to a pressure in the coronary sinus (and thus also in the aortic root) of 40-45 mmHg. Pressure and flow of cardioplegic infusion were registered after 5, 15, 25, 35, and 45 minutes of delivery. If the heart started to fibrillate, high-potassium cardioplegia was reintroduced until total arrest.

After 45 minutes of reperfusion, immediately before start of weaning from CBP, arterial and coronary sinus blood samples were drawn for troponin T measurements, and the coronary sinus catheter was removed.

Study III (Continuous vs. intermittent warm blood cardioplegia)

Pigs were randomised to continuous (n=8) or intermittent (n=10) warm (34°C) blood cardioplegia. The hearts were not fibrillated and continued to beat for up to 10 minutes. After the ischaemic period cardioplegic perfusion was begun, given continuously or intermittently. During delivery of cardioplegia, cardioplegic flow was continuously adjusted to maintain an aortic root pressure of 75 to 80 mmHg in both groups. In the continuous group, an initial dose of 12ml/kg high-potassium cardioplegia was given, followed by a continuous infusion of a low-potassium cardioplegia. In the intermittent group, an infusion of 12ml/kg high potassium cardioplegia was given and then repeated every 10 minutes after stop of the prior bolus. During the last 2 minutes before unclamping, cardioplegia was given in the intermittent group irrespective of the time from the prior bolus. Cardioplegic flow was registered at 5, 15, 25, 35, and 45 minutes of delivery in the continuous group and during times of delivery in the intermittent group.

Study IV (Segmental vs. global LV volume)

Six pigs were operated. After preparation the animals were stabilised for at least 30 minutes. Following calibrating procedures haemodynamic data were acquired at baseline, during occlusion of vena cava and occlusion of the descending aorta. To intentionally create an apical myocardial infarction the left anterior descending coronary artery (LAD) was ligated immediately distal to the second diagonal branch. After 60 minutes of apical ischaemia haemodynamic data were again acquired.

Table I. *Content of cardioplegic solution mixed 1:4 with blood (study I-III)*

Component (mmol)	*High* K^+ Solution	*Low* K^+ Solution
KCl	101	30
$MgSO_4$	16	15
Glucose 5 g	28	28
THAM	20	20
Normal (0.9%) saline solution to a volume of (ml)	1000	1000

THAM, *Tris hydroxymethyl-aminomethane (=Trometamol).*

Study V (Heart rate vs. cardiac function)

Eleven pigs were operated. After preparation the animals were stabilised for at least 30 minutes. After correcting the volume signal for blood conductivity, baseline hemodynamic data were recorded including measurements of CO by thermodilution. Mechanical data were collected as described above followed by correction measurements for parallel conductance. This data set was called "baseline 1", and was followed by atrial pacing to 100 beats per minute (bpm). After 5 minutes of pacing haemodynamic and mechanical data were again recorded. The pacemaker was turned off, and following 10 minutes of heart rate stabilisation a "baseline 2" was recorded. These procedures were repeated at 120, 140 and 160 bpm giving eight sets of recordings (baseline 1 to 4 with corresponding increments in heart rate). The time span for each set was 20-30 minutes.

Statistical analysis

Paired or unpaired t-tests, and one- or two-way ANOVA with repeated measures were used in study **I** and **II** respectively. Data in these studies were reported as mean with quartile or 90%-95% confidence limits.

In analysing cardioplegic flow, regression coefficients for individual slopes were given in study **I** and **III,** and ANOVA with repeated measures was used in study **II**.

In study **III, IV** and **V** Wilcoxon matched pairs test was used for within group analysis. Data were presented as median with quartile limits or minimum to maximum range.

In study **III** due to a skewed randomisation, treatment effects (baseline - post-bypass M_W) became correlated to baseline values. To accomplish an unbiased estimate of the difference between continuous and intermittent cardioplegia, avoiding a "regression to the mean - effect", only post-bypass values were considered in the analysis of hemodynamic and mechanical data. Two pigs, in the intermittent group, dying of cardiac failure were assigned worst rank for the analysis of mechanical data and the Mann-Whitney U test was used for the intergroup comparison. In presenting a 95% confidence interval for the difference between post-bypass M_W , a "trimmed mean" was used to compensate for the two non-surviving pigs in the intermittent group, and thus two pigs were excluded in the upper tail of this group.

To correct for changing baseline values in study **V,** the differences between the value at paced heart rate and the corresponding baseline value were analysed using the Wilcoxon matched pairs test. For analysing any trend with increasing heart rate increments, linear and second order polynomial regression were used. The individual regression coefficients were analysed using the 2-sided Sign test. A p-value < 0.05 was considered significant in all studies. Statistics used in tables showing study I-III together are given in each table separately.

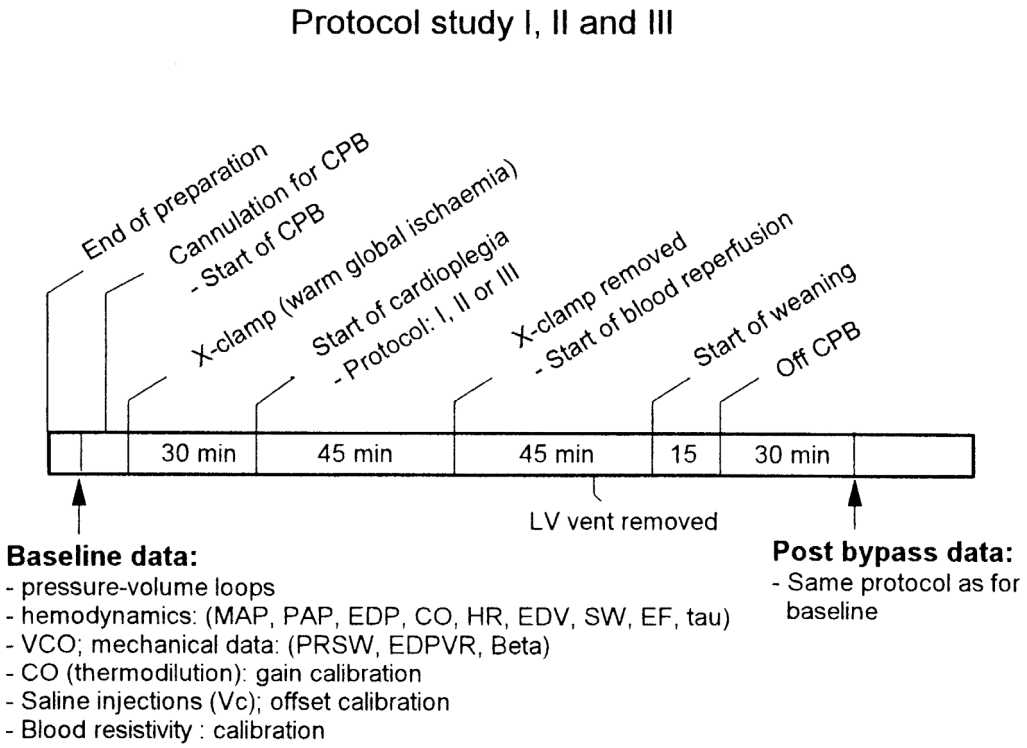

Fig 8. *Study protocol for study I, II, and III (the cardioplegia studies). For detailed protocol see text. CBP, cardiopulmonary bypass; X-clamp, cross clamping aorta; VCO, vena cava occlusion. For other abbreviations see page 10.*

Results

Results from study **I-III** are based on the same pig model. Continuos, antegrade, warm, blood cardioplegia was the cardioplegia against which the different regimens of cardioplegia were compared. This "standard cardioplegia" was tested with regard to cardioplegic temperature and modes of delivery. In study **I** against continuous, antegrade, cold cardioplegia, in study **II** against continuos, simultaneous antgrade-retrograde, warm cardioplegia, and in study **III** against intermittent, antegrade, warm cardioplegia. However, the cardioplegic delivery pressures were different in the 3 studies. Results from the methodological studies **IV** and **V** are accounted for separately.

Results study I-III - Cardioplegia studies

Subjects

In study **I**, 2 pigs in each group were excluded giving 10 pigs in the cold and 11 in the warm group for final analysis. In the cold group two pigs weaned from CPB, but one died of ventricular fibrillation and one of pulmonary hypertension before post-bypass measurement. In the warm group two pigs could not wean from CPB and died of cardiac failure.

In study **II**, 2 pigs in each group were excluded giving 8 pigs in the antegrade and 9 the simultaneous antegrade-retrograde group for final analysis. These animals died of cardiac failure after weaning from CPB, or were in cardiogenic chock at the time for post-bypass measurement making it impossible to acquire mechanical data.

In study **III**, 1 pig in the continuous group were excluded, but two pigs dying in the intermittent group were included giving 7 pigs in the continuous and 10 in the intermittent group for final analysis. The excluded pig had a baseline troponin T value several standard deviations above the mean for both groups, 1.57 versus 0.19 µg/L (standard deviation 0.11), indicating preoperative cardiac ischaemia. Two pigs given intermittent cardioplegia died of cardiac failure after weaning from CBP, but before post-bypass measurement. For inclusion of these pigs see statistical analysis and discussion.

Cardioplegic flow and coronary vascular resistance

In study **I** warm cardioplegia resulted in a constantly increasing coronary vascular resistance, during the 45 minutes of cardioplegic delivery, but the resistance was unchanged with cold cardioplegia. Regression coefficients being 6.0 (95%CI; 3.4 to 8.5) (warm group) and 0.9 (95%CI; -0.4 to 2.2) mmHg $x10^{-3}$ /ml (cold group), respectively (p=0.001 between regression coefficients). For cardioplegic flow and delivery pressures see table II.

In study **I** mean cardioplegic flow during the 45 minutes of delivery was similar with cold and warm cardioplegia (Table II). As with coronary vascular resistance there was a difference over time (interaction) with warm cardioplegic flow declining more than the cold flow. In study **II** cardioplegic flow was higher in the antegrade than in the simultaneous antegrade-retrograde group. Also here an interaction was seen with antegrade cardioplegic flow changing more over time than simultaneous flow (Table II). In the retrograde-antegrade group 28 (25-

31)% of total cardioplegic flow was directed to the coronary sinus, and this ratio did not change over time. In study **III** total amount of delivered cardioplegia to each animal in the intermittent and continuous group were 2.5 (2.4-2.6) and 13.4 (10.1-15.4) litres, respectively. Intermittent cardioplegia was delivered five times during totally 17 (16-21) % of the cross-clamp time.

Table II. *Cardioplegic flow in study I-III*

		CPP	*Cardioplegic flow (ml/minute)*			*p Values (ANOVA)*	
		(mmHg)	*Mean (quartile limits)*				
Study	*Group (subjects)*		*Mean*	*5 min*	*45 min*	*Within group - over time*	*Between groups/ interaction: group x time*
I	Cold (n= 10)	55-60	143 (104-174)	154 (114-189)	134 (110-154)	0.2	>0.7/ <0.001
	Warm (n= 11)		137 (124-155)	182 (160-198)	109 (73-130)	<0.001	
II	Ante (n= 8)	70-80	165 (151-179)	205 (194-215)	147 (131-165)	0.001	0.03/ 0.003
	Sim (n= 9)	40-45	120 (104-137)	131 (100-140)	113 (100-140)	0.02	
III	Contin (n= 7)	75-80	275 (250-326)	325 (290-378)	239 (180-320)	<0.001	0.2/ 0.03
	Interm (n= 10)		323 (250-359)	332* (300-360)	284** (218-320)	<0.001	

Cardioplegic flow presented as mean for the whole period of cardioplegic delivery , and at 5, and at 45 minutes of delivery. Study I. *Cold* and *Warm* continuous antegrade blood cardioplegia; Study II. *Ante, Sim*, Antegrade and Simultaneous antegrade-retrograde continuous warm cardioplegia; Study III. *Contin, Interm*; Continuous and Intermittent warm antegrade cardioplegia. *CPP,* cardioplegic perfusion pressure. Flow in the intermittent group is given for the initial *, and terminal ** infusions. *ANOVA,* analysis of variance.

When comparing the "warm group" in study **I**, the "antegrade group" in study **II**, and the "continuous group" in study **III,** differing only in cardioplegic delivery pressure, flow declined similarly over time in all 3 groups (time: p< 0.001, interaction: p= 0.7; ANOVA). For these groups cardioplegic flow was significantly higher in study **III** with no differences between study **I** and **II**.

Haemodynamics

In none of the 3 studies were intergroup differences observed in post-bypass haemodynamics (MAP, systolic PAP, EDP, EDV, SW, HR, CO, or EF). In study **III** a larger increase in heart rate after intermittent cardioplegia almost reached statistical significance, 125 (117-132) beats per minute vs. 141 (126-160) beats per minute in the continuous and intermittent group respectively, p=0.054. Baseline mean/median heart rate was consistent between the studies ranging only from 82 to 85 beats per minute. Heart rate increased significantly in all studies to 122 to141 beats per minute.

Global left ventricular function

*In study **I*** , M_w as an index of global left ventricular contractility, was not different with cold and warm cardioplegia (Table III, Fig. 9). Also recovery of M_w was not significantly different [81(68-84) % vs. 99(70-120) % of baseline with cold and warm cardioplegia respectively] (p= 0.19). This despite a significant decline in the

cold, but not in the warm group (Table III) (se discussion).

*In study **II***, M_w decreased after ischaemia and cardioplegia, but with no difference between the groups (Table III).

*In study **III***, M_w decreased after intermittent, but not after continuous cardioplegia. A 95% confidence interval for the difference in post-bypass M_w was 2-62 erg/ml x 10^3, indicating a p-value ≈ 0.04, though p= 0.079 when comparing post-bypass values by a conservative rank test (Table III, Fig. 10).

Table III. *Global left ventricular function and chamber stiffness*

		M_w		*p Values*	*E_{ed}*		*p Values*
Study	*Group*	*Baseline (pre)*	*Post-bypass*	*Within group/ Between groups (Δ pre-pos)**	*Baseline (pre)*	*Post-bypass*	*Within group/ Between groups (Δ pre-pos)**
I	Cold	94 (85-103)	73 (55-87)	0.03	0.37 (0.26-0.46)	0.84 (0.43-1.04)	0.01
	Warm	110 (80-132)	109 (71-175)	>0.5/ 0.25	0.32 (0.22-0.37)	0.70 (0.43-0.98)	0.008/ >0.5
II	Ante	126 (100-156)	75 (59-83)	0.004	0.25 (0.20-0.28)	0.60 (0.39-0.84)	0.009
	Sim	122 (116-125)	95 (72-110)	0.02/ 0.13	0.30 (0.23-0.30)	0.53 (0.32-0.68)	0.02/ 0.4
III	Contin	95 (76-130)	91 (90-104)	>0.5	0.17 (0.14-0.20)	0.27 (0.22-0.33)	0.018/
	Interm	122 (100-128)	64 (23-93)	0.005/ 0.08 or 0.04**	0.15 (0.12-0.22)	0.39 (0.25-1.08)	0.005/ 0.20

M_w , slope of preload recruitable stroke work relation (erg/ml*10^3); *E_{ed} ,* slope of end-diastolic pressure-volume relation (mmHg/ml). Study I. *Cold* and *Warm* continuous antegrade blood cardioplegia; Study II. *Ante, Sim*, Antegrade and Simultaneous antegrade-retrograde continuous warm blood cardioplegia; Study III. *Contin, Interm*; Continuous and Intermittent antegrade warm blood cardioplegia. Study I and II, mean values (quartile limits); study III, medians (quartile limits). In group I and II paired and unpaired t-tests. In study III non-parametric statistics. * in study III between group analysis is only performed post-bypass. ** p-value after trimmed mean due to two pigs dying before post-bypass measurement in the intermittent group (see statistical analysis).

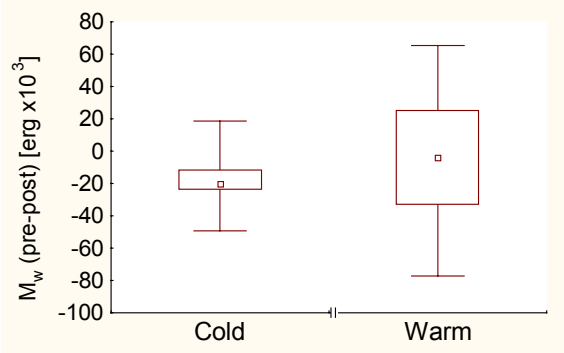

Fig 9. *Study I. Box plots of the difference between baseline (pre) and post-bypass M_w in the cold, and warm continuous cardioplegia group, p= 0.25. The box shows the quartile limits, the point the median, and the whiskers the range of data.*

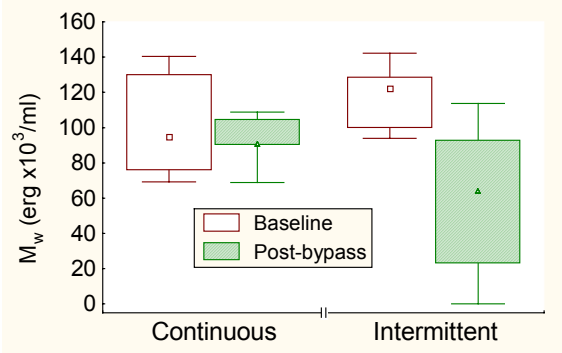

Fig 10. *Study III. Box plots of M_w at baseline and post-bypass in the continuous and intermittent warm car-dioplegia group. For p values se text.*

Diastolic function

Chamber stiffness. The regression coefficient E_{ed} ,of the end-diastolic pressure volume relation (EDPVR), increased after cardioplegia in all 3 studies indicating increased chamber stiffness. There were no differences between the groups within the 3 studies (Table III). For the stiffness constant (β), there were also no differences between the groups. The change in β showed a large spread and increased significantly only in the warm group in study I; from 0.06 (0.04-0.09) at baseline to 0.09 (0.07-0.13) post-bypass (p=0.02).

Early relaxation, tau (ι). In study II ι did not change. In study III ι decreased significantly in both groups, but without intergroup difference. From 39 (38-52) and 38 (36-49) ms in the continuous and intermittent group respectively at baseline to 34 (32-35) and 33 (31-36) ms post-bypass. Tau was not calculated in study I.

Troponin T and myocardial water content (Study II and III)

In study **II** and **III** troponin T concentrations increased post-bypass in all groups indicating severe myocardial ischemia, but without differences within the studies (Table IV). In study **II** troponin T was higher in the coronary sinus than in arterial blood after 45 minutes of ischaemia (p=0.001), but without intergroup difference (p=0.8). Troponin T was significantly higher in study II versus study III at baseline (p< 0.001), but not after 90 minutes of reperfusion (p=0.21). Troponin T was not measured in study **I.**

Myocardial water content was significantly higher after simultaneous antegrade-retrograde warm continuous cardioplegia than after only antegrade administration (Table IV).

Table IV. *Troponin T and myocardial water content.*

		Troponin T (μg/litre)		*p-value*			*p-value*
Study	*Group*	*Baseline*	*Post-bypass*	*Within groups*	*Between groups (post)*	*Water content (%)wet weight*	*Between groups*
II	Ante	0.40 (0.26-0.48)	11.2 (3.6-17.6)	0.012	>0.5	80.1 (79.6-80.7)	0.01
	Sim	0.50 (0.48-0.70)	12.4 (8.5-19.8)	0.012		81.1 (80.7-81.5)	
III	Contin	0.18 (0.16-0.20)	3.8 (1.5-12.0)	0.018	0.25	81.3 (80.2-82.0)	0.16
	Interm	0.15 (0.10-0.21)	8.0 (6.0-10.4)	0.012		80.5 (80.3-80.7)	

Study II, *Ante, Sim*, Antegrade and Simultaneous antegrade-retrograde continuous warm blood cardioplegia; Study III. *Contin, Interm*; Continuous and Intermittent antegrade warm blood cardioplegia. Post-bypass after global ischemia and 90 minutes of reperfusion. Values are medians and quartile limits.

Results study IV - Global vs. segmental volumes

Haemodynamic data and baseline volumes.

When analysing baseline data in 6 subjects the agreement between the normalised segmental and the global volume curve, was best for segment number 3 and 4, with segment 3 (located at mid-ventricular level) being slightly better (Fig. 11 and 12). Baseline stroke volume for segment number 3 constituted 34 (14 - 39)% [median (minimum to maximum range)] of global stroke volume with a variability over 10 beats of 1.3 (0.9 - 1.6) %-units in individual subjects. Stroke volume for segment number 4 constituted 29 (20 - 44)% with an interbeat variability of 1.1 (0.4 - 3.1) %-units. The median average absolute difference for segment 3 over a cardiac cycle was 4 (1 - 8)% (Table V). For segment 3 the peak difference in four of the six subjects never exceeded 9% of global stroke volume. However, in subject #5 and #6 the peak difference for short periods in mid-systole was approximately 10% and 18% respectively, and almost 30% in mid-, respective early diastole (Table V, Fig. 12).

Apical myocardial ischaemia

After 60 minutes of apical ischaemia a marked dyskinesia appeared in the two most apical segments (1 and 2) represented by a larger difference between segmental and global volume (Fig. 12). However, this lack of agreement was not seen in the adjacent segment number 3 where the mean absolute difference was in the range 2 - 3% with no peak over 7% (Table V). In one pig no ischaemic data was recorded due to ventricular fibrillation and death after ligation of LAD.

Preload reduction

With an end-systolic LV pressure declining to 60% of "preload"-baseline, during occlusion of vena cava, the mean absolute volume difference for segment number 3 was 3 - 7% in five of the six subjects. In subject #5 a mean difference of 39% was seen. This was due to a disagreement in early diastole, possibly caused by dyskinesia due to the decreasing LV volume (Table V and Fig. 13).

Afterload increase

During occlusion of the descending aorta only the "afterload"-baseline and two additional beats were analysed since LV end-systolic pressure reached its maximum already within a few beats. The median mean difference increased from 3 (2 – 10)% at "afterload"-baseline to 7 (3 – 12)% at the 3rd beat. The peak difference, located to the "isovolumetric relaxation period", was temporarily high in subject #3 - #5 (Table V and Fig. 13)

Table V. *The difference in curve shape between the normalised mid-segmental (segment 3) and global left ventricular volume.*

	Steady state		*Decreased preload**			*Increased*		
Subjects	*Baseline*	*Ischaemia*	*Baseline*	*80%*	*60%*	*Baseline*	*2nd beat*	*3rd beat*
#1	3%(7)	-	3%(9)	5%(9)	6%(12)	3%(8)	3%(8)	3%(7)
#2	1%(4)	2%(6)	2%(7)	2%(7)	3%(7)	2%(4)	2%(5)	4%(12)
#3	4%(8)	2%(7)	5%(10)	8%(15)	7%(14)	3%(9)	3%(8)	7%(21)
#4	4%(9)	2%(6)	4%(10)	4%(9)	5%(13)	3%(8)	6%(18)	8%(33)
#5	8%(27)	3%(6)	13%(44)	33%(71)	39%(78)	10%(31)	12%(26)	12%(24)
#6	8%(28)	2%(7)	8%(29)	5%(17)	4%(12)	4%(11)	4%(10)	7%(13)

Steady state at baseline and after 60 minutes of regional apical ischemia; * Vena cava occlusion to *80%* and *60%* of initial systolic left ventricular pressure, **aortic occlusion. Values are mean differences over ten cardiac cycles (peak differences in parenthesis) in per cent of global stroke volume.

General observations

During the heart cycle short episodes of increased difference between segmental and global volume appeared. In two of the six subjects the difference occasionally peaked over 10% at baseline, in the same two subjects over 15% during vena cava occlusion and in three subjects the difference peaked over 20% during aortic occlusion (Table V). When analysing these peak differences they were caused either by a relative delay in segmental diastolic filling, initiated by a further decrease in segmental volume at the end of the "isovolumetric relaxation period", or an extra volume peak early in this period. Also a slight earlier ejection in segmental volume relative to global volume initiated an increased differenc. There was also dyskinetic movements in the segments during vena cava occlusion as a consequence of the rapidly decreasing volume.

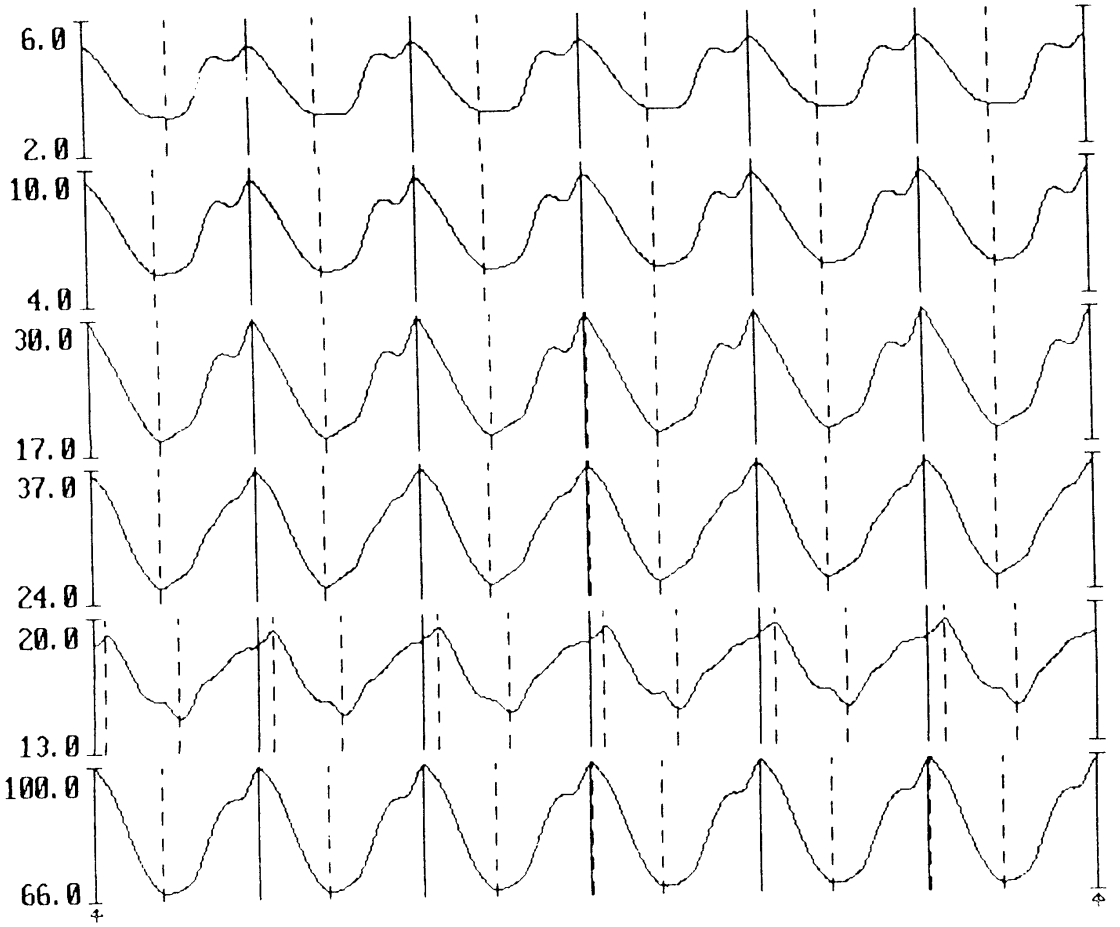

Fig 11. *This figure shows global and segmental volume curves from the left ventricle acquired with the conductance catheter in one pig. At the top the most apical volume segment (segment 1), at row three the mid-cardiac segment (segment 3), and at row five the most basal segment (segment 5). At the bottom the summarised global volume curve. End-diastole and end-systole are marked. Note the change of shape in volume curve from cardiac apex to the base and the temporal delay in segment 5. Also note the similarity in the shape between the volume curve of segment 3 and the global volume curve.*

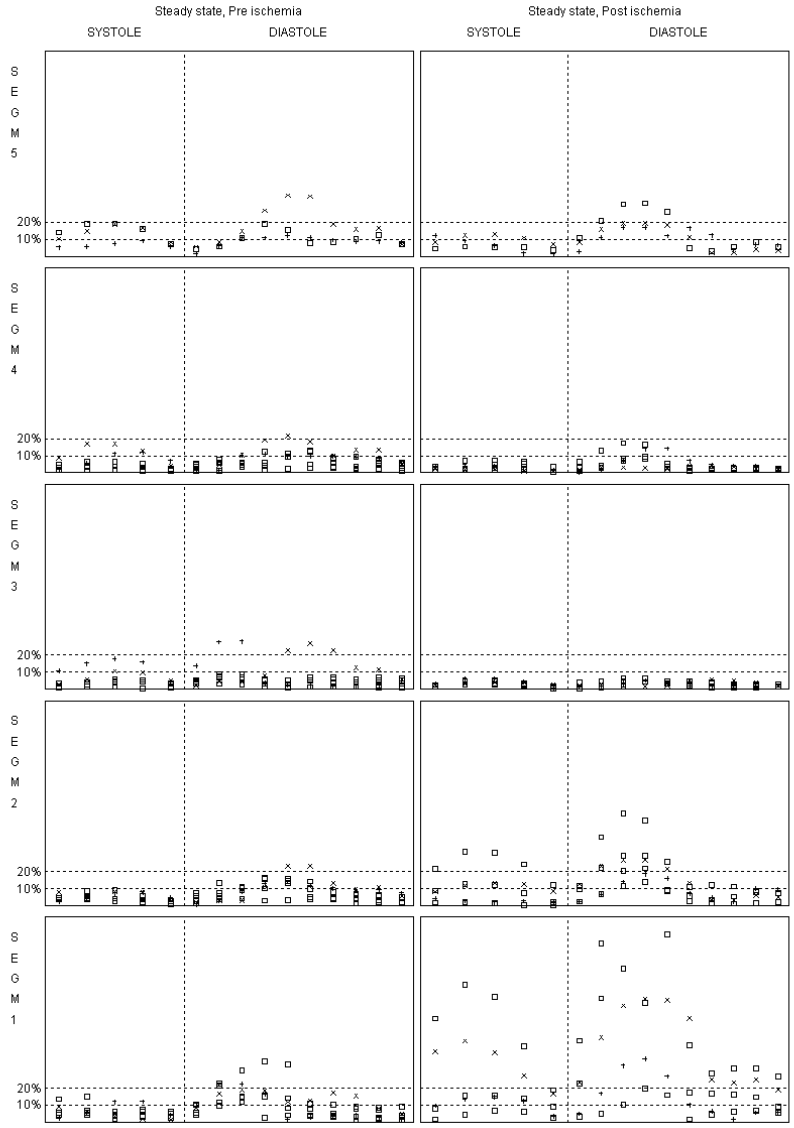

Fig 12. *The maximal absolute difference in curve shape between normalised segmental and global volume as percent of global stroke volume, calculated in individual subjects at baseline (n=6) and after 60 minutes of apical ischaemia (n=5), at five timepoints in systole and ten in diastole. Segment 5 (top panel) is located at the heart base, segment 3 at mid-cardiac level and segment 1 at the apex (bottom panel). Subject #6 is marked (+), #5 (x), and #1 to #4 ( ).*

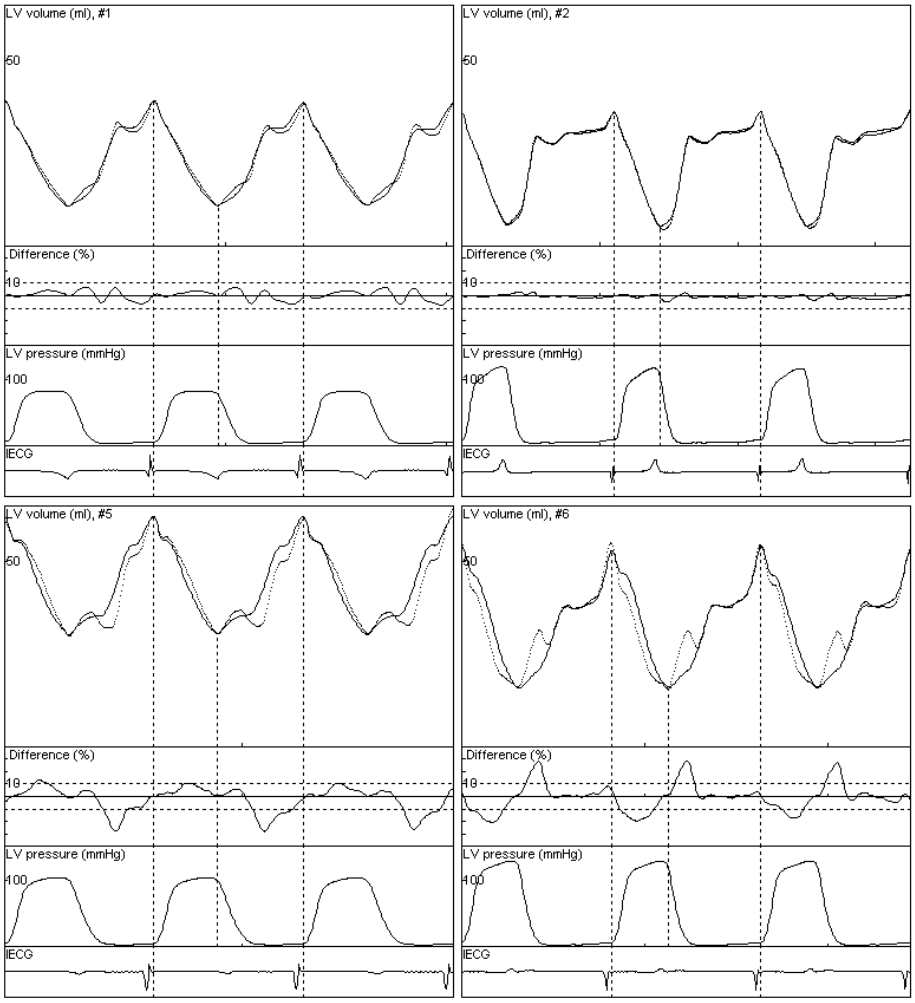

Fig 13. *Acquisitions from the mid-cardiac volume segment (number 3).* ***Upper left,*** *at baseline in subject #1;* ***Upper right,*** *at baseline in subject #5;* ***Lower left,*** *during preload reduction by occlusion of vena cava inferior in subject #1;* ***Lower right,*** *afterload increase by occlusion of the descending aorta in subject #1. In each panel from above: global and normalised segmental volume; the volume difference between the curves in percent of global stroke volume; left ventricular pressure; and intracardial ECG.*

Results study V
- Heart rate vs. cardiac function

Baseline haemodynamic and mechanical data

Baseline heart rate increased during the experiments from 72 (70-78) beats per minute (bpm) at "baseline 1" to 88 (79-95) bpm at "baseline 4" (p=0.02). Although baseline ESP and EDP did not change throughout the experiments, baseline EDV and SV declined (p=0.05 and p=0.01 respectively) (Table VI). Baseline M_w remained unchanged as did diastolic chamber stiffness (β and E_{ed}). Baseline systolic function (E_{es}) increased between "baseline 1" and "baseline 4" (p=0.01) (Table VI).

Paced haemodynamic data

Most variables changed linearly or curvilinearly with increasing heart rate increments (Fig 14.). DFT declined almost parallel with the declining RR-interval, but as a fraction of the RR-interval, DFT declined from 60 (59-62)% at 72 bpm ("baseline 1") to 32 (29-33)% at 160 bpm (p=0.002). The systolic time declined less than DFT and exceeded DFT at heart rates of more than 100 bpm (Fig.16)

Paced mechanical data (Fig. 15)

M_w was unchanged up to 140 bpm, but decreased 10% (-Δ8.5 (-13 to 3) mmHg) from "baseline 4" to 160 bpm (p=0.02). E_{es} increased from "baseline 1" to 100 bpm (p= 0.008), Δ1.3 (0.6-2.1) mmHg/ml, without any further change. E_{ed} started to increase at 140 bpm, but significantly first at 160 bpm, Δ0.13 (0.10 – 0.18) mmHg/ml, corresponding to a 69% increase from "baseline 4" (p=0.007). For β the increase started already at 120 bpm, but was significant only at 140 bpm, Δ0.026 (0.022-0.040) (p=0.005), where it peaked. The volume intercepts for PRSW (V_w) and EDPVR (V_{ed}) were not influenced by heart rate (p>0.2 for linear regression). The ESPVR intercept (V_0) increased up to 100 bpm (p=0.04), with no further change (p=0.11).

Pressure-volume loops

A general observation in all pigs was that with increasing heart rate the pressure-volume loop moved progressively upwards with the left lower corner being increasingly blunt (Fig. 18). The mitral valve opening pressure (MVOP) started to increase at 140 bpm, being even higher at 160 bpm (Fig. 17). At "baseline 4" MVOP was 8 (8-8) mmHg and at the corresponding increment to 160 bpm MVOP increased to 20 (15-27) mmHg (p=0.005). When heart rate increased MVOP also became increasingly higher than EDP, significantly at heart rates above 100 bpm. Thus above 100 bpm LV pressure declined during the whole LV filling.

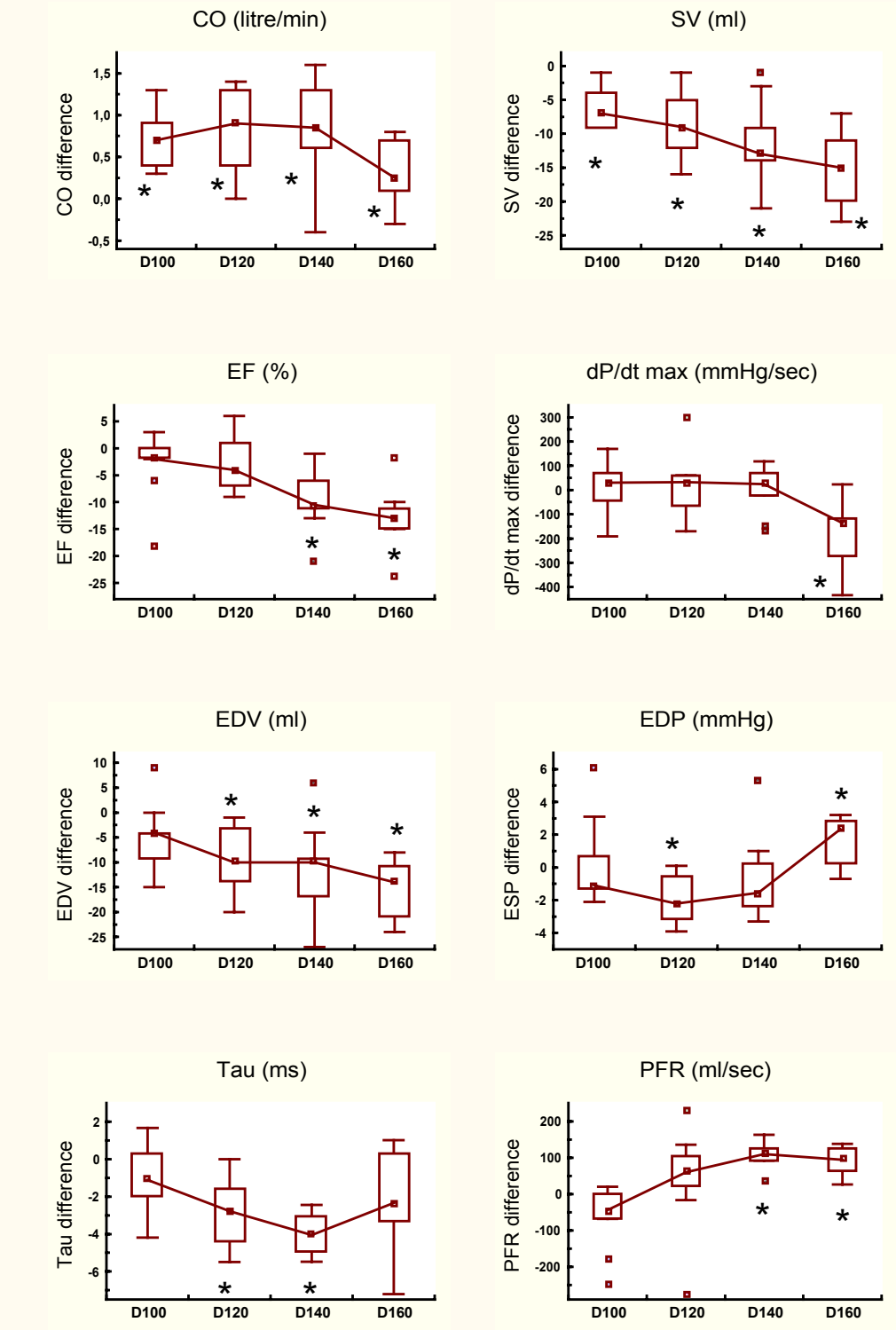

Fig 14. *Haemodynamics: The difference between baseline value and value at respective heart rate increment from 100 to160 beats per minute (D100 to D160) is shown. CO, cardiac output; SV, stroke volume; EF, ejection fraction; dP/dt max; maximum dP/dt; EDV, end-diastolic volume; EDP, end-diastolic pressure; Tau, time constant of left ventricular pressure relaxation; PFR, peak filling rate. Box plots with median (point), quartile interval (box), non-outlier range (whiskers), and outliers (dots). * denotes p<0.05 vs. baseline at each heart rate increment.*

Table VI. *Haemodynamic data. Baselines values*

	Baseline 1 (before HR 100)		*Baseline 4 (before HR 160)*		
n= 9-11	*Median*	*25% - 75%*	*Median*	*25% - 75%*	*p-value*
			General variables		
HR	72	70 - 78	88	79 - 95	0.02
CO	3.4	3.0 - 3.7	3.0	3.0 - 3.4	0.05
SV	50	41 - 54	33	31 - 43	0.01
			Systolic variables		
EF	72	61 - 75	70	67 - 83	0.16
dP/dt	1426	1343 - 1604	1474	1292 - 1644	>0.2
			Diastolic variables		
EDP	7.2	6.5 – 9.4	6.2	4.8 – 8.1	>0.2
EDV	75	57 - 80	45	39 – 63	0.05
τ	35	34 – 37	36	34 – 39	0.09
PFR	337	252 - 393	227	209 – 286	0.02
DFT	516	445 - 525	364	300 – 430	0.02
			Mechanical variables		
M_w	80	76 –101	85	79-95	>0.2
E_{es}	3.5	2.0 – 4.3	5.6	3.9 – 7.4	0.01
E_{ed}	0.18	0.16 - 0.20	0.19	0.18-0.27	0.16
β	0.037	0.031 – 0.041	0.064	0.049 – 0.084	0.09

HR, heart rate (beats/min); *CO*, cardiac output (litre/min); *SV*, stroke volume (ml); *EF*, ejection fraction (%); *dP/dt*, peak dP/dt (mmHg/sec); *EDV*, end-diastolic LV volume (ml); *EDP*, end-diastolic LV pressure (mmHg); *τ*, time constant of isovolumic pressure relaxation (msec); *PFR*, peak filling rate (ml/sec); *DFT*, diastolic filling time (msec); M_W, slope of preload recruitable stroke work (PRSW), (mmHg); E_{es} , slope of end-systolic pressure-volume relation (ESPVR), (mmHg/ml); E_{ed}, slope of end-diastolic pressure-volume relation (EDPVR), (mmHg/ml); *β*, the stiffness constant (unit less). 25%-75%, quartile interval.

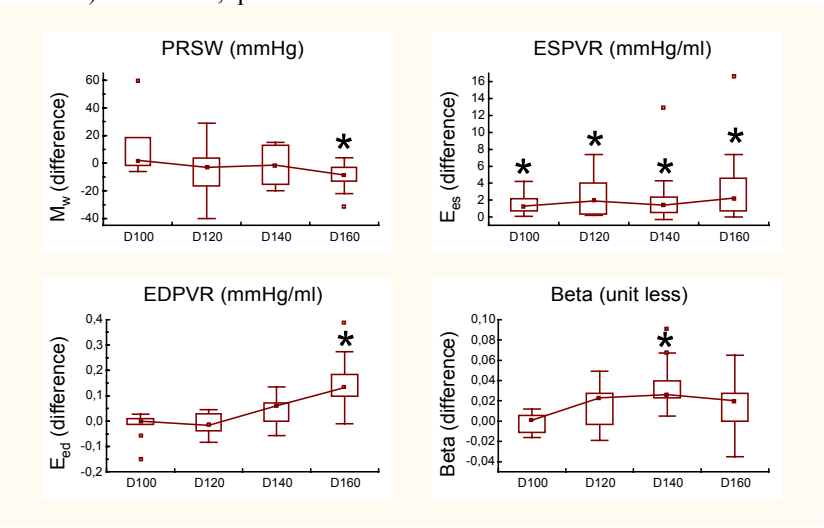

Fig 15. *Mechanical data. The difference between baseline value and value at respective heart rate increment from 100 to160 beats per minute (D100 to D160) is shown. PRSW, preload-recruitable stroke work; ESPVR, end-systolic pressure-volume relation; EDPVR, end-diastolic pressure-volume relation; Beta, the stiffness constant. Box plots with median (point), quartile interval (box), non-outlier range (whiskers), and outliers (dots). * denotes p<0.05 vs. baseline at each heart rate increment.*

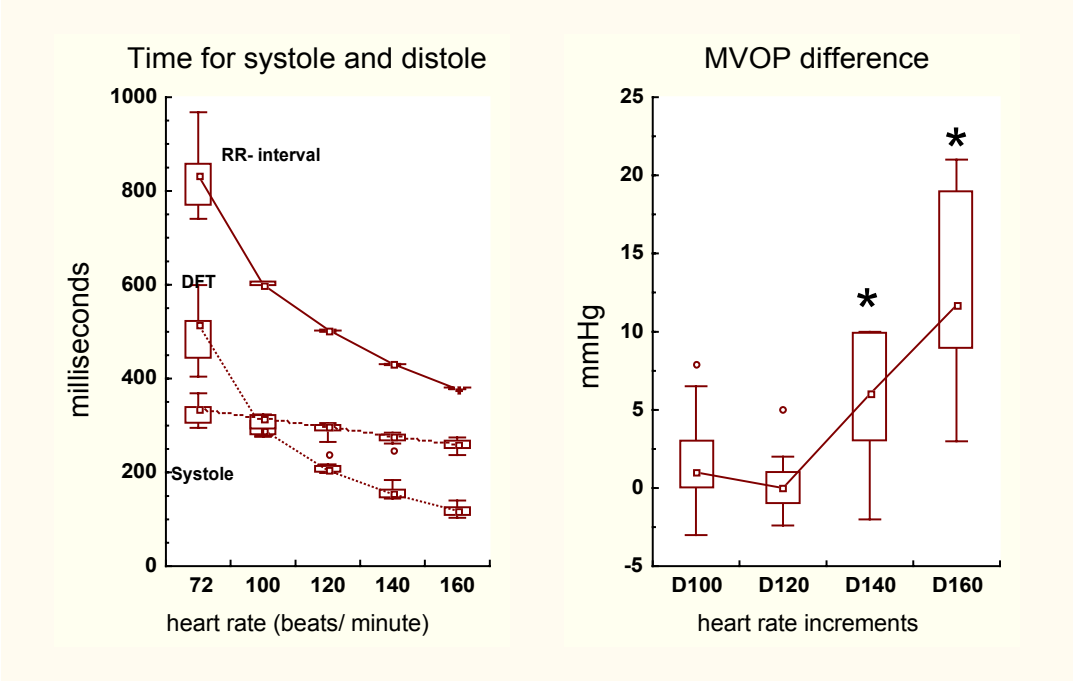

Fig 16. *RR-interval (beat-to-beat interval), DFT (diastolic filling time), and Systole (duration of systole) with increasing heart rate. Box plots with median (point), quartile interval (box), non-outlier range (whiskers), and outliers (dots).*

Fig 17. *MVOP(mitral valve opening pressure). The difference between baseline value and value at respective heart rate increment from 100 to160 beats per minute (D100 to D160) is shown. * denotes $p<0.05$ vs. baseline heart rate for each heart rate increment. Box plots with median (point), quartile interval (box), non-outlier range (whiskers), and outliers (dots)*

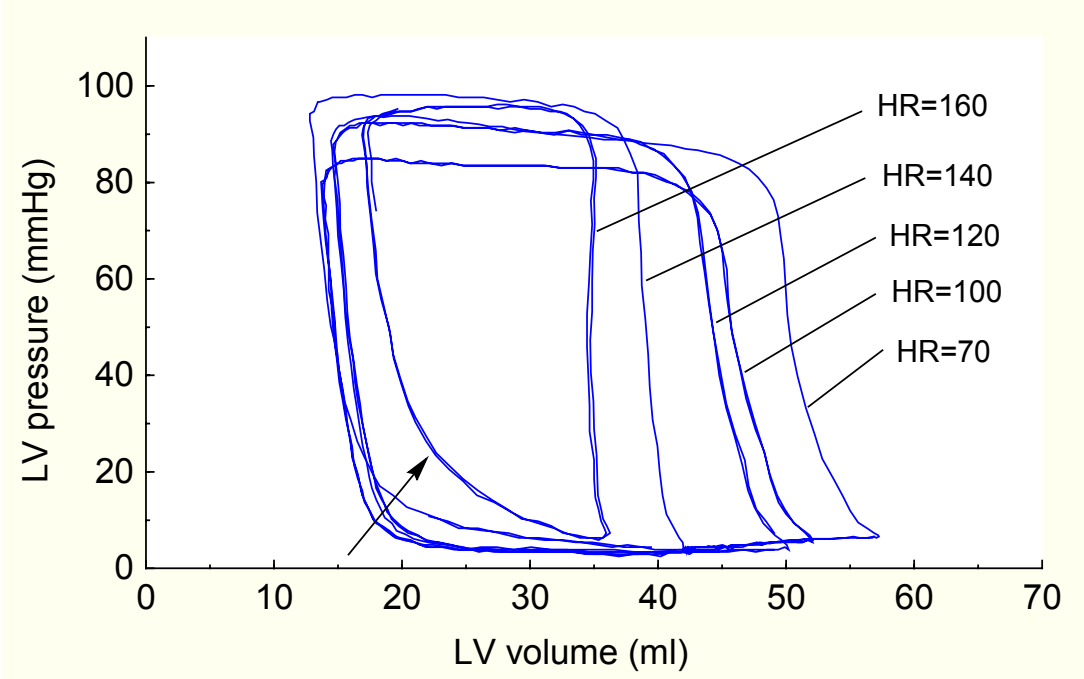

Fig 18. *Pressure-volume loops in one study pig showing loops at baseline heart rate (HR) = 70 beats per minute, and at paced heart rates up to 160 beats per minute. Note the increasing bluntness of the left lower corner as the heart rate increases and the LV pressure increases at the start of LV filling (indicated by the arrow). The LV pressure then declines during the whole filling.*

Discussion

Cardioplegia studies

The model

In the three cardioplegia studies (I to III) the same model was used. This model was partly copied from Jacob Vinten-Johansen´s group [Vinten-Johansen 1993]. It mimics the use of blood cardioplegia as resuscitation of the ischaemic myocardium. By using a model with unprotected warm, global, ischaemia, the myocardium was maximally challenged. In many cardioplegia studies post-cardioplegic or post-ischaemic endpoints have been measured with the animal still on cardiopulmonary bypass (CBP) [Rosenkranz 1982, Liu 1993, Asai 1995] or during right-heart bypass [Vinten-Johansen 1991, Brown 1993, Bufkin 1994, Misare 1993]. Although this is a "safer" model with less losses due to cardiac failure, we decided to do the post-cardioplegic measurements in the fully working and loaded heart after having weaned from CBP. This in order to get clinically more relevant endpoints. However this puts considerable and additional strain on the model, also being influenced by right ventricular function and pulmonary circulation. In pilot studies we tested the length of warm, unprotected global ischaemia, and found that 30 minutes was the longest ischaemic time possible without a high post-bypass mortality (versus 40 minutes of ischaemia). However, in study II the outcome was worse than in study I and III (the continuous group). This was probably due to a larger ischaemic insult added during the dissection of coronary sinus, when the beating heart had to be partly compressed. This was also reflected in the higher baseline troponin T in study II compared with baseline in study III.

By using a model like this the idea was to increase the sensitivity of the model since myocardial protection and cardioplegia was not studied in a normal, but in a severely compromised heart. Differences between groups otherwise to small to be detected might be revealed in this more severe model. However, the most important reason for using a preceding episode of unprotected ischaemia in order to "injure" the myocardium was to make the experiments shorter. If using high-quality myocardial protection without any preceding ischaemia, the time for cardioplegic delivery and cardiopulmonary bypass must be prolonged for maybe 4-6 hours in pigs with a healthy heart (personal observation) in order to induce a similar post-cardioplegic dysfunction. With our model the animals are kept in a better condition for haemodynamic studies and detrimental effects of prolonged CPB per se is better avoided.

The used cardioplegia was a cardioplegia with a simple composition without addition of aspartate and glutamate. The reason for this was that we wanted to used the same cardioplegic formula as used clinically at that time in our department.

Since the main variable of the investigation was PRSW being an index of global LV function, but also a surrogate index for "contractility", a model of global and not regional ischaemia was used. Global and regional ischaemia are shown to differently affect ESPVR [Sunagawa 1983]. Although not studied, we suppose that this might also be the case for PRSW. Theoretically, a regional ischaemic insult could be described to affect global function in two cardiac compartments, one with unaffected and normally working myocytes

having normal contractility and a second compartment with ischaemically deranged myocytes having a disturbed contractility [Swan 1972, Suga 1974, Suga 1988]. Using a global index on a regional insult will work, but the measured variable will then be even further away from "contractility" on the myocyte level. In our studies (I to III), with global ischaemia, the global myocardium will functionally represent regional events as after a coronary occlusion, but it will also represent the globally compromised heart after a long cardioplegic arrest. In study **I** (warm vs. cold cardioplegia) a further reason to use global and not regional ischemia was to avoid any negative bias to the warm group (study I) due to a possible greater vulnerability for nonhomogeneous cardioplegic distribution. This was also the reason for choosing antegrade and not retrograde perfusion.

Is continuous warm cardioplegia better than cold cardioplegia?

Study **I** ("warm versus cold cardioplegia") was designed to specifically isolate the effect of cardioplegic temperature on the recovery of post-bypass LV function. In this study no systematic difference was found between cold and warm cardioplegia. These results indicate that in the continuously perfused and vented cardioplegic heart the temperature of the perfusate may be of minor importance for the recovery of left ventricular function. One might speculate that positive clinical effects observed with warm cardioplegia might be attributed to the fact that this cardioplegia usually have been given more or less continuously. Theoretical advantages with warm cardioplegia may be counteracted by an inability to provide aerobic metabolism in all parts of the myocardium due a non homogeneous distribution of cardioplegia to which the cold heart is more resistant. Beside this, the warm heart may also be more susceptible to insufficient cardioplegic flow and oedema formation [Aldea 1990, Misare 1993, Stahl 1994, Van Camp 1995, Mehlhorn 1995].

There is only one study except ours which have investigating the effect of cardioplegic temperature *per se* on myocardial functional recovery. Bufkin and colleagues [Bufkin 1994] studied continuous retrograde cardioplegia at 18°, 28° or 37°C after LAD occlusion (with antegrade induction). After 90 min of reperfusion PRSW was higher (better cardiac function) in the 18°C and 28° C groups than in the 37°C group. However, in this study both the regional ischaemia and the retrograde delivery of cardioplegia may have contributed to the worse outcome in the normothermic group. In our study the cold perfusate had the temperature of 6-7°C, which is the standard temperature for intermittent cold blood cardioplegia at our clinic. Several investigators have shown good results with tepid cardioplegia (28-29° C) [Bufkin 1994, Hayashida 1994], and this may perhaps combine some advantages of aerobic metabolism with safety aspects and myocardial protection using hypothermia with safety also for intermittent admin-istration. The clinical role of tepid cardioplegia has not yet been defined.

Continuous cold blood cardioplegia has never been widely used among cardiac surgeons because of the inconvenience of a continuous perfusion without any evident advantages over intermittent cold techniques. However, at our clinic already in 1981 Bomfim and colleagues [Bomfim 1981], compared continuous cold antegrade and single dose blood cardioplegia during aortic surgery. They showed a decrease in lactate release and a normal lactate extraction 30 minutes after declamping with continuous cardioplegia. CK-MB and myoglobin release was significantly lower in the continuous group and myocardial ATP and CP decreased less. Khuri and associates [Khuri 1988] reported no change in myocardial pH during aortic clamping with continuous cold blood cardioplegia, but a decline with multidose perfusion. Recently Louagie and co-workers [Louagie 1997] reported that continuous retrograde cold blood cardioplegia results in better left and right ventricular stroke work index during the first twenty hours following coronary artery bypass grafting than

historical controls using intermittent ante- or retrograde cold blood cardioplegia.

Cardioplegic flow

With cold cardioplegia the initial coronary vascular resistance tended to be higher than with warm cardioplegia. This is consistent with other investigators comparing cold and warm *intermittent* cardioplegia [Hayashida 1995]. However, in our study the coronary vascular resistance, during continuous infusion of warm cardioplegia, increased constantly during the 45 minutes of delivery, but remained unchanged with cold cardioplegia. This may be explained by an increasing endothelial dysfunction in conjunction with warm continuous cardioplegia. This may offer an additional explanation for the discrepancy between theoretical advantages and in some studies a worse outcome using warm cardioplegia. A increasing inhomogenity in the distribution of warm cardioplegia initiated by increasing vascular resistance may further increase the risk for ischaemic injury with warm cardioplegia.

Is it safe to give antegrade and retrograde cardioplegia simultaneously?

In study **II** ("antegrade vs. simultaneous cardioplegia") an ischaemic insult was induced in both groups with cardiac dysfunction and increased release of troponin T. There was no evidence of a systematic difference in post bypass global left ventricular function, diastolic function, or haemodynamics between the groups. Neither was there a difference in the release of troponin T. After warm simultaneous cardioplegic delivery a small, but significant increase in myocardial water content was detected. However, despite this the simultaneous delivery did not induce any additional myocardial damage compared to antegrade cardioplegia.

This study did not demonstrate any significant *advantage* of simultaneous cardioplegia, which was not expected since antegrade cardioplegia provides adequate myocardial protection in a model like ours without coronary stenoses. However, even in a coronary circulation without critical stenoses, antegrade and retrograde delivery of cardioplegia may perfuse complementary vascular beds, and thus optimise the cardioplegic distribution. [Aldea 1994, Gates 1995, Gates 1996]. A magnetic resonance study in isolated Langendorf perfused pig hearts indicates that with simultaneous antegrade-retrograde cardioplegia the elevated coronary sinus pressure is responsible for a redistribution of the antegrade flow from adjacent areas to jeopardised myocardium (area of occluded LAD). This redistribution is shown to be more important than the retrograde perfusion through the coronary sinus [Tian 1998].

Mean cardioplegic flow in the antegrade group was higher than in the antegrade-retrograde group. The obvious explanation for this difference is the chosen higher perfusion pressure with antegrade cardioplegia. To achieve a clinically relevant model the cardioplegia pressure in the antegrade group was set to 70-80 mmHg. In the simultaneous antegrade-retrograde group cardioplegia was delivered by one pump head and through a Y-connection. Thus with simultaneous cardioplegia the coronary sinus pressure (40-45 mmHg) was the perfusion pressure also in the aortic root. This set-up is simple, easy, and convenient to use in the operating room. In a study on explanted human hearts antegrade cardioplegia with a perfusion pressure of 80 mmHg was infused simultaneously with retrograde cardioplegia at a pressure of 40 mmHg. Still approximately 30% of capillary flow was found to be the contribution of retrograde cardioplegia, compared with 28%of total flow in our study.

With simultaneous cardioplegia there was a small, but significant increase in water content. The water content is in agreement with previous findings in isolated, perfused pig hearts (about 80%) [Weng 1992], and in dogs subjected to global ischemia and reperfusion where myocardial water content increased from 82 to 84% [Spotnitz 1994]. Myocardial oedema per se may reduce both systolic and diastolic cardiac function. [Spotnitz 1994, Mehlhorn 1992,

Weng 1992]. Even if simultaneous cardioplegia may be safe in the ”non-ischaemic” myocardium [Ihnken 1994], the anticipated increase in microvascular hydrostatic pressures might be deleterious to the myocardium already injured by acute ischemia. The used model with 30 minutes of unprotected ischemia before 45 minutes of warm, continuous, simultaneous antegrade-retrograde cardioplegia will provoke a strong oedemagenic insult. The tendency to induce oedema is probably considerably less when simultaneous cardioplegia is given intermittently or when the pre-cardioplegic insult is less.

Should warm intermittent cardioplegia be used?

In study **III** (“continuous versus intermittent warm cardioplegia”) the use of intermittent warm blood cardioplegia resulted in a reduced left ventricular function measured as PRSW compared to continuous cardioplegia. Diastolic function deteriorated without difference between the treatments. Despite the obvious risk with warm ischemia only a few investigators [Matsuura 1993, Ko 1993] have in addition to us demonstrated detrimental effects of intermittent warm blood cardioplegia with ischaemic intervals of 10 minutes or less. The study of Matsuura et al using retrograde cardioplegia, is the only study except ours, where intermittent warm cardioplegia was given after an ischaemic insult [Matsuura 1993]. It might be that protocols of intermittent blood cardioplegia used by most investigators provide sufficient protection of the non-compromised myocardium, but have limitations in the metabolically deprived heart.

A problem when comparing different studies is that ”intermittent warm blood cardioplegia“ is not a defined entity, but represents a wide range of different regimens in the hands of different investigators. Especially with warm intermittent cardioplegia different ischaemic times and lengths of “catch-up” reperfusion will be critical for the development of an ischaemic insult. Most investigators have used warm blood cardioplegia for 30 to 60 % of the cross-clamp time. Others for only about 12% but are still reporting good results [Landymore 1992, Calafiore 1995, Mezzetti 1995, Caputo 1998].

Experimental data on the efficacy of intermittent warm blood cardioplegia are quite divergent with two studies showing detrimental effects with interruptions of cardioplegia for 7 to 10 minutes intervals [Matsuura 1993, Ko 1993]. Conversely, Landymore et al [Landymore 1994] demonstrated that intermittent perfusion with warm cardioplegia for approximately 1.5 minutes every 15 minutes, is well tolerated. Other investigators also report good results, but with “catch-up” reperfusions for 5 to 15 minutes between ischaemic intervals [Tönz 1996, Torracca 1996, Tian 1995, Carias de Oliveira].

Most clinical investigators report superior or equal results using intermittent warm, but compared to intermittent cold blood cardioplegia, as long as the ischaemic interval does not exceed 10 to 15 minutes. [Pelletier 1994, Landymore 1996]. Calafiore et al [Calafiore 1995] studied 250 coronary bypass patients which were given warm antegrade cardioplegia with interruptions for up to 15 minutes. Despite cardioplegic delivery for only 12% of the cross-clamp time, excellent results were reported in comparison with cold cardioplegia. The Warm Heart Investigators [Lichtenstein 1995] reported in an analysis of 720 patients, that interruptions of cardioplegic delivery exceeding 13 minutes were associated with increased risk for adverse outcome. In our study there were no interruptions exceeding 10 minutes.

Mezzetti et al [Mezzetti 1995] randomised 30 patients undergoing mitral valve surgery to warm or cold intermittent cardioplegia. Despite warm cardioplegic delivery during only 9.5 % of the cross-clamp time, post-bypass stroke work index decreased in the cold, but not in the warm group. In the warm group there was also a faster recovery of lactate extraction and a decrease in the release of oxidative metabolites suggesting a protective effect from ischaemia-reperfusion injury. However, in the study of Ali et al [Ali 1994] a cross-clamp time of more than 90 minutes, warm cardioplegia delivered every 15 minutes was associated

with significantly more ECG changes and low cardiac output.

Intermittent cardioplegia was in our study delivered during 17 % of the cross-clamp time, which in this model was inadequate for optimal protection. Although rather large amounts of cardioplegia were delivered in the intermittent group, the total time for "catch-up" reperfusion was evidently too short in relation to the ischaemic insult. Delivery of cardioplegia for 17% of the cross-clamp time is less than in most other studies, but longer than in some studies showing good results of intermittent, warm blood cardioplegia [Calafiore 1995, Mezzetti 1995]

In this study we used a cardioplegic temperature of 34°C, which some surgeons may regard as tepid, whereas classically intermittent warm cardioplegia has a temperature of 37°C [Lichtenstein 1995, Calafiore 1995]. We prefer clinically to let the CPB temperature drift to about 34°C, and therefore decided to give cardioplegia at the same temperature. That intermittent cardioplegia was inferior to continuous administration at 34°C, suggests that intermittent cardioplegia at 37°C would have been even more harmful.

Obviously the cumulative time off cardioplegia must have an upper limit and attention must be paid to "catch-up" perfusion taking place to avoid further ischaemic damage. An adequate time must also be permitted for the "catch-up" perfusion since oxygen uptake in the warm heart will be limited by the time and not the dose of cardioplegia. In the normothermic cardioplegic heart without an oxygen debt the oxygen demand is approximately 1-2 ml O_2/100g per minute [Buckberg 1977, Bernard 1961]. However, in the ischaemic and cardioplegic heart the oxygen uptake may increase up to 6 ml O_2/100g per minute [Rosenkranz 1986]. In a normal 300g heart the oxygen uptake over 10 minutes may then increase to 180 ml O_2 (6 x 3 x 10) if constant uptake. Theoretically a blood cardioplegic infusion will contain approximately 6 ml O2/100 ml of cardioplegic solution (if Hb =8g/100 ml; SaO2= 100%; myocardial oxygen extraction = 60% [Baim 1982]; and oxygen content in haemoglobin = 1.34 ml O_2/g Hb, (8 x 1 x 0.6 x 1.34)). Hence a cardioplegic infusion of 3000ml over 10 minutes has to be given to completely repay the oxygen dept of 180 ml O_2. If the infusion is given retrogradely with perhaps only 50 % nutritive flow [Ardehali 1995, Gates 1993] the infusion rate has to be doubled (and with hypertrophied hearts even higher flows). This example illustrates that a regimen of myocardial protection providing excellent results in the normal, unstressed myocardium, may be insufficient as resuscitation after ischaemia. We believe it is important to acknowledge this fact and to define the borders where the *specific* regimen of warm intermittent cardioplegia could be used, before adopting it in the clinic.

Our model proved to be beyond the limit of what intermittent warm blood cardioplegia could accomplish after a severe ischaemic insult. A longer "catch-up" period had probably improved the outcome as the continuous cardioplegia actually resuscitated the myocardium with a 96% recovery of global systolic function (PRSW). However, this study defines an outer border of what is "too intermittent" for intermittent warm blood cardioplegia

Statistical considerations

Generally, when comparing different treatments (as modes of cardioplegia) the study groups (the two groups of pigs) are hopefully representative samples drawn from a general population (in this case the population of 40 kg pigs). We evaluate the treatment effect by applying the results to a statistical model in order to draw a conclusion about the population from which the study pigs are taken (=statistical interference). The normal level of significance, generally set to <0.05 means that it is a probability of less than 5% that i.e. two groups are similar when we claim that they are different (this is called alfa or type I-error). If $p> 0.05$ we are generally not allowed to claim that a difference exists. A p-value > 0.05 thus means that a difference could not be demonstrated which is not the same as claiming the non-existents of a difference. The difference between i.e. two groups might have been to

small to detect because the variability within the groups was too large in the relation to the amount of pigs studied. If stating that no difference exist there is the risk of committing an beta error (type II-error) which is the probability that i.e. two group are different when we claim that no difference exists. From this we can then calculate the probability that we have made a correct decision (the power).

Inherited in all large animal research, as performed in this thesis, is the problem with small study groups, especially when studying possible differences between two or several groups. It is often not possible to use more than a few animals in each group and studies with more than 10-15 animals in each treatment arm are rarely seen, and consequently the statistical power may be low.

In study **I** and **II** no statistical difference between groups were found, but accordingly clinically relevant differences may have remained undetected. In study **I** recovery of PRSW was 81% and 99% in the cold and warm group respectively. This difference was not statistically significant (Fig. 9). However, we do not know the clinical significance of this difference. Although 99% recovery in the warm group the larger variability of PRSW after warm cardioplegia, including the pigs with both the highest and lowest PRSW, may have clinical implications (Fig. 9).

In study **II**, if any finding remained undetected, it might indeed be that the simultaneous administration was the best one, as preload recruitable stroke work and preload recruitable stroke work area tended to change less in the simultaneous group. In an initial clinical study where 155 patients were given simultaneous cardioplegia no adverse effects were observed [Ihnken 1994]

Clinical implications

Based on study I, II and III what can be said about administration of blood cardioplegia? *First,* with continuos cardioplegia there is a potential for an almost 100 % recovery of global cardiac function (PRSW) even after a severe myocardial damage (warm global total ischemia) when resuscitation with blood cardioplegia is started within 30 minutes. *Second,* to use warm intermittent cardioplegic administration in the protocol used is detrimental in situations like this when myocardial oxygen demand is elevated. Even though this method is clinically used only by a small minority of cardiac surgeons in Europe this study defines a border where intermittent cardioplegia should not be used and where considerably longer catch-up periods is required (probably coming close to the continuous perfusion). *Third,* since there was no demonstrated difference in outcome with continuous cold and warm cardioplegia we prefer cold perfusion (when not used as induction or terminal reperfusion) due to the added safety. It is safer with respect to non-optimal homogeneity in the cardioplegic distribution, to inadequate cardioplegic flow or inadequate time for cardioplegic perfusion and it is safer to use intermittently. *Fourth,* even though we could not prove that simultaneous cardioplegia is better than antegrade perfusion there was no detectable difference in postcardioplegic cardiac function between the two modes of delivery. Due to the theoretical advantages simultaneous cardioplegia could be recommended.

So what is the practical outcome in the operating theatre? With regard to these studies I still use cold intermittent cardioplegia, but give it in larger doses, more often and over longer time intervals, but still not during the construction of coronary anastomoses. There is no danger in giving large amounts of blood cardioplegia as long as the potassium level is monitored. I do feel that the optimal cardioplegia is given as a continuous perfusion. However, when cardioplegia is cold it can safely be interrupted and the safety net is present. With warm cardioplegia the safety margins are significantly reduced. Consequently, the reason to use warm cardioplegia should be a strong scientific foundation that it provides superior protection. If retrograde cardioplegia is otherwise indicated I often use simultaneous antegrade/retrograde cardioplegia, however being aware of any distension of the left ventricle if there is no LV vent.

How much global volume information is gained from a cm thick mid-cardiac volume segment?

The invasiveness of the conductance catheter limits its use for volume measurements in humans. The old technique with non-invasive impedance cardiography [Kubicek 1974], building on the same principle as the conductance catheter, was only to measure stroke volume and is shown to be inexact [Lamberts 1984]. One solution might be to simplify the catheter, however, still requiring a position in the left or right ventricle. In this study (**IV**) we wanted to investigate if volume information obtained from only a single segment of the conductance catheter (instead of multiple segments along the LV long axis) were able to provide information about the global volume. If so, this may allow the construction of smaller conductance catheters not having to cover the whole LV long axis. Results form this study might also indicate how well area-changes measured by echocardiography correlate to changes in the total left ventricular volume.

Our results from study **IV** ("segmental versus global left ventricular volume") indicate a good agreement between the shape of the volume curve of a volume segment (length: 0.7 to 1.4 cm) located at mid-ventricular level, and the curve shape of the global LV volume. The absolute mean difference over a cardiac cycle, between normalised segmental (at mid-ventricular level) and global volume, was in the range 1 - 12% at baseline (after 60 min-utes of apical ischaemia, during preload reduc-tion, or during increasing afterload), except in one pig during preload reduction where the difference was 39%. Differences in curve shape were located to limited parts of the heart cycle or were just a consequence of a temporal de-lay. During apical ischaemia the developed dyskinesia resulted in a large disagreement between the global volume and the ischaemic two apical segments. However, this disagreement was not seen in the adjacent mid-ventricular segment. The contribution from this mid-ventricular segment to the global volume was highly constant within individual animals. Thus changes in this volume segment seem to be a good estimate and description of changes in global volume.

As opposed to comparisons where dimensions are used for volume calculations as with ventriculography or echocardiography, the conclusion from this study is not based on an assumed geometric model, but from the linear relationship between the intraventricular blood volume and the measured conductance which might decrease errors in the comparison. After calibrating the conductance volume for parallel conductance and for cardiac output measured by e.g. thermodilution, the absolute volume may be calculated with reasonable accuracy using a single mid-ventricular volume segment

However, there are some limitations with this study:

Global and segmental volumes were measured concomitantly using the same method. Global volume was derived from the individual volume segments, with 14 to 39% of the global volume influenced by the mid-ventricular segment, thus creating an inter-dependence in the comparison.

The relation between "true" and measured volume (the slope factor) may vary between different segments within and between subjects. Van der Velde and co-workers [Van der Velde 1992] compared conductance catheter derived segmental and global LV volumes with the corresponding cine-CT volumes. High correlation coefficients were found for the comparison, except for segment five (at the heart base). Between different animals there were significant variability in slope factors for segmental, but not for global volumes. However, since that study the conductance catheter has been modified. In our study we used the present standard with "dual excitation" increasing the homogeneity of the intracavitary electrical field leading to less variability in segmental slope factors and improving its linearity over a larger volume range [Steendijk 1992]. Except for calculations of general hemody-

namic data the slope factors in our study were approximated to one, since segmental slope factors were not possible to calculate.

Segmental parallel conductance was not calculated, because we did not want to introduce a confounding factor also unnecessary due to normalisation to global volume.

Because of different heart sizes the active length of the catheter had to be changed, and in some animals the length of the mid-ventricular segment had to be increased (from 0.7 to 1.4 cm), which influenced the relative part of total volume constituted by the this segment (Fig. 6). However, the total evaluation of our data does not imply this had any influence on our conclusion.

Cardiac function indices versus heart rate

In the cardioplegia studies (study I-III) the an-imals showed to have higher heart rates at the post-bypass measurement than at baseline, most probably due to the ischaemic insult. Since we were concerned about the validity of the PRSW and EDPVR relationships during tachycardia we wanted to study these indices, as well as other indices and pressure-volume loops with increasing heart rate.

Hemodynamics and methodological considerations

During the course of the experiments baseline EDV declined despite unchanged baseline EDP, ESP, and ESV. To correct for this decrease, the various parameters were analysed as differences between the value at paced heart rate and the value at the preceding baseline heart rate. Despite decreasing EDV from "baseline 1" to "baseline 4", there were linear increasing negative differences for EDV and SV with increasing heart rate. With unchanged baseline this negative increase may have been even more pronounced.

DFT declined inversely with increasing heart rate. The shorter DFT impaired LV filling and consequently EDV, SV, SW and EF became negatively related to heart rate as earlier shown [Braunwald 1992]. CO showed a parabolic course with maximum at 120 to140 bpm, as previously described [Berglund 1958]. However, in humans CO is otherwise shown to be unaffected by increasing pacing rate when studied under optimal filling condition in supine [Bevegård 1967]. Contrary to other animal studies [Glower 1985, Klautz 1997, Davies 1997], investigating mechanical LV function, the baseline heart rate in this study was of the same magnitude as in humans.

Global (PRSW) LV function

In our study PRSW was independent of heart rate up to 140 bpm. Except for a slight decrease at 160 bpm our results agree with prior studies [Glower 1985, Klautz 1997, Davies 1997]. A decrease of PRSW of approximately 10% was also seen in humans during pacing at 150 bpm [Liu 1993], but in that study preceded by a 20% increase of PRSW between 71 and 120 bpm. The baseline PRSW was almost similar in our pigs and in humans [Liu 1993], PRSW being 80 and 82 mmHg respectively. These values are considerably higher than in dogs (49 mmHg) [Glower 1985] and piglets (38 mmHg) [Davies 1997], but in these studies the animals were heavily beta-blocked to attenuate autonomic reflexes. In a piglet study [Klautz 1997] not using beta-blockers PRSW was 68 mmHg.

Systolic (ESPVR) LV function

In our study ESPVR increased approximately 37% between 72 and 100 bpm indicating a force-frequency relation. This is in opposition to one piglet study using beta-blockers [Davies 1997], showing no heart rate dependency of ESPVR. However, in another piglet study without beta-blockers [Klautz 1997] ESPVR increased 44%, but at 200 bpm and from a baseline of 140 bpm. In conscious dogs with autonomic blockade [Freeman 1987, Spratt 1987] and in a humans [Liu 1993] ESPVR increase 50 - 80% up to 160 bpm. In the study

by Maughan et al [Maughan 1985] using isolated, afterload-controlled hearts, ESPVR increased from 60 to 120 bpm, followed by a plateau and a small further increase at 200 bpm. In our study the increasing baseline ESPVR (table VI), was probably due to the increasing baseline heart rate being an expression of the force-frequency relation. The behaviour of peak dP/dt also indicated the presence of a force-frequency relation. Though peak dP/dt is strongly preload dependent [Little 1985], it remained unchanged up to 140 bpm despite constantly decreasing EDV.

Further comments about PRSW and ESPVR

The differences between the referred studies may be explained by species differences with different operating ranges of heart rates, and by differences between the new-born and the adult myocardium. Furthermore the different behaviour of PRSW (global function) and ESPVR (systolic function) may be explained by the global nature of PRSW, also being influenced by diastolic characteristics, as opposed to ESPVR being a pure systolic function index. This probably makes ESPVR more sensible to the myocardial force-frequency relation as shown in our study. With increasing heart rate diastolic function deteriorated (see discussion below) which probably influenced the PRSW relationship at 160 bpm. However, PRSW might be a more "robust" parameter than ESPVR because PRSW is independent of absolute volume calibrations (the unit for PRSW is mmHg and for ESPVR mmHg/ml). The tendency of ESPVR to be curvilinear [Burkhoff 1987, Kass 1989] is also a source of error (see Materials and Methods).

Diastolic function - chamber stiffness (EDPVR, beta) and relaxation (tau, PFR)

With increasing heart rate EDP declined, but increased again at 160 bpm despite a continuous declining EDV. Diastolic chamber stiffness measured as β increased at 140 bpm and EDPVR increased at 160 bpm and thereby probably influenced PRSW as discussed above. However, in a human study [Liu 1993] end-diastolic compliance was unchanged up to 150 bpm. It has been shown that diastolic stiffness increases during exercise, but then as a result of decreasing minimum diastolic pressure with end-diastolic pressure and volume being mainly unchanged [Nonogi 1988]. In our study the mechanism of increased stiffness was probably different with EDV and EDP being changed. Unlike conditions during exercise, pacing-induced tachycardia reduces EDV and SV due to a limiting venous return [Bevegård 1997]. As also shown by others [Freeman 1987, Karliner 1977] the time constant of LV pressure decay (ι) decreased with increasing pacing rate, indicating increasing rate of early relaxation. Although ι in our study decreased up to 140 bpm, this decrease was small, and relaxation velocity was probably too slow to compensate for the declining DFT. This may have caused both incomplete filling (small EDV) and incomplete relaxation at end-diastole causing a stiffer end-diastolic chamber with increased EDPVR, β, and EDP. There might also have been some pacing-induced ischaemia due to reduced coronary perfusion time. Increased EDP reduces subendocardial perfusion pressure and in combination with decreased diastolic perfusion time, a vicious circle may be initiated. Other factors possibly influencing diastolic relaxation and stiffness are increased vascular turgor [Salisbury 1960] and viscoelastic properties of the myocardium [Nikolic 1990].

Adrenergic augmentation of the force-frequency-relation

In addition to the inability to increase the venous return, lack of adrenergic stimulation is another important difference between our anaesthetised, paced pigs and exercise-induced tachycardia. It has been shown that adrenergic stimulation not only influences myocardial contractility and the systolic force-frequency relation, but also influences the diastolic function, augmenting relaxation velocity at any given heart rate [Kambayashi 1992, Ross 1995]. The importance of a diastolic force-frequency relation has recently been shown in

muscle strips from failing human myocardium, where insufficient up-regulation of sarcolemmal Na^{+}-Ca^{2+} exchanger increases diastolic stiffness [Hasenfuss 1999]. During tachycardia the diastolic force-frequency relation may probably be responsible for a down and leftward movement of the diastolic pressure-volume relation with unchanged left atrial pressure, increasing the atrio-ventricular pressure gradient [Cheng 1992, Miyazaki 1990, Aroesty 1985] and PFR [Ishida 1986]. Probably due to lack of adrenergic augmentation the reaction in our paced animals was completely different. Instead of the left and downward movement the diastolic portion of the pressure-volume loop moved upwards when heart rate increased. This probably affected PFR, which did not increase more than approximately 100 ml/s and levelled off at 140 bpm. A somewhat similar reaction, but with probably different mechanism, is seen in patients with angina pectoris where pacing-induced tachycardia leads to an upward shift of the diastolic portion of the pressure-volume loop with PFR declining at the highest heart rate [Aroesty 1985].

Pressure-volume loops vs. heart rate

No isovolumic phases observed

As seen in figure 19 the pressure-volume loops are leaning to the left. Neither the "isovolumic contraction", nor the "isovolumic relaxation" are isovolumic. This phenomenon has earlier been observed, not only with the conductance catheter, but also with implanted markers, sonomicrometry, and angiography [Baan 1988, Ruttley 1974, Hansen 1991, Ingels 1996]. Results from a study by Nolan et al [Nolan 1969] implies that the movements of the AV-plane may lead to falsely monitored changes of LV volume during the isovolumic phases of the heart cycle. When the geometry of the LV cavity is changing during systole from ellipsoid to spherical this is probably not fully registrated by the different methods used for measurements of LV volume.

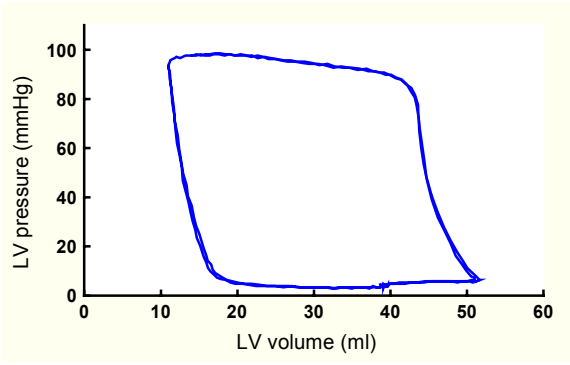

Fig 19. *A pressure-volume loop from a study pig (heart rate 76 beats per minute). As normally seen the loop is leaning to the left. The "isovolumic" contraction and relaxation is not registrated as isovolumic.*

Pressure-volume loops during paced tachycardia

At a heart rate of 140 to160 bpm the pressure-volume loop not only moved upwards, but there was also a blunting of the left lower corner indicating an increased pressure at the opening of the mitral valve (Figs. 18, 20-22). Normally, as seen in figure 19; LV pressure remains quite constant or increase when LV volume increases during filling. However, at these heart rates LV pressure decreased during the whole LV filling (Figs. 20, 22). This behaviour fulfils the criterion of a suction mechanism [Katz 1930] – a decreasing pressure (positive or negative) with concomitantly increasing volume. This "suction" is probably different from the suction seen with negative LV pressures, generated by a diastolic recoil of the ventricular wall [Robinson 1986], and existing when end-systolic LV volume decreases below its "equilibrium volume" as during exercise or inotropic stimulation with increased heart rate [Ingels 1996, Sabbah 1981, Udelson 1990] (see Fig 3. where the minimum diastolic pressure is –0.5 mmHg in the loop most to the left). With infusion of isoproterenol Udelson et al [Udelson 1990] induced a left and downward movement of the pressure-volume loop resulting in negative

(subatmospheric) pressures during early diastolic filling. When the heart later was paced to the same heart rate as induced by isoproterenol (130 bpm), no left-downward movement was seen. Not commented on in that study [Udelson 1990], but clearly seen in their figure 2, is the increased LV pressure at the start for LV filing (in comparison with the pressure during the isoproterenol infusion). As seen in our study, the LV volume then increases during the decline of LV pressure.

This type of "suction" may be important for LV filling when diastolic filling time and early relaxation are being to short or not facilitated by adrenergic stimulation as during paced tachycardia when the flow velocity is increased. LV filling without positive transmitral pressure gradients has earlier been demonstrated by Courtois et al [Courtois 1988], showing the existence of intraventricular pressure gradients with lower minimum LV pressure at the apex than at the base. The existence of physiological reversed pressure gradients during early diastolic filling has also been shown by Van der Werf [Van der Werf 1984]. Courtois also demonstrated a continuing forward transmitral flow despite a reversed atrial-ventricular pressure gradient, which were shown to exist during short parts of early diastolic filling. Consequently, flow may be directed against its pressure gradient (but during decelerating flow) due to surplus of kinetic energy, in this case directed against apex. A possible mechanism for LV filling is the one described by Nolan et al [Nolan 1969] where kinetic energy is generated by the AV-plane moving apically during systole [McDonald 1970], thus accelerating atrial fluid against apex already before opening of the mitral valves. At high frequencies this mechanism might gain increased importance as shown in an in vitro pump model by Lundbäck [Lundbäck 1986].

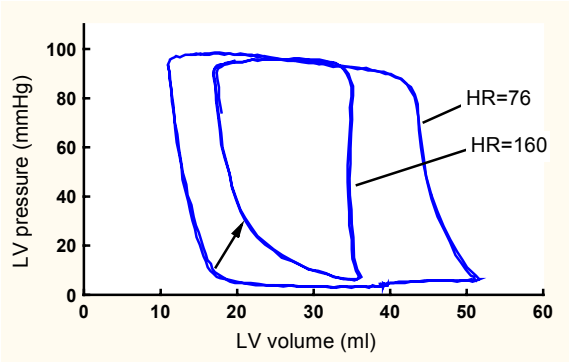

Fig 20. *Pressure-volume loops in one pig at a paced heart rate of 76 (the same loop as in figure 18 and 20) and at 160 beats per minute. At 160 bpm the stroke volume is seen to decrease. The end-diastolic volume has decreased from approximately 52 ml to 36 ml. In this animal end-diastolic pressure is almost unchanged and end-systolic volume has increased, but the maximum systolic LV pressure is mainly unchanged. This figure is to show that, with increased heart rate, the LV pressure will be increased at the point when the mitral valve opens and filling of the left ventricle begins (indicated by the arrow). Also note the continuous decline of LV pressure during the whole filling.*

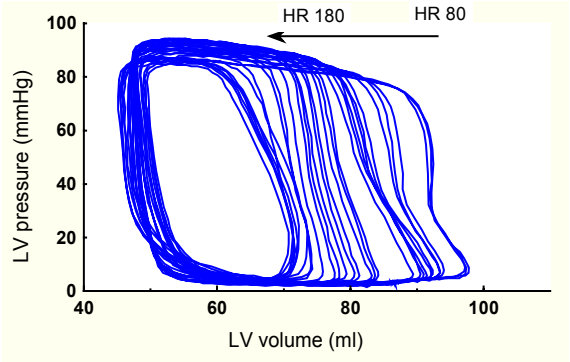

Fig 21. *Pressure-volume loops when pacing rate was continuously increased from 80 to 180 beats per minute in one animal. The figure shows the gradually change of shape of the loops with increasing pacing rate. The behaviour of the loops are principally the same as in figure 20, although this is not a steady state acquisition as the one in figure 20. Note that end-diastolic pressure first decreases and then increases at the highest heart rates (see Fig.14).*

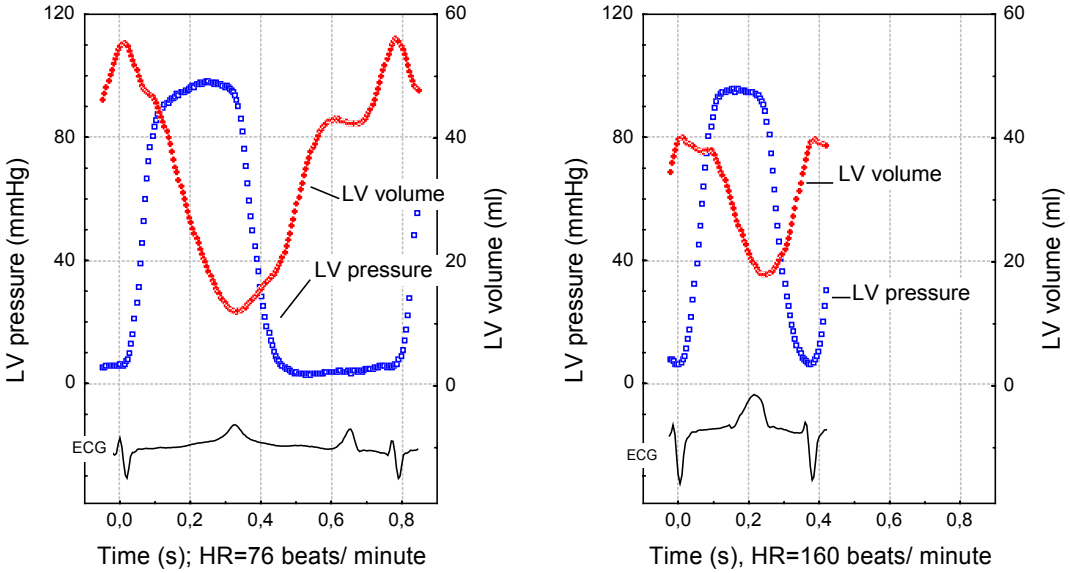

Fig 22. *Left ventricular (LV) pressure and volume curves during one heart beat with ECG, at baseline(left) and during paced heart rate to 160 beats per minute (right). These are the same beats as seen in figure 20. There is 4 ms between the points in the curves indicating the relative speed of the heart beat during different parts of the cardiac cycle.* **Left panel:** *heart rate 76 beats per minute. Note the atrial contribution to LV filling and the small plateau in the volume curve before the real ejection (not clearly explained).* **Right panel:** *heart rate 160 beats per minute. Note that diastole has shortened more than systole and that atrial systole has disappeared due to the shortened diastole. The pressure wave has almost taken the shape of a sinus wave. Compared with the pressure wave at 76 beats per minute, dP/dtmax looks almost unchanged despite the decrease in end-diastolic volume thus indicating a positive force-frequency effect. Also note that LV pressure declines during the whole LV filling. The rapid increase in filling still starts at approximately 20 ml, but now at a higher LV pressure compared with the pressure at 76 beats per minute.*

Conclusions and practical consequenses of study V

1. In the adult pig ESPVR was rate dependent up to 100 bpm, possibly a reflection of the force-frequency relation, and ESPVR should be used with caution when heart rate is different from baseline.
2. No pacing-induced systolic dysfunction was observed up to maximum pacing rate at 160 bpm.
3. With increasing heart rate diastolic chamber stiffness measured as EDPVR increased from 140 bpm, and the stiffness constant (ß) increased from 120 bpm, but significantly at 160 and 140 bpm respectively.
4. PRSW was rate independent up to 140 bpm.
5. The deterioration of PRSW at160 bpm was probably due to diastolic dysfunction (increased chamber stiffness).
6. At 140 to160 bpm the pressure-volume loop moved upwards with elevated pressure at the beginning of LV filling.
7. At 140 to 160 bpm the left ventricle was filled during a continuously declining LV pressure.
8. Despite the lack of negative LV pressures a suction mechanism might contribute to LV filling with increased heart rate during pacing.
9. Except for ESPVR the adult porcine model is stable with respect to increased heart rate and mechanical function indices up to approximately 140 bpm.

10. With regard to study I-III, the heart rate post-bypass was not high enough to influence the evaluation of cardiac function with PRSW. However, β (the stiffness coefficient), and to a certain extent also EDPVR was possibly affected by the tachycardia, especially in the intermittent group of study III. However, there was no statistical difference between groups with regard to β and EDPVR, although the highest values were seen in the intermittent group. ESPVR was not used in study I-III because its curvilinearity (discussed in "materials and methods").

The conductance catheter method and its limitations

The conductance catheter was used for on-line registration of volume throughout study **I-V**. Conductance catheter-derived stroke volume has been validated against stroke volume measured by electromagnetic flow probes and thermodilution in open–chest dogs [Baan 81, Baan84] and in humans by comparison against ventriculography and cine CT [Baan 84, Van der Velde92]. The volume measured with the conductance catheter has also been compared against the volume in intraventricular balloons in isolated ejecting dog hearts[Burkhoff 85]. These studies have shown a high linear correlation between the volume measurements. However, the relation between the conductance catheter-derived volume and the absolute volume is not 1:1. There is a volume offset due to parallel volume from structures surrounding the left ventricle which has to be subtracted. To obtain absolute volume the conductance catheter-derived volume has also to be corrected against another method, for instance stroke volume measured by thermodilution (gain calibration). Both the parallel volume and the conductance catheter-derived volume may at large volumes be nonlinearly related to the "real" volume [Boltwood 1989, Applegate 1989]. However, within the usual volume range the conductance volume is linearly related to volumes measured by other techniques. A problem with volume measurements are the lack of a golden standard. Techniques used for comparisons as sonomicrometry, ventriculography, CT or casted models of the LV cavity are thereby also not fully validated. In study **III** to **V** a conductance catheter with dual excitation field was used [Steendijk 92]. This was a technical improvement of the conductance catheter improving the homogeneity of the electrical field and theoretically improving the linearity of conductance catheter measured volume.

Conclusions

The following conclusions are based on studies (I-V) in the adult, anaesthetised, and open chest, porcine.

- When blood cardioplegia is given continuously the temperature of the cardioplegic solution is of minor importance for the acute post-cardioplegic cardiac function. Since warm continuous cardioplegia is not proved better than cold cardioplegia the latter is advocated due to the added safety of hypothermia.

- Warm blood cardioplegia results in an increasing coronary vascular resistance during the time of delivery which might have implications for its myocardial distribution.

- Intermittent warm blood cardioplegia is detrimental for post-cardioplegic cardiac function when used in the ischaemically compromised heart, and should be avoided in these situations.

- Blood cardioplegia given continuously has the potential to resuscitate the acute and severely damaged myocardium.

- Simultaneous antegrade and retrograde continuos blood cardioplegia does not, in comparison with continuous antegrade perfusion, impair post-cardioplegic cardiac function despite a small increase in myocardial water content. Simultaneous cardioplegia has the potential to be an optimal technique for cardioplegic delivery.

- A conductance catheter only registrating volume changes in a 0.7 to 1.5 cm broad volume segment of the left ventricle gives a good description of the acute changes in global left ventricular volume. This implies that the conductance catheter might be simplified with a potential for increased clinical use.

- ESPVR is rate dependent up to 100 bpm and should by used with caution when heart rate is changing from baseline. No pacing induced systolic dysfunction was observed up to 160 bpm.

- EDPVR is increasingly rate dependent from 140 bpm indicating a pacing induced increase in diastolic chamber stiffness.

- PRSW is rate independent up to 140 bpm and deteriorates at 160 bpm, probably due to increased chamber stiffness.

- During pacing at heart rates from 140 bpm and above the pressure–volume loop moves upwards with increasing LV pressure at mitral valve opening, followed by a continuous decline in LV pressure during filling of the left ventricle.

- A possible suction mechanism, without negative LV pressures, might contribute to LV filling when heart rate is increased during pacing at rest.

Acknowledgements

Jarle Vaage, Associate professor, my tutor, for introducing me into the field of experimental research and inviting me to this work. For his always positive way of thinking, his never ending enthusiasm and encouragement. For sharing me his scientific knowledge and learning me about cardioplegia and scientific writing.

Kjell Rådegran, Professor of Cardiothoracic Surgery, for his genuine interest in the project and generous support, for without, this project had never been performed.

Lars-Åke Brodin, Associate professor, clinical physiologist, for the idea to paper IV, and pushing the study forward. For his always bubbling enthusiasm in discussions about cardiovascular dynamics.

Håkan Kronander, M.Sc., Ph.D- student at the Division of Medical Engineering. My co-author in paper IV. For developing the necessary computer algorithm for comparison of volume curves and for doing the computer analysis and graphic presentations in this work.

Shigeto Takeshima, M.D. (co-author paper I and II), and Tsutomu Kawakami, M.D. (co-author paper III), guest researchers from Japan, and Emil Söderqvist, M.Sc., Ph.D-student at the Division of Medical Engineering (co-author paper IV) for their great help in the experimental laboratory.

Håkan Elmqvist, Professor of Medical Engineering, for supporting study IV.

Ulf Brodin, MSc, statistician at the Institution for Medical Statistics, Karolinska Institutet. For statistical help with paper III.

Chief perfusionist Ove Johansson and the perfusionist team of the Department of Thoracic Surgery: Bengt Ander (also for running the heart-lung machine in late evenings), Carl Duffke, Peter Kronlund, Lena Sahlén, Christer Tirén, and Mikael Utahs for their invaluable assistance.

Jan Baan, Professor of Clinical Physiology, Department of Cardiology, University Hospital Leiden, Holland, for his warm hospitality during my stay in Leiden giving me the possibility to learn the conductance catheter technique.

Alf Holmgren, Professor emeritus of Clinical Physiology, for interesting discussions and remarks about cardiac work and cardiovascular interactions.

Mrs Kerstin Librén and Mrs Britt Lingberth, Crafoord Laboratorium, for friendly assistance and help during the surgical experiments.

Arne Olsson, M.D., clinical physiologist, for help with illustrations.

My parents Suse and Bo, my sisters Charlotta and Elisabeth with families for their lifelong continuing support and encouragement.

These studies were supported by grants from the Swedish Heart Lung Foundation, from the Swedish Medical Research Council (grant no. 11235), Karolinska Institutet, and from the Karolinska Hospital.

References

1. Aldea GS, Austin Jr RE, Flynn AE, Coggins DL, Husseini W, Hoffman JIE. Heterogeneous delivery of cardioplegic solution in the absence of coronary artery disease. J Thorac Cardiovasc Surg 1990;99: 345-53.
2. Aldea GS, Hou D, Fonger JD, Shemin RJ. Inhomogeneous and complementary antegrade and retrograde delivery of cardioplegic solution in the absence of coronary artery obstruction. J Thorac Cardiovasc Surg 1994; 107:499-504.
3. Ali IM, Kinley CE. The safety of intermittent warm blood cardioplegia. Eur J CardioThorac Surg 1994; 8:554-6.
4. Allen P, Lillehei CW. Use of induced cardiac arrest in open-heart surgery. Minnesota Med 1957;40: 672.
5. Applegate RJ, Cheng CP, Little WC. Simultaneous conductance catheter and dimension assessment of left ventricular volume in the intact animal. Circulation 1990;81:638-648
6. Ardehali A, Laks H, Drinkwater DC Jr, Gates RN, Kaczer E. Ventricular effluent of retrograde cardioplegia in human hearts has traversed capillary beds. Ann Thorac Surg 1995;60:78-83.
7. Aroesty. JM, McKay RG, Heller GV, Royal HD, Als AV, Grossman W. Simultaneous assessment of left ventricular systolic and diastolic dysfunction during pacing-induced ischemia. Circulation 1985;71:889-900.
8. Astrup P, Engel K, Severinghaus JW, Munson E. The influence of temperature and pH on dissociation curve of oxyhemoglobin of human blood. Can J Clin Lab Invest 1965; 17:515-23.
9. Baan J,Jong TT, Kerkhof PL, Moene RJ, van Dijk AD, van der Velde ET, Koops J. Continuous stroke volume and cardiac out-put from intraventricular dimensions obtained with impedance catheter. Cardiovasc Res 1981;15:328-34.
10. Baan J, Van der Velde ET, De Bruin HG, et al. Continuous measurement of left ventricular volume in animals and humans by conductance catheter. Circulation 1984; 70: 812-23.
11. Baan J, Van der Velde ET, Steendijk P, Koops J. Calibration and application of the conductance catheter for ventricular volume measurement. Automedica 1989;11:357-65.
12. Baan J, Van der Velde ET. Sensitivity of left ventricular end-systolic pressure-volume relation to type of loading intervention in dogs. Circ Res 1988; 62: 1247-58.
13. Baim DS, Rothman MT, Harrison DC. Simultaneous measurement of coronary venous blood flow and oxygen saturation during transient alterations in myocardial oxygen supply and demand. Am J Cardiol 1982;49: 743-752.
14. Barner HB, Laks H, Codd JE, Standeven J, Pennington DG, Hahn JAW, Willman VL. Cold blood as the vehicle for potassium cardioplegia. Ann Thorac Surg 1979;28:509-16.
15. Berglund E, Borst HG, Duff F, Schreiner GL. Effect of heart rate on cardiac work, myocardial oxygen consumption and coronary blood flow in dog. Acta Physiol Scand 1958; 42: 185-198.
16. Bernard WF, Schwarz HF, Mallick, Netar NP. Elective hypothermic cardiac arrest in normothermic animals. Ann Surg 1961;153: 43-51.
17. Bevegård S, Jonsson B, Karlöf I, Lagergren H, Sowton. Effect of changes in ventricular rate on cardiac output and central pressures at rest and during exercise in patients with artificial pacemakers. Cardiovasc Res 1967; 1:21-33.
18. Beyersdorf F, Mitrev Z, Sarai K, Eckel L, Klepzig H, Maul FD, Ihnken K, Satter P. Changing patterns of patients undergoing emergency surgical revascularization for acute coronary occlusion. Importance of myocardial protection techniques. J Thorac Cardiovasc Surg 1993;106:137-48.
19. Beyersdorf F. Protection of evolving myocardial infarction and failed PTCA. Ann Thorac Surg 1995; 60:833-8

20. Bigelow WG, Lindsay WK, Greenwood WF. Hypothermia: its possible role in cardiac surgery. Ann Surg 1950;132:849-66.
21. Bing OHL, LaRaia PJ, Gaasch WH, Spadaro J, Franklin A, Weintraub RM. Independernt protection provided by red blood cells during cardioplegia. Circulation 1982;66(suppl I): I81-4.
22. Bjork VO, Fors B. Induced cardiac arrest. J Thorac Cardiovasc Surgery 1961;41:387-94.
23. Blix M. Die Lange und die Spannung des Muskels. Skandin Arch Physiol 1895;5:173.
24. Bodenhamer RM, DeBoer LWV, Geffin GA. Enhanced myocardial protection during ischemic arrest. Oxygenation of a crystalloid cardioplegic solution. J Thorac Cardiovasc Surg 1983; 85:769-80.
25. Boltewood CM, Appleyard RF, Glantz SA. Left ventricular volume measurements by conductance catheter in intact dogs. Circulation 1989; 80:1360-77.
26. Bomfim V, Kaijser L, Bendz R, Sylven C, Morillo F, Olin C. Myocardial protection during aortic valve replacement. Scand J Thorac Cardiovasc Surg 1981;15:141-7.
27. Bomfim V, Kaijser L, Bendz R, Sylvén C, Olin C. Myocardial protection during aortic valve replacement. Cardiac metabolism and enzyme reease following hypothermic cardioplegia. Scand J Thorac Cardiovasc Surg 1980;14:43-49.
28. Bonchek LI, Burlingame MW, Vazales BE, Lundy EF, Gassmann CJ. Applicability of noncardioplegic coronary bypass to high risk patients; selection of patients, technique, and clinical experience in 3000 patients. J Thorac Cardiovasc Surg 1992;103:230-7.
29. Bowditch HP. Über die Eigenthumlichkeiten der Reizbarkeit, welche die Muskelfasern des Herzens zeigen. Arb Physiol Anstalt zu Leipzig 1871;6:139-176
30. Braimbridge MW, Chayen J, Bitensky L, Hearse DJ, Jynge P, Cankovic-Darracott S. Cold cardioplegia or continuous coronary perfusion? J Thorac Cardiovasc Surg 1977; 74:900-906.
31. Braunwald E, Sonnenblick EH, Ross J Jr. Normal and abnormal circulatory function: Mechanisms of cardiac contraction and relaxation. In: Heart disease: a textbook of cardiovascular medicine. Ed. Braunwald E. 1992, 4th ed 351-392. Philadephia. WB Saunders company.
32. Bretschneider HJ, Hubner G, Knoll D, Lohr B, Nordbeck H, Spiekermann PG. Myocardial resis-tance and tolerance to ischemia: physiological and biochemical basis. J Cardiovasc Surg (Torino) 1975;16:241-260.
33. Bretschneider HJ. Überlenszeit und Weiderbelebungszeit des Herzens bei Normo- und Hypothermie. Verh Dtsch Ges Kreisl – Forsch 1964;30: 11-34.
34. Brewer DL, Bilbro RH, Bartel AG. Myocardial infarction as a complication of coronary bypass surgery. Circulation 1973; 47:58-64
35. Brown III WM, Jay JL, Gott JP, et al. Warm blood cardioplegia: Superior protection after acute myocardial ischemia. Ann Thorac Surg 1993;55:32-42.
36. Brown JM, Grosso MA, Terada LS, Beehler CJ, Toth KM, Whitman GJ, Harken AH, Repine JE. Erythrocytes decrease myocardial hydrogen per-oxide levels and reperfusion injury. Am J Physiol 1989;256):H584-8.
37. Buckberg GD, Beyersdorf F, Allen BS, Robertson JM. Integrated myocardial management: Background and initial application. J Card Surg 1995;10: 68-89.
38. Buckberg GD, Brazier JR, Nelson RL, Goldstein SM, McConnell DH, Cooper N. Studies of the effects of hypothermia on regional myocardial blood flow and metabolism during cardiopulmonary bypass I. J Thorac Cardiovasc Surg 1977;73:87-94
39. Buckberg GD. A proposed "solution" to the cardioplegic controversy. J Thorac Cardiovasc Surg. 1979; 77:803-15.
40. Buckberg GD. Myocardial temperature management during aortic clamping for cardiac surgery. J Thorac Cardiovasc Surg 1991;102: 895-903.
41. Buckberg GD. Oxygenated Cardioplegia: Blood is a many splendored thing. Ann Thorac Surg 1990;50: 175-7.
42. Bufkin BL, Mellitt RJ, Gott JP, Huang AH, PanChih, Guyton RA. Aerobic blood cardioplegia for revascularization of acute infarct: Effects of delivery temperature. Ann Thorac Surg 1994;58.953-60.
43. Burkhoff D, Sugiura S, Yue DT, Sagawa K. Contractility - dependent curvilinearity of end-systolic pressure-volume relations. Am J Physiol 1987; 252:H1218-1227.
44. Burkhoff D, Van Der Velde ET, Kass D, Baan J, Maughan WL, Sagawa K. Accuracy of volume measurement by conductance catheter in isolated, ejecting canine hearts. Circulation 1985;72:440-447.
45. Calafiore AM, Teodori G, Mezzetti A, Bosco G, Verna AM, Di Giammarco G, Lapenna D. Inter-mittent antegrade warm blood cardioplegia. Ann Thorac Surg 1995;59:398-402.
46. Caldarone CA, Krukenkamp IB, Misare BD, Levitsky S. Perfusion deficits with retrograde warm blood cardioplegia. Ann Thorac Surg 1994;57:403-6.
47. Caputo M, Bryan AJ, Calafiore AM, Suleiman M-S, Angelini GD. Intermittent antegrade hyperkalaemic warm blood cardioplegia

supplemented with magnesium prevents myocardial substrate derangement in patients undergoing coronary artery bypass surgery. Eur J Cardio-thorac Surg 1998;14: 596-601.
48. Carias de Oliveira N, Boeve TJ, Torchiana DF, Kantor HL, Titus JS, Schmidt CJ, Lu CZ, Kim J, Daggett WM, Geffin GA. Ischemic intervals during warm blood cardioplegia in the canine heart evaluated by phosphorus 31-magnetic resonance spectroscopy. J Thorac Cardiovasc Surg 1997;114: 1070-80.
49. Carrier M, Grégoire J, Khalil A, Thai P, La-tour J-G, Pelletier LC. Myocardial distri-bution of retrograde cardioplegic solution assessed by myocardial thallium 201 uptake. J Thorac Cardiovasc Surg 1994;108:1115-8.
50. Chambers DJ, Hearse DJ. Developments in cardioprotection: ”polarized” arrest as an al-ternative to ”depolarized” arrest. Ann Thorac Surg 1999;68: 1960-6.
51. Cheng CP, Igarashi Y, Little WC. Mechanism of augmented rate of left ventricular filling during exercise. Circ Res 1992;70:9-19.
52. Christakis GT, Koch JP, Deemar KA, et al. A randomized study of the systemic effects of warm heart surgery. Ann Thorac Surg 1992; 54:449-59.
53. Conti VR, Bertranou E, Blackstone EH, Kirklin JW, Digerness SB. Cold cardioplegia versus hypothermia as myocardial protection. Randomized clinical study. J Thorac Cardiovasc Surg 1978;76: 577-89.
54. Cooley DA, Reul GJ, Wukasch DC. Ischemic contracture of the heart: “Stone Heart”. Am J Cardiol 1972;29:575-577.
55. Courtois M, Kovács Jr SJ, Ludbrook PA. Transmitral pressure-flow velocity relation. Circulation 1988;78:661-671.
56. Covell JW, Ross J Jr., Taylor R, Sonnenblick EH, Braunwald E. Effects of increasing frequency of contraction on force velocity relation of left ventricle. Cardiovasc Res 1967;1:2-8.
57. Crafoord C, Norberg B, Senning Å. Clinical studies in extracorporeal circulation with the heart-lung machine. Acta Chir Scand 1957;112; 200.
58. Dabforth WH, Naegle S, Bing RJ. Effect of ischemia and reoxygenation on glycolytic reac-tions and ATP in heart muscle. Circ Res 1960; 8:965-71.
59. Daggett WM, Randolph JD, Jacobs M. The superiority of cold oxygenated dilute blood cardioplegia. Ann Thorac Surg 1987;43:397.
60. Davies MJ, Quarterman RL, Tsui SSL, Jacobs MT, Davis JM, Elliot MJ, Ungerleider RM, Gaynor JW. Comparison of three indexes of contractility in the neonatal left ventricle. CVE 1997;2:180-190.
61. Drinkwater DC Jr, Cushen CK, Laks H, Buckberg GD. The use of combined ante-grade-retrograde infusion of blood cardio-plegic solution in pediatric patients under-going heart operations. J Thorac Cardiovasc Surg 1992;104:1349-55.
62. Ebert PA, Greenfield LJ, Austen WG, Morrow AG. Experimental comparison of methods for protecting the heart during aortic occlusion. Ann Surg 1962;155:25-.
63. Eising GP, Hammond HK, Helmer GA, Gilpin E, Ross J Jr. Force-frequency relations during heart failure in the pig. Am J Physiol 1994;267;H2516-H2522.
64. Engelman RM, Rousou JH, Dobbs W, Pels MA, Longo F. The superiority of blood cardioplegia in myocardial preservation. Cir-culation. 1980;62(2 Pt 2):I62-6.
65. Fick A. Mechanische Arbeit und Warme-entwick-lung bei der Muskelthatigkeit. Leipzig 1882, F.A. Brackhaus.
66. Foglia RP, Steed DL, Follette DM, DeLand E, Buckberg GD. Iatrogenic myocardial edema with potassium cardioplegia. J Thorac Cardiovasc Surg 1979;78:217-22.
67. Follette D, Steed D, Foglia R, Fey K, Buckberg GD. Reduction of post ischemic myocardial damage by maintaining arrest during initial reperfusion. Surg Forum 1977;28:281-3.
68. Follette DM, Fey K, Buckberg GD, Helly JJ Jr, Steed DL, Foglia RP, Maloney JV Jr. Reducing postischemic damage by temporary modification of reperfusate calcium, potas-sium, pH, and osmolarity. J Thorac Cardio-vasc Surg 1981;82:221-38.
69. Follette DM, Mulder DG, Maloney JV, Buckberg GD. Advantages of blood cardio-plegia over contin-uous perfusion or inter-mittent ischemia. J Thorac Cardiovasc Surg 1978;76:604-17.
70. Frank O. Zur Dynamik des Herzmuskels. Zeitschrift für Biologie. 1895;32:370-447. [Translated by: Chapman CB, Wasserman E. On the dynamics of heart muscle. Am Heart J 1959;58:282-319,467-78.]
71. Freeman GL, Little WC, O´Rourke RA. Influence of heart rate on left ventricular per-formance in conscious dogs. Circ Res 1987; 61:455-64.
72. Fremes SE, Christakis GT, Weisel RD, Mickle DA, Madonik MM, Ivanov J, Harding R, Seawright SJ, Houle S, McLaughlin PR. et al. A clinical trial of blood and crystalloid cardioplegia. J Thorac Cardiovas Surg 1984; 88:726-41.
73. Gates RN, Laks H, Drinkwater DC Jr, Arde-hali A, Aharon AS, Zaragoza AM, Chang PA. Can improved microvascular perfusion be achieved using both antegrade and retrograde

cardioplegia? Ann Thorac Surg 1995;60: 1308-11.
74. Gates RN, Laks H, Drinkwater DC Jr, et al. Can improved microvascular perfusion be achieved using both antegrade and retrograde cardioplegia? Ann Thorac Surg 1995;60: 1308-11.
75. Gates RN, Laks H, Drinkwater DC, et al. Gross and microvascular distribution of retrograde cardioplegia in explanted human hearts. Ann Thorac Surg 1993;56:410-7.
76. Gates RN, Laks H, Drinkwater DC, et al. The microvascular distribution of cardioplegic solution in the piglet heart. - Retrograde versus antegrade delivery. J Thorac Cardiovasc Surg 1993;105:845-53.
77. Gates RN, Lee J, Laks H, Drinkwater DC Jr, Rhudis E, Aharon AS, Chung JY, Chang PA. Evidence of improved microvascular perfusion when using antegrade and retrograde cardioplegia. Ann Thorac Surg 1996;62:1388-91.
78. Gertz EW, Wisneski JA, Stanley WC, Neese RA. Myocardial substrate utlilization during exercise in humans. Dual carbon-labeled carbohydrate isotope experiments. J Clin Invest 1988;82:2017-25.
79. Gibbon JH. Application of a mechanical heart and lung apparatus to cardiac surgery. Minn Med 1954; 37:171-180.
80. Gleason WL, Braunwald E. Studies on the first derivative of the ventricular pulse in man. J Clin Invest 1962;41:80-.
81. Glower DD, Spratt JA, Snow ND, Kabas JS, Davis JW, Olsen CO, Tyson GS, Sabiston Jr DC, Rankin JS. Linearity of the Frank-Starling relationship in the intact heart: the concept of preload recruitable stroke work. Circulation 1985;71:994-1009.
82. Gorcsan J III, Denault A, Mandarino WA, Pinsky MR. Left ventricular pressure-volume relations with transesophageal echocardiographic automated border detection: Comparison with conductance-catheter technique. Am Heart J 1996;131:544-52.
83. Gorcsan J III, Gasior TA, Mandarino WA, Deneault LG, Hattler BG, Pinsky MR. Online estimation of changes in left ventricular stroke volume by trans-esophageal echocardiographic automated boarder detection in patients undergoing coronary bypass grafting. Am J Cardiol 1993; 72: 721-7.
84. Gorscan J III, Morita S, Mandarino WA et al. Two-dimensional echocardiographic automated border detection accurately reflects changes in left ventricular volume. J Am Soc Echocardiogr 1993; 6: 482-9.
85. Gott VL, Gonzales JL, Zuhdi MN, Varco RL, Lillehei CW. Retrograde perfusion of the coronary sinus for direct vision aortic surgery. Surg Gynecol Obstet 1957;94:319-328.
86. Gott VL, Gonzalez JL, Paneth M, Varco RL, Sellers RD, Lillehei CW. Cardiac retroperfusion with induced asystole for open surgery upon the aortic valve or coronary arteries. Proc Soc Exp Biol Med 1957;94:689-692.
87. Gutierrez G, Warley AR, Dantzker DR. Oxygen delivery and utilization in hypothermic dogs. J Appl Physiol 1986;60:751-7.
88. Guyton AC. Determination of cardiac output by equating venous return curves with cardiac respone curves. Physiol Rev 1955;35:123-129.
89. Haan C, Lazar HL, Bernhard S, Rivers S, Zallnick J, Shemin RJ. Superiority of retrograde cardioplegia after acute coronary occlusion. Ann Thorac Surg 1991;51:408-12.
90. Hansen DE, Daughters II GT, Alderman EL, Ingels NB, Stinson EB, Miller DC. Effect of volume loading, pressure loading, and inotropic stimulation on left ventricular torsion in humans. Circulation 1991;83:1315-1326.
91. Hasenfuss G. Schillinger W. Lehnart SE, Preuss M, Pieske B, Maier LS, Prestle J, Minami K, Just H. Relationship between Na^{+}-Ca^{2+}-exchanger protein levels and diastolic function of failing human myocardium. Circulation 1999;99:641-8.
92. Hayashida N, Ikonomidis JS, Weisel RD, Shirai T, Ivanov J, Carson SM, Mohabeer MK, Tumiati L, Mickle DAG. The optimal cardioplegic temperature. Ann Thorac Surg 1994;58:961-71.
93. Hayashida N, Ikonomidis JS, Weisel RD. Adequate distribution of warm cardioplegic solution. J Thorac Cardiovasc Surg 1995; 110:800-12.
94. Hayashida N, Weisel RD, Shirai T, Ikonomidis JS, Ivanov J, Carson SM, Mohabeer MK. Tumiati LC. Mickle DA. Tepid antegrade and retrograde cardioplegia. Ann Thorac Surg 1995;59:723-9.
95. Hearse DJ, Humphrey SM, Chain EB. Abrupt reoxygenation of the anoxic potassium-arrested perfused rat heart: A study of myocardial enzyme release. J Mol Cell Cardiol 1973;5:395-407.
96. Hearse DJ, Stewart DA, Braimbridge MV. Cellular protection during myocardial ischemia: the development and characterization of a procedure for the induction of reversible ischemic arrest. Circulation 1976;54: 193-202.
97. Hearse DJ. Reperfusion of the ischemic myocardium. J Mol Cell Cardiol 1977;9:605-16.
98. Heitmiller RF, DeBoer LWV, Geffin GA, Toal KW, Fallon JT,. Drop LJ, Teplick RS, O'Keefe DD, Daggett WM. Myocardial recovery after hypothermic arrest: A com-

parison of oxygenated crystalloid to blood cardioplegia. The role of calcium. Circulation 1985;72(suppl II):II241-53.
99. Henson DE, Najafi H, Callaghan R, Coogan P, Julian OC, Eisenstein R. Myocardial lesions follow-ing open heart surgery. Arch Pathol Lab Med 1969;88:423-30.
100. Heyndrickx GR,Millard RW, McRitchie RJ, Maroko PR, Vatner SF. Regional myocardial functional and electrophysiological alterations after brief coronary artery occlusion in conscious dogs. Journal of Clinical Investigation 1975;56:978-85.
101. Hilton CJ, Teubl W, Acker M, et al. Inadequate cardioplegic protection with obstructed coronary arteries. Ann Thorac Surg 1979;281:323-34.
102. Hoelscher B, Just OH, Merker HJ. Studies by electron microscope on various forms of cardiac arrest in dog and rabbit. Surgery 1961;49:492-9.
103. Hoelscher B. Studies by electron microscope on the effects of magnesium chloride-procaine amide or potassium citrate on the myocardium in induced arrest. J Cardiovasc Surg (Torino). 1967;8:163-166.
104. Hottenrott CE, Towers B, Kurkji HJ, Maloney JV Jr, Buckberg GD. The hazard of ventricular fibrillation in hypertrophied ventricles during cardiopulmonary bypass. J Thorac Cardiovasc Surg 1973; 66:742-53.
105. Hufnagel CA, Conrad PW, Schanno J, Pifarre R. Profound cardiac hypothermia. Ann Surg 1961; 153:790.
106. Hultgren HN, Miyagawa M, Buch W, Angell WW. Ischemic myocardial injury during cardiopulmonary bypass surgery. Am Heart J 1973;85: 167- 76.
107. Ibrahim MF, Venn GE, Young CP, Chambers DJ. A clinical comparative study between crystalloid and blood-based St Thomas' hospital cardioplegic solution. Eur J Cardio-Thorac Surg 1999;15: 75-83.
108. Ihnken K, Morita K, Buckberg GD, Aharon A, Laks H, Beyersdorf F, Salerno TA. Simultaneous arterial and coronary sinus cardioplegic perfusion: an experimental and clinical study. Thorac Cardiovasc Surg 1994;42:141-7.
109. Ihnken K, Morita K, Buckberg GD, Aharon A, Laks H, Panos AL, Drinkwater DC, Chugh R, Del Rizzo D, Salerno TA. The safety of simultaneous arterial and coronary sinus perfusion: experimental background and initial clinical results. J Card Surg 1994;9:15-25.
110. Illes RW, Silverman NA, Krukenkamp IB, Yusen RD, Chausow DD, Levitsky S. The efficacy of blood cardioplegia is not due to oxygen delivery. J Thorac Cardiovasc Surg 1989;98:1051-6.
111. Ingels Jr NB, Daughters GT, Nikolic SD, DeAnda A, Moon MR, Bolger AF, Komeda M, Derby GC, Yellin EL, Miller DC. Left ventricular diastolic suction with zero left atrial pressure in open-chest dogs. Am J Physiol 1996;270:H1217-H1224.
112. Ishida Y, Meisner JS, Tsujioka K, Gallo JI, Yoran C, Frater RW, Yellin EL. Left ventricular filling dynamics: influence of left ventricular relaxation and left atrial pressure. Circulation 1986;74:187-96.
113. Kaijser L, Jansson E, Schmidt W, Bomfim V. Myocardial energy depletion during profound hypothermic cardioplegia for cardiac operations. J Thorac Cardiovasc Surg 1985;80:896-900.
114. Kambayashi M, Miura T, Oh BH, Rockman HA, Murata K, Ross Jr J. Enhancement of the force-frequency effect on myocardial contractility by adrenergic stimulation in conscious dogs. Circulation 1992;86:572-80.
115. Karliner JS, LeWinter MM, Mahler F, Engler R, O´Rourke RA. Pharmacologic and hemodynamic influences on the rate of isovolumic left ventricular relaxation in normal conscious dogs. J Clin Invest 1977,60:511-521.
116. Kass DA, Beyar R, Lankford E, Heard M, Maughan WL, Sagawa K. Influence of contractile state on in situ end-systolic pressure-volume relation. Circulation 1989;79:167-178.
117. Kass DA, Maughan WL. From "Emax" to pressure-volume relations: a broader view. Circulation 1988; 77:1203-1212.
118. Katz LN. The role played by the ventricular relaxation process in filling of the ventricle. Am J Physiol 1930;95:542-553.
119. Khuri SF, Warner KG, Josa M, Butler M, Hayes A, Hanson R, Siouffi S, Barsamian EM. The superiority of continuous blood cardioplegia in the metabolic protection of the hypertrophied human heart. J Thorac Cardiovasc Surg 1988;95:442-54.
120. Kirklin JW, Barratt-Boyes BG. Cardiac Surgery 2nd edition 1993. In Myocardial management during cardiac surgery with cardiopulmonary bypass. Churchill Livingstolne Inc., New York.
121. Klautz RJM, Baan J, Teitel DF. The effect of sarcoplasmatic reticulum blockade on the force-frequency relationship and systolic contraction patterns in the newborn pig heart. Pflug Arch-Eur J Physiol 1997;435:130-6.
122. Ko W, Zelano J, Fahey AL, Berman F, Isom OW, Krieger KH. Ischemic tolerance of the arrested heart during warm cardioplegia. Eur J Cardio Thorac Surg 1993;7:295-9.

123. Koch-Weser J, Blinks JR. The influence of the interval between beats on myocardial contractility. Pharmacol Rev. 1963;15:601-652.
124. Krukenkamp IB, Levitsky S. Myocardial oxygen consumption after fibrillation in the non-hypertrophied porcine ventricle. Ann Thorac Surg 1991;52:949-54.
125. Krukenkamp IB, Silverman NA, Levitsky S. The effect of cardioplegic oxygenation on the correlation between the linearized Frank-Starling relationship and myocardial energetics in the ejecting postischemic heart. Circulation 1987;76(5 Pt 2): V122-8.
126. Kubicek WG, Kottke J, Ramos MU, Patterson RP, Witsoe DA, Labree JW, Remole W, Layman TE. Schoening H. Garamela JT. The Minnesota impedance cardiograph - theory and applications. Biomed Eng 1974;9:410-6.
127. Lakatta EG. Starling's law of the heart is explained by an intimate interaction of muscle length and myofilament calcium activation. J Am Coll Cardiol 1987;10;1157-1164.
128. Lam CR, Gahagan T, Sergeant C, Green E. Clinical experience with induced cardiac arrest during intracardiac surgical procedures. Ann Surg 1957;146: 439-49.
129. Lam CR, Gahagan T, Sergeant K, Green E. Experiences in the use of cardioplegia (induced cardiac arrest) in the repair of intraventricular septal defects. J Thorac Surg 1957;34:509-20
130. Lam CR, Geoghegan T, Lepore A. Induced cardiac arrest for intracardiac surgical procedures. J Thorac Surg 1955;30;620.
131. Lambert R. Impedance cardiography (Thesis). Groningen, The Netherlands, 1984.
132. Landymore RW, Marble AE, Eng P, MacAulay MA, Fris J. Myocardial oxygen consumption and lactate production during antegrade warm blood cardioplegia. Eur J Cardio-Thorac Surg 1992;6:372-6.
133. Landymore RW, Marble AE, Fris J. Effect of intermittent delivery of warm blood cardioplegia on myocardial recovery. Ann Thorac Surg 1994;57: 1267-72.
134. Landymore RW, Murphy JT, Hall R, Islam M. Randomized trial comparing intermittent antegrade warm blood cardioplegia with multidose cold blood cardioplegia for coronary artery by-pass. Eur J Cardio-Thorac Surg 1996;10:179-84.
135. Lewis FJ, Taufic M. Closure of atrial septal defects with the aid of hypothermia. Experimental accomplishments and the report of one successful case. Surgery 1953;33:52-59.
136. Lichtenstein SV, Abel JG, Panos A, Slutsky AS, Salerno TA. Warm heart surgery: experience with long cross-clamp times. Ann Thorac Surg 1991;52: 1009-13.
137. Lichtenstein SV, Abel JG, Salerno TA. Warm heart surgery and results of operation for recent myocardial infarction. Ann Thorac Surg 1991;52:455-8.
138. Lichtenstein SV, Abel JG, Slutsky AS. Warm retro-grade cardioplegia. Protection of the right ventricle in mitral valve operations. J Thorac Cardiovasc Surg 1992;104:374-80.
139. Lichtenstein SV, Abel JG. Warm heart surgery: Theory and current practice. In: Karp RB, Laks H, Wechsler AS, editors. Advances in cardiac surgery. vol 3. St Louis: Mosby, 1992; 135-155.
140. Lichtenstein SV, Ashe KA, Dalati HE, Cusimano RJ, Panos A, Slutsky AS. Warm heart surgery. J Thorac Cardiovasc Surg 1991;101: 269-74.
141. Lichtenstein SV, Naylor CD, Feindel CM, Sykora K, Abel JG, Slutsky AS, Mazer CD, Christakis GT, Goldman BS, Fremes SE, for The warm heart investigators. Intermittent warm blood cardioplegia. Circulation 1995;92 [Suppl]:II341-6.
142. Liechtenstein SV, El Dalati H, Panos A, Slutsky AS. Long cross clamp times with warm heart surgery [Letter]. Lancet 1989; 1:1443.
143. Lillehei CW, DeWall RA, Gott VL, Gott VL, Varco RL. The direct vision correction of calcific aortic stenosis by means of a pump oxygenator and retrograde coronary sinus perfusion. Dis Chest 1956;30: 123-132.
144. Little WC. The left ventricular dP/dt_{max}-end-diastolic volume relation in closed-chest dogs. Circ Res 1985;56;808-815.
145. Liu C-P, Ting C-T, Lawrence W, Maughan WL, Chang M-S, Kass DA. Diminished contractile response to increased heart rate in intact human left ventricular hypertrophy Circulation 1993;88: 1893-1906.
146. Longimuir IS. The effect of hypothermia on the affinity of tissues for oxygen. Life Sci 1962;7: 297-300.
147. Louagie YAG, Gonzalez E, Jacques J, Malhomme B, Broka S, Buche M, Eucher PM, Schoevaerdts J-C. Assessment of continuous cold blood cardio-plegia in coronary artery bypass grafting. Ann Thorac Surg 1997;63:689-96.
148. Louiagie YAG, Gonzalez E, Jacques J, et al. Assessment of continuous cold blood cardioplegia in coronary artery bypass grafting. Ann Thorac Surg 1997;63:689-96.
149. Lundbäck S. Cardiac pumping and function of the ventricular septum. Thesis Karolinska Institutet. Acta Physiol Scand 1986;550:1-101.
150. Maddaus M. Ali IS. Birnbaum PL. Panos AL. Salerno TA. Coronary artery surgery without cardiopulmonary bypass: usefulness of the

surgical blower-humidifier. J Card Surg 1992;7:348-50.
151. Magovern GJ Jr, Flaherty JT, Gott VL, Bulkley BH, Gardner TJ. Failure of blood cardioplegia to protect myocardium at lower temperatures. Circulation 1982;66:I60-7.
152. Mahler F, Yoran C, Ross J Jr. Inotropic effect of tachycardia and poststimulation potentiation in the conscious dog. Am J Physiol 1974;227:569-575.
153. Martin DR, Scott DF, Downer GL, Belzer FO. Primary cause of unsuccessful liver and heart presarvation: cold sensitivity of the ATP-ase system. Ann Surg 1972;175:111-7.
154. Martin TD, Craver JM, Gott JP, et al. Prospective, randomized trial of retrograde warm blood cardioplegia: myocardial benefit and neurologic threat. Ann Thorac Surg 1994; 57:298-304.
155. Matsuura H, Lazar HL, Yang XM, Rivers S, Treanor PR, Shemin RJ. Detrimental effects of interrupting warm blood cardioplegia during coronary revascularization. J Thorac Cardiovasc Surg 1993;106:357-61.
156. Maughan WL, Sunagawa K, Burkhoff D, Graves Jr WL, Hunter WC, Sagawa K. Effect of heart rate on canine end-systolic pressure-volume relationship. Circulation 1985;72:654-659.
157. McCord JN. Oxygen derived free radicals in postischemic tissue injury. N Engl J Med 1985;312: 159-63.
158. McDonald IG. The shape and movements of the human ventricle during systole. Am J Cardiol 1970; 26:221-230.
159. Mehlhorn U, Allen SJ, Adams DI, Davis KL, Gogola GR, deVivie ER, Laine GA. Normothermic continuous antegrade blood cardioplegia does not prevent myocardial edema and cardiac dysfunction. Circulation 1995;92: 1940-6.
160. Mehlhorn U, Sauer H, Kuhn-Regnier F, Sudkamp M, Dhein S, Eberhardt F, Grond S, Horst M, Hekmat K, Geissler HJ, Warters RD, Allen SJ, Rainer de Vivie E. Myocardial beta-blockade as an alternative to cardioplegic arrest during coronary artery surgery. Cardiovascular Surgery 1999; 7: 549-57.
161. Melros DG, Dreyer B, Bentall HH. Elective cardiac arrest. Lancet 1955;2:21-22.
162. Menasché P, Subayi J-B, Veyssié L, Le Dref O, Chevret S, Piwnica A. Efficacy of coronary sinus cardioplegia in patients with complete coronary artery occlusions. Ann Thorac Surg 1991;51:418-23.
163. Mezzetti A, Calafiore AM, Lapenna D, Deslauriers R, Tian G, Salerno TA, Verna AM, Bosco G, Pierdomenico SD, Caccurullo F. Intermittent antegrade warm cardioplegia reduces oxidative stress and improves metabolism of the ischemicreperfused human myocardium. J Thorac Cardiovasc Surg 1995; 109:787-95.
164. Mirsky I. Assessment of diastolic function: suggested methods and future considerations. Circulation 1984;69:836-41.
165. Misare BD, Krukenkamp IB, Lazer ZP, Levitsky S. Recovery of postischemic contractile function is depressed by antegrade warm continuos blood cardioplegia. J Thorac Cardiovasc Surg 1993;105: 37-44.
166. Mitchell JH, Wallace AG, Skinner NS Jr. Intrinsic effects of heart rate on left ventricular performance. Am J Physiol 1963;205:41-48.
167. Miyamoto ATM, Robinson L, Matloff JM, Norman JR. Perioperative infarction: Effects of cardiopulmonary bypass on collateral circulation in an acute canine model. Circulation 1978;58(suppl):I-147.
168. Miyazaki S, Guth BD, Miura T, Indolfi C, Schulz R, Ross J Jr. Changes of left ventricular diastolic function in exercising dogs without and with ischemia. Circulation 1990;81:1058-70.
169. Morales AR, Fine G, Taber RE. Cardiac surgery and myocardial necrosis. Arch Pathol Lab Med 1967;83:71-9.
170. Najafi H, Henson D, Dye WS, Javid H, Hunter JA, Najafi H. Henson D. Dye WS. Javid H. Hunter JA. Callaghan R. Eisenstein R. Julian OC. Left ventricular hemorrhagic necrosis. Ann Thorac Surg 1969:7:550-61.
171. Nelson R, Fey K, Follette DM. Critical importance of intermittent infusion of cardioplegic solution during aortic cross-clamping. Surg Forum 1976;26:241-3.
172. Nikolic SD, Tamura K, Tamura T, Dahm M, Frater RWM, Yellin EL. Diastolic viscous properties of the intact canine left ventricle. Circ Res 1990;67:352-359.
173. Nolan SP, Dixon Jr SH, Fisher RD, Morrow AG. The influence of atrial contraction and mitral valve mechanics on ventricular filling. Am Heart J 1969; 77:784-791.
174. Nonogi H, Hess OM, Ritter M, Krayenbuehl HP. Diastolic properties of the normal left ventricle during supine exercise. Br Heart J 1988;60:30-8.
175. Okamoto F, Allen BS, Buckberg GD, Bugyi H, Leaf J. Studies of controlled reperfusion after ischemia. XIV. Reperfusion conditions. Importance of ensuring gentle versus sudden reperfusion during relief of coronary occlusion. J Thorac Cardiovasc Surg 1986;92:613-20.
176. Pabst TS III, Flanigan DP, Buchbinder D. Reduced intimal injury to canine arteries with controlled application of vessel loops. J Surg Res 1989;47:235-41.

177. Partington MT, Acar C, Buckberg GD, Julia PL, Kofsky ER, Bugyi HI. Studies of retrograde cardioplegia. I. Capillary blood flow distribution to myo-cardium supplied by open and occluded arteries. J Thorac Cardiovasc Surg 1989;97:605-12.
178. Partington MT, Acar C, Buckberg GD, Julia PL. Studies of retrograde cardioplegia. II. Advantages of antegrade/retrograde cardioplegia to optimize distribution in jeopardized myocardium. J Thorac Cardiovasc Surg 1989; 97:613-22.
179. Patterson SW, Piper H, Starling EH. The regulation of the heart beat. J Physiol (London). 1914;48; 465-613.
180. Patterson, SW, Starling EH: On the mech anical factors which determine the output of the ventricles. J Physiol (London) 1914;48: 357-379
181. Pelletier LC, Carrier M, Leclerc Y, Cartier R, Wesolowska E, Solymoss BC. Intermittent ante- grade warm versus cold blood cardioplegia: A prospective, randomized study. Ann Thorac Surg 1994;58:41-9.
182. Penrod KE. Cardiac oxygenation during severe hypothermia in dog. Am J Physiol 1951;164:79-85.
183. Perez JE, Waggoner AD, Barzilai B, Melton HE Jr, Miller JG, Sobel BE. On-line assessment of ventricular function by automated boundary detection and ultrasound backscatter imaging. J Am Coll Cardiol 1992; 19: 313-20.
184. Preusse CJ, Gebhard MM, Schnabel A, Ulbricht LJ, Bretschneider HJ. Post-ischemic myocardial function after pre-ischemic application of pro-pranolol or verapamil. J Cardiovasc Surg 1984;25:158-64.
185. Rankin JS, McHale PA, Arentzen CE, Ling D, Greenfield JC Jr, Anderson RW. The three-dimensional dynamic geometry of the left ventricle in the conscious dog. Circulation Research 1976; 39: 304-13.
186. Reeves RB, Park JS, Lapennas GN, Olszowka AJ. Oxygen affinity and Bohr coefficients of dog blood. J Appl Physiol 1982;53:87-95.
187. Reeves RB. An imidazole alphastat hypothesis for vertebrate acid-base regulation. Tissue carbondioxide content and body temperature in bull frogs. Respir Physiol 1972;14:219-27.
188. Roberts WC, Bulkley BH, Morrow AG. Pathologic anatomy of cardiac valve replacement: a study of 224 necropsy patients. Prog Cardiovasc Dis 1973; 15:539-87.
189. Robertson JM, Buckberg GD, Vinten-Johansen J, Leaf JD. Comparison of distribution beyond coronary stenoses of blood and asanguineous cardioplegic solutions. J Thorac Cardiovasc Surg 1983; 86:80-6.
190. Robinson TF, Factor SM, Sonnenblick EH. The heart as a suction pump. Sc Am. 1986;254:84-91.
191. Rosenkranz ER, Buckberg GD, Laks H, Mulder DG. Warm induction of cardioplegia with glutamate-enriched blood in coronary patients with cardiogenic chock who are dependent on inotropic drugs and intra-aortic balloon support. J Thoracic Cardiovascu Surg 1983;86:507-18.
192. Rosenkranz ER, Okamoto F, Buckberg GD, Robertson JM, Vinten-Johansen J, Bugyi HI. Safety of prolonged aortic clamping with blood cardioplegia. III. Aspartate enrichment of glutamate blood cardioplegia in energy-depleted hearts after ischemic and reperfusion injury. J Thorac Cardiovasc Surg 1986;91:428-35.
193. Rosenkranz ER, Vinten-Johansen J, Buckberg GD, Okamoto F, Edwards H, Bugyi H. Benefits of normothermic induction of blood cardioplegia in energy-depleted hearts with maintenance of arrest by multidose cold blood cardioplegic infusions. J Thorac Cardiovasc Surg. 1982;84:667-677.
194. Ross J Jr, Covell JW, Sonnenblick EH, Braunwald E. Contractilie state of the heart charaterized by force-velocity relations in variably afterloaded and isovolumic beats. Circ Res 1966;17:163-169.
195. Ross J Jr, Miura T, Kambayashi M, Eising GP, Ryu KH. Adrenergic control of the force-frequency relation. Circulation 1995;92:2327-32.
196. Ruttley MS, Adams DF, Cohn PF, Abrams HL. Shape and volume changes during "isovolumic relaxation" in normal and asynergic ventricles. Circulation 1974;50:306-16.
197. Sabbah HN, Stein PD. Negative diastolic pressure in the intact canine right ventricle. Evidence of diastolic suction. Circ Res 1981;49:108-13.
198. Sagawa K, Maughan L, Suga H, Sunagawa K. Overview of the pressure-volume approach. Chapter 1. In: Cardiac contraction and the pressure-volume relationship. Oxford University Press, Inc. New York. 1988.
199. Sagawa K, Suga H, Shoukas AA, Bakalar K. End-systolic pressure-volume ratio: a new index of contractility. Am J Cardiol 1977;40:748-753.
200. Sagawa K. The ventricular pressure-volume diagram revisited. Circ Res 1978;43:677-87.
201. Sakai T, Kuihaara S. Effect of rapid cooling on the mechanical and electrical response in ventricular muscle of the guinea pig. J Physiol (Lond) 1985;361:361-78.
202. Salerno TA, Houck JP, Barrozo CA, Panos A, Christakis GT, Abel JG, Lichtenstein SV. Retrograde continuous warm blood cardioplegia:

a new concept in myocardial protection. Ann Thorac Surg 1991;51:245-.
203. Salisbury PF, Cross CE, Rieben PA. Influence of coronary artery pressure upon myocardial elasticity. Circ Res 1960;8:794-800.
204. Sapsford RN, Blackstone EH, Kirklin JW, Karp RB, Kouchoukos NT, Pacifico AD, Roe CR, Bradley EL. Coronary perfusion versus cold ischemic arrest during aortic valve surgery: A randomized study. Circulation 1974; 49:1190-9.
205. Sarnoff SJ, Berglunf E. Starling´s law of the heart studied by means of simultaneous right and left ventricular function curves in the dog. Circulation 1954;9:706.
206. Schlensak C, Doenst T, Beyersdorf F. Clinical experience with blood cardioplegia. Thorac Cardiovasc Surg 1998;46:282-287.
207. Schlensak C, Doenst T, Kobba J, Beyersdorf F. Protection of acutely ischemic myocardium by controlled reperfusion. Ann Thorac Surg 1999;68: 1967-70.
208. Schubert T, Vetter H, Owen P, Reichart B, Opie LH. Adenosine cardioplegia. Adenosine versus potassium cardioplegia: Effects on cardiac arrest and postischemic recovery in the isolated rat heart. J Thorac Cardiovasc Surg 1989;98:1057-65.
209. Seed WA. Interval-force processes in the intact animal and human heart. In: Noble MIM, Seed WA, eds. The interval-fore relationship of the heart: Bowditch revisited. Cambridge: University press, 1992:317-54.
210. Senning Ä. Ventricular fibrillation during extracorporeal circulation. Used as a method to prevent air-embolism and to facilitate intracardiac opera-tions. Acta Chir Scand 1952; 171(suppl):1.
211. Shumway NE, Lower RE, Stofer RC. Selective hypothermia of the heart in anoxic cardiac arrest. Surg Gynecol Obstet 1959;109: 750-754.
212. Sodums MT, Badke FR, Starling MR, Little WC, O´Rourke RA. Evaluation of left ventricular contractile performance utilizing end-sytolic pressure-volume relationships in conscious dogs. Circ Res 1990;54:731-739.
213. Solorzano J, Taitelbaum G, Chiu C-JR. Retrograde coronary sinus perfusion for myocardial protection during cardiopulmonary bypass. Ann Thorac Surg 1978;25:201-8.
214. Sonnenblick EH, Braunwald E, Williams JF Jr, Glick G. Effects of exercise on myocardial force-velocity relations in intact unanesthetized man: relative role of changes in heart rate, sympathetic activity, and ventricular dimensions. J Clin Invest 1965;44;2051-2062.
215. Sonnenblick EH. Force-velocity relations in mammalian heart muscle. Am J Physiol 1962;202: 931-939.
216. Spotnitz HM, Hsu DT. Myocardial edema: Importance in the study of left ventricular function. In: Karp RB, Laks H, Wechsler AS, eds. Advances in cardiac surgery. vol 5. St. Louis: Mosby, 1994: 1-25.
217. Spratt JA, Tyson GS, Glower DD, Davis JW, Muhlbaier LH, Olsen CO, Rankin JS. End-systolic pressure-volume relationship in conscious dogs. Circulation 1987;75:1295-309.
218. Stahl RF, Soller BR, Hsi C, BelleIsle J, Vander Salm TJ. Decreasing myocardial pH reflects ischemia during continuous warm retrograde car-dioplegic arrest. Ann Thorac Surg 1994;58:1645-50.
219. Starling EH. The Linacre Lecture on the Law of the Heart (Cambridge, 1915). London, Longmans, Green and Company, 1918.
220. Steendijk P, Van der Velde ET, Baan J. Single and dual excitation of the conductance-volume catheter analysed in a spheroidal mathematical model of the canine left ventricle. Eur Heart J 1992; 13: 28-34.
221. Stein DG, Drinkwater DC Jr, Laks H, Permut LC, Sangwan S, Chait HI, Child JS, Bhuta S. Cardiac preservation in patients undergoing transplantation. A clinical trial comparing University of Wisconsin solution and Stanford solution. J Thorac Cardiovasc Surg 1991;102:657-65.
222. Su JB, Crozatier B. Preload-induced curvilinearity of left ventricular end-systolic pressure-volume relations. Circulaion 1989;79:431-440.
223. Suga H, Kitabatake A, Sagawa K. End-systolic pressure determines stroke volume from fixed end-diastolic volume in the isolated canine left ventricle nder a constant contractile state. Cir Res 1979;44:238-249.
224. Suga H, Sagawa K, Shoukas AA. Load indepen-dence of the instantaneous pressure-volume ratio of the canine left ventricle and effects of epinephrine and heart rate on the ratio. Circ Res 1973;32:314-22.
225. Suga H, Sagawa K. Instantaneous pressure-volume relationships and their ratio in excised, supported canine left ventricle. Circ Res 1974;35: 117-126.
226. Suga H, Sagawa K. Two-compartment model of regionally ischemic ventricle (appendix 3-1); chapter 3, Physiological determinants of the ventricular pressure-volume relationship: s133-136 and 165-167. In: Sagawa S, Maughan L, Suga H, Sunagawa K. Cardiac contraction and the pressure-volume relationship. Oxford University Press, Inc., New York 1988.

227. Suga H. Left ventricular time-varying pressure/ volume ratio in systole as an index of myocardial inotropism. Jpn Heart J 1971;12:153-160.
228. Sunagawa K, Maughan WL, Sagawa K. Effect of regional ischemia on the left ventricular end-systolic pressure-volume relationship of isolated canine hearts. Circ Res 1983;52:170-178.
229. Swan HJ, Forrester JS, Diamond G, Chatterjee K, Parmley WW. Hemodynamic spectrum of myocar-dial infarction and cardiogenic shock. A conceptual model. Circulation 1972;45:1097-110.
230. Takamoto S, Levine FH, LaRaia PJ, Adzick NS, Fallon JT, Austen WG, Buckley MJ. Comparison of single-dose and multiple-dose crystalloid and blood potassium cardioplegia during prolonged hypothermic aortic occlusion. J Thorac Cardiovasc Surg 1980;79:19-28.
231. Teoh KH, Christakis GT, Weisel RD, Frames SE, Mickle DAG, Romaschin AD, et al. Accelerated myocardial metabolic recovery with terminal warm blood cardioplegia. J Thorac Cardiovasc Surg 1986;91:888-895.
232. Teoh KH, Panos AL, Harmantas AA, Lichtenstein SV, Salerno TA. Optimal visualization of coronary artery anastomoses by gas jet. Ann Thorac Surg 1991;52:564.
233. The warm heart investigators. Randomised trial of normothermic versus hypothermic coronary bypass surgery. Lancet 1994;343: 559-63.
234. Tian G, Shen J, Sun J, Xiang B, Oriaku GI, Zhezong L, Scarth G, Somorjai R, Saunders JK, Salerno TA, Deslauriers R. Does simultaneous antegrade/retrograde cardioplegia improve myocardial perfusion in the areas at risk? A magnetic resonance perfusion imaging study in isolated pig hearts. J Thorac Cardiovasr Surg 1998;115:913-24.
235. Tian G, Xiang B, Butler KW, Calafiore AM, Mezzetti A, Salerno TA, Deslauriers R. A 31P-nuclear magnetic resonance study of intermittent warm blood cardioplegia. J Thorac Cardiovasc Surg 1995;109:1155-63.
236. Torracca L, Pasini E, Curello S, Ceconi C, Coletti G, Alfieri O, Solfrini R, Ferrari R. Continuous versus intermittent warm blood cardioplegia: Func-tional and energetics changes. Ann Thorac Surg 1996;62:1172-9.
237. Tyers GFO, Todd GJ, Niebauer IM. The mechanism of myocardial damage following potassium citrate (Melrose) cardioplegia. Surgery 1975;78: 45-53.
238. Tönz M, Krogmann ON, Hess OM, Leskosek B, Mihaljevic T, von Segesser LK, Turina MI. Effect of intermittent warm blood cardioplegia on functional recovery after prolonged cardiac arrest. Ann Thorac Surg 1996; 62:1146-51.
239. Udelson JE, Bacharach SL, Cannon III RO, Bonow RO. Minimum left ventricular pressure during ß-adrenergic stimulation in human subjects. Evidence for elastic recoil and diastolic "suction" in the normal heart. Circulation 1990;82:1174-1182.
240. Waldhausen JA, Braunwald NS, Bloodwell RD, Cornell WP, Morrow AG. Left ventricular function following elective cardiac arrest. J Thorac Cardiovasc Surg 1960;39:799-807.
241. Van Camp JR, Brunsting III LA, Childs KF, Bolling SF. Functional recovery after ischemia: Warm versus cold cardioplegia. Ann Thorac Surg 1995; 59:795-803.
242. Van der Velde ET, Burkhoff D, Steendijk P, Karsdon J, Sagawa K, Baan J. Nonlinearity and load sensitivity of end-systolic pressure-volume relation of canine left ventricle in vivo. Circulation 1991;83:315-27.
243. Van der Velde ET, Van Dijk AD, Steendijk P, et al. Left ventricular segmental volume by conductance catheter and cine-CT. Eur Heart J 1992; 13: 15-21.
244. Van der Werf F, Minten J, Carmeliet P, De Geest H, Kesteloot H. The genesis of the third and fourth heart sounds: A pressure-flow study in dogs. J Clin Invest 1984;73:1400-7.
245. Vandenberg BF, Rath LS, Stuhlmuller P, Melton HE Jr, Skorton DJ. Estimation of left ventricular cavity area with an on-line, semiautomated echocardiographic edge detection system. Circulation 1992; 86: 159-66.
246. Weng Z-C, Nicolosi AC, Detwiler PW, Hsu DT, Schierman SW, Goldstein AH, Spotnitz HM. Effects of crystalloid, blood, and University of Wisconsin perfusates on weight, water content, and left ventricular compliance in an edema-prone, isolated porcine heart model. J Thorac Cardiovasc Surg 1992;103: 504-13.
247. Vine DL, Dodge HT, Frimer M, Stewart DK, Caldwell J. Quantitative measurement of left ventricular volumes in man from radiopaque epicardial markers. Circulation 1976; 54: 391-8.
248. Vinten-Johansen J, Julian JS, Yokoyama H, Johnston WE, Smith TD, McGee DS, Cordell AR. Efficacy of myocardial protection with hypothermic blood cardioplegia depends on oxygen. Ann Thorac Surg 1991;52:939-48.
249. Vinten-Johansen J, Nakanishi K, Zhao ZQ, McGee DS, Tan P. Acadesine improves surgical myocardial protection with blood cardioplegia in ischemically injured canine hearts. Circulation 1993;88:350-8.
250. Woodworth RS. Maximal contraction, "staircase" contraction, refractory period, and com-

pensatory pause of the heart. Am J Physiol. 1902;8:213-49.

251. Wyatt HL, Meerbaum S, Heng MK, Gueret P, Corday E. Cross-sectional echocardiography. III. Analysis of mathematic models for quantifying volume of symmetric and asymmetric left ventricles. Am Heart J 1980; 100: 821-8.
252. Yau TM, Ikonomidis JS, Weisel RS, et al. Ventricular function after normothermic versus hypothermic cardioplegia. J Thorac Cardiovasc Surg 1993;105:833-44.
253. Yau TM, Weisel RD, Mickle DAG, Komeda M, Ivanov J, Carson S, Mohabeer MK, Tumiati LC. Alternative techniques of cardioplegia. Circulation 1992;86[suppl II]:377-84.
254. Zughaib ME, McCay PB, Jeroudi MO, Hartley C, Sun J-Z, Sekili S, Li X-Y, Bolli R. Moderate ischemic injury and myocardial sunning. In: Ischemia-reperfusion in cardiac surgery. Ed. Piper HM, Preusse CJ. 1993;142: 3-40. Kluwer Academic Publishers,Dordrecht, The Netherlands