THE COMPOSITION, MECHANISMS OF ACTION AND INFUSION PARAMETERS OF CARDIOPLEGIC SOLUTIONS AS DETERMINANTS OF RECOVERY IN RABBIT ISOLATED HEARTS (LANGENDORFF)

BY

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ABSTRACT

This project was designed to develop new solutions to provide periods of cardioplegia longer and safer than those currently used. It was conducted on 188 rabbit, 8 rat and 5 guinea-pig hearts, mainly as isolated perfused preparations (Langendorff), with some recordings of intracellular potentials on rabbit isolated atria, to elucidate the actions of potassium, calcium, sodium nitroprusside and lignocaine as components of cardioplegic solutions, upon force and frequencey of ventricular beat, total coronary flow, rate of contraction, volume of coronary flow and the suppression of electrical activity. Similarities between the mechanisms responsible for escape beats and a newly observed ability of coronary occlusion to correct ventricular fibrillation have also emerged and been examined.

These basic experiments have led to the formulation of five new extracellular type cardioplegic solutions. These and the widely used St. Thomas's Solution Number One have been compared using 28 parameters during the induction and maintenance of two periods of cardioplegia followed by recovery periods of 30 minutes and 5 hours respectively. The solutions have been found to differ in their abilities to induce and maintain arrest and to influence recovery immediately after cardioplegia. No differences were found between the final extents of

recovery achieved after arrest with the various solutions. It was concluded that modification of extracellular type solutions was unlikely to result in a major increase in the safe duration of cardioplegia, as required for the transportation of donor to distent surgical centres. Controlled experiments and a review of the literature were therefore employed to identify factors responsible for poor viability after periods of arrest relevant to the transportation of donor hearts and an alternative type solution has been formulated to conserve intracellular ion and water balance. This is infused continuously, to meet minimal metabolic needs, at approximately 0.5-1% of normal perfusion rates and in a specially designed apparatus at $4^{o}C$. Rabbit hearts so treated for 6 hours after 30 minutes without perfusion ($37^{o}C$), more than sufficient to induce arrest, recovered 75% of their amplitude of contraction. When the same cardioplegic infusion was used to induce and maintain arrest for a full 24 hours, amplitude of contraction subsequently recovered to 61%. In both series all hearts recovered sinus rhythm and almost normal coronary flow.

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ABBREVIATIONS

AMP	Adenosine Monophosphate
ANOVA	Analysis of Variance
APD	Action Potential Duration
AVN	Atrio-Ventricular Node
Ca^{2+}	Calcium ion
$CaCl_2$	Calcium Chloride
^oC	Degrees Centigrade
cm	Centimetre
ECG	Electro-cardiogram
ERP	Effective Refractory Period
GMP	Guanosine Monophosphate
h	Hour(s)
H^+	Hydrogen ion
Hz	Hertz (cycles per second)
ISME(s)	Ion Sensitive Microelectrode(s)
K^+	Potassium ion
KCl	Potassium chloride
M	Molar (1 mole of solute/L solution)
mM	milli Molar
Mg^{2+}	Magnesium ion
$MgCl_2$	Magnesium chloride
min	minute(s)
mol	Mole (molecular weight in grams)
Na^+	Sodium ion
NaCl	Sodium chloride
RIH	Rabbit Isolated (Perfused) Heart
s	Second(s)
SAN	Sino-Atrial Node
s.e.m.	Standard error of the mean
SNP	Sodium Nitroprusside
V	Volt(s)
mV	Milli Volt(s)
VF	Ventricular Fibrillation

SECTION ONE

GENERAL INTRODUCTION and METHODS

A. GENERAL INTRODUCTION

Elective cardiac arrest, now generally referred to as cardioplegia, was introduced during the 1950's as a technique to limit cardiac ischaemic damage and contribute to appropriate operating conditions during open heart surgery. It was whilst discussing this area of cardiac research in a B.Sc. tutorial three years ago, that Professor Baker encouraged the suggestion of methods which might reduce ischaemic damage by reversibly inhibiting cardiac metabolism more completely than was then possible. My responses at that time have since developed into the ideas presented in this thesis and recent review articles have also centred on this aspect of cardioplegia (Hearse 1988, Ip and Levin 1988). The history of elective cardiac arrest is also the history of open heart surgery, both have been described in detail elsewhere (Melrose, 1978, Hearse, Braimbridge and Jynge 1981), and a short account is now presented.

1. Initial Success and Subsequent Failure of Cardioplegia

Regular human open heart surgery probably began with Dennis, Spreng, Nelson and Karlson (1951), Lewis and Taufic (1953) and Lillehei, Cohen, Warden and Varco (1955). Although at that time general surgical techniques were relatively advanced, to begin with only simple cardiac procedures could be attempted because it

was essential to prevent excessive blood loss and ensure a clear operating field by clamping the great vessels of the heart prior to opening any of its chambers, this caused interruptions of the circulation which could be tolerated only briefly by the central nervous system. Even with the protective effects of systemic hypothermia, operations could last safely no more than 15 minutes (Bigelow, Mustard and Evans 1954). Gibbon (1954), as a result of 20 years of dedication, removed this restriction by introducing an effective system for maintaining a circulation of oxygenated blood independently of the heart. Gibbon's pump-oxygenator was accepted eagerly by surgeons in America (Kirklin, DuShane, Patrick, Donald, Hetzel, Harshbarger and Wood 1955. Kirklin, Donald, Harshbarger, Hetzel, Patrick, Swan and Wood 1956) and in England, Melrose continued to develop what became known as the British Heart-lung Machine (Melrose and Aird 1953, Melrose 1955). Despite these advances, cardiac surgery had still to be performed under conditions which remained far from the recognised ideal which was described later by Melrose (1978) as operating on "hearts from which all blood flow is excluded and which are quite flaccid". Isolation from the artificial circulation did indeed exclude from the heart all blood, except for a little received via collaterals with mediastinal vessels, but the ensuing ischaemic cardiac arrest was not only slow in onset but was often incomplete. More importantly, cardiac recovery

from even relatively short periods of bypass was not good, revealing a new and only slightly less rigid restriction on the duration and hence the complexity of open heart operations.

Melrose, working with Cleland, Bentall and Dreyer in Professor Airds' team of pioneering heart surgeons at the Hammersmith hospital, London, suggested that rapid elective arrest would not only provide a flaccid heart more quickly but might conserve within it energy reserves sufficient to maintain homeostasis and so enable survival of the ischaemic heart for longer periods. The most obvious choice of natural arresting agent would seem to be acetylcholine, but this had already been shown not to work in experimental cardiac surgery performed on dogs (Bjork 1948). In deciding what they should use, Melrose approached Baker who had designed a highly efficient Langendorff type of perfusion apparatus (Baker 1951) and had considerable experience with isolated perfused hearts from animals and also human foetuses (Baker 1953). Baker proposed potassium chloride, basing this recommendation on Ringer's (1883) work in frog hearts, expanded by Hooker (1929), and his own observations on rabbit hearts, especially in relation to cardiac glycosides (Baker 1947). In the discussion which followed, Melrose suggested that as Ringer had shown also that lowering calcium concentration caused arrest, potassium citrate might be a better choice because the citrate moiety would

chelate calcium and so reduce the available concentration in the extracellular fluid.

A Preliminary Communication reporting on the use of potassium citrate as an experimental cardioplegic was published in the Lancet on 2 July 1955 (Melrose, Dreyer, Bentall and Baker 1955). Thirty three dogs had been anaesthetised and placed on cardiac bypass with Melrose's machine before injections of potassium citrate were made into the root of the aorta, which had been clamped distally. The authors felt that although these experiments were encouraging "questions of duration and reversibility of action of potassium citrate and the need for stimulants might profitably be studied further on isolated-heart preparations". From the preliminary short series of experiments made by Baker on the isolated perfused hearts of five rabbits, a guinea-pig, a kitten and a puppy it was clear that recovery from periods without perfusion was indeed more complete if hearts had been arrested with potassium citrate beforehand. An effective concentration in Locke's solution was identified as 1mg/ml, but it was noted that the slight atrial flickering that sometimes remained could be prevented if the concentration was raised to 5mg/ml. The method used originally on the dog hearts in situ was revised and a new procedure proposed at the end of that same publication in the Lancet (Melrose et al. 1955). Although it must be said that the success of the new method and the extent of their experience with it are

impossible to determine from this report, it was adopted almost unchanged for clinical use. The Melrose technique, as it became known, had been developed by the surgeons involved and who acted, under the constraints of surgery, principally to avoid the infusion of any significant extra volume of solution. After total bypass had been accomplished, arrest was achieved by clamping the aorta and simultaneously injecting into its root, 30-100ml of blood containing potassium citrate at a concentration of 200mM (Gerbode and Melrose 1958). Meantime, in 60 rabbit isolated hearts a detailed investigation had been completed which not only confirmed the earlier isolated heart work but suggested that potassium citrate did not appear to damage human isolated foetal hearts when used as a cardioplegic and that the ratio of potassium to citrate in the tri-potassium salt used was indeed the most appropriate (Baker, Bentall, Dreyer and Melrose 1957). Notwithstanding the appealing theoretical arguments in favour of citrate, in this study it was the observation that there were less episodes of ventricular fibrillation during recovery from potassium citrate which commended it slightly over potassium chloride as an arresting agent (Baker et al. 1957). Ventricular fibrillation had already been reported to be the norm in dogs during recovery from potassium chloride cardioplegia, in a paper delivered by Lam, Geoghegan and Lepore at the Annual Meeting of the American Association for Thoracic Surgery on 24th April 1955, a few months

before the work of Melrose and colleagues was published. Lam had injected 1ml/kg body weight of a 5% solution of potassium chloride into the left ventricle and squeezed it into the coronaries, the aorta being clamped distal to their origin, to achieve arrest within about 30 seconds. This approach was not adopted, but the Melrose technique was, and used successfully it gained the recommendations of other surgeons (Effler, Knight, Groves and Kolff 1957. Kolff, Effler, Groves and Moraca 1957. Gerbode and Melrose 1958). Lam later adopted acetylcholine and used it extensively clinically (Lam, Gahagan, Mota and Green 1959) but the arrested hearts gave troublesome and unexpected escape beats, often in response to handling, and as a result acetylcholine was not adopted widely either, although some interest continued in the use of acetylcholine experimentally (Daicoff, Lancaster, Stewart, Siderius and Moulder 1960, Isselhard and Merguet 1962). Shortly afterwards, at the time when it was first demonstrated that the membrane potential of rabbit atria responded to changes in the extracellular potassium concentration in a manner which could be predicted accurately by the Nernst equation (Vaughan-Williams 1958), it became clear that Melrose technique of potassium citrate cardioplegia was not always safe clinically. Areas of necrosis were found in the hearts of patients who had died following potassium citrate cardioplegia (McFarland, Thomas, Gilbert and Morrow 1960, Waldhausen, Braunwald, Bloodwell, Cornell and Morrow

1960), supporting similar observations previously made in dogs (Helmsworth 1959). The Melrose technique as used clinically was obviously very similar to that used in the dog experiments and which had yielded unsatisfactory results originally. The principles of the method, namely injecting small volumes of blood containing very high concentrations of potassium citrate into the root of the clamped aorta, had not been changed when it was modified and they remained very different from those used by Baker in the highly successful isolated heart experiments. In those the effective concentrations of potassium citrate were contained within otherwise physiological perfusion solutions given at normal flow rates, thus attempting an even myocardial distribution at the chosen concentration. When boluses of potassium citrate were used they were delivered in the freely running solution and perfusion was not stopped until cardiac arrest had occurred. These crucial differences probably account for the clinical events which followed. The reports of the potential danger of the Melrose technique, coupled with an awareness that the problems were not due to the choice of patients nor restricted to one surgical centre, led surgeons to abandon cardioplegia, and return to ischaemic arrest or in some cases ventricular fibrillation (Glenn, Toole, Longo, Hume and Gentsch 1960) to provide their desired operating conditions, with topical hypothermia to protect the heart (Shumway and Lower 1960). In addition to being mechanically inconvenient, ventricular

fibrillation was a strange choice when it was already known to be damaging to the heart and therefore not a reasonable alternative to elective arrest.

2. Reconsideration of Cardioplegic Formulations

After the initial clinical success with the Melrose technique it was described as "the most valuable adjunct to open-heart surgery since the advent of the cross-circulation approach" (Effler et al. 1957). So, although effectively exiled by the subsequent failure of that particular technique, the potential benefits of elective arrest were appreciated and ensured the re-emergence of cardioplegic solutions, but at first in a radically different form.

a. Intracellular type cardioplegic solutions.

Bretschneider (1964 in German), proposed that perfusing a heart with a solution containing very little or even no Na^+ or Ca^{2+} would be an ideal way to induce arrest. The ionic concentrations of the various forms of Bretschneider's solution consequently resemble intracellular fluid, except for the potassium concentration, which is almost extracellular (5.37mM). To make the final solution isotonic or hypertonic in relation to the fluid normally present in the extracellular space, histidine, sorbitol or mannitol was added. The original solution also contains high

concentrations of procaine (up to 10mM), magnesium (2mM) and glucose (5.05mM), but there have been many modifications since. The first reports of the clinical use of Bretschneider's solution appeared in 1967 from Sondergaard and Senn (1967), also Reidemeister, Heberer and Bretschneider (1967) which was the first account in English. However, in an environment already suspicious of and in some cases hostile to pharmacological cardioplegia, the development and application of intracellular type solutions was quickly stifled. It was reported (Zimmerman, Hulsmann, Snijder, Wisse, Durrer 1967) that returning calcium to rat hearts previously perfused with a calcium-free solution, surprisingly did not restore mechanical activity, but instead caused a permanent hypercontracted state. Zimmerman termed this dramatic phenomenon the "calcium paradox" and immediately the whole basis of the calcium-free cardioplegic solutions was called into question. Yet Kirsch's solution, although the most extreme of the intracellular type, containing only magnesium aspartate (161mM), procaine (11mM) and sorbitol (247mM), was used successfully by its originator (Kirsch, Rodewald and Kalmar 1972) and Bretschneider's original solution, likewise without sodium or calcium, also continued in use successfully (Rygg and Petersen 1978). The relevance of the calcium paradox to clinical cardioplegia is further obscured by the differences between experimental and clinical situations, but it is generally agreed that the

results obtained with intracellular type solutions are unpredictable and dependent on many variables which are difficult to control. These facts, apparent confusion over the exact composition of the successful solutions (Hearse, Braimbridge and Jynge 1981), possibly the paucity of publications in English and the introduction in 1975 of an effective alternative simultaneously in England and America, have since confined intracellular type solutions almost exclusively to the centres in which it developed.

b. Extracellular type cardioplegic solutions

It was the re-emergence of clinical cardioplegia in Germany and a belief in the theory behind cardioplegia generally, that stimulated several investigations into the mechanisms responsible for the damage caused by the Melrose technique. When used clinically, potassium citrate was given in high concentrations (200mM) and small volumes (30-100ml) into the aortic roots of otherwise non-perfused hearts. It is highly likely that within the myocardium areas existed which had received too little potassium citrate and others where the concentration was too high, possibly leading to osmotic damage or toxicity specific to the ions present. It has been suggested that the solutions of potassium citrate were so concentrated that they damaged the glass ampoules that contained them and as a result fragments of silicate

were infused along with the solution, but there appears to be no account of fragments having been found during histological examination, possibly because they were lost during preparation of the slides. Similarly, since potassium citrate did not remain in solution easily at the chosen concentration of 250mg/ml and at 4°C (Melrose 1978), the cloudiness seen in the solutions may not have been silicates but simply crystals of potassium citrate, which would almost certainly be washed out during the preparation of histological specimens. However, it is difficult to reconcile these two explanations with the clinical observation that it was the fresh solutions which were damaging and the cloudy ones which were not. Possibly the patients received less potassium citrate from the cloudy solutions because some of it had precipitated and remained behind in the ampoules or infusion apparatus. Whatever the exact cause of the damage, it is generally agreed to have originated in the concentration of potassium citrate used and the way in which it was administered clinically. By the early 1970's animal studies had again confirmed that potassium chloride and citrate were safe and effective if used in isotonic infusion solutions at concentrations between 10 and 50mM (Tyers, Todd, Niebauer, Manley and Waldhausen 1975,). The solutions so produced were in many respects the same as extracellular fluid, the major exception being the slightly higher potassium concentration, and

accordingly they became known as extracellular type solutions.

Clinical extracellular type cardioplegia returned in 1975 in the form of St. Thomas' Solution Number One (Hearse, Stewart and Braimbridge 1976), in which potassium chloride (20mM) was combined with those components of Bretshneider's solution considered to be useful, namely magnesium and procaine, in a solution based on that of Ringer. Similar solutions were introduced simultaneously but independently in America (Tyers et al. 1975, Gay 1975, Roe, Hutchinson, Fishman, Ullyot and Smith 1977)

Extracellular type solutions typically contain potassium concentrations raised to almost the same extent as in the original rabbit heart experiments (Baker et al. 1957), lowered calcium concentrations, in accordance with the idea behind the suggestion of citrate, and drugs to combat arrhythmias such as ventricular fibrillation, the property which really forwarded the use of potassium citrate in preference to potassium chloride. Cardioplegia has indeed come full circle.

3. Contemporary Cardioplegia

The successfully used St. Thomas' Hospital formulation has been associated with a worldwide return to the use of cardioplegia. A similar solution, St. Thomas' Solution Number Two, was introduced principally to conform with the requirements of the USA Food and Drug Administration

(Hearse, Braimbridge and Jynge 1981) but very recent reports have suggested that in rat hearts it provides protection superior to that of Number One (Ledingham, Braimbridge and Hearse 1987). These two solutions have been subjected to much laboratory investigation and are typical of the extracellular type solutions used throughout the world with great success in operations to replace aortic valves or to correct congenital defects, but less so in other aspects of cardiac surgery. An enormous effort has been directed at improving these, and other solutions, not least by their formulators, but the complexity of the search for improvements was by 1980 referred to as "approaching chaotic", (McGoon 1980). In 1985 it was suggested that in the quest for ideal myocardial protection "repetition, misdirection and at least unfamiliarity with what has already been done may be the current status of our work in this area", (McGoon 1985). This view led The Journal of Thoracic and Cardiovascular Surgery, in which the majority of papers dealing with the topic are published, to take the unusual step, "in hopes of improving the resources of the editors and reviewers, --- and in the belief that the results of this special survey might also be of value to the readership", of issuing a catalogue of original scientific literature published in English between 1979 and early 1984 dealing with "interoperative myocardial protection" (McGoon 1985). Interestingly, although myocardial protection spans a broad range of

pharmacology, physiology and biochemistry, of the 241 references included only one was published in a basic science journal. Despite the enormous effort, clinical cardioplegic solutions have remained largely unchanged since their re-introduction. Some interest has, however, been shown in the use of potassium-enriched blood (Buckberg 1979), the original cardioplegic technique, but this complicated approach does not appear to offer any advantage in terms of post-operative recovery over the simple crystalloid solutions (Goldstein, Salter, Murphy, Abd-Elfattah, Morris and Wechsler (1985). There is no agreement as to the best solution (Kaiser 1985) but crystalloid extracellular type solutions are by far the most widely used. Generally, cardioplegia is least effective when there is some pre-existing cardiac pathology (Yamamoto, Manning, Braimbridge and Hearse 1983), especially coronary artery stenosis which impedes swift and uniform delivery of solution (Myers, Weiss, Kirsh, Shepard and Shlaffer 1986) and in paediatric heart surgery or when long durations of arrest (exceeding 2 hours) are required during complex operations. Perhaps the area in which improved cardioplegia would have the greatest impact is that of cardiac transplantation. At present the shortage of donor hearts is so severe that between 25 and 35 per cent of patients awaiting a transplant die before a suitable heart becomes available. Recently John Wallwork, a heart-lung transplant surgeon at Papworth Hospital, commented in the national press

(The Independent, 1st August 1988) that because of the shortage of human donor hearts the use of animal or artificial hearts was the "right line of approach". There are major technical and ethical difficulties to be overcome before such an approach could be used clinically. However, the shortage is not due only to the small number of potential donors but also to the present restrictive requirements that donor hearts must be beating at the time of acquisition and have almost always to be used within 4 hours of removal from the donor (Green 1984, Darracott-Cankovic, Wheeldon, Cory-Pearce, Wallwork and English 1987). In marked contrast, it has long been possible to keep excised human kidneys, of which there is no real shortage, in a viable condition for up to 30 hours before transplantation (Collins, Bravo-Shurgarman and Teraskai 1969). More recently human liver and pancreases have been stored for similar periods, (Belzer and Southard 1988). Furthermore, all these organs can be taken from cadavers as well as from the preferable but comparatively rare, donors finally diagnosed as brain dead but in whom the circulation is maintained by life-support machines. The presently available cardioplegic solutions do not enable the conditions under which donor hearts are acquired and stored to be as flexible as those for other organs, but this was not the aim of their formulators. Although the first heart transplant was performed in dogs by Carrel and Guthrie in 1905 and the first human heart transplant

by Barnard in 1967, the initial excitement had waned in the face of immunological complications by the time that cardioplegia, as applied to reparative surgery, was able to return tentatively to clinical use. At that time some very interesting work on the resuscitation and short term storage of canine hearts had been reported by Cooper (1975) but general opinion was well expressed by the highly experienced cardiac transplant team at Stanford who concluded "development of improved methods of immunosuppression will be the main factor that determines whether cardiac transplantation will have a wider application in the future for patients with terminal cardiac disease". No mention was made of the importance of the availability of donors nor of any role for cardiopolegic solutions (Hunt, Rider, Stinson, Griepp, Schroeder, Harrison and Shumway 1976). Only now, has the revival in cardiac transplantation which came during the early 1980's with the adoption of cyclosporin A as an immunosupressive, by its very success created its own shortage of suitable donors. Just as the introduction of the heart-lung machine had emphasised the need for cardioplegia, the success of heart transplantation has made it necessary to design a new generation of cardioplegic solutions and infusion protocols which will at least enable hearts from further afield to be utilised and possibly also hearts which have already stopped.

4. The Scope of This Project

The aim of this project is to formulate cardioplegic solutions and design infusion protocols with the potential to increase the duration of safe cardioplegia in its various clinical applications. To achieve this aim it is necessary to re-examine the actions and interactions between potassium and calcium as components of cardioplegic solutions, particularly in respect of their effects on coronary flow and the suppression of escape beats and electical activity, as these are areas which have been neglected in the past but appear to be highly relevant to the improvment of cardioplegia. It is also necessary to investigate the possible effects of the clinical vasodilator, sodium nitroprusside, in the light of the recent suggestion that it relaxes smooth muscle by causing the extrusion of calcium, because the accumulation of intracellular calcium is probably the single most important factor in the development of cardiac ischaemic damage. Once the individual effects and mechanisms of action of these established and potential components of cardioplegic solutions have been determined, they must be assessed in combination, lest harmful or beneficial interactions are not detected. The protocol of such experiments must mimic the eventual situation in which the solution is to be used for it is clear that solutions already formulated on the basis of only single 30 minute periods of arrest in fresh hearts

have proved to be less effective clinically when long periods of arrest are required or when there are cirtain forms of pre-existing cardiac pathology. Multiple periods of cardioplegia, may provide a model which is more representative of hearts with existing pathological changes. No cardioplegic solution in clinical use is agreed to be the best because such a choice is specific to individual situations, there have been comparativly few studies in which similar solutions have been compared using diverse but readily appreciable parameters side by side experimentally. The exact contribution of individual components to the overall effects of solutions and the form and advisability of modifications to meet particular requirements therefore remain unresolved. In the field of cardiac transplantation the aim is to formulate a solution and design an infusion protocol to allow the use even of donor hearts which have stopped beating and to enable the preservation of viable donor hearts for periods which would allow them to be taken safely to far distant transplant centres. This can be achieved only by further elucidation the mechanisms which serve to limit the viability of hearts stored conventionally and of those already arrested due to ischaemia.

B. GENERAL METHODOLOGY USED IN THIS STUDY

1. Choice of Experimental Model

The isolated perfused working rat heart has been the most widely used basic model in this field of research and the dog the most widely used when experiments involved surgical procedures (McGoon 1985). The relatively inexpensive rat model is used in order to make large-scale screening of the "millions" (McGoon 1985) of combinations of components, infusion procedures, durations of arrest and means of assessing the effects of cardioplegic solutions possible on economic grounds. However, more information and probably of greater relevance can be gained easily from carefully designed experiments on rabbit heart models, so diminishing the slight economic penalty of using this species.

a. Histology of the myocardium

The similarity of the rabbit and human heart in respect of fibre diameter, densitiy of capillaries and number per fibre were reported by Wearn (1939-41) and led to the use of a rabbit heart model as the first choice for experiments carried out in this field (Baker et al. 1957). In particular, Wearn reported striking similarities between the hearts of children and those of rabbits and also between rabbit and human hypertrophied hearts. These observations are particularly relevent

because present cardioplegic solutions which were formulated on rat models are known to be less effective in children and in hearts with existing pathology.

b. Electrophysiology.

The rabbit heart, rather than the rat heart, is generally held to be a closer model of the human heart in terms of electrophysiology. Most of our understanding of the electropharmacology of antidysrhythmic drugs is derived from experiments on rabbit isolated atria. The size of the heart is important in determining the wavelength of re-entry circuits and so the likelihood of arrhythmias arising from them. Although very much smaller than the human heart, the rabbit heart is usually bigger than that of the rat. The electrical properties of the rabbit heart are stable and with fine adjustments it is possible to produce a surface ECG recording which is very similar in appearance to a human limb lead ECG (such as I, II, or III).

c. Anomalies of Pharmacological Response.

The rat heart is well known to be very much less sensitive to cardiac glycosides than either the rabbit or the human heart. In view of what is known of the mode of action of these drugs it is therefore reasonable to conclude that some aspect of the relationship between intracellular sodium and calcium concentrations is

different, this may make experiments involving the importance of sodium and calcium regulation mechanisms difficult to interpret with respect to the human.

2. Langendorff Perfused Hearts

After the animals had been killed humanely and in accordance with the Animals (Scientific Procedures) Act 1986, Schedule 1, they were bled from the vessels in the neck. Their hearts were carefully excised and placed in gassed perfusion solution at room temperature where they were trimmed prior to cannulation of the aorta. Care was taken not to introduce air emboli into the coronary vasculature nor cut, squash or stretch the hearts. The time taken from killing the animal to perfusing the heart was approximately 2 minutes.

a. Details of Perfusion Apparatus

Hearts were perfused at a constant pressure of 65cm H_2O by the well known Langendorff method in an apparatus similar to that of Baker (1951) but with two seperate warming coils see fig. 1B.2. The apparatus ensured that with reasonable physiological limits a chosen cardiac temperature could be maintained independently of the coronary flow rate. The position of the drainage valves allowed any solution which had remained static within the warming coils to be discarded easily and without entering

the heart, this procedure was adopted whenever perfusion solutions were selected by means of the stopcock.

	RABBIT	RAT	GUINEA-PIG
Number used	188	8	5
Strain	New Zealand white	Wistar	Dunkin Hartly
Sex	male + female	male	male
Mass (within)	800 - 1000g	170 - 180g	450 - 500g
Method of Humane killing	Dislocation of the neck + exsanguination	Striking the back of the head + exsanguination	Dislocation of the neck + exsanguination
Tissues Used	Heart, atria,	Heart	Heart
Preparation	Langendorff constant pressure (65cmH_2O) perfusion		
	Atrial strips		
Perfusion and bathing solutions	McEwen's (1956)	Krebs'	Krebs'
Temperature	37°C 28°C (atria)	37°C	37°C
Composition (Analar)			
NaCl	130.00 mM	118.40	118.40
KCl	005.60	004.70	004.70
$NaHCO_3$	025.00	023.80	023.80
$CaCl_2$	002.18	002.56	002.56
NaH_2PO_4	000.92	----	----
KH_2PO_4	----	001.20	001.20
Glucose	011.10	011.10	011.10
Sucrose	013.10	----	----
pH	7.4	7.4	7.4
All solutions filtered through Whatman inline filters and gassed with 95% CO_2 + 5% CO_2 for 20 min.			

Table 1B.1

A summary of experimental models and the formulation of perfusion solutions used in this study.

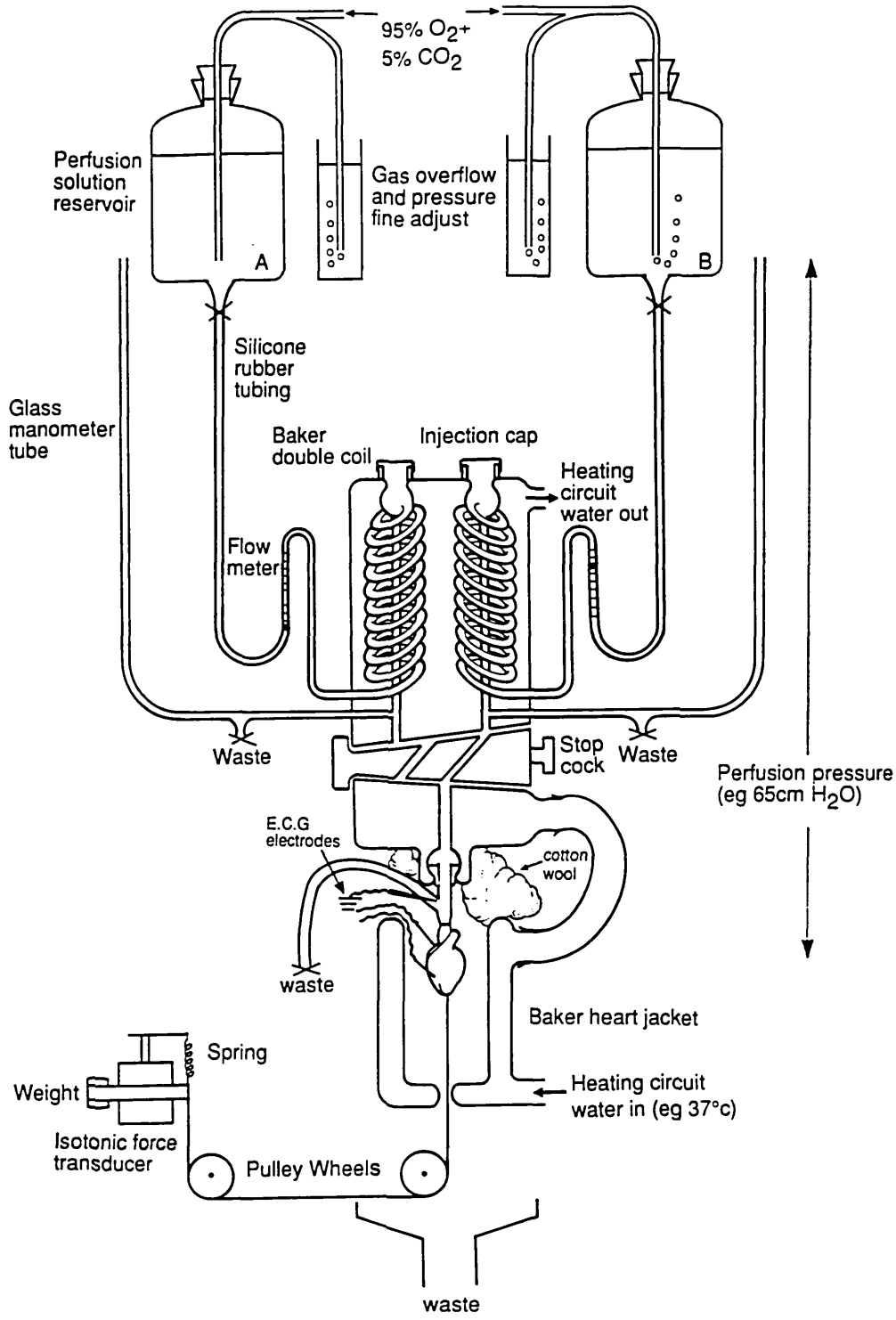


Figure 1B.2

Langendorff perfusion apparatus. The apparatus incorporates the Baker heart jacket (Baker 1951) and double warming coil (Baker, Aimer Products Catalogue).

Purely isometric recording was not adopted because this was found to shorten the life of the preparation. Instead, semi-isotonic recordings were made by means of a stainless steel hook placed in the interventricular septum at the apex of the ventricles. This was connected to a Biosciences T3 isotonic transducer with a diastolic load of 5g, rising by means of a spring to 6g by the end of systole. The height of oscillograph tracings (see typical tracing, fig. 1B. 5) were therefore proportional to the amplitude of contraction. This form of recording was adopted in order to obstruct the normal movement of the heart as little as possible and on the understanding that the value of the results lies mainly in the comparison of before and after recordings for each heart. Results are therefore expressed as arbitrary units or as percentage change.

Heart rate was calculated from the tracing (the accuracy of time markers and chart speed was checked periodically) and is expressed as beats per minute or as percentage change. Electrical activity was picked up by two saline wick electrodes (fig. 1B.4) one placed on the surface of the right atrium and the other midway down the anterior surface of the interventricular septum. Only the tips of the electrodes were in contact with the heart and they were sufficiently flexible not to alter its movement. Minor rearrangements from these positions were sometimes made to yield clearly distinguishable components of the E.C.G.

Electrical and mechanical signals were amplified by a Biosciences CD20 amplifier and recorded on a Biosciences Oscillograph.

The coronary flow was recorded intermittently from the flowmeter, the accuracy of which had been checked, on the imput side of the perfusion circuit and is expressed as percentage change or ml per minute.

Where specific forms of additional intrumentation were required for particular experiments these were arranged about the heart in accordance with fig. 1B.3.

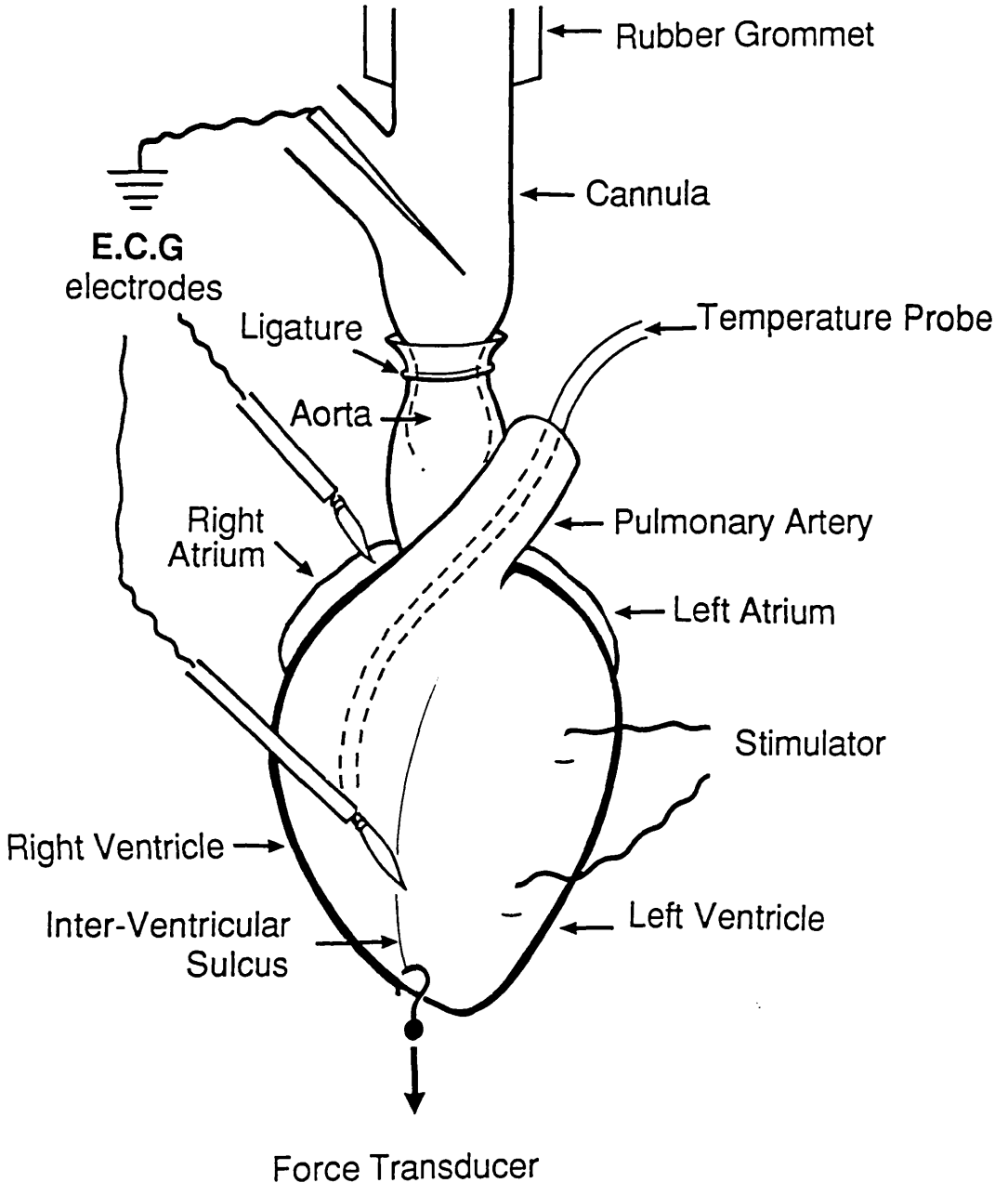


Figure 1B.3

Isolated heart instrumentation

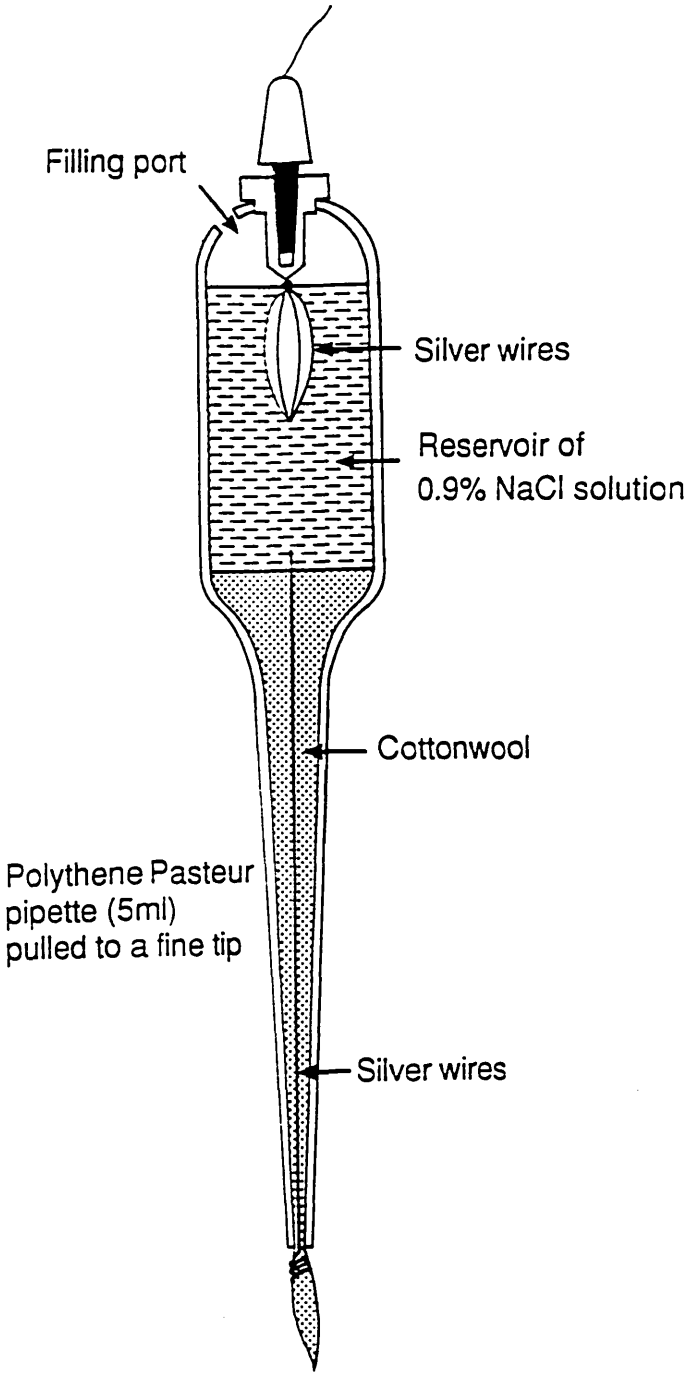


Figure 1B.4

Saline wick electrode

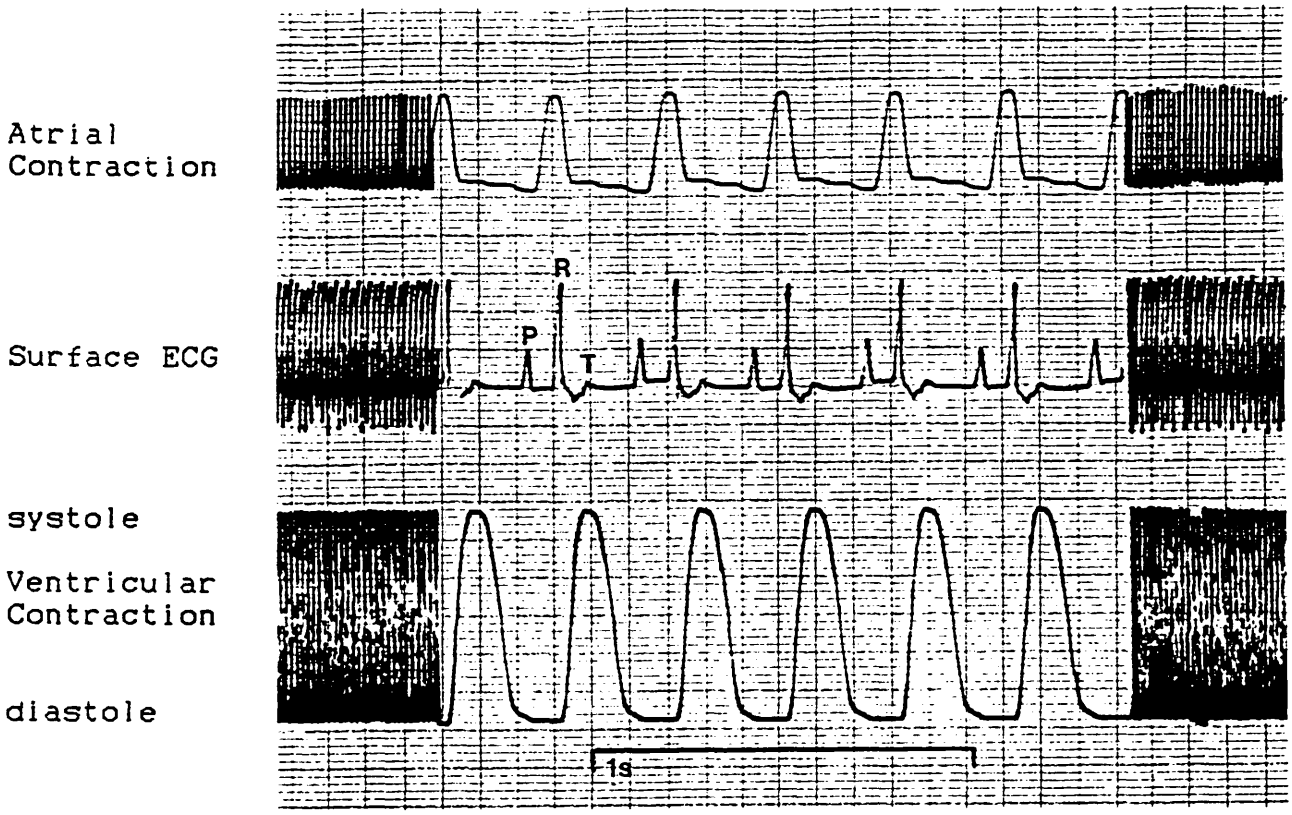


Figure 1B.5

Typical tracing from rabbit isolated perfused heart experiment. Heart from a NZW rabbit (889g) perfused with McEwen's (1956) solution, 65cm H_2O pressure and 37°C. Semi-isotonic contraction recorded by hook in apex of ventricles attached to isotonic transducer with diastolic load 5g rising during systole to 6g by means of a spring. Semi-isotonic transducer with a load of 1g was attached to the left atrium. Surface ECG activity was recorded using saline wick electrodes, one on the right atrium and one on the anterior superior aspect of the left ventricle. The co-ordinated sequence of P wave, atrial contraction, QRS complex (as denoted by position of R, followed by ventricular contraction and then a T wave is clear. (Note, simultaneous recording of atrial and ventricular contraction was used only for the experiments described in Section 2G, but ventricular and electrical activiy were recorded in all experiments).

b. Stability of Rabbit Isolated Perfused Hearts

The rat isolated heart is given to abnormalities of rhythm and generally does not survive isolation as well as the rabbit heart. Periods of hypothermic cardioplegia lasting only 3 hours are described as "long" in rat heart models (Ledingham, Braimbridge, and Hearse 1987) and a protocol of 30 minutes cardioplegia followed by 30 minutes of recovery has been adopted widely. This is inappropriate to the acquisition of information relevant to the improvement of donor heart storage, which may need to reach 24 hours before any impact is made on the current shortage. The rabbit isolated heart remains reasonably stable over long periods as can be seen from figure 1B.6, and is suited to the study of long periods of arrest which is at present carried out in very much more expensive dog and less frequently pig hearts in situ.

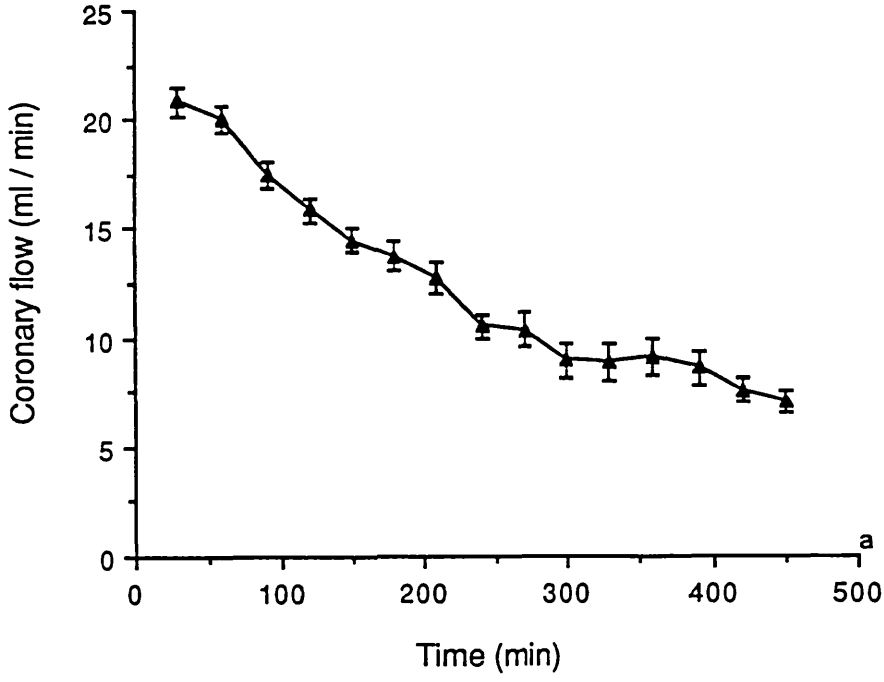
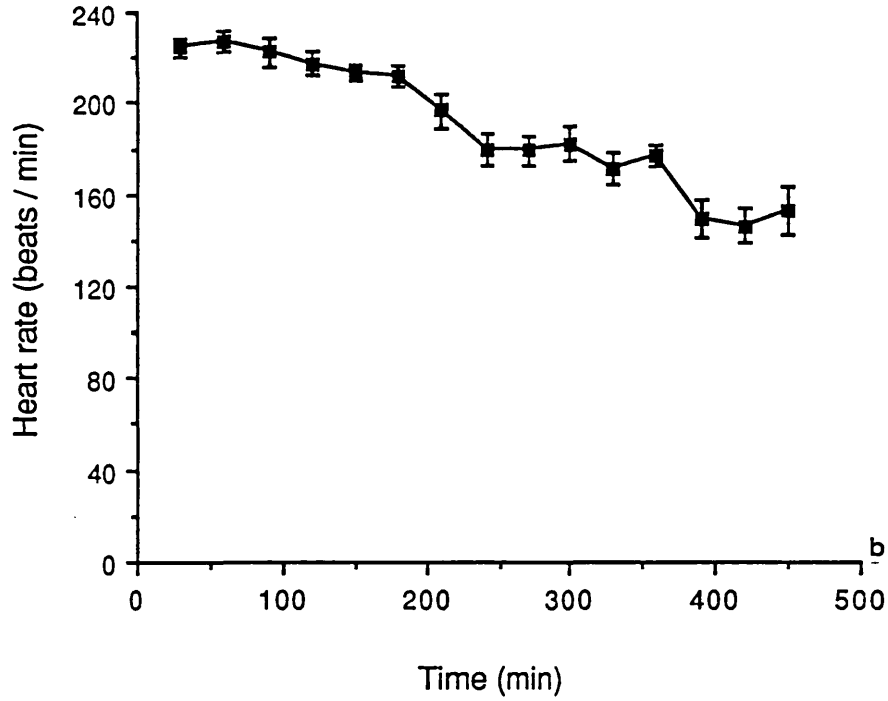


Figure 1B.6 (continued on following page)

The stability of rabbit isolated hearts during 450 minutes of perfusion. a) Coronary flow, b) Heart rate, c) Amplitude of contraction. (perfusion and recording parameters as for fig. 1B.5). Each point is the mean and the vertical bars the s.e. mean of 8 observations.



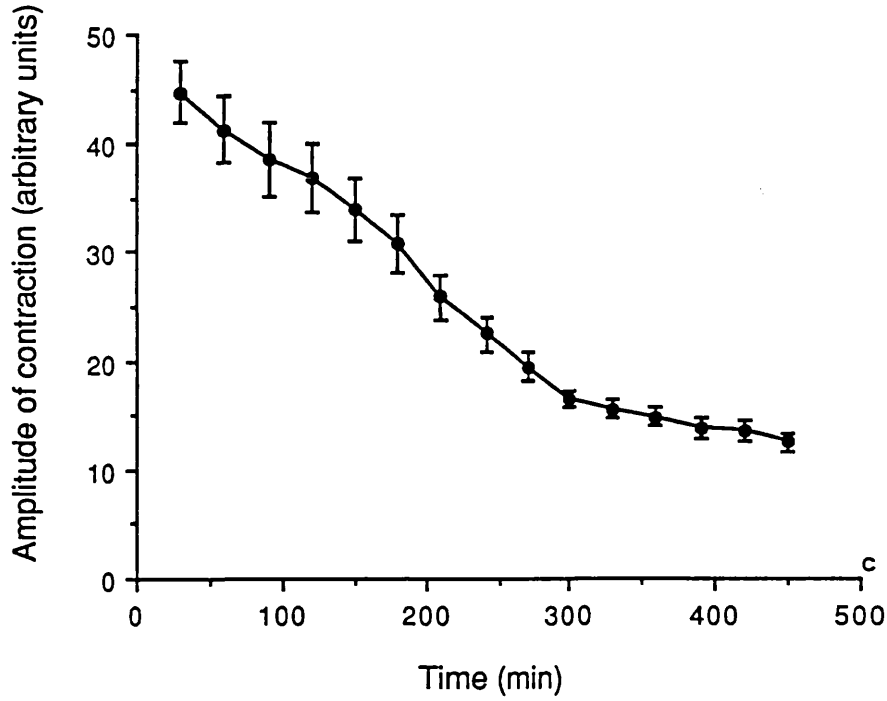


Figure 1B.6 (continued)

b) Heart rate, c) Amplitude of contraction (see previous page for details).

c. Limitations of the Langendorff Method

As with any other experimental technique, perfusion of an isolated heart by the Langendorff method has its limitations. For example, force-frequency and length-tension relationships can be determined more accurately from experiments on isolated strips of myocardium or single muscle cells. The electrophysiological properties of the myocardium and the contribution to them made by single ion species or via specific ion channels can likewise be studied more effectively with ion-selective, voltage clamp, or patch clamp techniques on isolated or cultured cell preparations. However, in the context of this study, where the primary aim is to formulate cardioplegic solutions to be used in whole and in effect isolated hearts, the Langendorff model is appropriate. In the preparation as described here, coronary flow can be increased greatly by coronary vasodilators. This would not be the case if the coronary vessels were already maximally vasodilated due to autoregulation in response to hypoxia. However, maintaining an adequate flow rate of oxygenated solution can lead to oedema formation and so to a reduction in the useful lifespan of the preparation. McEwen's solution was designed to overcome this problem specifically in rabbit Langendorff hearts and enables a useful preparation life span of 6-9 hours (McEwen 1956).

3. Recording of Intracellular Electrical Potentials

Apparatus capable of recording intracellular electrical potentials and intracellular ion activities has been assembled and is described below.

An antivibration surface was made from a steel desk with a sheet of chipboard (1.7cm thick) on top. This was separated by three bicycle tyre inner tubes from a concrete paving slab 60cm by 90cm by 5cm thick. The paving slab was covered with a sheet of rubber 0.5cm thick upon which instruments were mounted. This arrangement, coupled with the antivibration characteristics which are a design feature of the new Charing Cross Hospital (Fulham), provided sufficient stability to make recordings of good quality.

A custom built organ bath was mounted in the middle of the table and illuminated via optical fibres from a cold light unit. A dissecting microscope was available to view the preparation in greater detail. The rate of flow through the organ bath was gravity dependent and could therefore be controlled simply by adjusting the height of the reservoirs. Flow through the bath was maintained at 5ml/minute. The choice of solution could be selected by means of a two-way stopcock immediately before the organ bath. The volume of solution between stopcock and bath was less than 2ml. Solutions could be drained from immediately before the stopcock. Solution drained from the bath through a simple vertical pipe, the height of

which could be adjusted to contol the level of fluid in the bath.

The bath reference and recording electrodes were pulled from filamented capillary glass of 1mm diameter (Clarke electromedical) with a custom-made puller (Department of Pharmacology, University of Oxford) and filled with 3M KCl solution. The bath reference electrode was connected to the circuit by means of a silver wire and was positioned between the preparation and the bath outflow to prevent possible leakage from it from altering the composition of the fluid surrounding the tissue. Recording electrodes were positioned with a manual micromanipulator (Narishige) and connected by an electrode holder to a probe containing a head stage amplifier, and then to a WPI Duo Electrometer 773. A suitable chart recorder was not available during this study and it is for this reason that no original records of membrane potential have been presented. The values obtained in this study were read from the digital meter which is part of the WPI Duo Electrometer 773.

All items were earthed to a star earth and the whole assembly was enclosed in an earthed aluminium box. Holes in the box allowed operation of the micromanipulator and other functions with the box lid closed, thus ensuring good electrical shielding.

There were considerable problems in pulling electrodes sufficiently sharp to impale the very small atrial cells and record stable potentials. The effective electrodes

had high resistances (50-70Mohm) and very large tip potentials, upto -50mV. Tip potentials (see Adrian 1956) result from interactions between the components of the electrode filling solution and the glass at the tip of the electrode and as such are abolished if the tip is broken off. In general, tip potentials increase with increases in electrode resistance but they can vary greatly in apparently otherwise similar electrodes. The tip potential depends upon the ionic composition of the solution in which the electrode is immersed (if this solution is the same as the electrode filling solution there is no tip potential) and is not constant between extracellular fluid, eg. bathing solution, and intracellular fluid. Hence a degree of inaccuracy, proportional to the tip potential, exists when measuring absolute membrane potentials, which are usually underestimated as a consequence. The electrodes used in this study had large tip potentials and so the values given for absolute membrane potential must be viewed with some caution. To measure the full signal at the tip of the electrode and to draw only minimal current from the cell in which the electrode is placed, it is necessary to use an amplifier with an input resistance at least 100-1000 times greater than that of the electrode. As an input with a resistance of 10^{15}ohm was available during this study it was thought appropriate to use this.

4. Manner of Statistical Analysis of Results

All experiments were repeated, in random order unless stated otherwise, in six, or more usually eight individual preparations except where events did not always occur, in which case there were 6 or 8 opportunities for such events to occur. The arithmetical mean was calculated and used in all figures. The standard error of the arithmetical mean (s.e.m.) was calculated as the sample standard deviation divided by the square root of the sample size. Although all experiments were done on hearts from a very narrow weight range of animals of the same species and under carefully controlled conditions, data were treated as paired only if they derived from a single preparation. Except where stated otherwise, statistical analysis was carried out on the raw data (even when percentage change is presented in a figure).

To test the possible statistical significance between two means students paired and unpaired "t" Tests were used depending on whether the data were paired or independent. Analysis of variance was used to test the possible significance of differences between 3 or more means. Analysis of Variance by Ranks (Freidman) was used for ranked data.

Possible correlations were tested using Chi square (with Yeates correction) for independent samples and MacNemar's test for paired data.

SECTION TWO

THE EFFECTS AND MECHANISMS OF ACTION OF CARDIOPROTECTIVE COMPOUNDS AND PROCEDURES

INTRODUCTION

The major requirements of a cardioplegic solution are that it should, i) induce and maintain cardiac arrest, ii) protect the arrested heart iii) ensure adequate speed and extent of recovery. A single component of a solution may have specific or limited properties which may not be immediately apparent or could be obscured by the inadequacies of another. It is therefore necessary to investigate each component in a manner appropriate to the property which it is likely to confer before a complex solution is formulated.

SERIES A

EFFECTS OF SOLUTIONS WITH REDUCED $CaCl_2$ CONCENTRATIONS ON RABBIT ISOLATED HEARTS, 37°C.

Introduction

The initial causes of damage during cardioplegia and reperfusion are a matter of current debate, but it is clear that by the time such damage has become irreversible cytosolic calcium concentration is greatly increased and often phosphate or carbonate salts have been precipitated in the mitochondria and elsewhere (Henry, Shuchleib, Davis, Weiss and Sobel, 1977). A calcium overload is characterised also by hypercontracted muscle fibres (Ruigrok Burgersdyk and Zimmerman 1975),

enzyme leakage (Ruigrok and Zimmerman 1979) and massive membrane and cytosolic disruption (Yates and Dhalla 1975). The control of cytosolic calcium concentration is therefore an important aspect of solution design and has been attempted by blocking the mechanisms which admit calcium and by removing the inward gradient for this ion by reducing its extracellular concentration. However, the first approach is impossible to achieve completely and the second is potentially dangerous.

After some initial excitement that calcium channel blockers might prevent calcium overload it has been shown in rat isolated hearts that these agents do not enhance the protection provided by St.Thomas' cardioplegic solution at the clinically relevant temperature of 20°C (Yamamoto et al 1983, Fukunami and Hearse 1985). Cardioplegia at 20°C without a channel blocker is better than cardioplegia at 37°C with one and consequently interest in these agents has waned in this field of research. The blockade of highly specific channels is in any case an incomplete approach as it ignores many other possible means by which calcium may enter the cell.

The most effective means of preventing calcium influx during cardioplegia is to reduce the extracellular concentration of the ion. Ironically, to do this is to risk initiating a pathological calcium overload during reperfusion by causing the calcium paradox, when return of calcium to a previously calcium-depleted heart results in a total and permanent loss of function (Zimmerman and

Hulsmann 1967). Preventing calcium influx will contribute also to the induction of arrest. It is necessary to confirm that a proposed reduction of calcium concentration is safe before it can form the basis of a successful cardioplegic solution.

Protocol

16 rabbit hearts were used.

8 hearts were perfused with solutions containing progressively less calcium. In order to see what effects, if any, these solutions had, a parallel series of 8 time matched-control hearts were perfused with standard McEwen's solution (see table 1B.1. for composition). The patterns of decline of performance were compared. A fully randomised study would have been impossible because the calcium paradox prevents further work with the same heart.

Each test solution was infused for 8 minutes with a 12 minute recovery period between.

The osmotic potentials of the solutions were not equalised because the differences were very small, with the osmolarity of the final solutions ranging only from 355 to 349.5mOsmol per litre (calculated assuming full dissociation).

The perfusion apparatus and preparation equipment were washed thoroughly with distilled water between test solutions to avoid contamination with calcium. The

distilled water used throughout this study was prepared by double glass-distillation and the maximum contamination of McEwen's solution by calcium contamination of Analar chemicals = 6.5 micro mol/L.

Results

Reducing calcium concentration from 2.18 to 0.73 mM caused a fall of about 10% in the amplitude of contraction (fig. 2A.2), 20% in the heart rate, from which a substantial recovery was made (fig. 2A.3) and had no affect on coronary flow (fig. 2A.1.).

It is obvious that the recovery curves are almost identical to the time-matched control curves until the point at which attempted recovery from perfusion with calcium-free solutions is reached.

Nominally calcium-free solutions resulted in complete loss of mechanical activity. Subsequent perfusion with a physiological calcium concentration did not restore functioning. Instead the hearts entered a permanent hypercontracted state typical of the calcium paradox.

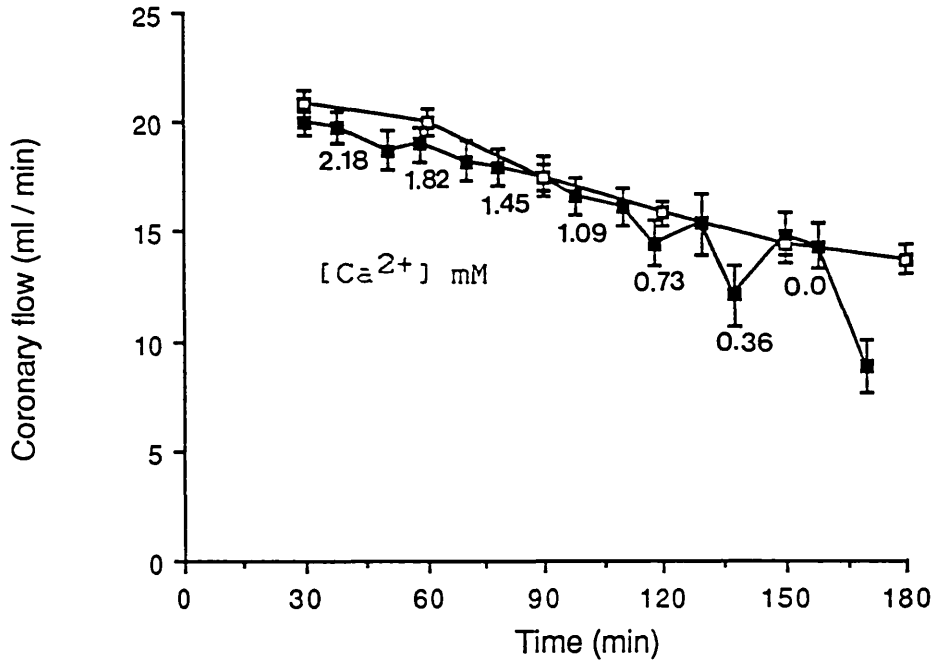


Figure 2A.1

Effects of changes of $CaCl_2$ concentration on coronary flow. Alternate points show the effect of 8 min infusions of solutions based on McEwen's (1956), but containing the $CaCl_2$ concentrations indicated, and the recovery achieved after 12 min of perfusion with standard McEwen's solution. The solutions were infused in order of decreasing calcium concentration and are presented on a scale against time in order that the recovery points can be compared with the time matched control group (open squares). Mean + s.e.m., n = 8 in each group, 16 in total.

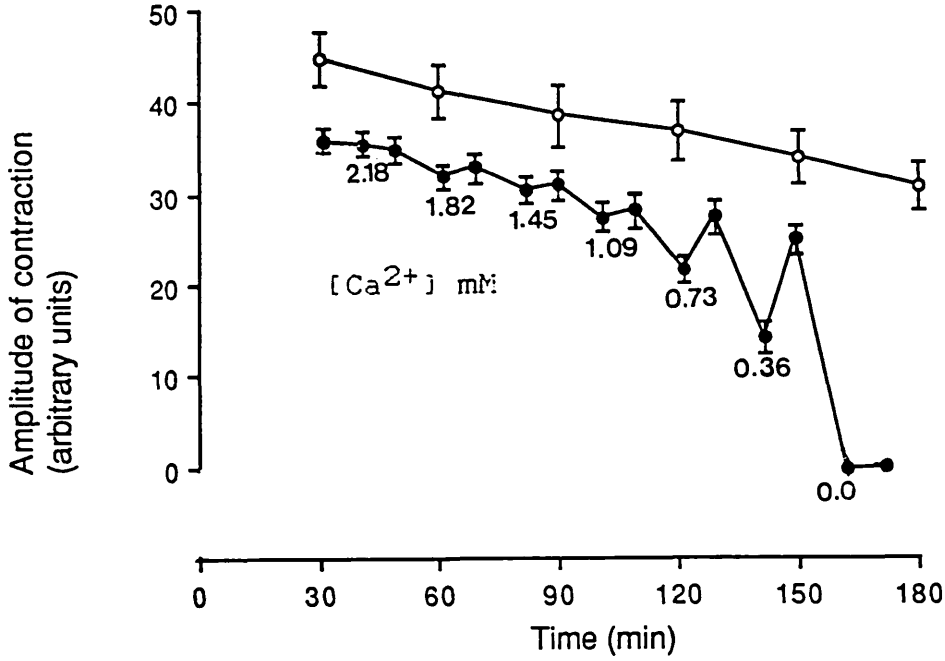


Figure 2A.2

Effects of changes of $CaCl_2$ concentration on amplitude of contraction. Alternate points show the effects of 8 min infusions of solutions based on McEwen's (1956), but containing the $CaCl_2$ concentrations indicated, and the recovery achieved after 12 min perfusion with standard McEwen's solution. The solutions were infused in order of decreasing calcium concentration and are presented on a scale against time in order that the recovery points can be compared with the time matched control group (open circles). Mean + s.e.m., n = 8 in each group, 16 in total.

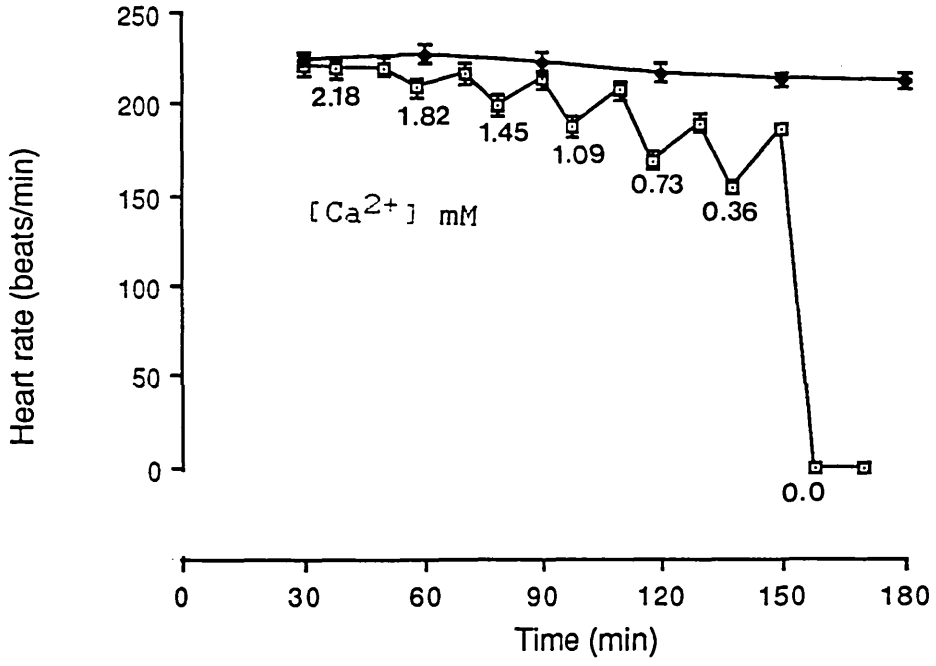


Figure 2A.3

Effects of changes of $CaCl_2$ concentration on heart rate. Alternate points show the effects of 8 min infusions of solutions based on McEwen's (1956) but containing the $CaCl_2$ concentrations indicated, and the recovery achieved 12 min of perfusion with standard McEwen's solution. The solutions were infused in order of decreasing calcium concentration and are presented on a scale against time in order that the recovery points can be compared with the time matched control group (closed diamonds). Mean + s.e. m., n = 8 in each group, 16 in total.

SERIES B

THE CARDIAC AND CARDIOPROTECTIVE EFFECTS OF SODIUM NITROPRUSSIDE IN RABBIT ISOLATED PERFUSED HEARTS, 37°C.

Introduction

It has been suggested that only 25% of the calcium necessary for contraction originates in the extracellular space and that the balance is released from intracellular stores (Fabiato and Fabiato 1979). Similarly, the increase in cytosolic calcium concentration associated with myocardial damage could originate intracellularly where regulation is not possible with currently available cardioplegic solutions.

In smooth muscle it has been suggested that sodium nitroprusside causes relaxation by acting intracellularly to reduce cytosolic calcium concentration (Zsoter, Henein and Wolchinsky 1977). Such an action might also prevent the development of potentially damaging calcium overloads within vascular smooth muscle during cardioplegia and so contribute to an often forgotten aspect of cardioprotective solutions. If the same effect were mediated also on myocardium, sodium nitroprusside might provide a significant advance in myocardial protection as well.

Protocol

36 rabbit Langendorff hearts were used.

After the usual 30 minute settling down period hearts were perfused for two minutes with either standard McEwen's solution (as a control), or McEwen's solution containing sodium nitroprusside (10^{-7} to 10^{-3} M). All perfusion was then stopped for thirty minutes. Perfusion with standard McEwen's solution was resumed for a thirty minute recovery period. The sequence of infusion, period without flow and 30 minute recovery period was followed a total of five times on each heart, using the same test solution each time.

There were 6 hearts in each of six treatment groups and the experiments were carried out in a random order.

Results

Infusions containing sodium nitroprusside increased coronary flow. The effects of the first 2 minute infusion period are shown (fig. 2B.1.), also increases of heart rate occurred at some concentrations.

There were neither significant nor consistent changes in the amplitude of contraction or the time until onset of arrest once coronary perfusion was stopped.

Generally, the greater proportion of the recovery of amplitude of contraction was achieved within the first five minutes of reperfusion and reached its maximum within fifteen minutes.

The coronary flow was always highest at the beginning of reperfusion, especially when high concentrations of sodium nitroprusside had been present (fig. 2B.4). The flow and heart rate both declined slightly during the recovery period.

Coronary flow in the early stages of reperfusion was greater in hearts which had received sodium nitroprusside and the effect appears to be concentration dependent. The recoveries of coronary flow and amplitude of contraction were good for all groups following the first period without flow. Recovery from the second to the fifth period without flow were almost twice as good when sodium nitroprusside (10^{-4}, and 10^{-3}M) had been present as compared with the standard McEwen's solution control ($P<0.05$). The recoveries from the 2nd and 4th periods without flow were intermediate between their numerical neighbours and have been omitted from the graphs presented only to make the figure less congested and thereby easier to read (fig. 2B.2).

There were no differences between the groups in the extent of recovery of heart rate.

In the study as a whole, there were 45 episodes of ventricular fibrillation of which 41 began within the first 5 minutes of reperfusion and only 6 reverted to normal rhythm spontaneously within the 30 min recovery periods. Sodium nitroprusside (10^{-4} and 10^{-3} M) reduced the proportion of hearts fibrillating ($P<0.05$, Chi Square) and the number of episodes of fibrillation

(P<0.05, Chi Square) as compared to the control group (fig. 2B.3.), but had no antiarrhythmic effect when infused during fibrillation.

Stopping the perfusion during VF resulted in a correction of that state in all hearts some 2-4 minutes later. This phenomenon is here termed an "anti-fibrillatory paradox" and investigated further in sections 2F and 2G, in which typical tracings are presented.

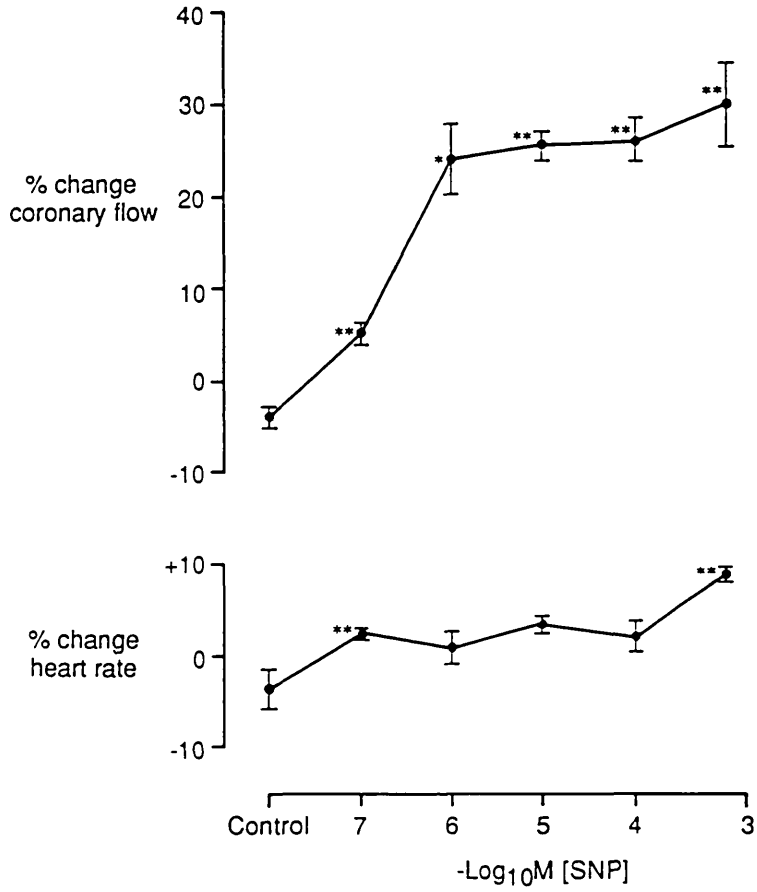


Figure 2B.1

Effects of sodium nitroprusside on heart rate and coronary flow. The percentage changes in coronary flow and heart rate immediately after the first 2 min infusion of McEwen's solution + sodium nitroprusside are shown. Each point is the mean and the bars the s.e.m. of 6 observations in each group (n = 36 in total). Values significantly different from the control are indicated * P<0.05, ** P<0.01. (unpaired "t" test).

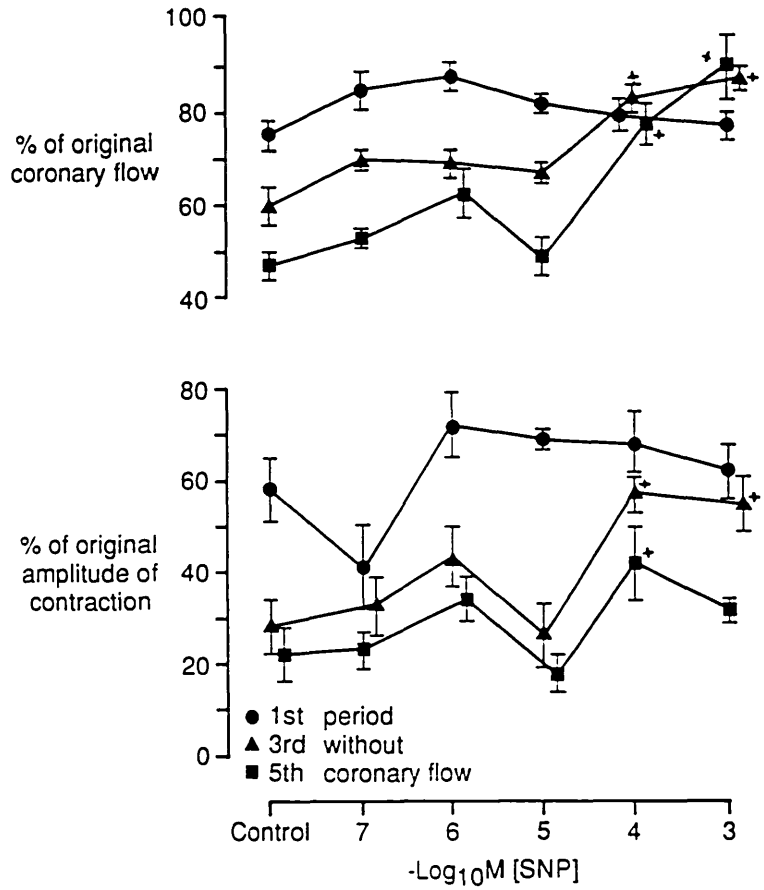


Figure 2B.2

Extent of recovery of coronary flow and amplitude of contraction after periods without perfusion preceded by infusions of sodium nitroprusside. Recovery 30 min after the first (circles), 3rd (triangles) and 5th (squares) 30 min periods without perfusion are expressed as percentages of the values recorded 30 min after setting up the hearts. Flow free periods were preceded by a 2 min infusion of McEwen's solution (control) or McEwen's solution + sodium nitroprusside. Each point is the mean and the bars the s.e.m. of 6 observations in each group (n = 36 in total). Values significantly different from the control are indicated * $P<0.05$, (unpaired "t" test).

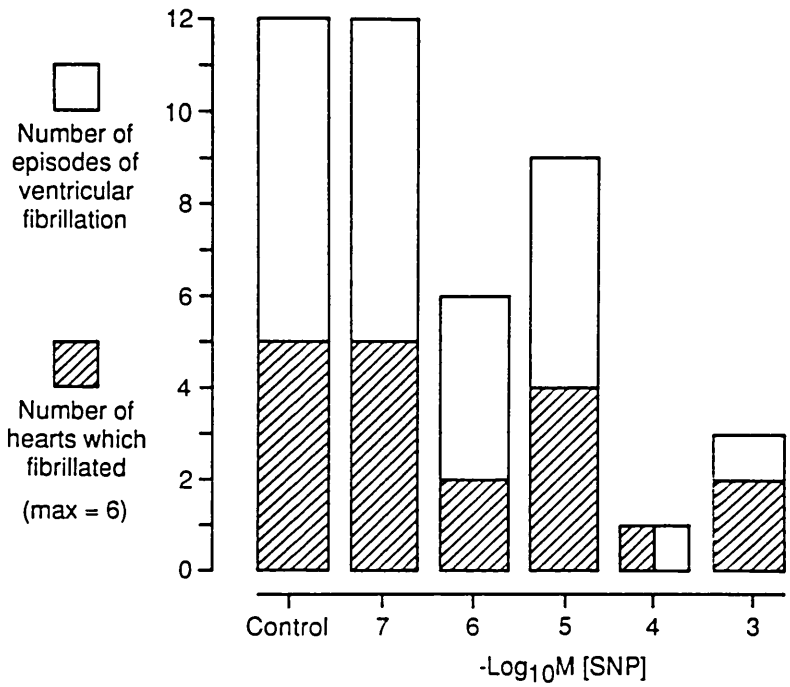


Figure 2B.3

Effect of pretreatment with sodium nitroprusside on the incidence of ventricular fibrillation during reperfusion. The number of hearts (open) (max=6) which fibrillated and the number of episodes (hatched) of fibrillation during reperfusion are shown. Each of the five, 30 min periods without perfusion was preceded by a two minute infusion of McEwen's solution (control) and McEwen's solution + sodium nitroprusside.

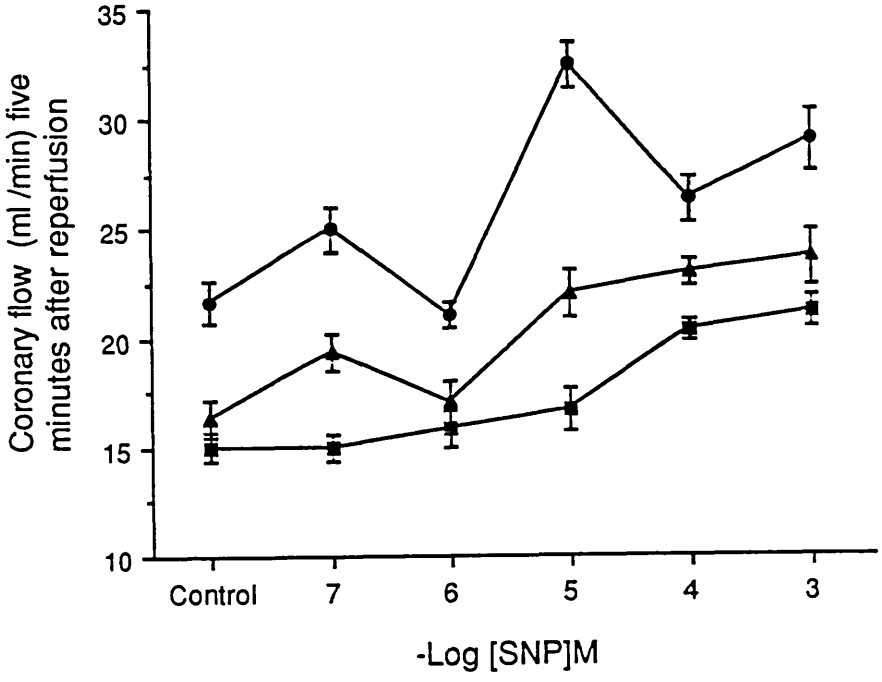


Figure 2B.4

Effect of sodium nitroprusside given before periods without perfusion on the coronary flow early in reperfusion. The coronary flow after 5 min of reperfusion following the first (circles), 3rd (triangles) and 5th (squares) 30 min periods without perfusion is shown. Each flow free period was preceded by a 2 min infusion of McEwen's solution (control) or McEwen's solution with added sodium nitroprusside. Each point is the mean and the bars the s.e m. of 6 observations (n = 36 in total).

SERIES C

INTERACTIONS BETWEEN THE EFFECTS OF SODIUM NITROPRUSSIDE AND CALCIUM ON RABBIT ISOLATED HEARTS, 37°C.

Introduction

Sodium nitroprusside relaxes vascular smooth muscle and appears to act directly on the myocardium. It is probable that these effects are related to the intracellular availability of calcium and could therefore be mediated by actions affecting the entry of calcium or the intracellular localisation of the ion. If the latter is the case it suggests that apparently cardioprotective effects of sodium nitroprusside may be additive to those of present cardioplegic solutions.

Protocol

Six rabbit hearts were set up as usual and left for 30 minutes to settle down.

Solutions containing concentrations of calcium chloride ranging from 0.36mM to 2.18mM, in the presence and absence of sodium nitroprusside (10^{-4}M) but otherwise identical with McEwen's solution, were infused in a random order for 4 minutes with a 10 minute recovery period between. (see section 2A for possible levels of calcium contamination).

Results

The fall in heart rate, fall in amplitude of contraction and the apparent lack of effect on the coronary flow of reducing calcium concentrations in this randomised study are in agreement with the results presented in section 2A.

Similarly, the increase in flow and heart rate, coupled with an apparent lack of an effect on the amplitude of contraction seen in the presence of sodium nitroprusside are in agreement with the results presented in section 2B.

Sodium nitroprusside had no effect on the response of amplitude of contraction to changes in calcium concentration. The marked increase in flow and slight increases in heart rate produced by sodium nitroprusside appear to be independent of calcium concentration.

The regression coefficients for each graph were for amplitude of contraction 0.86 and 0.85, for heart rate, 0.89 and 0.77, and for coronary flow 0.06 and 0.11, in the presence and absence of sodium nitroprusside respectively.

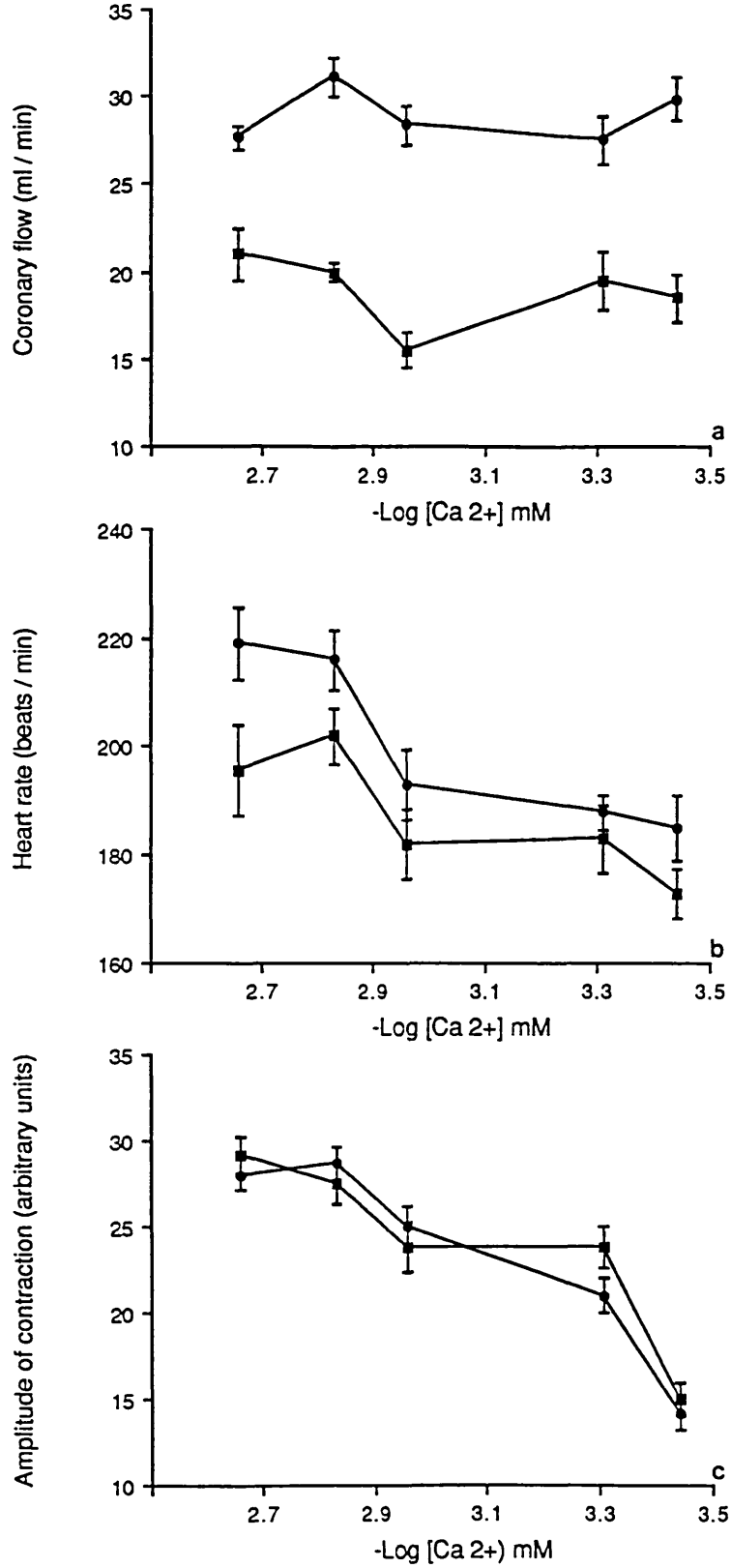


Figure 2C.1

The effects of sodium nitroprusside on the response of coronary flow, amplitude of contraction and heart rate to changes in $CaCl_2$ concentration. Solutions based on McEwen's solution but containing different calcium chloride concentrations in the presence (circles) and absence (squares) of sodium nitroprusside (10^{-4}M) were infused for 4 min in a random order with 10 min between each. Each point is the mean and the bars the s.e m. of 6 observations, n=6.

SERIES D

EFFECT OF SODIUM NITROPRUSSIDE ON THE RESTING MEMBRANE POTENTIAL OF RABBIT ISOLATED ATRIAL TISSUE, 28°C.

Introduction

Sodium nitroprusside has been shown in several studies to hyperpolarise vascular smooth muscle (Haueusler and Thorens 1976. Cheung and MacKay 1985), but the effect on myocardium is less clear. The following experiments were done to see what effect, if any, sodium nitroprusside has on the membrane potential of rabbit atrial strips.

Protocol

8 rabbits were killed in the usual way and their hearts excised. They were placed immediately in cold gassed McEwen's solution where the atria were removed. The atria were transferred to fresh solution which was bubbled continuously and in which they remained for 30 minutes. They were then separated and a section comprising most of the inferior wall of the left atrium was placed, epicardial surface uppermost, in the organ bath. The preparation was left for a further 15 minutes with the flow rate of solution through the bath constant at 5ml min. Membrane potentials were then recorded in at least 5 different cells in the same area of the preparation. Perfusion was then changed to McEwen's

solution to which had been added sodium nitroprusside. The flow rate through the bath was maintained at 5ml/min and with the lid of the shielding box down bright light was excluded. The solution was left to bathe the preparation for 5 minutes before membrane potentials were recorded (in the same area as the control values). Between 5 and 10 impalements were made for each concentration on each preparation and at least 5 control values were recorded between each of the test solutions, which were tested in a random order. This same protocol and the same preparations were used also to determine the effect of two cardioplegic solutions on resting membrane potential. The results of these experiments are given in the discussions of Section 3 and Section 4.

Results

Incubation with sodium nitroprusside caused significant ($P < 0.05$, ANOVA) increases in membrane potential (fig. 2D.1). The accuracy of the technique used to determine membrane potential is discussed in the general methods section (1B.3).

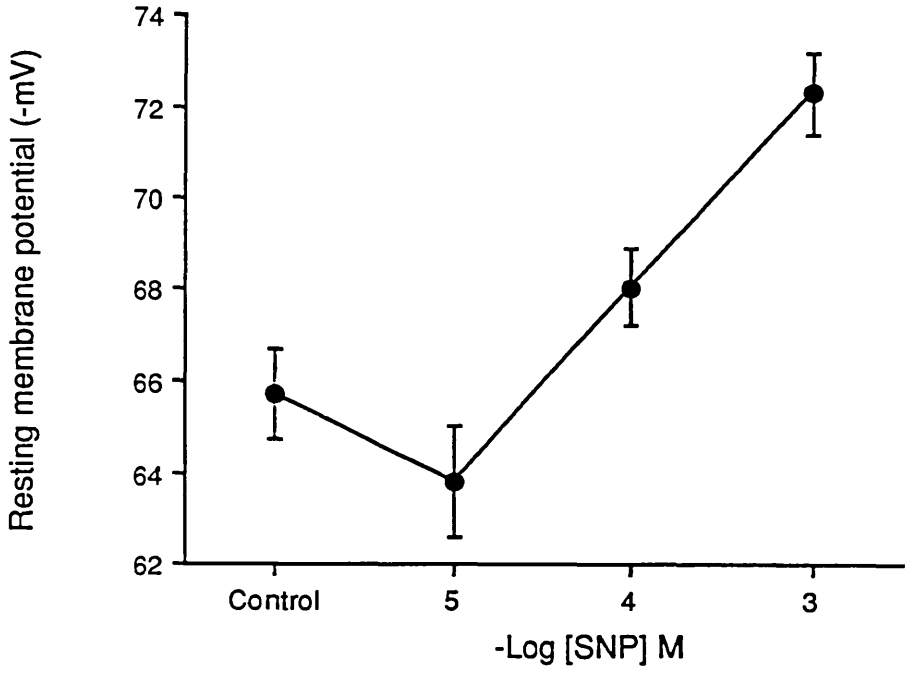


fig. 2D.1

The effect of sodium nitroprusside on the resting membrane potential of rabbit isolated atrial tissue, 28°C. Each point is the mean and the bars the s.e.m. of values obtained in 8 preparations (each value being the mean of 5 seperate impalements per preperation). The control and sodium nitroprusside experiments were conducted in a random order.

SERIES E

THE CARDIOPLEGIC EFFECTS OF RAISED KCL CONCENTRATIONS AND THEIR MODIFICATION BY SODIUM NITROPRUSSIDE COMBINED WITH A LOWERED $CaCl_2$ CONCENTRATION ON RABBIT LANGENDORFF HEARTS, 37°C.

Introduction

The effects of changes in the concentration of potassium salts in solutions perfusing isolated hearts have been studied extensively since Ringer (1883) identified the importance of this ion. However, in the field of cardioplegia it is mainly the induction of arrest which has been investigated and other important effects on electrical activity and the suppression of "escape beats" have not. As the widely used term "myocardial protection" suggests, the possible effects of cardioplegic solutions on the coronary vasculature have received scant attention. The effects of changes in ionic concentrations are complex. The possible interactions between potassium and the concentrations of calcium and sodium nitroprusside, suggested from earlier experiments to be beneficial, may be unfavourable and must therefore be investigated also.

Protocol

8 rabbit hearts were used.

Following the usual thirty minute settling period each heart was perfused with solutions containing potassium concentrations ranging from 14 mM to 26mM in steps of 2mM. The solutions were based on that of McEwen, but did not contain sucrose or glucose. The reasons for this omission were twofold.

i) The osmotic space so created allowed the extra potassium to be accommodated with a maximum increase in osmolarity of only 16 mOsmols. A reduction in the sodium chloride concentration, which is customary in the equalisation of osmotic potentials, was not made because of the importance of sodium concentration to cardiac electrophysiology and to the development of the calcium paradox (see discussion).

ii) Glucose has been shown to be a harmful component of cardioplegic solutions (Hearse, Stewart and Braimbridge 1978).

Two types of solution were tested. i) Basic. These contained the sodium salts and calcium chloride in the concentrations found in McEwen's solution but had elevated concentrations of potassium chloride. ii) Combined. These differed from the Basic solutions in containing sodium nitroprusside (10^{-4}M), a concentration identified by the previous experiments as possessing some cardioprotective properties (see Section 2B) and had a

lower calcium concentration (0.73mM instead of 2.18), shown by earlier experiments to be safe and likely to be cardioprotective (see Section 2A).

4 hearts received all the Basic followed by all the Combined solutions. The other four hearts received the Combined solutions first. The order in which the different potassium concentrations were infused was different for each heart but retained for the Basic and Combined solutions.

Results

As potassium concentration increased so did the number of hearts arrested. All hearts receiving a potassium concentration of 22mM in Basic or 16mM in the Combined solutions were arrested (fig. 2E.2).

The time to arrest decreased with increasing potassium concentration, but reached a minimum of about 25 seconds for the Basic solutions and 20 seconds for the Combined solutions (fig. 2E.1).

Ventricular mechanical arrest was not always followed by electrical silence but it was more common when higher concentration of potassium were infused (fig. 2E.2).

Increasing the potassium concentration increased the number of hearts arrested and initially the incidence of escape beats. This is because by definition escape can occur only after arrest. A typical tracing showing escape beats is presented in Section 2G. Increasing the

potassium concentration past the point at which all hearts were arrested reduced the proportion that escaped. However, even at a concentration of 26mM potassium, only 50% of the sample infused with the Basic solution were arrested without escape beats (fig. 2E.2) whereas the Combined solution with a potassium concentration of only 20mM arrested the whole sample without escape beats. The amplitude of the escape beats was remarkably constant at approximately 35 per cent of the amplitude of contraction measured immediately before infusion of the Basic solutions containing 20, 22, 24, 26 mM potassium. With the Basic solutions the time to escape after arrest increased from 32 seconds (s.e.m. 8s) with 20mM potassium to 60 seconds (s.e.m. 30s) with 26 mM potassium, but there were too few escape beats following infusion of the Combined solutions to enable statistical comparisons to be made. The mechanisms responsible for escape beats are investigated further in Section 2G. For any particular concentration of potassium there was a decrease in coronary flow which progressed during the four minutes of infusion (fig. 2E.3) and its extent was seemingly dependent on the concentration of potassium. In contrast, a sustained increase in coronary flow occurred during the 4 minutes of infusion of the Combined solutions.

The effects on the coronary flow are described in more detail for a potassium concentration of 24mM, (fig. 2E.3b). This concentration was tested because the

results of preliminary experiments suggested that such a concentration was likely to prove suitable for an extracellular type cardioplegic solution. It is clear that the two solutions have very different effects on the coronary flow. The increase in flow during induction of arrest was greatest with the Combined solution and although flow rate fell slightly during the 8 minute infusion periods it was at the end still greater than before infusion of the test solution. The Basic solution produced a smaller initial increase in the flow, which declined steadily so that by the end of the 8 min infusion period flow rate was less than before infusion of the test solution.

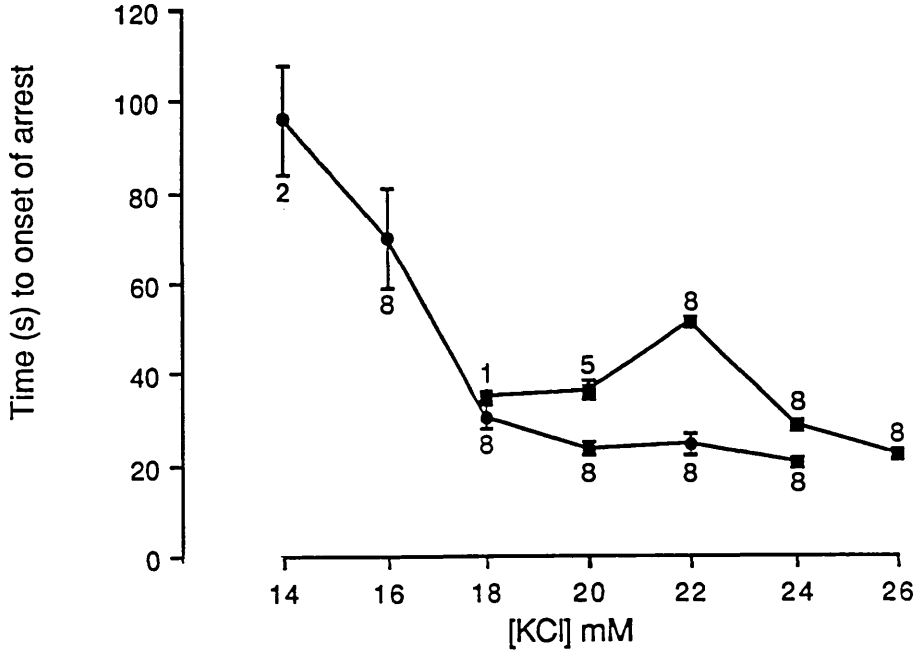
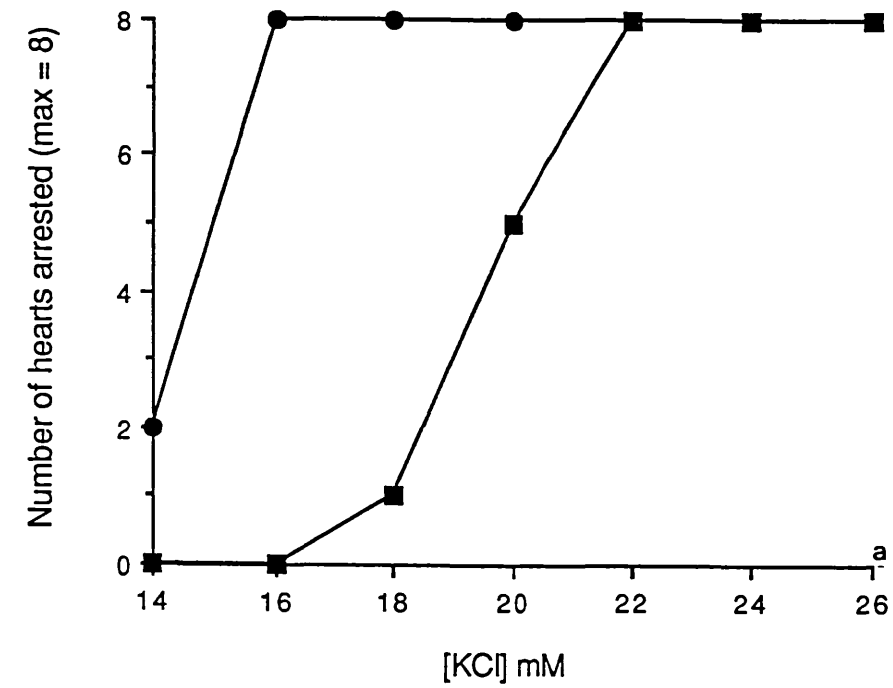
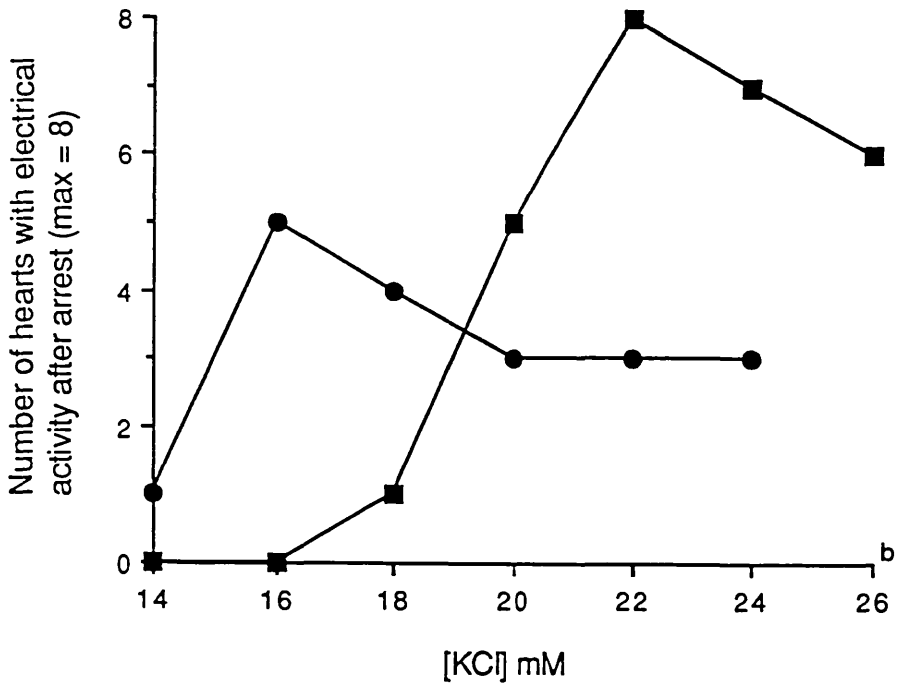


Figure 2E.1

The time to arrest following infusion of potassium-rich solutions. The time to arrest (s) during infusion of Basic (squares) and Combined (circles) potassium rich solutions is shown. Each point is the mean, and the vertical bars the s.e.m., the number of hearts is given in brackets, (maximum possible = 8).


Number of hearts arrested (max = 8)
8
6
4
2
0
14
16
18
20
22
24
26
[KCl] mM
a


Number of hearts with electrical activity after arrest (max = 8)
8
6
4
2
0
14
16
18
20
22
24
26
[KCl] mM
b

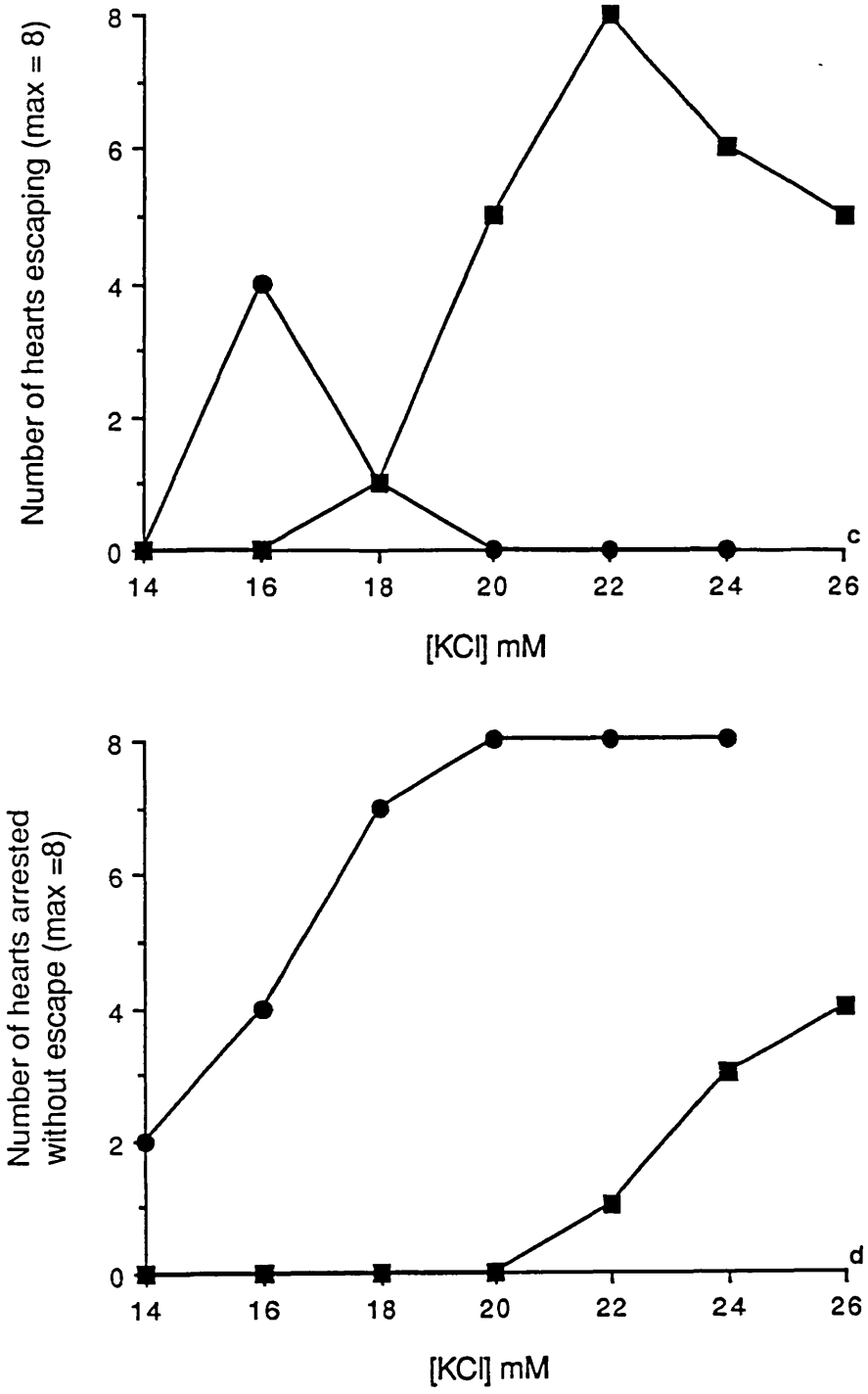


Figure 2E.2

The incidence of arrest, electrical activity and escape in hearts arrested with potassium-rich solutions. a) The number of hearts arrested, b) the number of hearts with electrical activity, c) the number of hearts escaping and d) the number of arrested hearts not escaping are shown. Combined (circles) and Basic (squares) solutions were infused for 4 minutes. Maximum possible = 8 (a difference of 7 is statistically significant, ($P<0.05$, MacNamar's test).

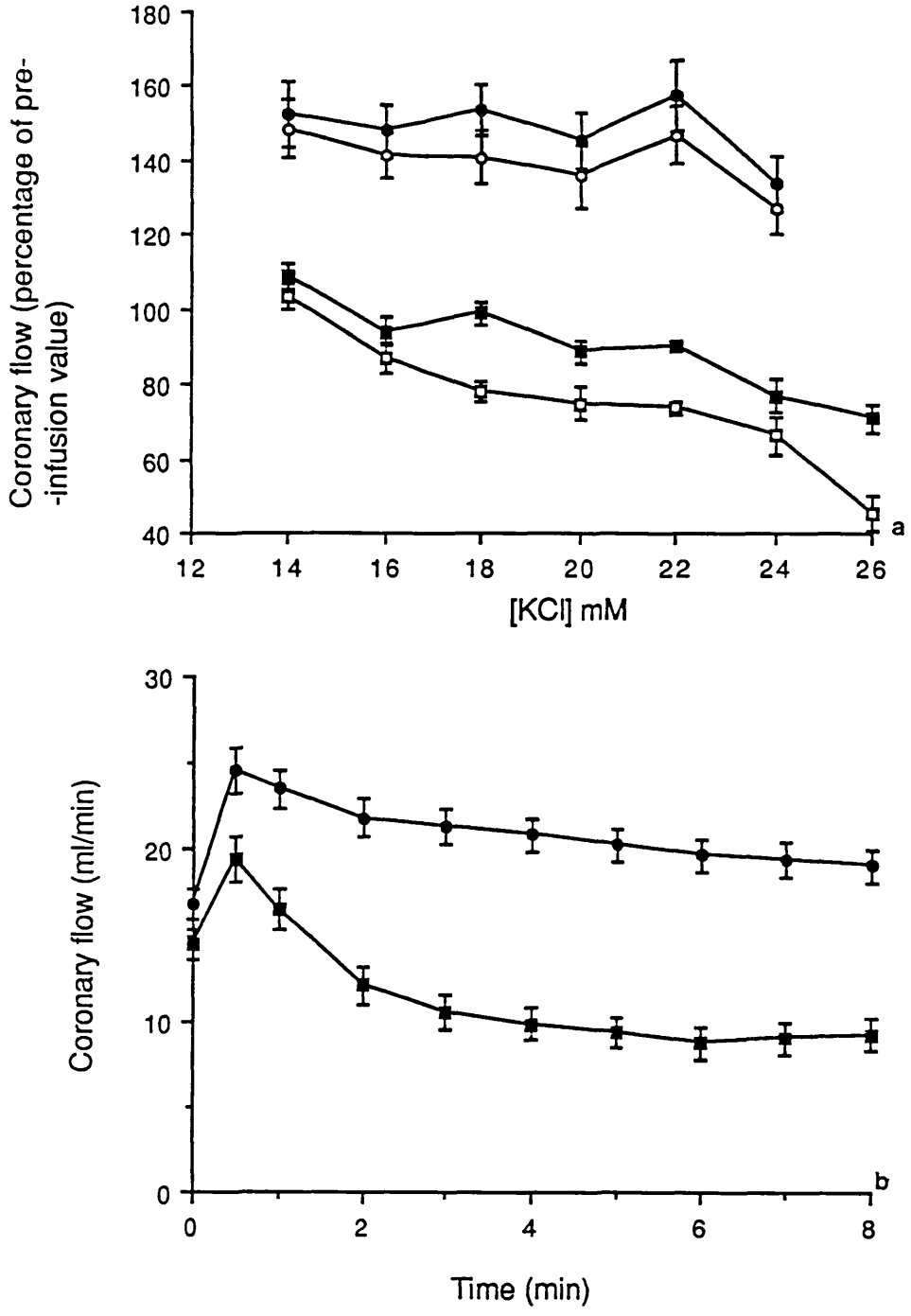


Figure 2E.3

The effect of potassium-rich solutions on coronary flow. a) The difference between coronary flow before and after 2 minutes (open) and 4 min (closed) infusion of Basic (squares) and Combined (circles) potassium rich solutions is expressed as a % change. b) the effect of 8 min infusion of solutions containing 24mM KCl is shown. Coronary flow immediately before infusion is shown as are the points at which diastolic arrest occurred. The Basic solution (squares) and Combined solution (circles) are shown. Each point is the mean and the vertical bars the s.e.m. of 8 observations, n = 8.

SERIES F

CARDIAC FUNCTIONING AFTER PROLONGED PERIODS OF VENTRICULAR FIBRILLATION AND A CHARACTERISATION OF THE ANTI-FIBRILLATORY PARADOX IN RABBIT LANGENDORFF HEARTS. 37°C.

Introduction

Ventricular fibrillation (VF) is an interesting phenomenon in that it prevents cardiac output but does not necessarily damage the heart directly, so reversion to normal rhythm may result in total recovery. However, the method by which VF is induced and the conditions under which it is maintained, particularly with regard to temperature and perfusion of the heart, are variables which complicate the calculation of a "safe" duration. Arrhythmias generally and ventricular fibrillation in particular are the consequence of changes in the regulation of transmembrane ionic movements. The mechanisms which predispose to ventricular fibrillation may therefore be regarded as those which cause membrane disturbances. The incidence of ventricular fibrillation and the ability to correct it is an indication of the extent to which these aspects of cell functioning have been protected and it provides information on the effectivness of protection, which is not related directly to the functioning of the contractile elements.

Protocol

8 Rabbit Langendorff hearts were used.

The hearts were set up in the usual manner. Ventricular fibrillation was induced by pacing at increasing frequency (1Hz to 100Hz) with square wave pulses of 24V amplitude and 10mS duration generated by a Fenton-Lewis stimulator (constant voltage output). The saline wick stimulating electrodes were placed one above the other 8mm apart on the left ventricle. For the purposes of this experiment VF was considered to be present when electrical activity was chaotic, co-ordinated beating was absent and continuous tension approached or exceeded that attained during systole (see typical tracings, fig. 2F.2 and 2H.3). Stimulation was then stopped and the VF left to persist for 1 to 32 minutes. The coronary flow was then turned off until co-ordinated electro-mechanical activity resumed and an apparently normal ventricular beat could be seen directly or as a typical deflection on the oscillograph. In one series of control experiments (n=8) coronary flow was stopped for 4 minutes, to provide an estimate of the loss of function resulting from the coronary occlusion required to correct the fibrillation. In another series (n=8) fibrillation was induced and immediately coronary flow suspended until the fibrillation was corrected. This was done to enable an estimate to be made of the possible effects that the induction as well as the correction procedure may

themselves exert on the parameters of function recorded. The hearts were left to recover for at least ten minutes or until the amplitude of contraction had reached a steady state of recovery, which was in every case less than 15 minutes, before VF was induced again. Different durations of ventricular fibrillation and the control experiments were performed in a random order.

Results

Coronary flow increased by the end of short periods of fibrillation (1-2min) and fell slightly by the end of long periods (4-32 min), whereas after VF had ceased it was decreased by a maxiumum of 15%, this occurring after only 4 minutes of VF ($P<0.05$, paired "t" test). Similarly, heart rate recovered after VF almost completely. In marked contrast, amplitude of contraction was affected greatly, even after short periods of VF (fig. 2F.1). Following termination and recovery from 2 minutes of VF amplitude was 15 per cent lower, after 8 minutes 44 per cent lower and after 32 minutes 65 per cent lower than immediately before VF ($P< 0.05$, ANOVA) (fig. 2F.1). There were no significant differences between the spontaneous heart rates before and after fibrillation.

The unexpected ability of an interruption in coronary flow to correct venticular fibrillation, seen in doing the experiments described in section 2B, was confirmed by

this series of experiments in which 62 out of 63 episodes of VF of greatly different duration were corrected by such an interruption. Furthermore, a relationship was seen in which longer durations of VF ultimately required longer interruptions in flow to correct them ($P<0.05$, ANOVA) (fig. 2F.3.).

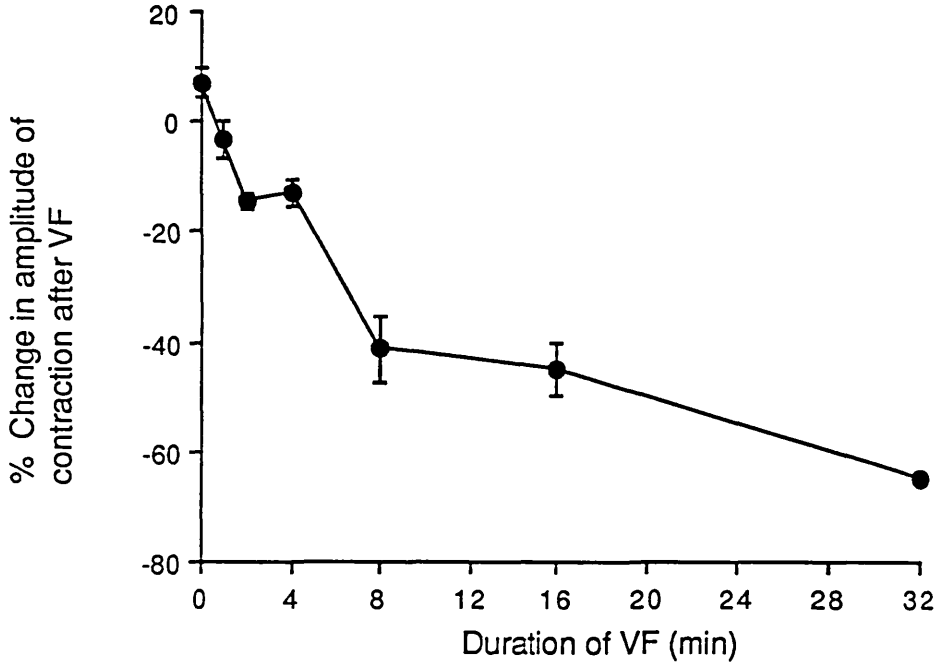


Figure 2F.1

The extent of recovery of amplitude of contraction after periods of ventricular fibrillation. The difference between the amplitude of contraction after recovery from ventricular fibrillation and immediately before is expressed as a percentage change. The effect of 4 min without perfusion and of fibrillation immediately followed by the correction procedure (given as VF duration 0 min) are shown also. Each point is the mean and the vertical bars the s.e.m. of 8 (*=7) observations.

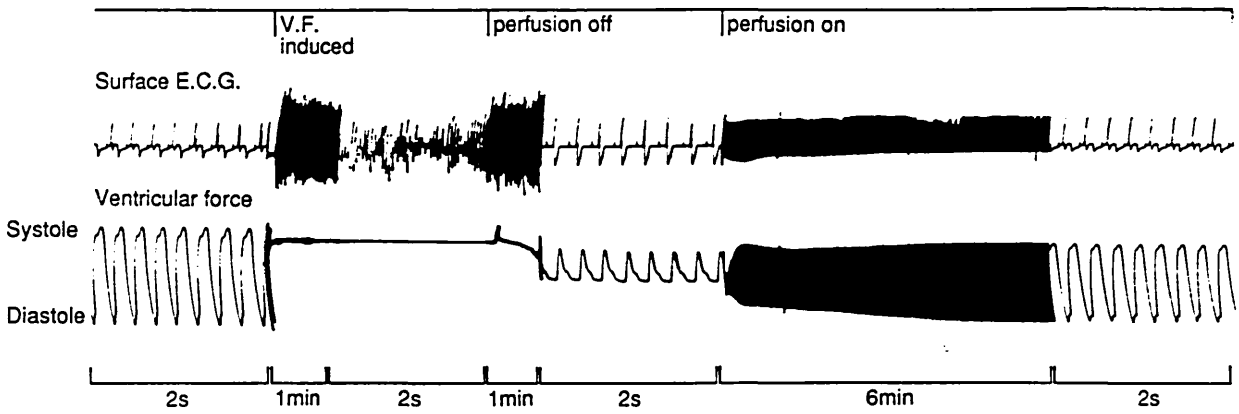


Figure 2F.2

Typical tracing of an "anti-fibrillatory paradox" in rabbit isolated heart, 37°C.

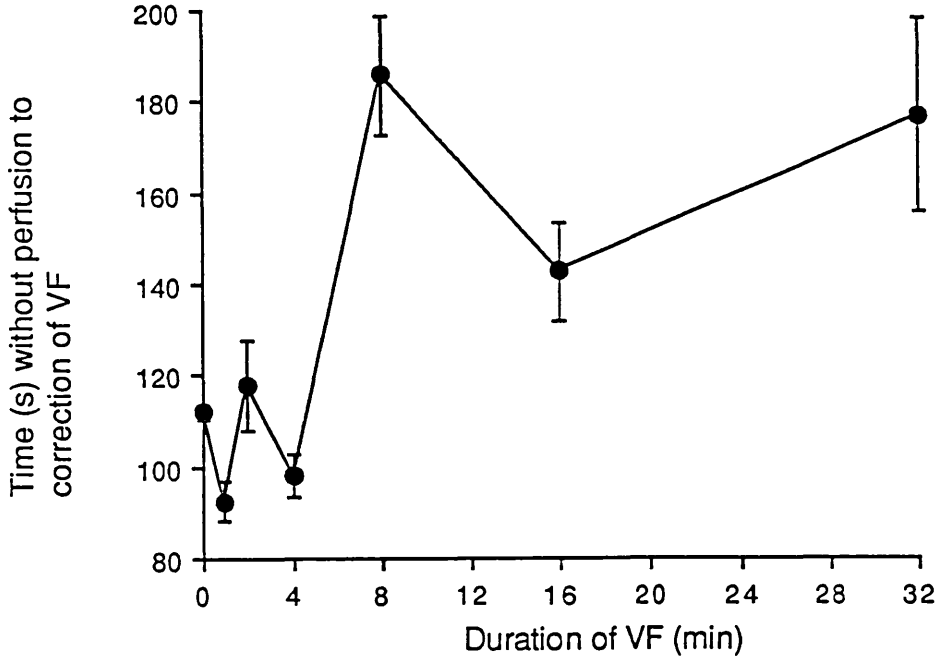


Figure 2F.3

The time without perfusion required to correct ventricular fibrillation. The time (s) after coronary occlusion during ventricular fibrillation to the appearance of a recognisable beat is shown for different durations of ventricular fibrillation. Each point is the mean and vertical bars the s.e.m. of 8 (*=7) observations.

SERIES G

MECHANISMS WHICH UNDERLIE THE ANTIFIBRILLATORY PARADOX AND ESCAPE BEATS IN RABBIT, RAT AND GUINEA-PIG ISOLATED HEARTS, 37°C.

Introduction

The potential of hearts to escape from arrest and the apparent ability of an interruption in coronary flow to correct ventricular fibrillation are important observations relevant to the provision of safe operating conditions and subsequent recovery of the heart. The previous experiments suggest a common causative mechanism which is investigated further in this short series of experiments.

Protocol

12 Rabbit, 5 guinea-pig and 8 rat hearts were used.
In this series of experiments the rabbit hearts were rotated in the vertical axis 90° anti-clockwise from the usual orientation within the perfusion apparatus to make it easier for attaching an isotonic transducer to the left atrium. This transducer had a load of 1g and was in addition to the usual semi-isotonic transducer attached to the apex of the ventricles. Two pairs of silver stimulating electrodes were attached, one pair to the right atrium and one to the left ventricle. Saline wicks

were not used for stimulation because of limited space and the need to maintain position exactly, but were used in the usual manner to record surface ECG activity. The arrangement was designed to allow atrial and ventricular mechanical activity to be monitored almost independently of each other and the effects of direct atrial or direct ventricular stimulation to be seen.

Each heart was left for the usual 30 minutes in which to settle down after being placed in the perfusion apparatus with the ventricular transducer attached. The atrial force transducer, the recording and both sets of stimulating electrodes were then positioned and the heart left for a further 15 minutes before the experiment proper was begun.

The rat and guinea-pig hearts were obtained in the manner described in Section One from animals being used for other purposes in the laboratory.

Results

The rabbit heart preparations were successful in enabling atrial and ventricular activity to be recorded simultaneously but independently of one another in the same heart (fig. 1B.5).

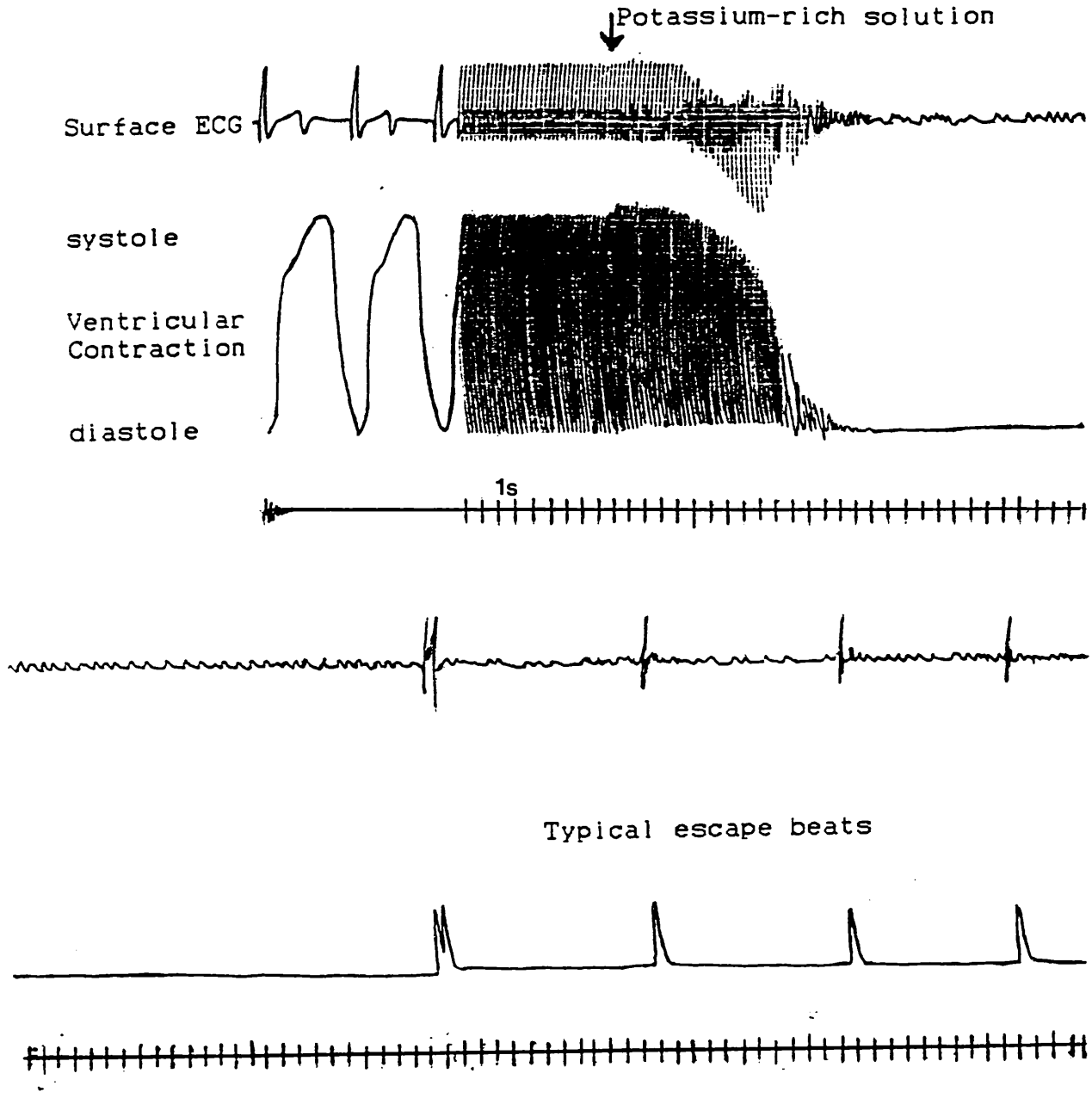


Figure 2G.1

Typical tracing of an escape beat after potassium-induced arrest of a rabbit isolated heart. Arrest was induced by a solution contining sodium salts as present in McEwen's solution, the usual calcium concentration and a potassium concentration raised to 22mM.

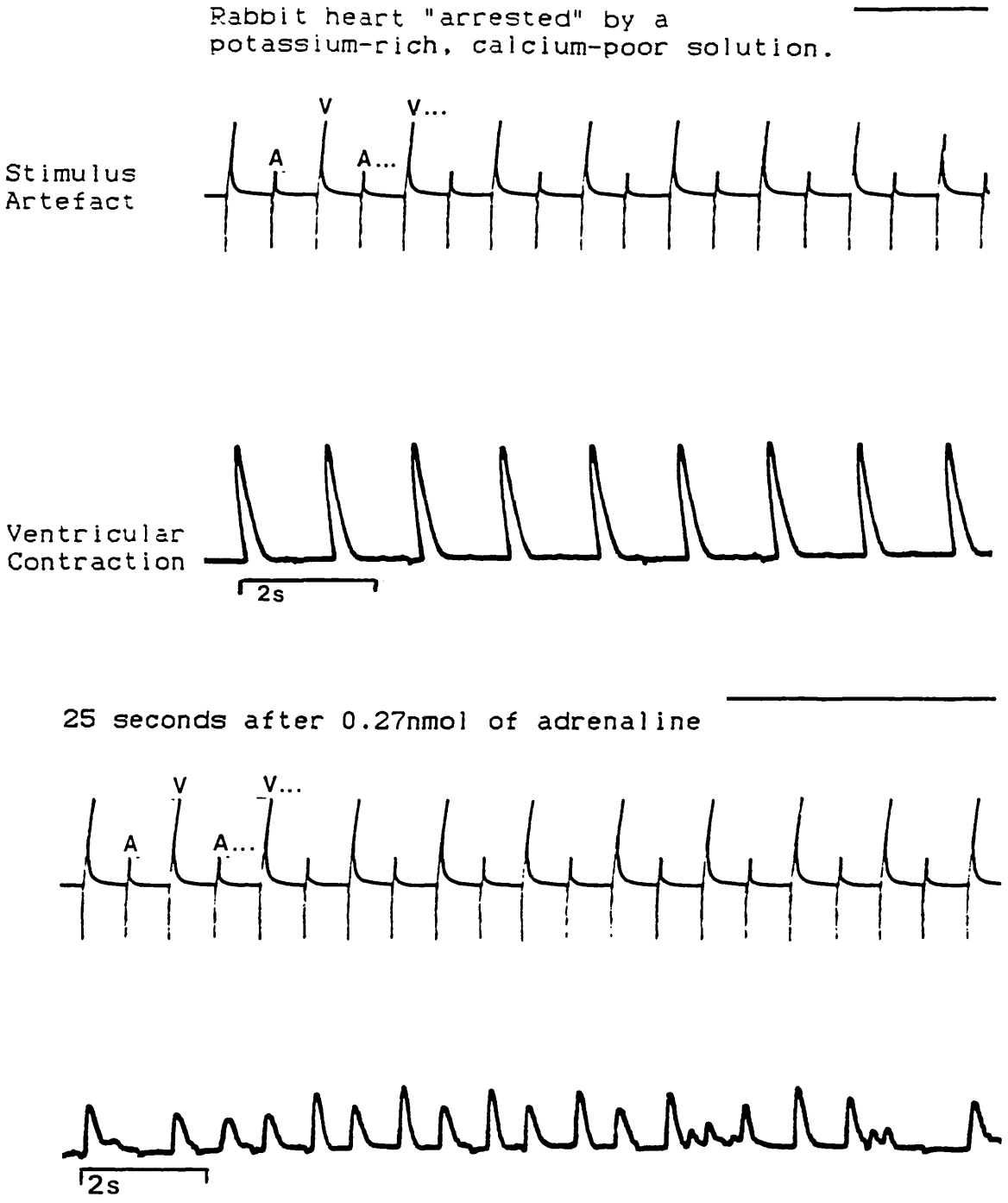


Figure 2G.2.

Typical response of ventricles to artificial electrical stimulation of the right atrium and left ventricle after arrest induced by potassium and low calcium and the modification of this response by adrenaline. A rabbit isolated heart was arrested by a solution containing sodium salts as present in McEwen's solution, 22mM KCl and a lowered $CaCl_2$ concentration (0.73mM). No escape beats were observed for two minutes. Electrical stimulation alternating between the right atrium (A) and the left ventrical (V) was started (the ability of either site of stimulation to evoke a ventricular response had been confirmed immediately beforehand). Adrenaline was given in a volume of 0.2ml and the coronary flow rate at that time was 18ml/min).

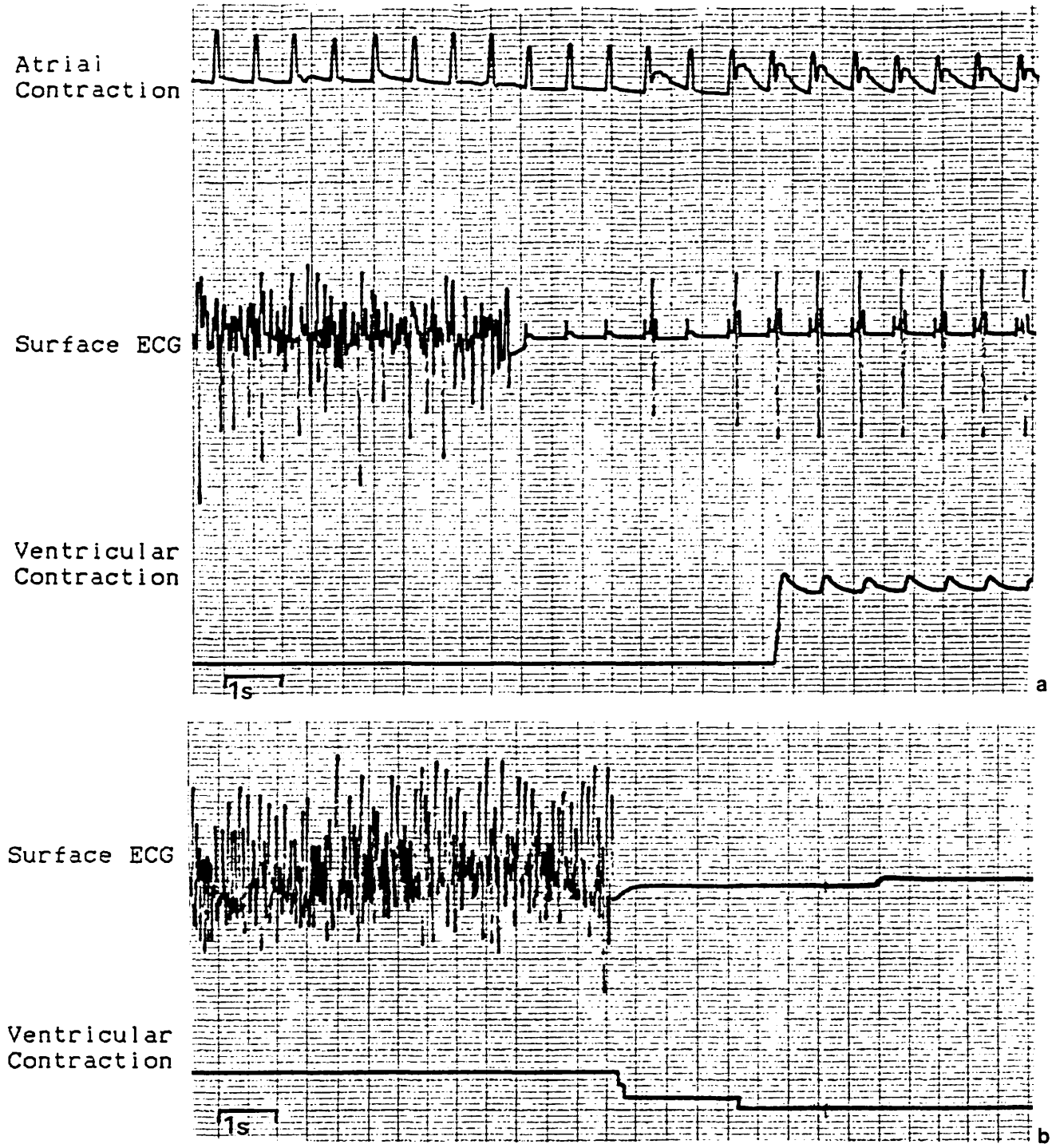


Figure 2G.3

Typical tracing showing the anti-fibrillatory paradox in the presence and absence of the atria. Ventricular fibrillation was induced by pacing the ventricles at increasing frequency (the pacing was stopped after fibrillation was induced). After fibrillation had persisted for 90s the coronary flow was stopped. The figure shows the recording approximately 90 seconds after the cessation of coronary flow. Part (a). a typical tracing from a rabbit whole heart b). a typical tracing obtained from a rabbit heart from which the atria had been removed prior to inducing the fibrillation.

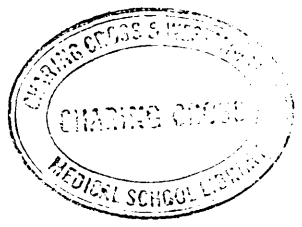
In all 8 rabbit hearts tested, infusion of a solution containing 22mM KCl without glucose or sucrose but otherwise identical to McEwen's solution, arrest was followed by escape beats (fig. 2G.1). In the same hearts, infusion of a similar solution in which the calcium concentration was only 0.73mM, there were no escape beats. The ventricles did respond to direct stimulation but not to stimulation of the atria or indeed any spontaneous atrial activity when it was present (fig. 2G.2). A ventricular response to atrial stimulation was restored by a dose of adrenaline at the bottom of the dose response curve for this agent (usually about 0.27nmol in 0.2ml 0.9% NaCl + ascorbic acid (pH 4) over two seconds into perfusion solution flowing at about 18ml/min).

In all 10 rabbit hearts the atria continued to beat, albeit dysrhythmically, during ventricular fibrillation. The atria were removed from 5 of the perfused rabbit hearts. This procedure did not subsequently prevent the induction of ventricular fibrillation. In the absence of the atria, stopping perfusion during fibrillation led as usual to complete electrical silence within 3 minutes. However, unlike the situation where atria were present, no ventricular beats occurred (fig. 2G.3). The ventricles did respond, in the continuing absence of perfusion, to direct electrical stimulation and in this respect the situation was very similar to that after arrest by a raised extracellular potassium concentration.

In all 5 appropriately instrumented rabbit hearts it was impossible to record a change in temperature from a probe (accurate to 0.1°C) positioned in the right ventricle before the correction of VF. This result confirms that when used as intended the Baker (1951) perfusion system maintains the heart at a given temperature independently of coronary flow rate and that the anti-fibrillatory paradox is not a consequence of a change in temperature associated with the cessation of perfusion.

In 6 rabbit hearts correction of fibrillation occurred within 3 minutes of the cessation of coronary flow. In the same hearts a 90% reduction in flow rate (from 10-20 to 1-2 ml/min, produced by clamping the input) did not correct VF even after 6 minutes and neither did perfusion at the full rate with McEwen's solution equilibrated with 95% N_2 + 5% CO_2 instead of the usual 95% O_2 + 5%CO_2.

In the 8 rat and 5 guinea-pig hearts perfused with Krebs' solution VF resulting from reperfusion or electrical pacing was corrected within 1-3 minutes of stopping perfusion and in a manner seemingly identical to that seen with rabbit hearts perfused with McEwen's solution.



H. DISCUSSION OF RESULTS OBTAINED IN SECTION TWO

1. Interactions Between Myocardial Ion Channels and Pumps

The following two figures and the description of the action potential sequence, have been included in this thesis so that any reader so needing may have immediate access to a representation of the basic background of the main mechanisms germane to the discussion stage now reached and to matters to be encountered further ahead in the text. Figure 2H.1 is presented as a summary of the interactions between various ion channels and pumps and indicates the main myocardial sites of action of cardioactive drugs. The co-ordinated activity of these pumps and channels gives rise to the action potentials depicted in fig. 2H.2 and regulates the intracellular concentration of ions. Failure to regulate their activity during cardioplegia may result in incomplete or unsustained arrest, damage during the arrest period and a slow, limited or arrhythmic recovery during reperfusion.

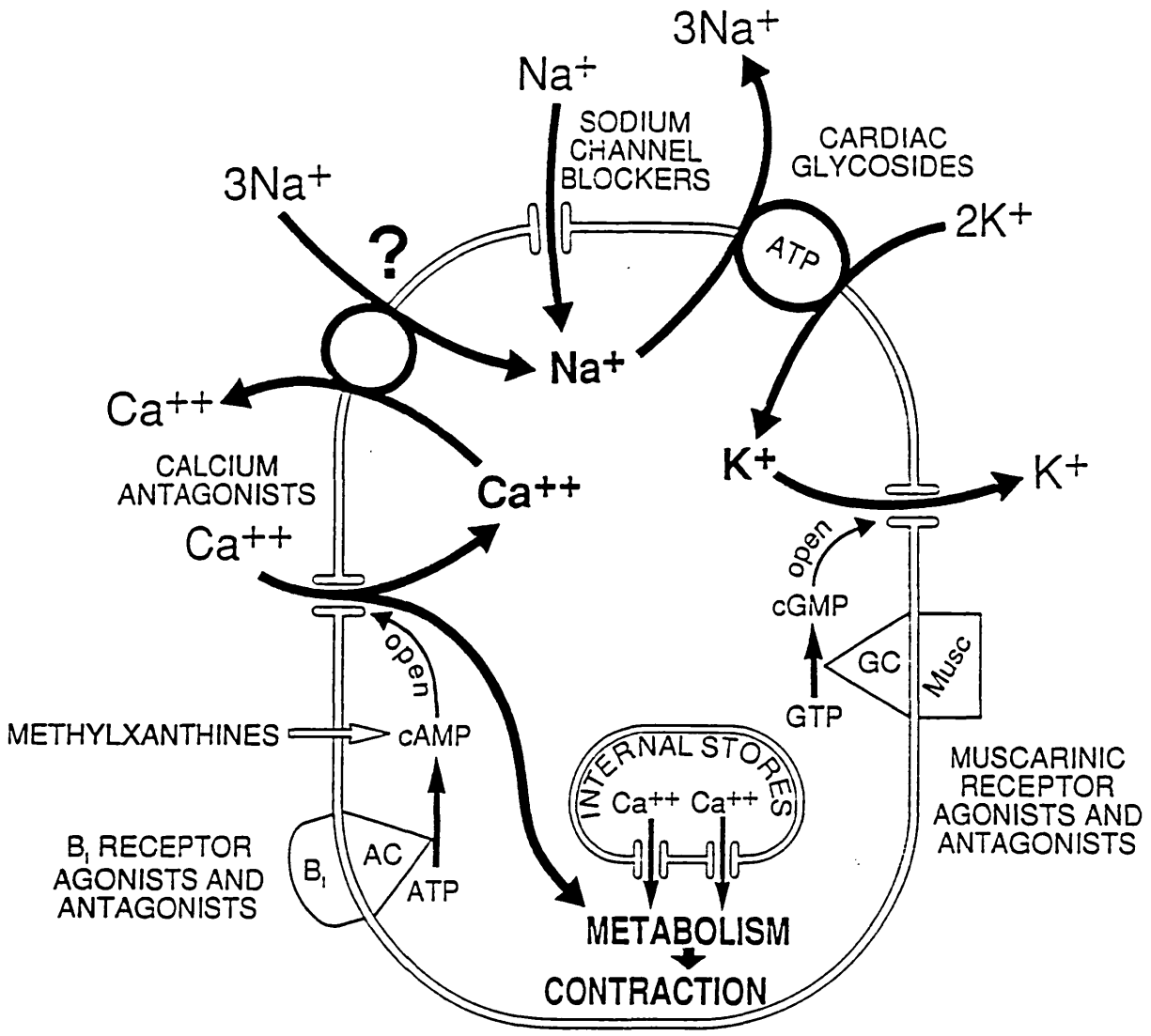


Figure 2H.1

Summary of myocardial ion channels and pumps and the major sites of action of cardiac drugs.

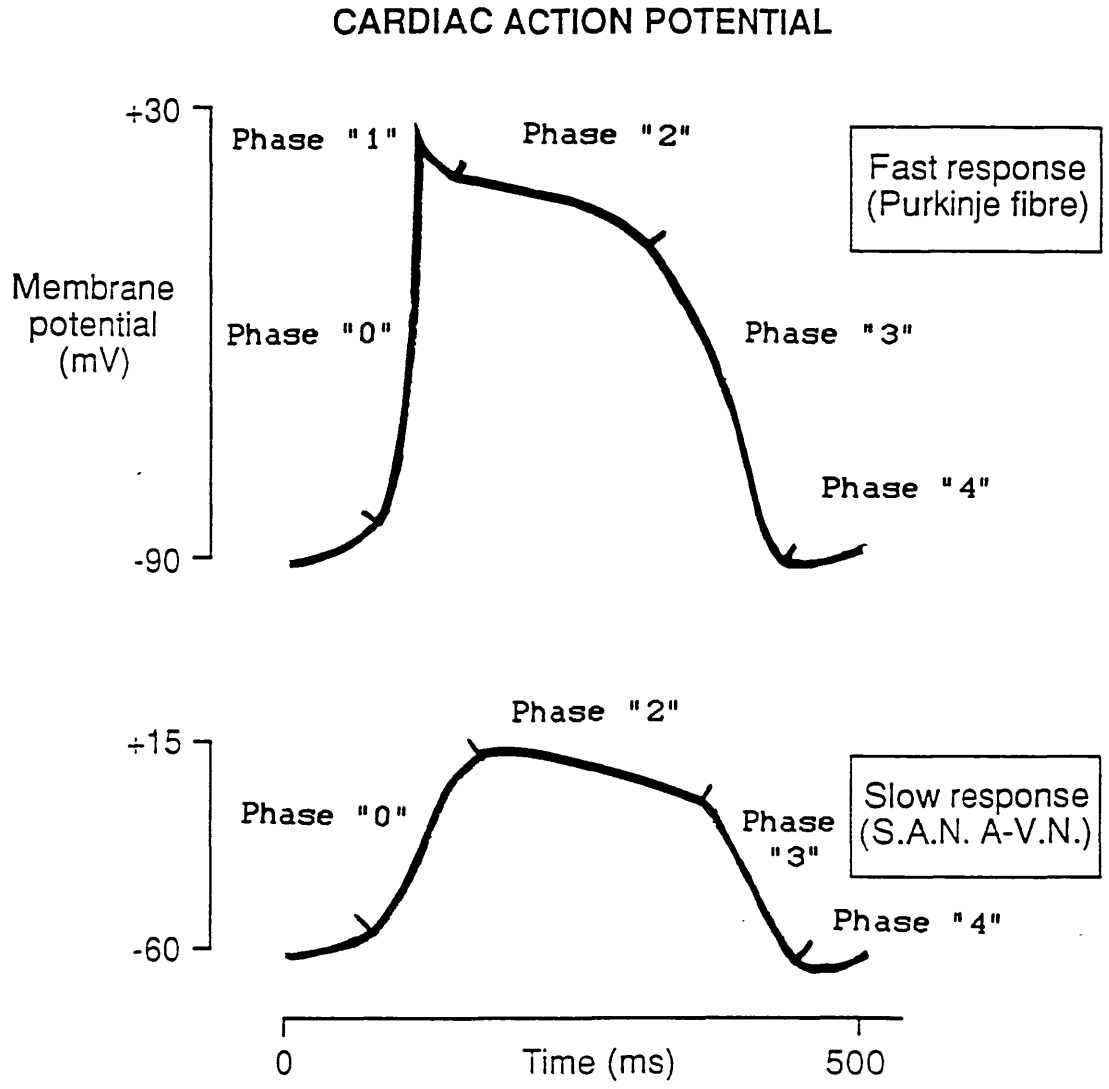


Figure 2H.2

Cardiac Action Potentials

In the Purkinje fibres and the plain myocardium the action potential sequence is generally accepted to be as follows. During diastole membrane potential is approximately -90mV and membrane sodium channels are in the resting state. If the tissue is stimulated, a "threshold" may be reached at approximately -70mV. Sodium channels then open allowing a rapid influx of sodium which depolarises the membrane at a rate of several hundred volts per second. This is referred to as Phase "0" and is sometimes followed by a slight repolarisation, Phase "1", the exact cause of which is unknown, but which may be due to inactivation of the fast inward sodium current or activation of a transient outward potassium current. The majority of sodium channels close when membrane potential reaches approximately -10mV, but some are open during the plateau phase of the action potential, Phase "2". This "window" current of sodium occurs because there is slight overlap between the activation and inactivation curves for sodium channels (Attwell, Cohen, Eisner, Ohba and Ojeda 1979). It is during Phase "2" that the main influx of calcium occurs through the L-type calcium channels and leads to release of additional calcium from the sarcoplasmic reticulum and thence to contraction. The L-type channels begin to open at membrane potentials of approximately -10mV and close in a time dependent manner during Phase "2". They begin to de-inactivate as the membrane repolarises to values negative to -20mV. Repolarisation

occurs during Phase "3" as a result of potassium efflux. The rate and extent of repolarisation are dependent on the extracellular potassium concentration and on membrane permeability. When the membrane has repolarised to approximately -40mV, the majority of the L-type calcium channels have re-entered the resting state and as membrane potential becomes negative to -50mV a second group of calcium channels, T-type, begin to de-inactivate also. Sodium channels begin to re-enter the resting state as the membrane repolarises to values negative to -60mV. The membrane continues to repolarises to a point usually well past threshold.

In the sino-atrial node and the atrio-ventricular node the maximum diastolic membrane potential is approximately -65mV. Phase "0" depolarisation is caused by an influx of calcium through L-type channels and proceeds more slowly than in the sodium dependent tissues. There is usually no Phase "1" repolarisation. Unlike in the plain myocardium the membrane potential attained by the end of Phase "3" does not remain constant, instead it drifts towards the threshold value, this is referred to as Phase "4" or pacemaker depolarisation. In sinus rhythm, the heart rate is determined primarily by the rate of pacemaker depolarisation in the sino-atrial node. It was suggested originally that pacemaker depolarisation was a consequence of inactivation of the outward repolarising current of potassium (Trautwein and Kassebaum 1961). More recently it has been suggested that an inward

calcium current is involved also. This was considered to be unlikely because depolarising calcium currents were thought to occur only at membrane potentials positive to -40mV, whereas the pacemaker current flows at potentials between -65 and -50mV. The discovery by Nilius, Hess, Lansman and Tsien (1985) in rat ventricle, of a transient, low magnitude calcium current flowing at potentials between -70mV and -20mV provided a mechanism by which calcium could be involved in pacemaker activity. They called the channel through which this transient calcium influx occurred, the T-Type calcium channel, to distinguish it from the L-type channel through which a longer and larger calcium influx occurs at less negative values. Two calcium currents with very similar characteristics were found in canine atrial and sino-atrial node cells by Bean (1985). Studying the relative importance of potassium efflux inactivation, and transient calcium influx in rabbit sino-atrial node cells has led to the following conclusion. The initial phase of pacemaker depolarisation is caused by inactivation of the repolarising outward potassium current, the membrane depolarisation which results leads to activation of T-type channels and thereby an influx of calcium. This further depolarisation activates a larger and more sustained influx of calcium through L-type channels (Hagiwara, Irisawa and Kameyama 1988). The contribution to spontaneous pacemaker activity made by a fourth current activated by membrane hyperpolarisation is

considered to be small because this current is activated only at potentials more negative than the normal maximum diastolic potential (Hagiwara and Irisawa 1989) .

The pharmacological properties of the two types of channel differ also (see Bean 1985, Nilius et al. 1985, and Hofmann, Nastainczyk, Rohrkasten, Schneider and Sieber 1987). The L-type channel is blocked by calcium channel antagonists such as nifedipine and by magnesium. The L-type channel is agonised by Bay K 8664 and the current flowing through it is increased by catecholamines. The T-type channel is not blocked by calcium channel antagonists nor by magnesium, but it is blocked by nickel. The current flowing through the T-Type channel does not appear to be increased by catecholamines (Hagiwara et al. 1988).

2. Consequences of a Lowered Extracellular Calcium Concentrations

a. Reduced Functional Activity

The fall in heart rate seen at lower calcium concentrations in both the randomised (fig. 2C.1) and the progressive (fig. 2A.3) studies are in agreement with other workers (Weiss, Surawicz and Rubenstein 1966). Similarly, the reductions in amplitude of contraction in both studies agree with each other and results obtained by other workers. These results are in sharp contrast to the almost total insensitivity of coronary flow rate to

the lowering of extracellular calcium concentrations either in the progressive (fig. 2A.1) or the randomised study (fig. 2C.1). Again, these results agree with those obtained by other workers with isolated hearts and are to be expected from the findings that under normal conditions isolated vascular smooth muscle does not relax when extracellular calcium concentration is reduced.

b. The Calcium Paradox

Return of standard perfusion to hearts perfused previously with calcium free solutions resulted in a loss of co-ordinated electro-mechanical activity. Interestingly, coronary flow was reduced also. This could be due to damage to the coronary vasculature as reported by Zimmerman et al. (1967) or be a consequence of the hypercontraction of the surrounding myocardium obstructing the vasculature, as occurs during ventricular fibrillation.

It is clear that whereas small reductions in the extracellular calcium concentration reduce heart rate and amplitude of contraction and must therefore be sufficient to limit the availability of calcium to the contractile elements, these effects are temporary. In this study only the solution with no deliberately added calcium caused total arrest and subsequently induced the calcium paradox. These observations suggest that a citrate-induced calcium paradox is unlikely to have been

the reason for the damage caused by Melrose's solution because citrate (as the sodium salt), even at a concentration 10 times that recommended for potassium citrate cardioplegia, was insufficient to arrest rabbit hearts (Baker et al. 1957). Only when hearts were perfused with solutions containing sodium citrate but no added calcium were phenomena seen which could have been mild manifestations of the calcium paradox (Baker personal communication).

From the results it can be concluded that a reduction in calcium concentration from 2.18mM to 0.73mM and 0.36mM were safe under the relatively severe conditions of 8 minutes of normothermic infusion of otherwise physiological perfusion solutions. Furthermore, both concentrations caused significant reductions in amplitude of contraction, showing that influx of calcium was attenuated and indicating a probable cardioprotective effect during cardioplegia.

3. Possible Mechanisms for the Calcium Paradox.

a. Membrane Disruption Leading to Increased Permeability to Calcium During Reperfusion

Calcium is required to maintain the structural integrity of the membrane. Perfusion with a calcium free solution removes calcium from the membrane glycocalyx, so altering its structure and causing for calcium an increased permeability which persists during reperfusion, thereby

allowing a damaging influx of the ion (Holland and Olson 1975. Frank, Langer, Nudd and Seraydarian 1977).

b. Sodium / Calcium Exchange During Reperfusion

It has been suggested that exposure to extracellular calcium concentrations (<50 microM) causes slow calcium channels to open via a feedback mechanism. Sodium enters through these open channels and an intracellular sodium overload develops, which is exchanged for calcium present in the reperfusion solution (Chapman, Rodrigo, Tunstall, Yeates and Busselen, 1984, Chapman, Fozzard, Friedlander and January 1986).

The factors which determine the development of the paradox provide evidence in support of both theories. These same factors are variables in the formulation and use of clinical cardioplegia solutions which could account for differences between the success of nominally calcium free solutions in different surgical centres and experimentally.

4. Factors Which Influence the Development of the Calcium Paradox

a. Volume and Duration of Calcium Free Infusion

The infusion regime is of great importance in the generation of the paradox. Small volumes or short durations (less than two minutes) of calcium-free

infusion do not appear to cause the paradox (Boink, Ruigrok, de Moes and Zimmerman 1980, Ruigrok and Poole-Wilson 1983). In the clinical setting, after an initial 2 minute infusion of cardioplegic solution, reinfusion may be necessary later in an operation to prevent premature restoration of mechanical activity, but the time for which the myocardium is actively perfused is comparatively short. In contrast, attempts to limit experimental investigations to the effects of calcium alone and remove any contribution made by anoxia or potassium-induced depolarisation have led to calcium-free solutions usually being infused continually for up to an hour. In such situations where the membrane is washed continually there is no equilibrium established between calcium in the membrane and that in extracellular fluid. It might reasonably be expected that under these circumstances more calcium will be removed from the membrane and the conditions which may produce the paradox thereby exaggerated. In rabbit hearts continuous perfusion with potassium citrate was much more damaging than an initial perfusion followed by an equivalent period without any coronary flow (Baker et al. 1957). The volume dependence of Bretshneider's solution and intracellular type solutions generally, supports the membrane disruption hypothesis of the calcium paradox.

b. Temperature

Hypothermia during the calcium-free, though not the reperfusion phase, protects against the paradox (Rich and Langer 1982). The time for which a solution must be infused before inducing the paradox is prolonged greatly if the temperature is reduced. At 37°C the paradox can be induced after only 4 minutes of calcium-free infusion but if the infusion is performed at 20°C 25 minutes are required (Boink, Ruigrok, Maas and Zimmerman 1980). These facts are compatible with both theories since the proteins and lipids which form the membrane and channels within it are less fluid and less susceptible to disruption when cold.

c. Similar Divalent Cations

The ability of divalent cations to uncouple electromechanical activity and to protect from the calcium paradox increases as their ionic radii approach that of calcium, the sequence $Ca^{++} > Cd^{++} > Mn^{++} > Co^{++} > Mg^{2+}$ is reported by Rich and Langer (1982). They suggest that the ability to protect from the calcium paradox is due either to blockade of calcium channels or an ability to take the place of calcium as it is washed from the glycocalyx. Blockade of the calcium channels might prevent sodium entering during the calcium-free stage and so delay the development of a sodium overload.

Similarly, non-ionic calcium channel blockers might be effective at preventing the calcium paradox.

d. Extracellular Sodium Concentration

It has been shown in a number of studies that reducing the extracellular sodium concentration during the calcium-free perfusion prevents the calcium paradox (Baker and Betmouni 1987). This observation strongly supports sodium-calcium exchange as the mechanism of the calcium paradox (see also Section 2H.9 and 4C.).

e. The Composition of Reperfusion Solutions

Interpretation of results from published experiments in this field is made more difficult by the wide range of calcium concentrations in the various perfusion solutions (1.3mM to 2.4mM). It is likely that an increase in membrane permeability to calcium will be more apparent if the extracellular calcium concentration is higher. In this study, lowering calcium concentration from 2.18 to 1.45mM reduced both heart rate (fig 2A.3, 2C.1) and amplitude of contraction (2A.2, 2C.1) significantly, showing that the reduction of the inward gradient was sufficient to alter myocardial functioning. This range is less than that present in reperfusion solutions and may explain the differences in the initiation and development of the paradox seen by different workers.

During surgery the volume of solution, rate of infusion, temperature, influence of other components of a solution and contamination, especially by calcium present in the patient's blood, are all variables whose contribution to the final outcome is difficult to assess and should not be relied upon to prevent the paradox. It is clear that the differences which exist between clinical cardioplegia and experiments designed to investigate the calcium paradox are such that extrapolation from one situation to the other is possibly misleading.

Although control of extracellular calcium influx is essential to successful cardioplegia, it is an incomplete answer to the problems of increased cytosolic calcium concentration because it does not directly regulate intracellular calcium movements. At present none of the proposed cardioprotective agents act intracellularly to limit the release of calcium from intracellular stores or to deal with an increasing cytosolic calcium concentration as it occurs. However, such an action has been proposed for sodium nitroprusside in vascular smooth muscle (Zsoter et al. 1977) and might account for the cardioprotective effects seen in this study (fig 2B.2, 2B.3, 2B.4) and the observation that a similar compound, sodium nitrite, enhanced significantly the recovery of isolated rabbit atria after bathing in anoxic solution (Penn 1965).

5. Mechanism of Action of Sodium Nitroprusside

a. Regulation of Calcium Movements

The increases in heart rate (fig. 2B.1, 2C.1) are similar to those reported for guinea-pig isolated atria superfused with 10^{-3} M sodium nitroprusside in Tyrode's solution for 3 mins (Mirro, Bailey and Watanabe 1979) and seen by the author in rabbit isolated atria (unpublished findings). In intact animals such increases have been attributed to reflexes triggered by a fall in blood pressure, caused by vasodilatation (Adams, Clarke, Edmonds-Seal, Foex, Prys-Roberts and Roberts 1974). The increased heart rate reported here cannot be due to such reflexes, but they could be due to liberation of a factor from residual nerve tissue or perhaps the vasculature.

In this preparation, reducing the extracellular calcium concentration even to zero did not have a great effect on the coronary flow (fig 2A.1, 2C.1), but even low concentrations of sodium nitroprusside caused marked vasodilatation (fig. 2B.1). Relaxation of vascular smooth muscle is usually investigated by first contracting the muscle by causing a calcium influx, any subsequent relaxation seen in response to a drug is then likely to have resulted from modification of that influx. The increase in coronary flow following infusion of sodium nitroprusside seen in this study was unaffected by variations in extracellular calcium concentration (fig.2C.1) and occurred even though the vascular smooth

muscle had not been deliberately precontracted. Similarly, lowering extracellular calcium decreased both heart rate (fig. 2A.3) and amplitude of contraction (fig.2A.2, 2C.1), but sodium nitroprusside had no affect on the amplitude of contraction and increased the heart rate in the presence of lowered calcium concentrations (fig. 2C.1). These results suggest that the mechanism of action of sodium nitroprusside is not related primarily to extracellular calcium.

b. Membrane Hyperpolarisation: Similarities With Muscarinic Receptor Agonists

Cheung and McKay (1985) have reported that sodium nitroprusside hyperpolarises the vascular smooth muscle of the rabbit and it can be seen from fig. 2D.1 that rabbit atrial tissue is hyperpolarised and the degree of this hyperpolarisation is similar to that reported for guinea-pig atria (Mirro et al. 1985). Repolarisation requires the net loss of positive charge from inside the cell and this could be achieved by extrusion of potassium or calcium. An increased rate of extrusion may speed repolarisation and shorten the action potential duration thereby increasing the heart rate slightly, but if extrusion of potassium results in hyperpolarisation, the action potential duration may be increased leading to a decreased heart rate. If the observed increase in heart rate (fig. 2B.1) were due to extrusion of calcium it might account for the apparent cardioprotective effects seen (fig. 2B.2). Potassium channels are thought to be

opened by cGMP produced by the action of guanylate cyclase on GTP in response to the binding of agonists to muscarinic receptors (Daugherty and Woodward 1985). Sodium nitroprusside increases the cGMP concentrations in cardiac tissue, particularly the atria (Mirro et al. 1979, Daugherty and Woodward 1985), where there are more muscarinic receptors. Muscarinic receptor agonists and sodium nitroprusside both cause hyperpolarisation, but the concentration response curve for sodium nitroprusside is very shallow (fig. 2B.1, 2B.2, 2B.3, 2B.4) unlike that of agents which mediate their effects directly at autonomic receptors. However, a more subtle modulation of the second messenger systems of those receptors could account for the effects of sodium nitroprusside seen in this study and in others.

6. Anti-arrhythmic Actions of Sodium Nitroprusside

At high concentrations (e.g $10^{-4,-3}$M) sodium nitroprusside reduced the incidence of ventricular fibrillation significantly (fig. 2B.3). Arrhythmias induced with barium chloride in anaesthetised rabbits have been corrected during infusion of sodium nitroprusside (Rabkin, Ohmae and Klass 1982). In that study, and this, it is impossible to identify the specific mechanism of antiarrhythmic action, which could be a directly mediated electrophysiological effect on the

myocardium, or a secondary mechanism probably on the vasculature.

a. Directly On The Myocardium

Isolated guinea-pig atria superfused with sodium nitroprusside (10^{-3} M) in Tyrode's solution for three minutes, exhibit an increased action potential duration (Mirro et al. 1979). Class III anti-arrhythmic drugs, as classified by Vaughan-Williams, act by prolonging the action potential and do so by slowing the repolarising efflux of potassium. The shortening of the action potential duration, described on theoretical grounds as a possible effect of cGMP, would be expected to be pro-arrhythmic. The action potential duration is determined by the balance between the rate of repolarisation and the extent of hyperpolarisation, both of which are governed by the membrane potassium permeability. Detailed electrophysiological investigations are required to determine which, if any, of the phases of the action potential are modified by sodium nitroprusside and in which cardiac tissues and under what conditions.

There has been much debate in the literature as to the role of the balance between the putatively pro-arrhythmic cAMP and anti-arrhythmic cGMP in the generation of ventricular fibrillation (Opie, Nathan and Lubbe 1979, Daugherty and Woodward 1985). Sodium nitroprusside

increases cGMP concentrations and it is possible that this underlies the anti-arrhythmic effect by a mechanism which is independent of potassium channel mechanisms described above. The question of a possible link between the two cannot be resolved satisfactorily because the techniques used to measure cGMP concentrations do not distinguish between different intracellular sites. Whole cell changes probably do not reflect similar changes at the regulatory sites of membrane channels. As it is the functioning of these channels which determines the development of arrhythmias this missing information is probably the most valuable.

b. Indirectly Via The Coronary Vasculature

The small increases in extracellular potassium concentration which occur during periods of ischaemia or non-perfusion inhibit repolarisation slightly, making the cell more excitable, increasing heart rate and predisposing to arrhythmias, especially if there is heterogeneity within the myocardium. In this study 91% of the episodes of VF began in the first few minutes of reperfusion and were therefore most probably a consequence of changes which occurred during the flow-free period. Sodium nitroprusside produced higher coronary flow during reperfusion (fig. 2B.4) and a lower incidence of VF (fig. 2B.3), but was ineffective as an anti-arrhythmic when infused into fibrillating hearts.

These results suggest that the anti-arrhythmic effects of sodium nitroprusside are a consequence of a more rapid and uniform washout of abnormal extracellular fluid.

7. Interactions Between Sodium Nitroprusside and Raised Extracellular Potassium Concentrations

a. Volume of Cardioplegic Solution Required to Induce Arrest

Arrest can be achieved only by partially replacing the contents of the extracellular space with the cardioplegic solution. The degree of replacement required depends on the composition of the cardioplegic solution and extent to which it can be diluted by the extracellular fluid without losing its effectivness. The rate at which replacement occurs is dependent on the coronary flow and the adequacy of its distribution throughout the myocardium. During infusion of a high-potassium cardioplegic solution in rabbit isolated hearts flow increases initially (fig. 2E.3), probably because the frequencey and extent to which systolic contractions occlude the vessels is reduced, but subsequently falls as the vessels constrict in response to the elevated potassium concentration. The area under the curves (fig. 2E.3) for the period up to ventricular arrest takes this complicated pattern into account and reveals that on average 8.5 ml of a solution containing 24mM of potassium had to be infused to induce arrest, but when sodium

nitroprusside (10^{-4}M) was added to the solution and the calcium concentration reduced from 2.18 to 0.73mM the same hearts were arrested after infusion of only 6.6 ml (fig. 2E.3). Leaving aside the apparent differences between the two groups, approximately 8ml of solution were required to induce arrest. The average mass of the hearts taken from NZW rabbits of 800 - 1000g was 4g. The known similarities between rabbit and human hearts in terms of capillaries per fibre and capillaries per unit area suggest that a similar relationship of 2ml of perfusion solution per gram of tissue might also apply and an average human heart of 300g should receive at least 600ml of cardioplegic solution to ensure complete and uniform arrest. Originally, 1000ml of St Thomas' Solution Number 1 were given to human hearts before surgery but this was associated with A-V block on reperfusion. This did not happen when only 500ml of solution were given (Hearse, Braimbridge and Jynge 1981).

8. Mechanism of Action of Raised Extracellular Potassium Concentrations

a. Vascular

Vascular smooth muscle has a variable membrane potential which responds to changes in the extracellular potassium concentration and, again like myocardium, contraction depends on the extracellular calcium concentration. The potassium concentrations of extracellular type

cardioplegic solutions (approximately 16 - 30mM) are sufficient to shift the membrane potential of vascular smooth muscle to a value which favours the sustained activation of calcium influx, leading to contraction, as revealed by a decrease in the coronary flow (fig. 2E.3), and possibly to pathological changes associated with calcium overload. Perfusion with solutions containing sodium nitroprusside and a lowered calcium concentration did not reduce coronary flow (fig. 2E.3), suggesting that they did not predispose towards calcium overload.

b. Myocardial

Increasing potassium concentration from 16 to 22mM was sufficient to increase the number of hearts arrested from 0 to 8 (ie. all hearts tested). When sodium nitroprusside was included and the calcium concentration reduced, the range was shifted and all hearts were arrested by 18mM KCl (fig. 2E.2). The mechanism by which arrest occurs is obviously very sensitive to potassium concentration. Increasing the extracellular potassium concentration causes potassium to enter the cell, so depolarising the membrane and triggering an action potential. This is of little functional importance because the isolated rabbit heart (at 37^{o}C) is in any case depolarised 3 to 4 times every second. The raised potassium concentration assumes its major importance only during repolarisation. The reduced outward gradient

prevents membrane repolarisation to a value at which sodium channels are de-inactivated, so arrest in diastole occurs. With the concentrations of potassium used in this study (16-26mM) membrane L-Type calcium channels will not be inactivated and may be involved in some of the electrical phenomena seen after arrest. The onset of arrest is dependent on the speed with which cardioplegic solution enters the extracellular space, the concentration of potassium and the preceding heart rate. This is because not all available sodium channels open during each action potential, those which do not are unaffected by changes in membrane potential and can participate in a subsequent action potential. At a faster heart rate the total population of channels will have participated in at least one action potential in a shorter time and the onset of arrest is quicker. This frequency depencence is similar to that seen with the Class I anti-arrhythmic drugs, as classified by Vaughan-Williams. (see Vaughan-Williams 1980, 1984).

9. The Relationship Between Potassium and Calcium in Cardioplegic Solutions

Under resting (diastolic) conditions the Na/Ca exchanger extrudes calcium, but if the cell is depolarised the exchanger reverses, causing an influx of calcium. The magnitude of this influx increases as the membrane potential approaches zero. Reversal occurs also when

there is an intracellular sodium overload, which may develop during calcium free perfusion (see also Section 4C.). Both depolarisation and intracellular sodium accumulation lead to tonic contraction of sheep Purkinje fibres (Vaughan-Jones, Eisner and Lederer 1985), due to a rise in intracellular calcium concentration. Such a rise may result eventually in pathological changes. The Na/Ca exchanger is of particular interest to cardioplegia because the cells are depolarised and may well be overloaded with sodium. In this study, where the hearts were perfused with potassium-rich, calcium-poor solutions for 8 minutes at 37^{o}C, full recovery occurred when perfusion with McEwen's solution was resumed, indicating that an excessive influx of calcium had not occurred, either during arrest (as a result of exchanger reversal due to depolarisation) or reperfusion (as a result of calcium entry in exchange for sodium).

The lowest potassium concentration at which the suppression of escape beats was not significantly dependent, statistically, on lower calcium concentration was 24mM. At this concentation there was complete arrest in the whole sample and the time to arrest was not as variable as with 20 and 22 mM potassium. It may appear as though this potassium concentration is unnecessarily high in comparison with that in other cardioplegic solutions, such as 16mM in St.Thomas' 2 and 20mM in St.Thomas' 1, but these solutions contain additional components (such as lignocaine, procaine, and magnesium)

which add to or potentiate the arresting effects of potassium. Potassium concentrations between 10 and 50 mM in isotonic solutions have been declared to be safe and effective, (Tyers et al. 1975). By selecting a concentration of potassium which on its own confers the majority of properties desired of a cardioplegic solution, less account need be taken of the biological variation in response to all contributing factors in the formulation of that solution and there is greater freedom to alter other aspects of it without compromising its basic function of arresting the heart.

10. Possible Causes of Escape Beats After Potassium-induced arrest

The results of this study indicate strongly that escape beats are caused by impulses passing through the A-V node and exciting ventricular tissue which is not entirely refractory, in other words it is proposed that arrest induced by small increases in potassium concentration is in part a consequence of A-V block rather than widespread inactivation of the contractile tissue. The evidence to support this hypothesis is as follows.

Arrest of the ventricles occurs first but electrical activity composed mainly of P waves continues and is often associated with visible flickering of the atria.

The atria were less quickly affected by changes to the potassium concentration of the perfusion solution,

possibly because a lower flow rate per unit mass of tissue delayed equilibration with the extracellular fluid or more likely because the consequences of raised potassium concentrations are different. The latter is particularly true of the S.A.N. but not of the plain atrial muscle which is essentially the same as that of the ventricles. Electrical activity was also reduced when the calcium concentration was lower. It is well known that the pacemaker tissue of the S.A.N. is dependent upon calcium and therefore more susceptible to alterations in the extracellular concentration of this ion. In all but three of the twenty three instances of escape beats seen during the experiments described in section 2E, there were obvious P waves after arrest and immediately before escape. Even though the concentration of calcium used in the experiment was only reduced sufficiently to decrease the force of contraction by 10% (fig. 2A.2) and the heart rate by 20% (fig. 2A.3) it totally abolished all escape beats. This suggests a mechanism which is more sensitive to calcium than a reduction of the availability of calcium to the contractile elements. The results of the experiments in section 2G. confirm that it is the reduction in the calcium concentration rather than the presence of sodium nitroprusside which accounts for the suppression of escape beats. It is clear from the description earlier of calcium channel activation and inactivation (Section 2H.1) that although closed at the membrane potential

resulting from an extracellular potassium concentration of 22mM (approximately -49mV) the L-Type channels possess the ability to open. The T-Type channels may also be beginning to de-inactivate at this membrane potential, but sodium channels remain inactivated. Escape beats could therefore result from a depolarising influx of calcium through L-Type calcium channels, this might explain their apparent sensitivity to the extracellular calcium concentration. The A-V node is dependent upon calcium to a greater extent than is the plain myocardium. Removal of some of the calcium may have been sufficient to add the final touches to A-V block induced only partially by elevated potassium concentrations.

11. Similarities Between the Mechanisms by Which Escape Beats and the Anti-fibrillatory Paradox Occur

The term "anti-fibrillatory paradox" can be used to describe the ability of an interruption in the coronary flow to correct VF in a consistent, predictable and dependable manner. The phenonenon is paradoxical because stopping coronary flow could under the normal circumstances of perfusion result in hypoxia, changes in pH and transmembrane ionic distributions, all of which are known to cause or support VF. The phenomenon has been seen in rabbit isolated hearts perfused with McEwen's solution as well as rat and guinea-pig isolated hearts perfused with Krebs' solution, all three species

tried so far. Furthermore, it occurs without regard for the initial causes of the fibrillation which have so far included, electrical pacing, reperfusion, and bolus injections of potassium chloride and adrenaline.

The possible involvement of a temperature change when perfusion stops has been ruled out and was in any case unlikely as the prevention of such changes was an important design feature of the Baker perfusion system, (Baker 1951). The phenomenon does not occur if the fibrillating heart is perfused with a solution equilibrated with nitrogen rather than oxygen or when an oxygenated solution is infused at only 10 percent of the usual rate. Both situations must render the tissue extremely hypoxic but do not correct the fibrillation. These results demonstrate that it is the cessation of flow which is important and indicate that a build up of a factor in the extracellular space is responsible for the phenomenon.

Both escape beats and the anti-fibrillatory paradox appear to require the presence of an atrial pacemaker. When there are no atria present and after either infusion of extra potassium or the cessation of VF, the ventricles gave no escape beats but did respond to direct electrical stimulation. It is known that potassium leaves the cells during hypoxia and increases the concentration in the extracellular space (Opie, Nathan and Lubbe 1979). This would in effect be equivalent to perfusing the heart with an increased extracellular potassium concentration, which

has in the past been used as anti-arrhythmic therapy (Montgomery, Prevedel and Swann 1954). Longer periods of VF required longer periods without flow to correct them. This suggests that the factor responsible for correction may also be lost during fibrillation and if the intracellular concentration is lower, the outward gradient is lower and consequently the rate of accumulation in the extracellular fluid during the flow free period is less. Potassium is lost both during fibrillation and hypoxia. At present it seems likely that the anti-fibrillatory paradox is caused by an accumulation of potassium in the extracellular space.

12. The Effects of Periods of Ventricular Fibrillation

Surface electrodes were used to pace the heart to avoid damage caused by embedded electrodes. VF was always corrected after less than 4 minutes without coronary flow and hearts recovered fully from the 4 minute control period without perfusion, in the absence of VF. The recovery was also near to 100% when VF was induced and the coronary flow immediately stopped. A decline in amplitude of contraction of 5% occurred over 30 minutes of perfusion under normal conditions (fig. 1B.6) of 10 % for 30 minutes without flow preceded by potassium-induced arrest (see Section 3), and of 30% after 30 minutes without flow (see Section 2B). These are slight changes when compared with the 65% fall seen after 32 minutes of

VF and perfusion. These findings suggest that failure to recover fully after VF was a consequence of the duration of that VF and not its induction, correction or perfusion of the organ.

The slight reductions in coronary flow seen by the end of periods of VF are presumabley due to tension developed by the myocardium compressing the coronary vasculature (Buckberg and Hottenrott 1975, Grover, Fewel, Ghidoni, Norton, Arom and Trinkle 1977). It is impossible to say from these experiments if the fall in flow is generalised or regional, but it might reasonably be expected that those vessels surrounded by the greatest muscle mass would be affected most. A localised effect is potentially the more dangerous because it may lead to a heterogeneity of extracellular ion concentrations and in turn perpetuate a fibrillation. The distribution of an anti-arrhythmic, should it be necessary, will also be restricted and it is advisable to have an anti-arrhythmic drug present in the cardioplegic solution so that it is evenly distributed before it is likely to be needed. Accordingly, most cardioplegic solutions in clinical use contain some form of anti-arrhythmic agent, usually lignocaine or procaine.

a. Mechanical

During VF there is little co-ordination between cells and as a consequence of the asynchronous contraction the

tension then applied to many cells may catch them in relaxation and be sufficient to affect the arrangement of their contractile elements and the intercalated discs, permanently and adversely.

b. Metabolic

Although the myocardium is generally thought of as not entering tetanus in the preparation used during this study, the tension recorded during fibrillation always approached and sometimes exceeded that achieved at the peak of systole during normal beating (fig. 2H.3).

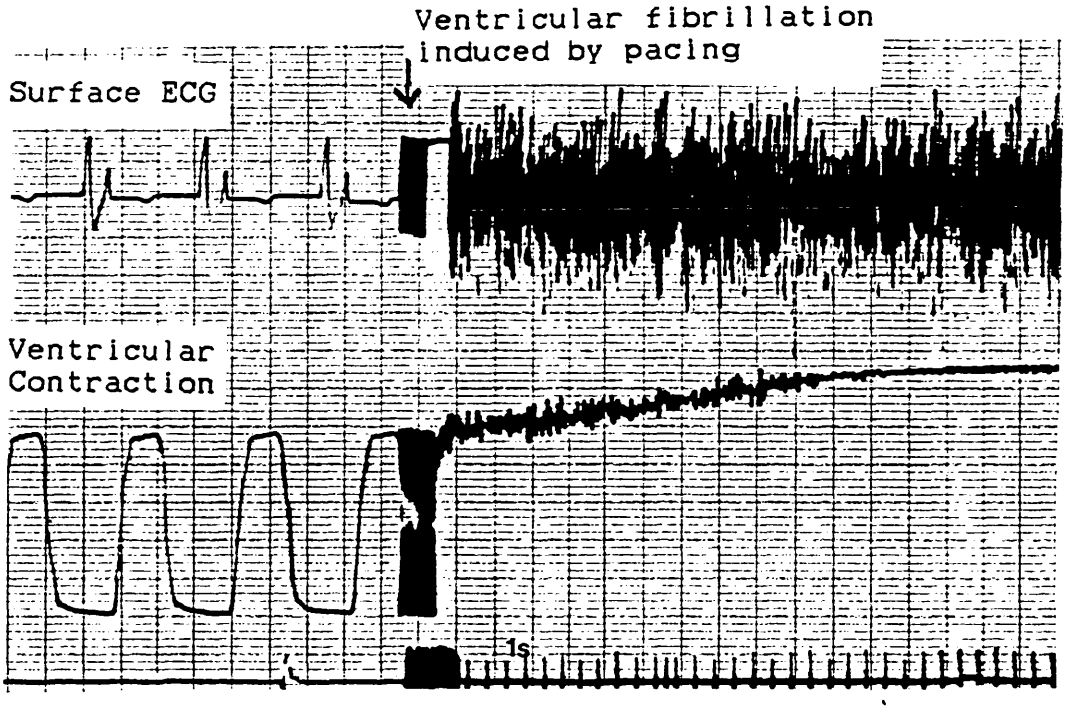


Figure 2H.3

A typical tracing of a rabbit isolated heart with ventricular fibrillation.

Integrating the area under a tracing of tension developed during fibrillation suggests that the heart does twice as

much work as when beating normally. In this situation the metabolic requirements will be greater and inadequacies of simple perfusion solutions more apparent, particularly as the coronary flow falls also.
Interestingly the heart rate, which also depends on many active metabolic processes, is affected little after periods of VF. This supports a view that mechanical damage accounts for the reduced amplitude of contraction.
Although short periods of VF do not appear (Section 2F) to inflict lasting damage on the isolated perfused heart, temporary interruption to a normal circulation can lead to brain damage and death within only a few minutes.

I. CONCLUSIONS DRAWN FROM SECTION TWO

The effects of sodium nitroprusside directly upon the myocardium are minimal, especially in comparison with its effects on the coronary vasculature. Infusion of a high concentration of sodium nitroprusside (10^{-4}M) prior to periods without perfusion was associated with improved recovery of amplitude of contraction and coronary flow, and a lower incidence of ventricular fibrillation when perfusion was restored. These effects may be regarded as evidence of cardioprotection and may result from a primary action upon the coronary vasculature.
Elevated extracellular potassium concentrations (16mM and higher) arrest rabbit isolated hearts in diastole

without, initially at least, rendering the ventricles entirely refractory to stimulation. Escape beats triggered by impulses originating in the atria are a common feature of potassium-induced (18-22mM) arrest but can be prevented by a simulataneous reduction in the extracellular calcium concentration from 2.18mM to 0.73mM. This lower calcium concentration, in an otherwise physiological perfusion solution, did not result in the calcium paradox after 8 minutes of infusion at 37°C, and can be regarded as functionally significant. The following concentrations have been selected as suitable for inclusion in extracellular type cardioplegic solutions and should be investigated in various combinations in an experimental model of cardioplegia, $CaCl_2$ 0.73mM, KCl 24mM, and sodium nitroprusside, 10^{-4}M. Periods of ventricular fibrillation, in the presence of perfusion, are damaging to the heart. Interrupting the coronary flow in a fibrillating isolated heart of rabbit, rat or guinea-pig, causes a return to co-ordinated electro-mechanical activity, from which almost full recovery is achievable once flow is restored. Build up of a factor, possibly potassium, in the extracellular space appears to be responsible for this phenomenon which is here termed an anti-fibrillatory paradox.

SECTION THREE

A COMPARISON OF FIVE NEW CARDIOPLEGIC SOLUTIONS and ST.THOMAS' SOLUTION NUMBER ONE IN RABBIT LANGENDORFF HEARTS. 37°C.

Introduction

In this series of experiments the compounds and concentrations identified as potentially beneficial by earlier experiments (Section Two) are tested in different combinations and the effects of each solution compared. Any component of a solution may confer upon the solution as a whole one or more desirable properties and be active in one or more of the stages of cardioplegia. The exact contribution made by any component is influenced by the rest of the solution, the conditions under which it is given and the condition of the tissue. The parameters chosen for investigation relate to the final extent of recovery after arrest, the induction and maintenance of arrest, and recovery immediately after arrest.

Protocol

56 rabbit Langendorff hearts were prepared in the usual way and left for thirty minutes in which to settle down. Reference values of heart rate, coronary flow and amplitude of contraction were then recorded for each heart and the means and s.e.m for each group of hearts are given in the table below.

One of six cardioplegic solutions (formulations given below) was then infused for 2 minutes, after which all forms of coronary perfusion were suspended for 30 minutes. The coronary flow was subsequently restored for 30 minutes, at the end of which the parameters of

function were recorded before the same cardioplegic solution was infused a second time for 2 minutes prior to 60 minutes without coronary flow. Following this second period of cardioplegia, perfusion was restored and the performance of each heart recorded for five hours.
One group of 8 hearts was perfused with McEwen's solution continuously to act as a control.

		Cardioplegic solutions					
		The Five New Cardioplegic Solutions (key used in figures and tables to identify new solutions)					St. Thomas' Solution Number 1.
Components (mM)	McEwen's Perfusion Solution	Na ↑K ↓Ca SNP ligno.	Na ↑K ↓Ca SNP	Na ↑K ↓Ca	Na ↑K Ca SNP	Na ↑K Ca	
NaCl	130.00	130.00	130.00	130.00	130.00	130.00	144.00
NaH_2PO_4	000.92	000.92	000.92	000.92	000.92	000.92	----
$NaHCO_3$	025.00	025.00	025.00	025.00	025.00	025.00	----
KCL	005.60	024.00	024.00	024.00	024.00	024.00	020.00
$CaCl_2$	002.18	000.73	000.73	000.73	002.18	002.18	002.2
Glucose	011.10	----	----	----	----	----	----
sucrose	013.10	----	----	----	----	----	----
SNP	----	000.10	000.10	----	000.10	----	----
Lignocaine	----	001.00	----	----	----	----	----
Procaine	----	----	----	----	----	----	001.00
$MgCl_2$	----	----	----	----	----	----	016.00
pH	7.4	7.4	7.4	7.4	7.4	7.4	5.5-7.0
	Filtered (Whatman Inline Filter) Gassed with 5% CO_2 + 95% O_2 for 20 min						----

Table 3A.1

The composition of McEwen's (1956) perfusion solution, St. Thomas' Cardioplegic Solution Number One and five new cardioplegic solutions. (note, maximum calcium contamination of new cardioplegic solutions, resulting from contamination of Analar grade chemicals, = 6.9micro mol/L)

Stock Solutions of sodium nitroprusside (SNP) 0.1M were prepared with distilled water on the morning of the experiment and protected from light.

A 1M stock solution of lignocaine (hydrochloride) was prepared with distilled water and used for the whole series of experiments.

St. Thomas' solution (Number One) was freshly prepared with a concentrate (Macarthys Ltd.) and Ringer's injection (Travenol) in accordance with instructions.

Those solutions which contained sodium nitroprusside and St.Thomas' solution were protected from light.

The experiments were conducted in a random order.

(In order to ensure the maximum value was derived from the carcasses various organs were used by other researchers).

Results

After the first and the second infusions of the six different cardioplegic solutions there were significant ($P<0.05$, ANOVA) differences between the time to onset of arrest (fig. 3A.2). The presence of a sodium channel blocker, either procaine in St. Thomas' Solution Number One, or lignocaine, was associated with a faster onset of arrest. Similarly, there were significant differences between groups for coronary flow during the infusion of the different cardioplegic solutions on both occasions ($P<0.05$, ANOVA). Flow rate was much higher when

solutions contained sodium nitroprusside, especially when lignocaine was present also. St.Thomas' Solution Number One also increased coronary flow substantially, most probably by vasodilatation produced by its procaine content. Of the new solutions, those without sodium nitroprusside decreased coronary flow in a manner expected from the experiments described in Section 2E. Escape beats during the induction of arrest were common during infusion of solutions with physiological calcium concentrations but non-existent when the solution had a lowered calcium concentration. These results are in agreement with those presented in Section 2E. Escape beats during the arrest and non-perfusion stage were also less common when arrest had been induced by solutions containing lowered calcium concentrations, but were only absent when there was a sodium channel blocker present as well (fig. 3A.3). The description of results will resume after fig. 3A.2 and 3A.3.

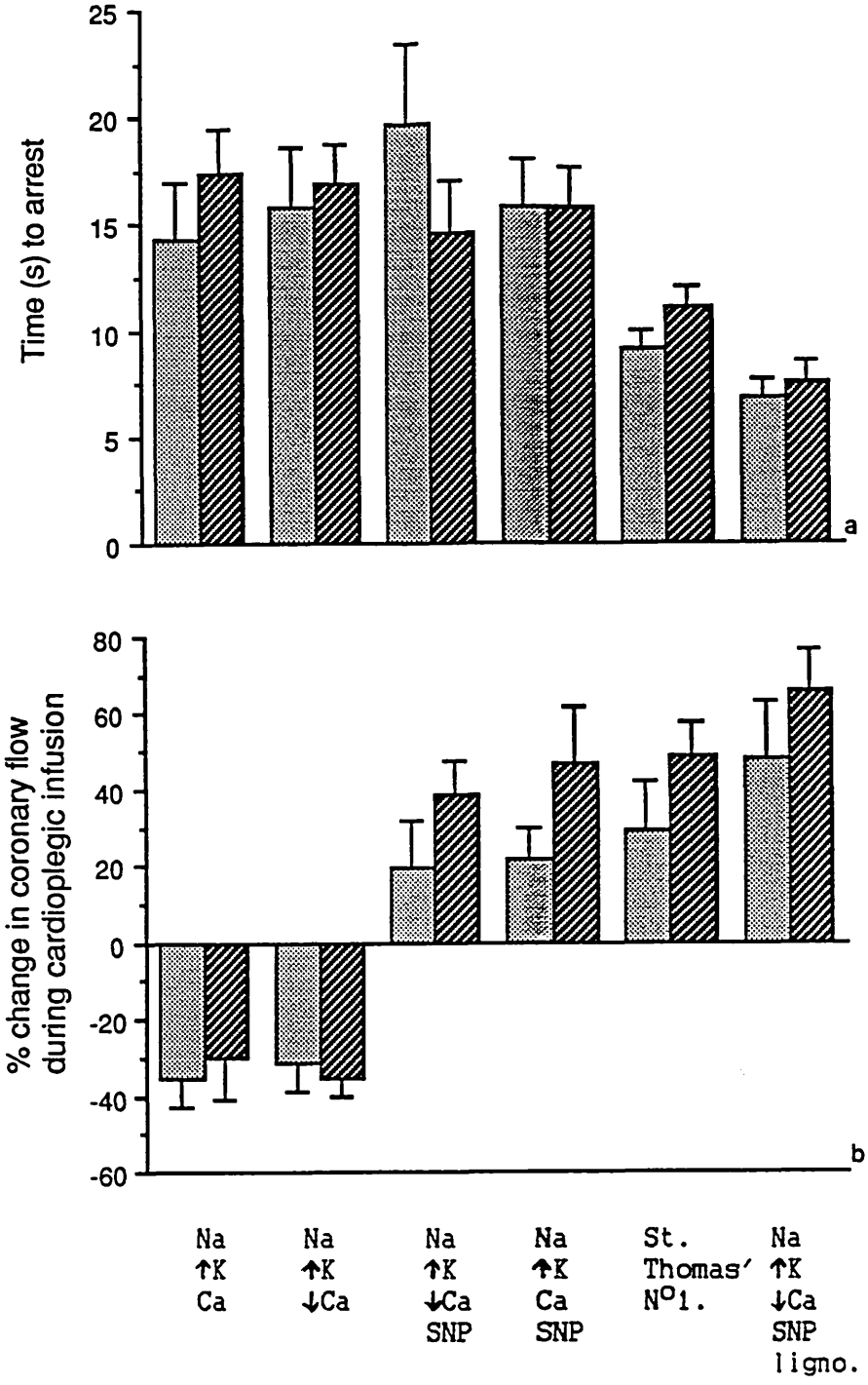


Figure 3A.2

The time to arrest and changes in coronary flow during infusion of six cardioplegic solutions. a) The time (s) to arrest following infusion of cardioplegic solution and B) coronary flow at the end of the 2 minute infusion period. Stippled boxes show the effect of the first infusion and hatched boxes the effects of the second infusion. Each column is the mean and the bars the s.e.m. of 8 observations. n = 8 for each group, 48 in total.

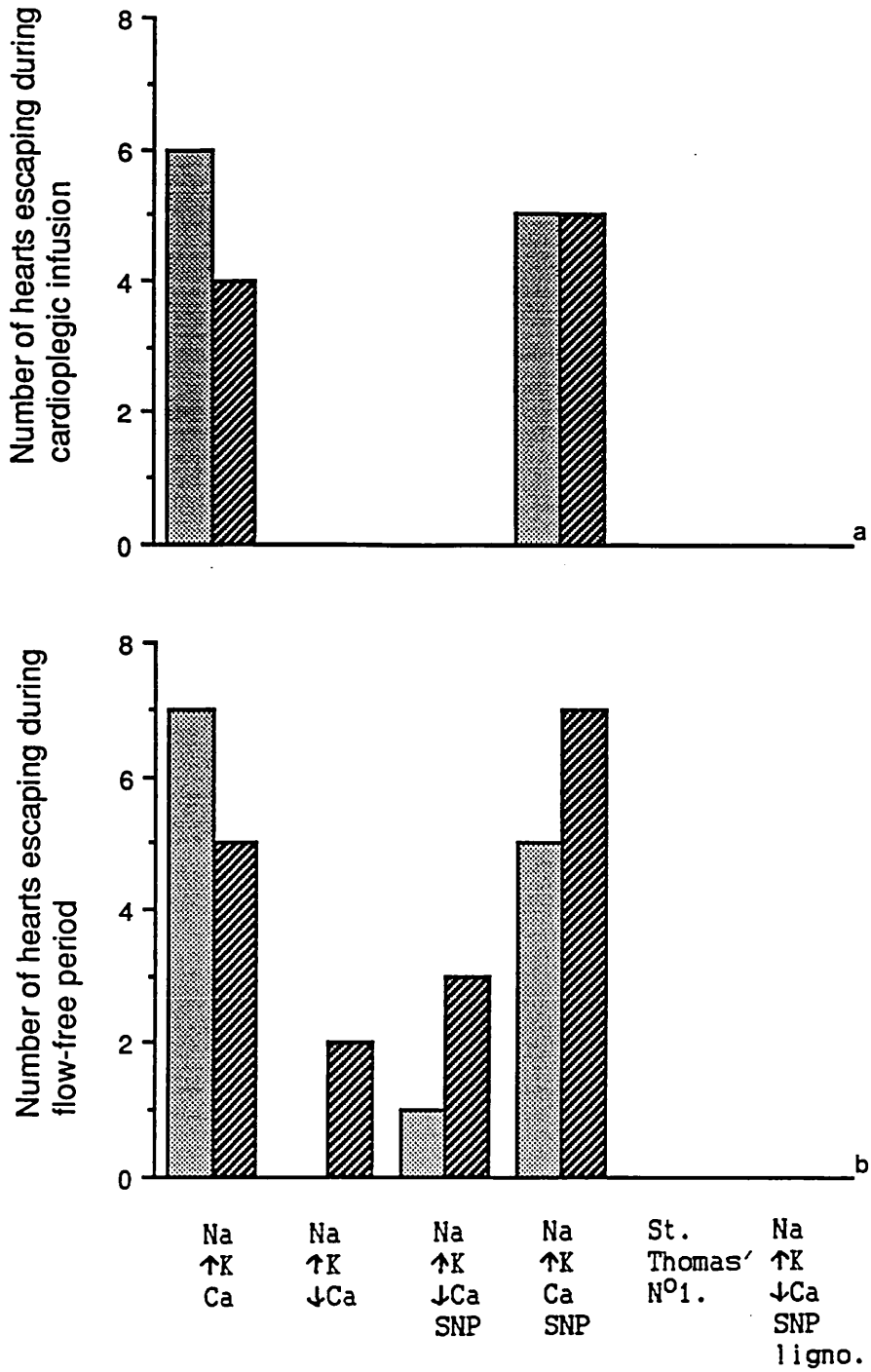


Figure 3A.3

The incidence of escape beats after infusion of six cardioplegic solutions. The number of hearts escaping a) during induction period and b) flow-free period. Stippled boxes = first arrest period and hatched boxes the second. The maximum possible in each group was 8.

Electrical activity during both periods of cardioplegia was common in all groups, except those which had received a sodium channel blocker (fig. 3A.4). Conversely, ventricular fibrillation during reperfusion was a phenomenon almost exclusively linked to the second period of reperfusion where, not surprisingly, the lowest incidence of VF was in hearts which had received a Class 1 anti-arrhythmic drug (fig. 3A.4). There was a similar pattern to the incidence of electrical activity during the second arrest period and the incidence of VF during reperfusion but the correlation coefficient was only 0.76. Electrical activity during the second period without perfusion and escape beats during the same period were correlated (coefficient = 0.97, $P<0.01$). There were significant ($P<0.05$, ANOVA) differences between the coronary flows in the different groups, during the first minute of reperfusion after both arrest periods (fig. 3A.5). These were lowest in the hearts which had not received sodium nitroprusside, the same hearts in which reperfusion ventricular fibrillation was commonest, and highest in those hearts which had received it. The flows during reperfusion were similar to the flows during infusion of the corresponding cardioplegic solutions.

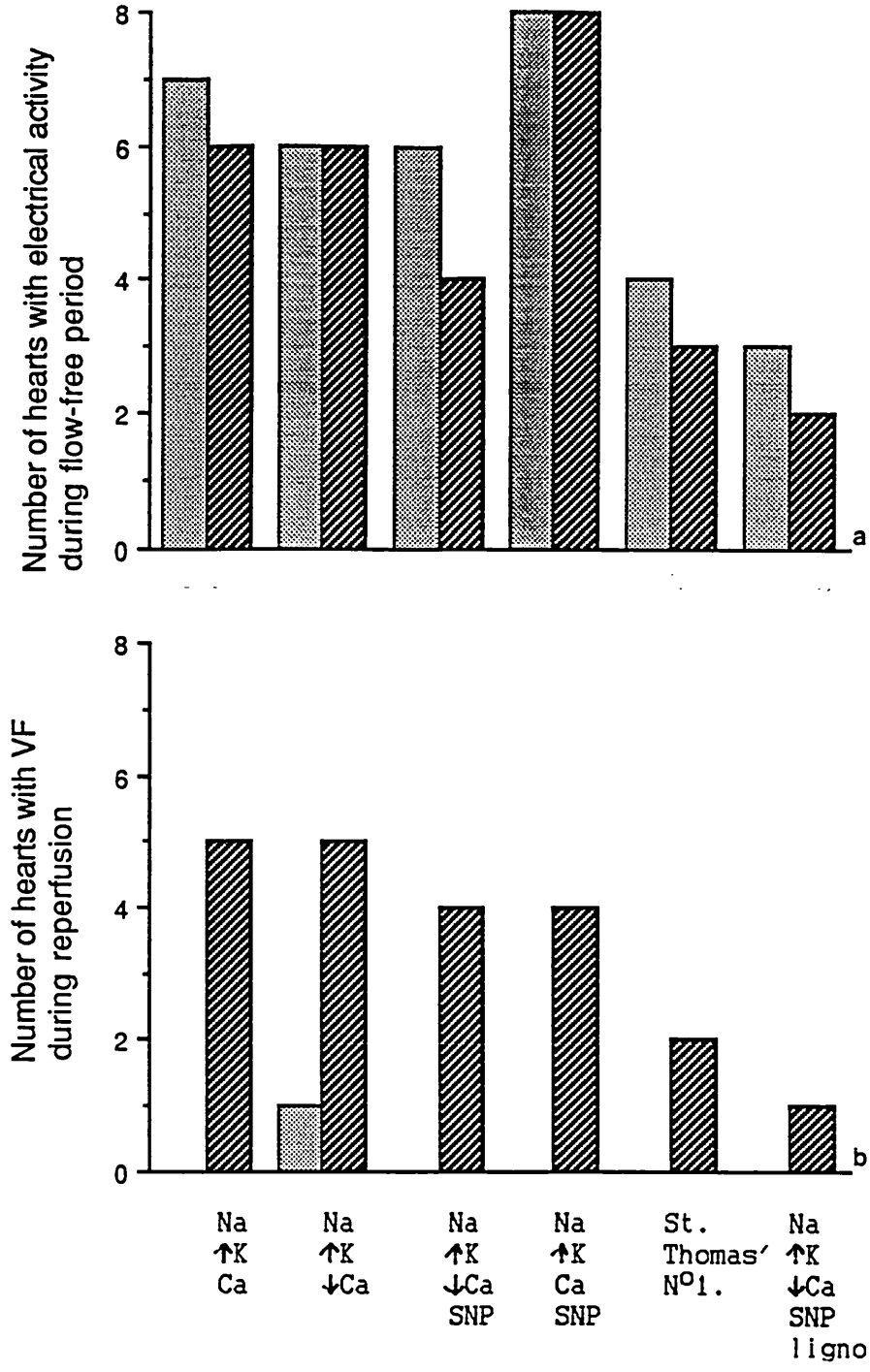


Figure 3A.4

The incidence of electrical activity during arrest induced by six cardioplegic solutions and of VF during reperfusion. a) The number of hearts with electrical activity during flow-free periods after cardioplegic infusions and (b) the number of hearts entering ventricular fibrillation during reperfusion. Stippled boxes = first period of cardioplegia and hatched boxes the second. Maximum = 8 in each group.

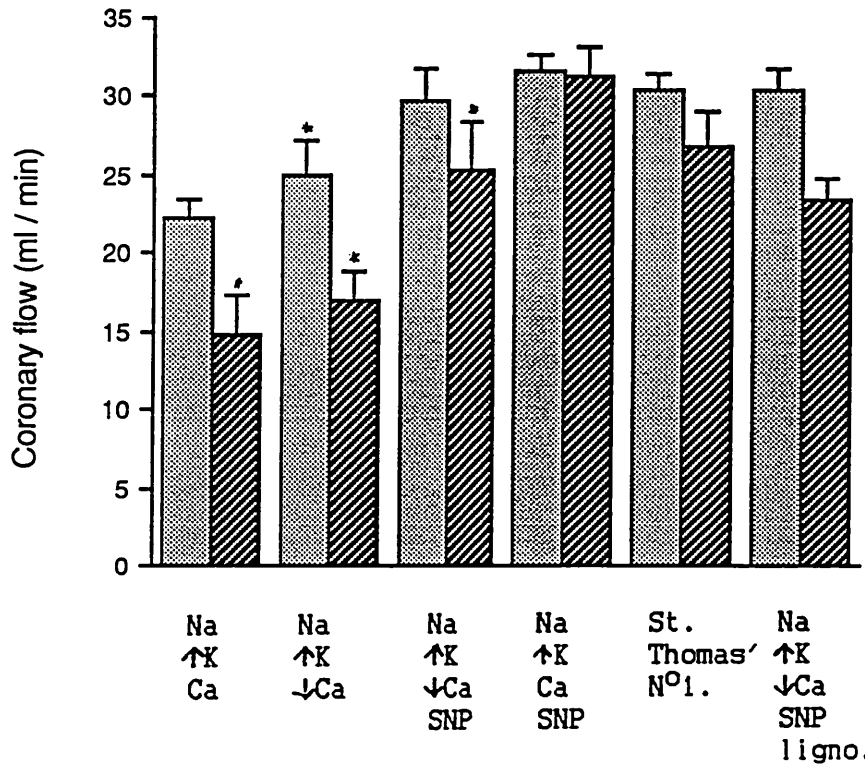


Figure 3A.5

Coronary flow during the first minute of reperfusion after cardioplegia induced by six solutions. The coronary flow in the first minute of reperfusion after the 30 minute (Stippled) and the 60 minute (hatched) period of cardioplegia are shown. Each column is the mean and the bars the s.e m. of 8 (*=7) observations.

There were significant (P<0.05), ANOVA) differences between the times taken to reach half though not full recovery of amplitude of contraction after the first period of arrest and significant differences (P<0.05, ANOVA) in the time taken to reach both half maximal and maximal recovery of contraction after the second period. After the first period of arrest half maximal recovery took the longest in hearts which had received sodium channel blockers (fig. 3A.6). In these hearts recovery was much more gradual and began with very small beats, whereas in the others the amplitude of the first beat during reperfusion reached almost half that eventually attained during recovery. Maximal recovery (fig. 3A.6) after the first period of arrest was delayed only in the hearts which had received St.Thomas' Solution Number One. There were considerable differences between groups during recovery from the second period of cardioplegia. In all cases recovery took much longer, the least increase being seen in the hearts which had received the solution containing lignocaine and sodium nitroprusside and the greatest being in the hearts which had received St. Thomas' Solution Number One. The maximum recoveries of amplitude of contraction (fig. 3A.7), heart rate (fig. 3A.9) and coronary flow (fig. 3A.7) eventually attained were not significantly different (P<0.05 level) at the three time-points chosen for analysis.

Amplitude of contraction recovered after the second period of arrest to a peak level and then declined. In

43 of the 48 hearts examined, amplitude of contraction fell to 50% of the peak level within the 5 hour observation period (in the remaining 5 hearts, A-V conduction delay had developed and the amplitudes of contraction were very variable making accurate assessment of the half-time impossible). The time taken for peak recovery of amplitude of contraction to fall by 50% is shown in fig. 3A.8.. Taken as a whole, the differences were not quite statistically significant ($P<0.05$, ANOVA), but the fastest decline 94min (s.e.m. = 12.66min) (that following St. Thomas's Solution Number One), was significantly different ($P<0.05$, unpaired "t" test) from the slowest decline, 183min (s.e.m. = 22min) that following arrest by the new solution containing lignocaine, sodium nitroprusside, lowered $CaCl_2$ concentration and elevated KCl concentration (fig. 3A.8).

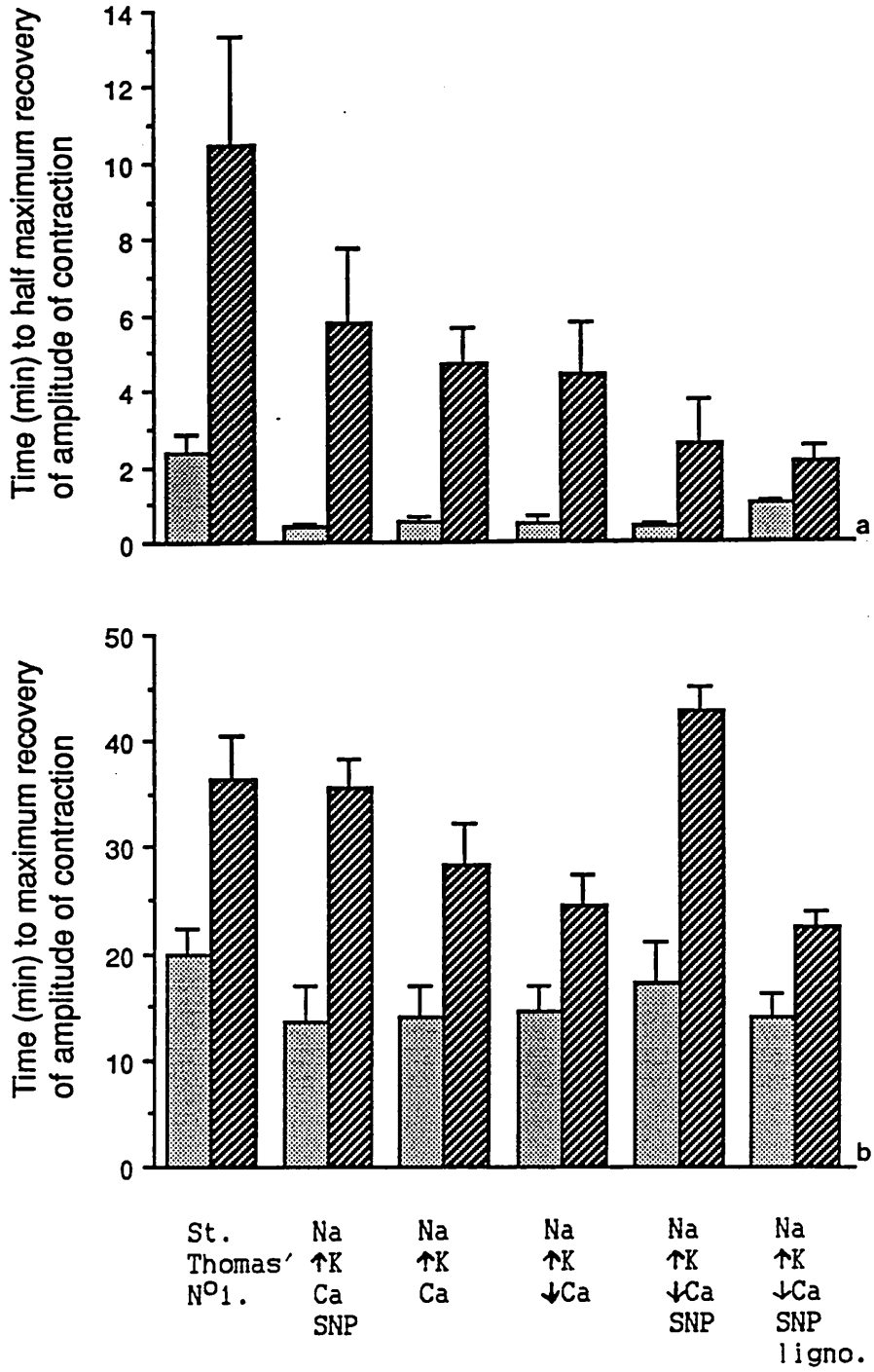


Figure 3A.6

Rate of recovery of amplitude of contraction following cardioplegia induced by six solutions. The time taken for amplitude of contraction to reach a) 50% and b) 100% of the maximum value attained after the 30 minute (stippled) and the 60 minute (hatched) periods of cardioplegia. Each column is the mean of 8 observations and the bars the s.e.m. n = 8 for each group, 48 in total.

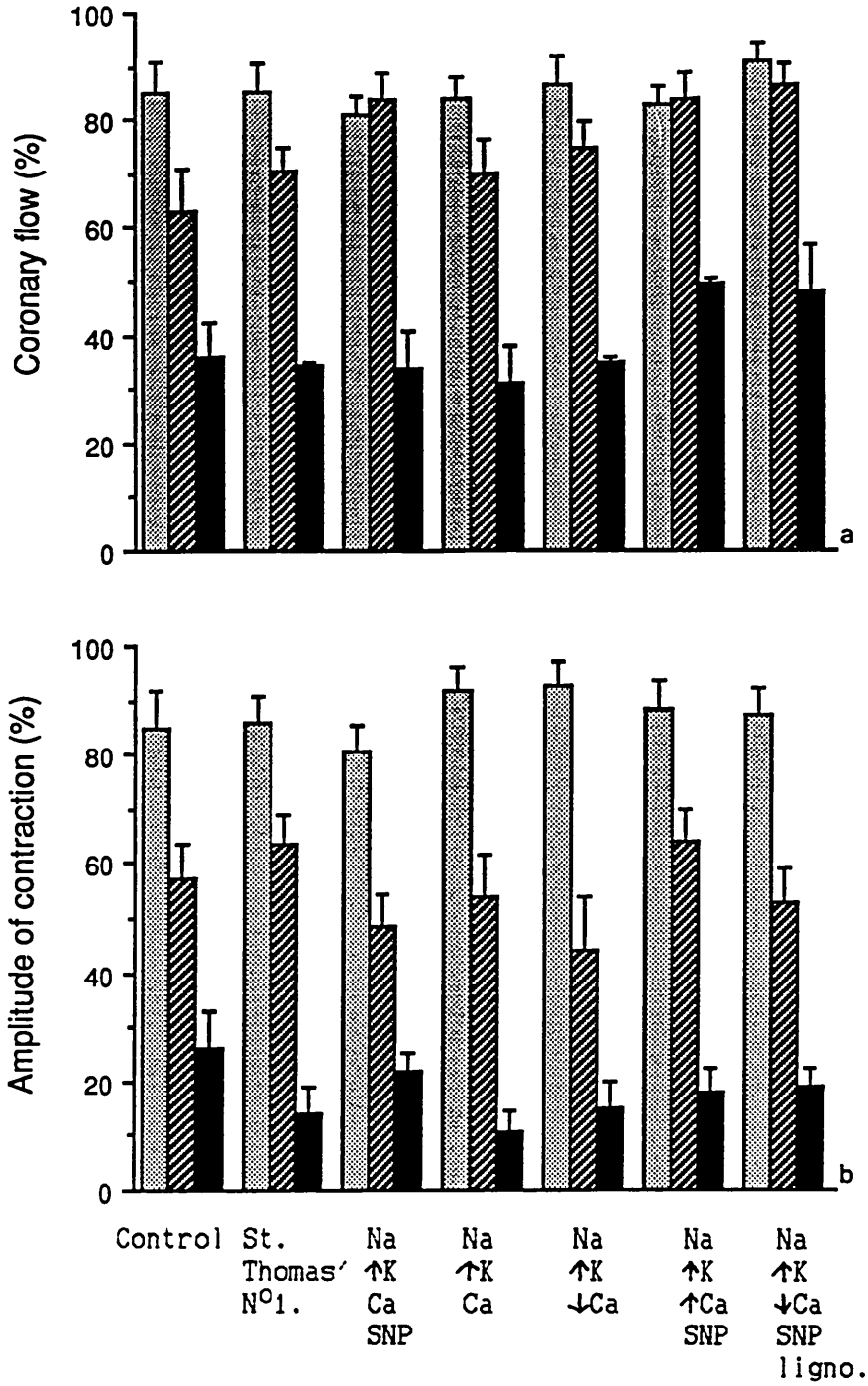


Figure 3A.7

The extent of recovery of coronary flow and amplitude of contraction after cardioplegia induced by six solutions. (a) coronary flow and (b) amplitude of contraction 30 minutes after the 30 minute period of cardioplegia (stippled), and 60 (hatched) and 210 (solid) minutes after the 60 minute period of cardioplegia are expressed as % of the reference values recorded 30 minutes after setting up the preparation. The control group was perfused for the whole period with McEwen's solution. Each column is the mean of 8 observations and the bars the s.e m. n = 8 for each group, 56 in total.

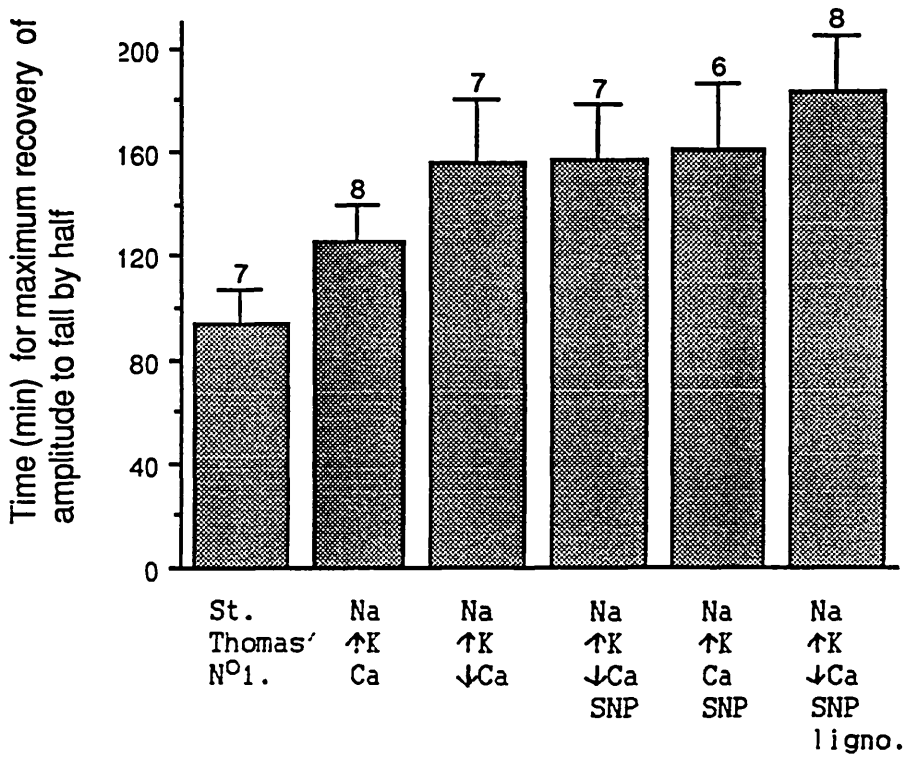


Figure 3A.8

The rate of decline of the maximum recovery of amplitude of contraction achieved after cardioplegia induced by six solutions. The time taken for the maximum recovery of amplitude of contraction attained after the 60 minute period of cardioplegia to decay to 50% is shown. Each column is the mean and the bars the s.e.m. of the number of observations indicated (maximum possible = 8). Where n is less than 8 this indicates that amplitude did not always fall by 50% over the 5 hour observation period.

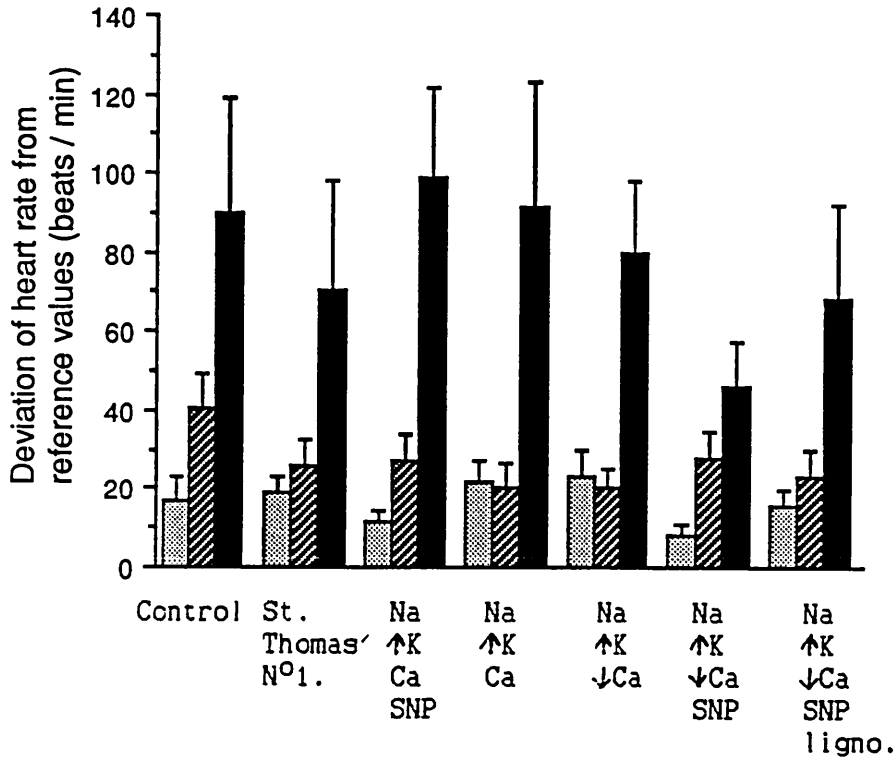


Figure 3A.9

Changes in heart rate after recovery from cardioplegia induced by six solutions. The deviation (regardless of direction) from the reference value (recorded 30 minutes after setting up the preparation) 30 min after the 30 min period of cardioplegia (stippled), and 60 (hatched) and 210 (closed) min after the 60 min period of cardioplegia. The control group was perfused throughout with McEwen's solution and deviation was recorded at the same time as the other groups. Each column is the mean and the bars the s.e.m of 8 observations, n = 8 for each group, 56 in total.

B. SOME PHARMACEUTICAL CONSIDERATIONS IN THE FORMULATION AND STORAGE OF ONE OF FIVE NEW CARDIOPLEGIC SOLUTIONS

Introduction

The water solubilities and methods of sterilisation of the components of the cardioplegic solution are given below.

Component	Solubility in Water	g/litre Cardioplegic solution	Method of Sterilisation
NaCl	1 : 3	7.60	Autoclave or Filtration
KCl	1 : 3	1.80	" "
$CaCl_2,2H_2O$	1 : 1.2	0.11	" "
Na_2HPO_4	1 : 12	0.14	" "
$NaHCO_3$	1 : 11	2.10	" "
Lignocaine hydrochloride	1 : 0.7	0.29	" "
SNP	"Freely"	0.03	" "

Table 3B.1

The solubilities in water and methods of sterilisation for various components of cardioplegic solutions. (Martindale - The Extra Pharmacopia, 28th edition).

In clinical practice, cardioplegic solutions are usually prepared at the time of use by the addition of a concentrated solution of the active principles to a sterilised volume of intravenous infusion fluid, such as Ringer's Injection in the case of St Thomas' Solution Number One.

A concentrated form of the major active principles in a volume of 20ml would be convenient for storage and could then be added to readily available infusion solutions to make up the final solution as and when needed. However, sodium bicarbonate, although reasonably soluble, cannot safely be included in the storage solution because it reacts with calcium chloride to precipitate calcium carbonate. This potentially dangerous reaction could lead to the introduction of particulate matter into the coronary arteries and might reduce the availability of calcium within the solution, possibly to a point at which it would be inadequate to protect from the calcium paradox. Similarly, high concentrations of sodium chloride cause the loss of solid matter from the walls of glass ampoules and could therefore result in damage similar to that which may have resulted from silicate fragments in the original Melrose solution.

Methods

The only observed interaction between the components occurred when a solution 500 times more concentrated than that to be infused was prepared, in which sodium nitroprusside and lignocaine formed visible clumps. A solution was prepared containing lignocaine, sodium nitroprusside, KCL, NaH_2PO_4, $CaCl_2$ at 50 times the concentration to be infused. 20ml of this concentrate is then to be added to 980ml of a solution containing $NaHCO_3$

and NaCl. The concentrate, which had a clear pink appearance, was decanted into fourteen 10ml ampoules which were then sealed in air. Seven were immediately covered in aluminium foil and the remainder left exposed to light. The groups were placed next to each other and left undisturbed at room temperature.

Results

After approximately 2 days the solution in all seven ampoules exposed to light had turned a pale yellow. The solution darkened a little over two weeks and become very slightly cloudy with the presence of a small amount of a fine white deposit, which redissolved on shaking. However, the solution in all the ampoules which had been wrapped in aluminium foil was still the original pink colour, clear and free of any visible deposits even after eight weeks, the duration of the experiment to date.

C. Discussion of Results Presented in Section Three

In general it is clear from the results presented that properties bestowed by specific components can be identified in the response to a whole solution. This suggests that it may not be necessary to use a model involving cardioplegia as applied clinically in order to investigate individual components. These results suggest that the widely adopted protocol of only a single 30 minute period of cardioplegia and a 30 minute recovery

period is inadequate and cannot reveal the effects of a solution fully. Furthermore, it appears that the evaluation of a solution should include experiments which mimic the intended clinical application as closely as possible because even slight variations may be sufficient to make a different solution more appropriate.

The protocol of these experiments was designed to mimic a heart in which there is some pre-existing pathology. The first period of arrest, which lasted only 30 minutes, can be viewed as a means of "damaging" the heart, also recovery after this period can be compared with results from a large body of literature. The second period of cardioplegia was perhaps more typical of the clinical situation in that it was longer and performed in hearts which had already survived one period of arrest. It is very clear from the results that the incidence of ventricular fibrillation during reperfusion and the time taken to recover from arrest are strikingly different after the second period of cardioplegia. These differences may be because the second period was longer than the first or simply because it was second, either way it is reasonable to say that it was more typical of the clinical situation. Had the durations been randomised, differences between the two periods might well have been masked.

1. Properties of an Ideal Cardioplegic Solution

The overall success of an open heart operation is determined by the effectivness of the cardioprotection and the quality of the surgery. Protection will be greater if the operation is completed quickly and the surgery more rapid and of a higher quality, if favourable operating conditions are maintained. In addition, as the desired operating conditions are produced by an effective suppression of metabolism, which is essential for a good recovery after cardioplegia, the ability to produce these conditions may indicate that recovery from periods of arrest longer than attempted in these experiments might also be greater. Accordingly, the requirements of a functional solution may be expanded and an ideal solution defined in some detail as one which

a. Induces diastolic arrest rapidly
b. Maintains electro-mechanical standstill
c. Can be easily and rapidly overcome by simple washout or pharmacological antagonism
d. Enables the heart to tolerate periods of little or no perfusion
e. Does not itself subsequently bring about significant reduction in cardiac performance
f. Is safe and effective under the likely conditions of infusion
g. Is easy to prepare and store in a stable form

2. Onset of Diastolic Arrest

The speed of onset of diastolic arrest (fig. 3A.2) clearly reveals which of the cardioplegic solutions tested contained a sodium channel blocker (lignocaine or procaine), arrest occurring within about 8 seconds of infusion whereas solutions without a sodium channel blocker took approximately 16 seconds.

The hearts which had received sodium nitroprusside had much higher coronary flows during the induction of arrest but were not arrested more quickly, suggesting that the rate of equilibration with the extracellular fluid was not the rate-limiting step in determining the onset of arrest in these experiments. From the beginning, it was felt that the more rapid the arrest the greater would be the energy reserves remaining in the heart and the better the tolerance of ischaemia. Over long periods of ischaemia, it is probable that differences in the speed of onset of arrest may affect the extent of recovery afterwards.

3. Maintenance of Arrest

During the infusion of the extracellular cardioplegic solutions used in this study the amplitude of contraction diminishes beat by beat until diastolic arrest occurs. Sometimes, single beats usually of amplitude approximately 30 per cent of that immediately before infusion, occur periodically afterwards. For the

purposes of this study any such beats occurring at least 10 seconds after the initial induction of diastolic arrest are termed "escape beats". Escape beats occurred in nearly all groups at some time, the exceptions being those which had received a sodium channel blocker. Electrical activity in the presence of mechanical arrest was seen during the original experiments with potassium citrate (Baker et al. 1957). The resting membrane potential recorded from isolated atrial tissue bathed ($28^{o}C$) with the solution used in these experiments and containing high potassium (24mM) and low calcium (0.73mM) was -43mV (s.e.m. = 3.13mV, n = 8 preparations, 40 impalements. For experimental method and protocol see Section 2D). This membrane potential is below that at which sodium channels are generally accepted to re-enter the activated but closed state, accounting for the arrest seen, but only just. It is extremely likely that with cardioplegic solutions which contain lower potassium concentrations, such as St. Thomas' Solution Number Two which contains only 16mM, the membrane potential will be very close indeed to the critical value and this must make electrical activity and possibly escape beats more likely. The success of this solution in causing arrest must therefore depend to some extent on the ability of a lower calcium concentration and the presence of magnesium to uncouple electromechanical activity. Electrical activity during the arrest period has recently been observed with specially designed electrodes in dog hearts

arrested in situ with clinical cardioplegic solutions. Hearts in which no electrical activity was recorded subsequently recovered more completely than those hearts in which electrical activity persisted during the arrest period (Landymore, Marble, Trillo, MacAulay, Faulkner and Cameron 1986). This is hardly surprising as electrical activity can result only from the movement of ions across membranes. These ions will then either remain where they are and possibly result in pathological changes, or they will stimulate active pumping mechanisms which will reduce energy reserves and tolerance of ischaemia. Furthermore, the experiments in Section Two suggest that the presence of electrical activity is also a prerequisite of escape beats, which are not only hazardous surgically, but also cause expenditure of energy. Unfortunately, such electrical activity is unlikely to be easy to detect during surgery. However, the above results and those presented in Section 2 confirm that in this simple rabbit heart model it is easy to detect some form of activity which might be analogous to that seen in dog hearts. Recently, it has been reported that electrical activity is diminished if the cardioplegic solution contains a calcium channel blocker such as verapamil (Landymore, Marble, Trillo, Faulkner, MacAulay and Cameron 1987), this observation is consistent with those made in this study which suggest that lowering extracellular calcium concentration reduces the occurrence of electrical activity and other

phenomena, such as escape beats, which appear to be associated with it.

4. Rate, Quality and Extent of Recovery

Ventricular fibrillation typically began within the first minute of reperfusion. In total, there were 22 cases of VF, of which 21 occurred during reperfusion after the second period of cardioplegia (fig. 3A.4). Not surprisingly, VF was least common in hearts receiving lignocaine, followed by those receiving St.Thomas' Solution Number One, containing procaine. The time taken for amplitude of contraction to recover by 50% of that finally attained was rapid, except in the hearts which had received solutions containing lignocaine and more especially the procaine-containing St. Thomas' Solution Number One (fig. 3A.6). In these hearts it took approximately 1 - 2 minutes to reach half maximal recovery as opposed to 30 seconds in hearts which had not received a sodium channel blocker. There was no correlation between half time to recovery and coronary flow early in reperfusion. The pattern of recovery of amplitude of contraction following the second period without coronary flow was very different from that after the first, taking much longer in all hearts. The greatest of these increases was in hearts which had received St. Thomas' solution Number One, taking 10 minutes the second time as opposed to 2 minutes on the

first occasion. The most likely reason for the delayed recovery, almost twice as long as any other group, is the procaine which is known to exert lasting negative inotropic effects, but it is possible that the magnesium component of the solution may also contribute. The recovery of amplitude of contraction to 50% of the maximum subsequently achieved was fastest and most similar in both recovery periods for those hearts which had received lignocaine, reduced calcium and sodium nitroprusside where recovery took 2 minutes the second time and one minute the first time.

Hearts which had received lignocaine took considerably longer to achieve half maximal recovery but reached maximum recovery at about the same time as the other groups, suggesting that a lingering negative inotropic effect of lignocaine had by then worn off. However, hearts which received St. Thomas' Solution Number One continued to lag behind the others right up to maximal recovery. This was most probably due to the procaine which this solution contains.

It took much longer for hearts to recover fully from the second period of cardioplegia than from the first, though proportionately the differences are not as marked as for half maximal recovery.

No differences were found between the groups for heart rate and amplitude of contraction, or coronary flow. This suggests that despite the differential effects on other parameters the level of protection during

cardioplegia provided by the various solutions was essentially the same. The amplitude of contraction of isolated hearts which had undergone no experiment, other than isolation and perfusion, fell by 50 % in approximately 240 minutes (fig. 1B.6c), but after cardioplegia the peak recovery of amplitude of contraction fell by 50% in 94min (s.e.m. = 12.66min) for hearts that had received St. Thomas's Solution Number One, and 183min (s.e.m. = 22min) for those that had received the new solution containing lignocaine, sodium nitroprusside, lowered $CaCl_2$ concentration and elevated KCl concentration (fig. 3A.8). This suggests that cardioplegia alters the ability of isolated hearts to tolerate perfusion. A likely explanation for the difference in the rate of decline of amplitude of contraction seen after cardioplegia induced by the two solutions would be a decrease of coronary flow, but there was no difference between groups at the time-points recorded. The difference in the rate of decline of contraction between the two groups and the observation that the most significant differences (although not significant at the $P<0.05$ level) between all groups for heart rate and amplitude of contraction were recorded at the end of the 5 hour observation period suggest that had the observation period been even longer, significant differences between the extents of recovery may have been seen.

5. Storage of Cardioplegic Solutions

Each component is very highly soluble in water and the required concentrations are very low, making precipitation unlikely. All non-gaseous components can easily be sterilised by filtration and autoclaving. In addition all components are in regular clinical use, which should make the introduction of this solution possible in a relatively short period of time.

The usual indication that a solution of sodium nitroprusside has decomposed enough to be unfit to use is the appearance of a blue colour. In this cardioplegic concentrate the cloudiness and deposits associated with the earlier change from pink to yellow necessitate that this change be accepted as the indication that a solution is unfit. Clinicians are acquainted with these colour changes through the widespread use of sodium nitroprusside in the management of acute hypertension and the induction of hypotension during surgery. St.Thomas' solution Number One contains procaine, which is also light sensitive, but unfortunately there is no colour change associated with its breakdown and therefore no simple means of assessing the condition of the solution.

The results of these simple experiments suggest that a convenient concentrated form of the solution can easily be prepared and that in the concentration suggested for storage the colour changes associated with the breakdown of sodium nitroprusside are unmistakable, but do not

occur if the solution is protected from light, even after several months.

C. CONCLUSIONS DRAWN FROM SECTION THREE

The effects of five new cardioplegic solutions and the widely used St. Thomas's Solution Number One, all examples of extracellular type solutions, were investigated in rabbit isolated hearts. No differences were found between the final extents of recovery achieved after cardioplegia with the various solutions. Differences were found for parameters relating to the induction and maintenence of arrest and recovery immediately thereafter. These differences may ultimately be of significance in determining the duration of an operation or the conditions under which it is performed, but the relative importances of both the parameters and the differences will vary between specific clinical situations and as such cannot reasonably be assessed from this experimental model. Therefore no conclusion can be drawn here as to which is the superior cardioplegic solution.

The absence of any demonstrable improvement in extent of recovery afforded by any of the six solutions suggests that within the confines of an extracellular type formulation protection is unlikely to be increased past that which is already available clinically in the form of

the St. Thomas's Hospital Solutions. A major increase in the duration of safe cardioplegia, sufficient to improve the availablity of donor hearts, may be achievable only by adopting an alternative approach to solution design and infusion.

SECTION FOUR

THE FORMULATION AND INFUSION PARAMETERS OF A NEW CARDIOPLEGIC SOLUTION FOR THE LONG-TERM STORAGE (eg. 24 HOURS) OF DONOR HEARTS BEATING OR ISCHAEMICALLY ARRESTED AT THE TIME OF ACQUISITION

Introduction

The five new cardioplegic solutions and St.Thomas's Solution Number One compared in Section Three, although different from each other, are all nonetheless typical of the extracellular type solutions already used sucessfully in reparative surgery. In the previous section, the final extents of recovery achieved after cardioplegia were not dependent upon which solution had been used to induce arrest. The extracellular type solutions in clinical use do not enable the transportation of donor hearts to distant transplant centres nor do they enable the use of hearts which have ceased to beat at the time of acquisition. The present shortage of suitable donor hearts would be alleviated if the criteria for removal and storage were more like those for kidneys, which can be kept in a viable state even if removed many hours after death and stored for up to 30 hours. The formulation of an effective cardioprotective solution specifically for the heart in transit, whether beating or not at the time of acquisition, requires an understanding of the ways in which their needs differ from those of hearts undergoing reparative surgery. Perfusion solutions and infusion parameters can then be designed accordingly.

A. REASONS FOR POOR VIABILITY AFTER PROLONGED CARDIOPLEGIA

The following information has been obtained from observing more than 460 periods of experimental arrest from 4 minutes to 48 hours in duration, performed under different conditions in 130 rabbit isolated perfused hearts.

1. Contracture During Cardioplegia

Immediately after arrest the heart is usually flaccid, but it becomes progressively more contracted and solid to the touch. This is due initially to rigor, as the intracellular concentration of ATP decreases, and then also to elevation of cytosolic calcium concentration (Smith and Allen 1988). Hypothermia and cardioplegic solutions containing calcium channel blockers, or a calcium concentration lower than that usually present in extracellular fluid, delay the development of contracture (Henry, Shuchlieb, Davis, Weiss and Sobel 1977). Severe contraction may be sufficient to alter permanently the arrangement of the contractile elements or the connections between adjacent cells. The entry of ions is accompanied by an influx of water leading to cell swelling which may contribute to the development of tension. Ion and water movements are likely to be more severe if the heart is perfused continuously with a solution really intended to be given as a single bolus repeated as may be necessary.

2. Ventricular Fibrillation During Reperfusion

Ventricular fibrillation occurred often during early reperfusion after 30 - 60 minutes of normothermic cardioplegia but rarely after longer periods. This state has been shown to be extremely damaging to rabbit isolated hearts even in the presence of perfusion (fig. 2F. 1) and should not be allowed to persist.

3. A-V Conduction Block During Reperfusion

The most usual electrical abnormality during recovery from more than an hour of cardioplegia was A-V conduction block of various degrees and durations, but usually associated with poor coronary flow. Increasing the perfusion pressure to 95cm H_2O from the usual 65cm H_2O increased coronary flow and corrected A-V conduction block.

Coronary flow and A-V conduction velocity, as indicated by the lengthening P-R interval on a surface ECG recording, are also diminished after about 4 hours of standard perfusion. In three hearts with severe A-V block (1 in 4 to 1 in 8) and coronary flows of less than 3ml/min, the distribution of perfusate was observed by adding a water soluble blue ink. Within 2 minutes the blue colour had spread evenly across the surface of the ventricles, but even after 20 minutes it was not visible in either atrium.

The functional borders of the A-V node are indistinct but extend into the right atrium (Meijler and Janse 1988), so it must be suspected from the above observations that the A-V node also is poorly supplied with perfusion solution and therefore hypoxic. This condition is well known to decrease conduction velocity in excitable tissues.

Interestingly, human hearts arrested for surgery with 1000ml of St. Thomas' solution Number One develop A-V block on reperfusion whereas those arrested with only 500ml do not (Hearse, Braimbridge and Jynge 1981). Both in these cases and the experimental settings described above, A-V block is associated with the infusion of large volumes of ionic solutions and could be a consequence of osmotic damage to the vessels supplying this region, or possibly the deposition of crystals even though the solutions are initially filtered. In either case the state of the blood supply to the atria and A-V node is of interest and it is worth reflecting that the atria normally receive non-coronary collateral perfusion from the mediastinum, which is obviously absent in the Langendorff preparation.

4. Failure To Re-establish Coronary Flow

The "no re-flow" phenomenon (Kloner, Ganote and Jennings 1974) aptly describes failure to re-establish perfusion after organ transplantation, or occlusion of major

vessels. In rabbit isolated hearts, after long periods of cardioplegia it is sometimes difficult to re-establish coronary perfusion. The two main mechanisms contributing to this situation appear to be as follows:

a. Due to vascular contraction or compression

The moderate elevation of the potassium concentration of extracellular type solutions may activate voltage dependent channels in vascular smooth muscle, allowing calcium entry and so inadvertently leading to constriction of the vessels and a reduced coronary flow. Over long periods, the calcium overload may result in permanent and severe constriction. The results presented in figure 3A.5 show that cardioplegic solutions with potassium concentrations of 24mM have lower coronary flow early in reperfusion than the equivalent solutions with added sodium nitroprusside (10^{-4}M).
A continuous contraction of the myocardium or swelling due to oedema (Lee, Willson, Domenech and MacGregor 1980) may compress the vessels running through it, so that even if they are not themselves damaged the flow through them will be reduced.

b. Due to blockage by cell debris and air emboli

In 4 rabbit isolated hearts exhibiting the "no-reflow" phenomenon, increasing perfusion pressure to 95cm H_2O

restored flow, which could then be adequately maintained at the usual pressure of 65 cm H_2O. The simultaneous recovery in force and rate of beating suggests that a temporary occlusion was responsible for the "no-reflow" phenomenon. This could be caused by adhesions between vessel walls or blockage possibly with cell fragments. The involvement of accumulated endothellal cell debris is supported by the observation that after recovery from prolonged cardioplegia, acetylcholine reduced coronary flow whereas in a freshly isolated heart an increase was usually seen. In response to acetylcholine, vascular endothelium releases a factor (Furchgott and Zawadzr 1980) now thought to be nitric oxide (Palmer, Ferrige and Moncada 1987) which relaxes the underlying smooth muscle, leading to vasodilatation. In the absence of an endothelium, acetylcholine acts directly on the vascular smooth muscle cells causing contraction and thereby constriction of the vessel. The reduced flow with acetylcholine after prolonged cardioplegia may therefore be viewed as pharmacological evidence of damage to the coronary endothelium.

It is possible also that air emboli may be introduced into the entrances of the coronary arteries and pushed further in when perfusion is restarted. In rabbit hearts which have been removed from the perfusion apparatus solution continues to drain from them for a considerable time, this dralnage, which could occur also

In excised human hearts, is likely to pull air into the entrances of the coronary arteries.

Recovery of the myocardium depends on replacing sufficient of the cardioplegic solution in the extracellular space with a physiological perfusion solution. This can occur only through the coronary vascular system. Restoration of coronary flow after arrest is therefore the most important factor for early recovery.

5. Failure To Establish Co-ordinated Electro-mechanical Activity During Reperfusion

One of the new cardioplegic solutions described in section 3 (containing lignocaine 1mM, sodium nitroprusside 10^{-4}M, $CaCl_2$ 0.73mM, NaCl 130mM, KCL 24mM, NaH_2PO_4 0.92mM, $NaHCO_3$ 25mM and equilibrated with 95% O_2 + 5% CO_2) was infused at 22°C for 2 minutes and then for 20 hours at 4°C and approximately 0.1ml min, in 6 rabbit isolated hearts. Reperfusion with McEwen's solution (37°C) was characterised by complete failure of the hearts to resume co-ordinated electrical activity, even in the presence of coronary flows adequate to support some activity in freshly isolated hearts (approximately 3-5ml/min). Interestingly, Burn and Bulbring (1949) have shown that isolated rabbit atria, which stop and fail to re-start spontaneously after several days in a physiological bathing solution, may be restarted by a

high concentration of acetylcholine. Similar results have been obtained in this laboratory. Burn and Bulbring found that the atria which had stopped and failed to restart were totally depleted of acetylcholine and were unable to synthesise it. They proposed that the addition of acetylcholine restored to the membrane a selective permeability to potassium, thereby allowing a functional membrane potential to be generated prior to the first beat. In addition to a high and selective permeability to potassium, the generation of a membrane potential requires a substantial potassium gradient across the membrane and loss of either may result in permanent depolarisation and thereby arrest. The potassium gradient is maintained by energy consumptive sodium/potassium ATPase pumps in the membrane. The energy reserves of a heart deprived of perfusion are soon sufficiently depleted to curtail active pumping and potassium leaves the cell down its concentration gradient as a consequence. The situation may be more extreme if the activity of the pumps is inhibited further by hypothermia. Eventually, intracellular potassium concentration may become so low that the membrane potential established when normal perfusion is restored will be incapable of supporting normal action potentials and the heart will fail to restart. It is unlikely that under normal conditions the duration of cardioplegia required for routine surgery is long enough to result in severe potassium depletion, but the time

often needed for transportation of donated hearts most certainly is. The danger of potassium depletion is particularly apparent if the cardioplegic solution is infused continuously throughout the arrest period. The preservation of intracellular potassium concentration is, therefore, of prime importance in this situation.
Likewise, if the sodium-potassium pump is inhibited, sodium will enter the cell, leading to a similar ionic redistribution and producing an intracellular sodium overload. This could form the basis from which an intracellular calcium overload might develop, especially during reperfusion. In addition, the generation and conduction of action potentials requires a rapid influx of sodium and this will to some extent be inhibited if the intracellular concentration is increased and so the inward gradient reduced.

6. Residual Effects of Cardioplegic Solutions

Recovery may be delayed by drugs such as procaine and slow calcium channel blockers which bind avidly to the myocardium and exert lasting negative inotropic or negative chronotropic effects. Such effects can be avoided if the solution is composed of simple ions which can be displaced from the extracellular space easily, so allowing rapid recovery on reperfusion.
It can readily be appreciated that for the most part the changes which restrict the viability of hearts after

long periods of arrest, or shorter periods under non-ideal conditions, are at least related to and probably caused by redistribution of ionic species and water during the arrest period and the early stages of reperfusion.

B. IMPORTANT DIFFERENCES BETWEEN THE HEART IN SITU AND IN TRANSIT

It is necessary to identify those aspects of cardioplegic solution design and infusion which are relevant only to the constraints imposed by open heart surgery, in order that they can be modified in accordance with the needs of the heart in transit. The most important of these differences appear to be as follows.

1. The Absence of Collateral Circulation

The myocardium is perfused via the coronary system predominantly, but receives also a contribution from a collateral circulation between vessels in the mediastinum (Brazier, Hottenrott and Buckberg, 1975). The importance of collateral circulation in experimental and a variety of clinical situations has been reviewed in detail recently (Schaper, Gorge, Winkler and Schaper 1988). The arrested heart in situ does not receive any direct coronary perfusion once the aorta is clamped. The systemic circulation is then necessarily maintained

by a machine and the heart continues to receive some perfusion via the collaterals.

The extent of collateral circulation varies between subjects, but usually displaces sufficient cardioplegic solution to necessitate reinfusion every 30 to 60 minutes in order to avoid a premature restoration of electromechanical activity (Hearse, Braimbridge and Jynge 1981). However, the required frequency of such reinfusions and the amount needed each time are unpredictable and the total volume of cardioplegic solution used during operations varies considerably. Whilst the protective effects of the extracellular type solutions are seemingly independent of the volume infused under normal surgical conditions, the same is not true of the intracellular type solutions developed by Bretshneider. These contain little or no sodium or calcium and although they provide very significant cardioprotection when used in small volumes and under optimum conditions (Preusse, Gebhard and Bretshneider 1979), in larger volumes they can become ineffective or harmful (Jynge, Hearse and Braimbridge 1978). During surgery it may be necessary to infuse a larger than expected volume of solution and in the case of the intracellular type solutions this may reduce the protection provided. These solutions are in this situation better avoided.

Cardiac surgery is usually conducted with some deliberate cooling of the heart, often by filling the

pericardium with cool saline or infusing a cold cardioplegic solution. Naturally, the collateral circulation warms the heart, thereby adding to the already considerable difficulties of maintaining more extreme myocardial hypothermia (Rosenfeldt and Watson 1979). The provision of extreme hypothermia cannot be guaranteed during surgery and should not therefore be relied upon to prevent the calcium paradox. Solutions which could otherwise induce the paradox, no matter how potentially protective they may be in other respects, should not be used.

Similarly, cardioplegic solution may enter the systemic circulation via the collateral anastomoses and consideration must therefore be given to the effects of the solution elsewhere in the body. Highly cardioprotective compounds, such as tetrodotoxin, which would be dangerous if allowed to enter the systemic circulation cannot be used in routine surgery but could possibly be incorporated into transit solutions for donor hearts.

The absence of collateral circulation from the heart in transit allows an optimum volume of cardioplegic solution to be used, the optimum protective temperature to be maintained, the danger of blood-born substances removed and allows incorporation of easily removable substances which would be harmful in other parts of the body.

2. Pre-existing Pathology and Tissue Variability

Hearts in need of surgery often have some pre-existing pathology which alters the functioning of their tissues, possibly accounting for the clinical observation that cardioplegic solutions are less effective in diseased hearts (Coughlin, Levitski, O'Donoghue, Williams, Wright, Roper and Feinberg, 1979). In particular the even distribution of solution may be impaired in hearts with coronary artery stenosis (Myers et al. 1986), leading to potentially dangerous heterogeneity of protection. However, the hearts chosen for transplantation are obviously healthy and furthermore they are drawn from a narrow age range of donors, mainly young adults. There is more known about the pharmacology, physiology and biochemistry of this group of the population than any other, making the task of formulation easier.

The reductions in tissue variability and the likely free distribution of solution within the myocardium allow more precise calculation of optimum concentrations of components and the mechanics of solution delivery such as rate, volume and pressure, to be made for the donor heart.

3. Possible Dangers of Delayed Recovery

In practice, highly protective solutions which inhibit metabolic processes profoundly are likely to slow

recovery and thus increase the duration of anaesthesia and cardiac bypass. For many patients, the dangers of so doing outweigh the likely benefits of improved cardioprotection.

A donor heart in which such profound metabolic inhibition had been achieved during transit could be allowed to recover during the course of the operation under the protection of a more conventional solution. Delayed recovery at the end of an operation would thereby be prevented. The exchange of solutions would also remove any potentially harmful components of the transit solution before they could enter the systemic circulation of the recipient.

4. Similarities Between the Needs of Donor Kidneys and Hearts in Transit

The formulation of a solution and the design of an infusion protocol must reflect the needs of the situation and also its constraints. With respect to protection requirements, the heart in transit differs greatly from the heart in situ and in many practical respects more closely resembles the donor kidney. Remarkably, excised kidneys perfused with cold Collins' solution can be stored safely for up to 30 hours (Collins, Bravo-shurgarman and Teraskai 1969) and it has recently become possible to store organs such as liver and pancreas for similar periods of time (Belzer and

Southard 1988). However, donor hearts arrested with cold extracellular type cardioplegic solutions are best used within 4 hours (Green 1984), although protection for 16 hours has been reported (English, Cooper, Medd and Wheeldon 1980). Interestingly, the heart and kidney are in some respects very similar. Many of the ion exchange mechanisms which control the generation of action potentials and regulate the cytosolic calcium concentration in the myocardium, are found also in the membranes of kidney cells. By inference, it must be possible to protect these aspects of myocardial functioning for substantially longer periods than at present. The same must be true also of general membrane structure, the maintenance of nuclear material and the ability to synthesise proteins. The apparent differences in the tolerable durations of cardiac and renal ischaemia are therefore very likely to result from the interactions between calcium, the membrane and the contractile proteins in the myocardium and the approaches made so far to the formulation and delivery of protective solutions.

C. THE FORMULATION AND INFUSION OF A CARDIOPLEGIC SOLUTION TO PROTECT HEARTS IN TRANSIT

The heart in transit is easily accessible and the myocardium is intact, making it easy to infuse exact volumes of cardioplegic solution under controlled

conditions throughout the arrest period. The potential benefits and dangers of such an approach are discussed in the following paragraphs.

1. The Protective Effects of Continuous Perfusion

Continuous perfusion will enable delivery of metabolic substrates, remove potentially harmful products of cell breakdown, maintain vascular patency and reduce the likelihood of introducing air emboli, thereby reducing the possiblility of the "no re-flow" phenomenon after cardioplegia.

As the period of arrest increases, some cells will die in spite of any attempt to prevent them from so doing, the enzymes and tissue factors then released into the extracellular space harm other cells and cellular fragments may accumulate to block small vessels. These potentially dangerous products of cellular degeneration will be removed if the extracellular fluid is continually replaced by fresh cardioplegic solution, which must itself be filtered to remove crystalloid fragments, the dangers of which are well known (Robinson, Braimbridge and Hearse 1984).

2. The Dangers of Continuous Infusion: Adverse Effects on Intracellular Ion Concentrations

As energy reserves are depleted, transmembrane ion movements cannot be controlled actively and the loss or

accumulation of ions is determined by the direction and magnitude of their concentration gradients. In a heart which receives a single bolus of cardioplegic solution, equilibria will be established by a redistribution of ions between intracellular and extracellular fluid. The relatively large intracellular volume ensures that changes in intracellular ion concentrations remain small. This is not the case if the extracellular fluid is continuously replaced. The establishing of equilibria then depends on the intracellular loss and gain of ions only and not on any changes that such ion movements make to the composition of the extracellular fluid, which is in effect fixed. Greater changes in the intracellular ion concentrations will therefore occur during constant perfusion and the formulation of the solution to be used in this manner must take this into account, to ensure that intracellular ion concentrations are not changed to an extent which threatens the future viability of the organ. In particular the following must be avoided.

a. Potassium depletion
b. Sodium accumulation
c. Calcium accumulation
d. Magnesium depletion
e. Osmotic imbalances

These ionic changes are the same as those which may occur "naturally" during ischaemia or a period without

perfusion. Thus continuous infusion of a solution designed to be given as single or multiple boli might potentiate the development of damage. The solutions in use today provide very good protection to the heart undergoing reparative surgery but are inappropriate to the protection of the heart in transit. It can readily be appreciated that the extracellular type solutions would, if infused over long periods, lead to potassium depletion and sodium and calcium accumulation. The intracellular type solutions would also lead to potassium depletion and possibly to manifestations of the calcium paradox. Furthermore, the concentrations of ions in these solutions are inadequate to adjust the ionic imbalances which begin to develop within only a few seconds of the heart stopping due to cessation of perfusion. It is a reasonable argument that a solution specifically designed to prevent ionic redistribution, will have some ability to restore ionic distribution if it has already changed. In other words, such a solution might retain significant protective effects in hearts which have stopped before the time of acquisition.

3. The Maintenance of Intracellular Potassium Concentration

Intracellular potassium concentration can be maintained independently of energy consumptive processes only by reducing the outward gradient for this ion or by

rendering the membrane totally impermeable to it. Potassium permeability during cardioplegia depends on the activity of pumps and channels, membrane potential, the concentrations of other ions or drugs, membrane integrity and temperature. The most simple and reliable approach, therefore, is to increase the extracellular potassium concentration until it resembles that within the cell, so removing the outward gradient.

In an enormous amount of literature dealing with cardioplegia there are very few reports of the use of potassium concentrations equivalent to that of intracellular fluid. Of these, probably the most dramatic was that of Reitz, Brady, Hickey and Michalis (1974). They successfully transplanted 9 out of 11 human hearts which had been arrested for 24 hours with a solution containing a potassium concentration of 120-140mM and designed originally to protect donated kidneys (Collins, Bravo-Shurgarman and Teraski 1969). However, this approach was not adopted by other groups and this apparent lack of interest in the kidney type solutions is understandable only in the context of the reintroduction of potassium cardioplegia.

4. Reasons for the Unpopularity of Intracellular Potassium Concentrations

The Melrose technique had originally fallen into disrepute when it became clear that it was not always

successful clinically and could be positively harmful (Helmsworth et al. 1959, McFarland et al. 1960). However, the belief that recovery from rapid arrest had to be better than that from slow asphyxiation by aortic clamping, the method which took over, prompted several investigations into Melrose's solution. Notable amongst these was a report given by Tyers at the 36th Annual Meeting of the Society of University Surgeons (1975) in America. He concluded that the potassium concentration (250mM) of the Melrose solution in clinical use was responsible for the damage it caused and confirmed that potassium, as the citrate or chloride salt, was indeed a safe and efficacious cardioplegic, but recommended that it be used in an isotonic solution at a concentration only between 10 and 50 mM (Tyers et al. 1975). In the discussion which followed, Gay, who had himself reported beneficial effects of potassium (10-50mM) cardioplegia (Gay and Ebert, 1973, Gay 1975 and Gay, Ebert and Kass 1975), asked him to comment on the excellent results obtained (Reitz et al. 1974) with the kidney type solutions. Tyers replied that like Gay he could not repeat the findings of the Reitz group at the National Institutes of Health and had found the kidney type solutions to be "equivalent to hitting the heart with a hammer". This remark was made in front of many whose work was about to culminate in the reintroduction of a safe form of potassium cardioplegia (notably by Levitski et al. 1971, 1975, a,b,c) and seems to have almost ended

published thoughts on using potassium concentrations as high as those found intracellularly. The excellent and consistent results obtained with the low potassium solutions have served as reinforcement and since then there has not been a cardioplegic solution in clinical use which contains a potassium concentration in excess of 40mM (Fisk, Gelfand and Callaghan 1977), 30mM (Conti, Bertranou, Blackstone, Kirklin and Digerness 1978), 25mM (Tyers, Mannly, Williams, Shaffer, Williams and Kurusz 1977), 25mM (Craver, Sams and Hatcher 1978). 20mM (Hearse et al. 1975), 16mM (Hearse et al. 1981).

5. Reasons for the Variable Results Obtained in Hearts With Intracellular Potassium Concentrations (Collins' Kidney Preservation Solution)

Collins' solution remains the preferred way of protecting donor kidneys (Belzer and Southard 1988). However, several aspects of its formulation could explain why there were variable and in some cases disastrous results when it was used in hearts. Of these, the total absence of calcium is the most likely, especially as the calcium paradox, which was known at the time this solution was tried in hearts (Zimmerman et al. 1967), can be prevented by contamination levels of calcium and is dependent on the temperature and volume of solution infused, making variable results between surgical centres quite probable. In addition, Collins'

solution contains dextrose and a concentration of magnesium which has been shown to be damaging to rat hearts, the commonest basic experimental model in this field and is considerably hypertonic, containing 430mOsm/KgH_2O, as opposed to 320mOsm/KgH_2O which is usual for cardioplegic solutions.

6. Calculation of an Optimum Potassium Concentration

In this context, an optimum potassium concentration is the one which prevents serious potassium depletion and does not favour calcium entry via sodium/calcium exchange.

Simple calculations using the Nernst equation reveal;

$$\text{Equilibrium Potential For Potassium} = \frac{RT}{zF} \ln \frac{[K^+]out}{[K^+]in}$$

Where, F = Faraday's Constant
R = Gas Constant
T = Absolute Temperature
z = Valency
ln = Natural Logarithm

In rabbit atria, membrane potential follows and is almost identical with the value predicted for the potassium equilibrium potential (Vaughan-Williams 1958). In rabbit ventricle, for extracellular potassium concentrations over 5mM, membrane potential is within

2mV of potassium equilibrium potential (Lee and Fozzard 1975). Membrane potential relates also to intracellular potassium activity in sheep Purkinje fibres (Sheu, Korth, Lathrop and Fozzard 1980) and guinea-pig atria (Baumgarten, Singer and Fozzard). From these observational studies it appears that the Nernst equation is of use in predicting the membrane potential produced by different intracellular potassium activities but the relationship appears to be less exact and has, for technical reasons, been investigated to a much lesser extent than that between extracellular potassium concentration and membrane potential

a. For the normal intracellular potassium concentration, $[K^+]i$ = 140mM and a normal extracellular potassium concentration, $[K^+]o$ = 4mM, T = 310 K (= 37°C),

membrane potential = -94mV.

Threshold for an action potential is -70mV, and it is reasonable to consider a membrane potential in excess of -70mV to be a "functional membrane potential".

b. The lowest $[K^+]i$ which will give rise to a membrane potential exceeding -70mV when $[K^+]o$ = 4mM and T = 310 K,

$$[K^+]i = 60mM.$$

On theoretical grounds $[K^+]i$ can fall to 60mM during cardioplegia without preventing a functional membrane potential from being established during reperfusion with a $[K^+]o$ of 4mM.

c. If $[K^+]o$ = 60mM, $[K^+]i$ = 140mM, and T = 310 K,

membrane potential = -22.4mV.

A solution was prepared which contained KCL, 60mM, NaCl, 40mM, $NaHCO_3$, 25mM, NaH_2PO_4 1mM and $MgCl_2$ 15mM and gassed with 95%O_2 + 5%CO_2 (this solution with added lignocaine is used in the experiments described later). It was used to bathe rabbit isolated atrial strips (for detailed method and protocol see Section 2D). The resting membrane potential recorded at 28°C was -21.2 mV (s.e.m. = 1.68mV, n = 8 preparations, 40 impalements). In the light of these simple calculations it is obvious that the potassium concentration of a cardioplegic solution can be much less than the intracellular concentration and still prevent potassium depletion from reaching a point where reperfusion membrane potentials are at risk.

The degree of depolarisation induced by a potassium concentration of 60mM is sufficient to prevent sodium channel de-inactivation but will not maximally activate calcium influx via sodium/calcium exchange. However, further manoeuvres will be necessary to prevent calcium influx by this mechanism.

7. The Importance of Infusion Protocol to the Calculation of Optimum Ionic Concentrations

Imagine a simplified cell in which active processes have been inhibited by energy depletion and hypothermia, and around which the extracellular fluid has been replaced by cardioplegic solution. Potassium leaving the cell enters the extracellular space, assuming free membrane permeability and equal volume for intra and extra cellular space, an equilibrium will be established across the cell membrane when potassium concentration is the mean of the two originals, ie. 100mM if the cardioplegic solution contained 60mM potassium (60+140 /2). From the arguments set out in section 4C.6b, an intracellular potassium concentration of 100mM is more than adequate to establish a functional membrane potential with normal extracellular fluid ($[K^+]o$ = 4mM). Similarly, during infusion of a conventional cardioplegic solution ($[K^+]o$ = 16mM), equilibrium would be established at 140+16 /2 = 78mM which is also adequate. However, if the conventional cardioplegic

solution passes through the myocardium continually, so that $[K^+]o$ is constant, intracellular potassium concentration would eventually be reduced to 16mM, which is obviously too small to re-establish a functional membrane potential.

Since depolarisation, and thereby the beginnings of a possible calcium overload, occurs when the potassium gradient is reduced, it is reasonable to argue that the lowest potassium concentration capable of arresting the heart, approximately 16mM (Hearse 1988), should be used because it will take longer for equilibrium to be established, so delaying depolarisation and a possible influx of calcium via sodium/calcium exchange. However, starting with an $[K^+]o$ = 16mM and allowing potassium to accumulate in the extracellular space will result in equilibrium for the loss of 62mmol X intracellular fluid volume (in litres) of potassium. If a solution containing 60mM potassium is continuously infused, 80mmol X intracellular fluid volume (in litres) of potassium would have to leave the cell to produce potassium equilibrium. Hence equilibrium would be established more quickly if a solution containing 16 mM of potassium was infused as a single bolus at the beginning of a period of cardioplegia, than if a solution containing 60mM potassium was infused continuously.

This is obviously a simplification and the real situation is affected also by other ionic species,

relative permeabilities and temperature. Hypothermia causes a phase-transition in the lipid components of the membrane and decreased permeability to ions. This is in addition to the physical effects of temperature on the mobility of ions, which also reduces membrane potential at lower temperatures (see Nernst equation).

8. The Prevention of Intracellular Accumulation of Sodium and Calcium

It is essential that calcium influx during the arrest period or during reperfusion is kept at a minimum. This may be achieved by inhibiting the direct and indirect mechanisms by which calcium may enter the cell. In rabbit hearts, a lowering of the calcium concentration in McEwen's solution from 2.18mM to 0.36mM, reduces amplitude of contraction by 60% (fig. 2A.2) and heart rate by 40% (fig. 2A.3) over 8 minutes at 37^{o}C without adversely affecting the recovery when the normal calcium concentration is restored. This may therefore be considered to be a safe and functionally significant reduction of the inward gradient for calcium. Calcium may enter also in response to depolarisation and this route may be activated by the high potassium concentration of a cardioplegic solution. In sheep Purkinje fibres, depolarisation-induced tonic contraction, thought to be mediated by sodium-calcium exchange increasing intracellular calcium concentration,

is inhibited by reducing extracellular calcium concentration (Vaughan-Jones, Eisner and Lederer 1985). Such contractions are inhibited also by reducing the sodium equilibrium potential (E_{Na}) (Vaughan-Jones, Eisner and Lederer 1985). An excessive influx of calcium, possibly leading to pathological changes, may occur during reperfusion in exchange for intracellular sodium accumulated during arrest. A build up of sodium could result from non specific entry or entry through L-Type calcium channels, which lose their selectivity if extracellular calcium concentration is reduced. The inward electrochemical gradient for sodium is determined by the sodium concentration gradient and the membrane potential (ie. Inward gradient = $E_m - E_{Na}$) hence if the membrane potential is reduced, for example by high potassium solutions, the inward sodium gradient is reduced also. If, in addition the extracellular sodium concentration is lowered the inward electrochemical gradient for sodium is reduced further. Thus, intracellular sodium accumulation is limited, thereby reducing potentially damaging calcium influx due to sodium-calcium exchange during reperfusion. In rat isolated hearts, infusion of calcium-free solutions results in a massive influx of calcium, leading to the calcium paradox, when the ion is returned to the perfusion solution, but if the sodium concentration of the calcium-free solution is reduced to 35mM the calcium paradox does not occur during reperfusion (Alto and

Della 1979). Similarly, in a model almost identical with that used in this study, an extracellular sodium concentration reduced from 156mM to 78mM has been shown to enable rabbit isolated hearts to tolerate 5 minutes of perfusion with calcium-free solutions at 37°C, conditions that otherwise result in the calcium paradox (Baker and Betmouni 1987). Low sodium/low calcium solutions have been used successfully as cardioplegic solutions during reparative surgery (Bretshneider 1964, Rygg and Petersen 1978), (see General Introduction).

9. The Maintenance of Intracellular Magnesium Concentration

This ion is involved in the activation and regulation of metabolic processes and the maintenance of membrane structure. Magnesium can also prevent the calcium paradox in situations where it might otherwise occur and it blocks calcium channels. These effects appear to be greater in rat than rabbit myocardium and although magnesium is an effective constituent of many cardioplegic solutions, both intracellular (Bretschneider 1964, Kirsch et al. 1972) and extracellular (Hearse, Stuart and Braimbridge 1978), the exact mechanism of protection is in both cases uncertain.

Sodium influx through what is now referred to as the L-type calcium channel can occur during infusion of

calcium-free solutions. If membrane potential is clamped at -80mV the sodium influx is inhibited by a magnesium concentration of 1-2mM in the extracellular fluid, but this manoeuvre is less effective when membrane potential is clamped at less negative values (-30 to -50mV) and doesnot prevent the calcium paradox if the membrane potential is clamped positive to -10mV (Rodrigo 1988). It is possible that a higher concentration of magnesium would be effective at preventing the calcium paradox when calcium depletion occurs at the less negative membrane potentials, for instance those associated with a high potassium cardioplegic solution.

A concentration equivalent to the total intracellular concentration (approximately 15mM) has been shown in rat hearts to be the optimum in a complex relationship between concentration in cardioplegic solutions and subsequent recovery after periods without perfusion (Hearse, Stewart and Braimbridge 1978). Similar concentrations, 10mM for rabbit ventricular septum and 16mM for rat septa (Shine and Douglas 1975), have been shown to reduce potassium efflux. The importance of magnesium to the functioning of membrane sodium-potassium pumps has long been appreciated (Scou, 1960), but the prevention of potassium efflux may be due also to magnesium acting on specific membrane potassium channels (Shine and Douglas 1974).

10. The Use of Pharmacological Agents

In addition to the ionic species discussed already, the proposed cardioplegic solution should contain drugs with proven cardioprotective properties relevant to this situation and discussed below.

a. Oxygen

The addition of oxygen to clinical crystalloid cardioplegic solutions has been shown to enhance the protection that they provide and is neither difficult nor expensive, (Guyton, Dorsey, Craver, Bone, Jones, Murphy, and Hatcher 1985). Saturating the solution with oxygen at low temperature may provide sufficient oxygen in the arrested and hypothermic state to support some metabolism and prevent damage to the endothelial cells. Oxygen is 1.8 times more soluble in ionic solutions at 5°C than in solutions at 37°C and so, if equilibration takes place at the lower temperature, the available oxygen in water almost doubles, assuming that partial pressure remains the same. The oxygen requirement of a heart at 37°C is reduced by 90 per cent if it is arrested chemically. If the temperature is then decreased to 5°C, a further reduction of 80 per cent is achieved (Hearse, Braimbridge and Jynge 1981).
The rabbit isolated hearts used in this study had a coronary flow of approximately 20ml/min. Chemically arrested hearts have a 90% lower oxygen requirement than

beating ones, so in an arrested heart a coronary flow of 2ml/min should be sufficient to provide the required amount of oxygen. If the temperature were reduced to 5°C the oxyygen requirement would fall by a further 80 per cent, to that provided by 0.4ml/min of perfusion solution. If that solution were equilibrated with oxygen at the lower temperature the available oxygen content would be doubled and the requirement of the heart met by only 0.2ml/min of perfusion solution. As the rabbit hearts used in this study appeared to function quite normally when coronary flow had fallen to 10ml/min it is reasonable to assume that as little as 0.1ml/min of a cardioplegic solution, equilibrated with oxygen and infused at 5°C, would be sufficient to meet the oxygen requirements of the arrested rabbit heart.

A danger exists in that if the temperature of the solution rises above that at which initial equilibration took place, oxygen will come out of solution and form emboli in the tissue. This danger is absent from the heart in transit because in this situation it is possible to maintain a desired temperature exactly, preferably slightly lower than the equilibration temperature.

b. Sodium hydrogen carbonate

(as a hydrogen ion buffer)

It is important that the solution passing through the heart has a pH close to the physiological norm. The buffering capacity of the solution can be sufficient either to maintain solution stability, as is the case with the buffered St. Thomas' Solution Number Two, or to buffer any changes caused by cardiac metabolism. This is achieved by a buffering capacity equivalent to that of blood (approximately equivalent to that provided by 25mM $NaHCO_3$) and enhances the protection provided by extracellular type cardioplegic solutions, although the effect is dependent on the potassium concentration and the chosen pH of the solution (Nido, Wilson, Mickle, Bush, Rebeyka, Klement, Harding and Tait, 1985).

c. Lignocaine

Lignocaine reduces the risk of reperfusion arrhythmias. It is known also that lignocaine reduces the loss of intracellular potassium (Opie, Nathan and Lubbe, 1979). It has been suggested also that lignocaine may block a subset of sodium channels which otherwise remain open at low membrane potential (plateau phase). Over long periods this action might be of benefit by preventing an intracellular sodium overload, a probable precursor of a more damaging calcium overload during reperfusion. A concentration of 1mM has been shown to be optimal in rat

hearts (Hearse, O'Brien and Braimbridge 1978) and was of great benefit when added to a low potassium extracellular type cardioplegic solution (see Section 3). Lignocaine is also a vasodilator, this property is very useful because it counteracts the vasoconstrictor effect of elevated potassium concentrations, allowing coronary flow to be maintained at a lower pressure and thereby reducing the risk of oedema formation.

11. Final Composition of a New Cardioplegic Solution for Use in Beating and Non-beating Donor Hearts

From the foregoing discussion a new cardioplegic solution to be given as a continuous infusion over long periods has been formulated. The composition in mmol per litre of this solution is given on the following page and practical experimental experience with it is described in Section 4D.

	mmol/l
NaCl	40
KCl	60
$NaHCO_3$	25
$CaCl_2$	0.36
$MgCl_2$	15
NaH_2PO_4	1
Lignocaine (hydrochloride)	1

Equilibrated with 95% O_2 + 05% CO_2

pH 7.3 at 22°C

Fig. 4C.1

The composition of a new cardioplegic solution for use in beating and non-beating donor hearts

12. Maintenance of Osmotic Balance and the Mechanics of Infusion

There have been several reasons proposed for the failure of the Melrose solution, but the hypertonicity of the solution used clinically was almost certainly one of the most important (Gay and Ebert 1973, Tyers et al. 1975). However, crystalloid cardioplegic solutions which have no colloidal component are liable to cause oedema (Schaper, Walter, Scheld and Henrlein 1985). The possible development of oedema is influenced greatly by the mechanics of infusion.

Fluid movements between vascular and interstitial compartments are controlled by osmolarity, membrane permeability and hydrostatic pressure. If there is little pressure within the coronary vasculature, fluid will enter from the interstitium and cells, leading to their dehydration. This will in itself be damaging but will also predispose to additional damage caused by rapid rehydration during reperfusion. A reduced hydrostatic pressure is of less importance when a solution has no oncotic component, because the inorganic ions commonly present in cardioplegic solutions are relatively permeant and will themselves cross vascular membranes to establish equilibria without excessive water movement. It remains impossible to maintain both specified ionic concentrations of the extracellular space and fluid movement without a hydrostatic pressure within the vasculature. Cardioplegic solution passes through the myocardium only if there is a pressure gradient. Simple experiments conducted in arrested rabbit hearts at $8^{o}C$, reveal that a flow can be maintained for at least 24 hours with an input pressure of as little as 3cm H_2O. It is common clinical practice to induce hypothermia by infusion of cold cardioplegic solutions, but in this study it was noted that cold (below $18^{o}C$) cardioplegic solutions resulted in a marked reduction in the coronary flow similar to that which occurs with cold physiological perfusion solutions (Anrep and Heausler 1929). The rate of equilibration

between extracellular fluid and cold cardioplegic solution and the extent of its distribution might be affected adversely, leading to heterogeneity within the myocardium and thereby leaving it inadequately protected. Depending on the type of solution used, it might be more satisfactory to dissociate the induction of arrest from the provision of hypothermia. This could be achieved by an initial infusion of cardioplegic solution at a normal temperature followed by a second infusion at the desired temperature.

D. PRACTICAL LABORATORY EXPERIENCE WITH A NEW CARDIOPLEGIC SOLUTION USED IN BEATING AND NON-BEATING RABBIT ISOLATED HEARTS

1. Methods

Three groups of six hearts each were perfused normally for 30 minutes before control values for heart rate, coronary flow and amplitude of contraction were recorded. The six hearts in Group A were then deprived of all perfusion (without prior arrest) for 30 min at 37^{o}C. Each was then removed from the perfusion apparatus and placed in a latex bag before immersion in saline (which was not in direct contact with the hearts) at 4^{o}C, for a period of 5 minutes to induce hypothermia rapidly. The hearts were then removed from their bags

and placed in a refrigerator at 4-6°C for 6 hours before normal perfusion was resumed.

The six hearts in Group B were also left without perfusion for 30 minutes at 37°C without prior arrest. These hearts were then perfused with cardioplegic solution for 2 minutes at a pressure of 40cm H_2O at room temperature (22°C) and then at 5cm H_2O pressure and 4 - 6 °C for a further 6 hours before being returned to the perfusion apparatus.

The remaining six hearts, Group C, were infused with cardioplegic solution for 2 minutes at 40cm H_2O pressure and room temperature (22°C) and then for 24 hours at 5cm H_2O pressure and 4-6°C before being returned to the perfusion apparatus.

After being returned to the perfusion apparatus the performances of all hearts were watched for 2 hours.

a. Solutions

Solutions were prepared with Analar grade chemicals and filtered through Whatman filter tubes (size B2). Maximum calcium contamination of solution resulting from contamination of Analar chemicals = 6.6micro mol/L. Both perfusion and cardioplegic solutions were gassed with 95% O_2 + 5% CO_2. In the case of the perfusion solution this was carried out at room temperature, but for the cardioplegic solution this was done at the

temperatures at which the solution was to be infused, that is room temperature and 4°C.

b. Infusion Apparatus

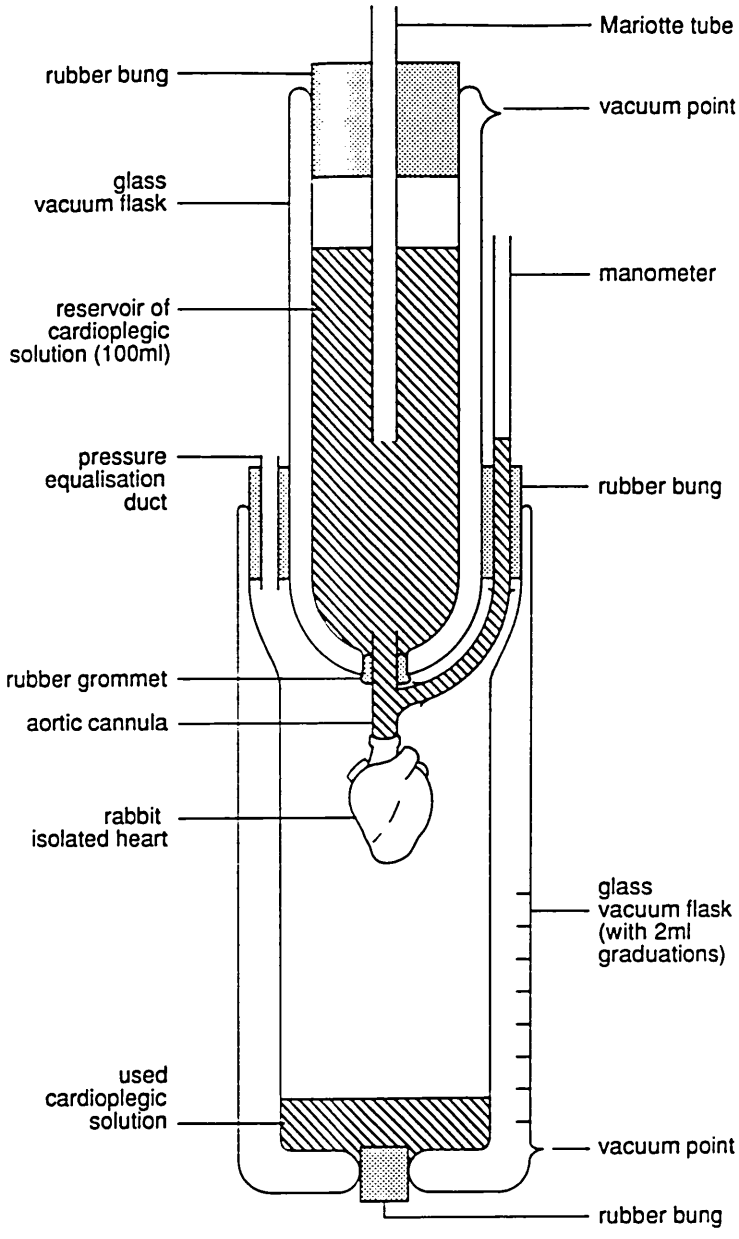


Fig. 4D.1

A prototype sterilisable "Portable Heart-Preserver"

The prototype is designed for a rabbit isolated heart but could easily be scaled up to accomodate a human heart, in which case a reservoir of 5L of cardioplegic solution rather than 100ml would be required.

The heart is suspended from a cannula positioned in the aorta to allow perfusion of the coronary arteries. In clinical practice the aim would be to use a section of the aorta not subsequently required for the transplant, so enabling later removal of any area damaged by the aortic cannula. In the flasks, the heart is not in contact with any surface and so the possibility of contact damage is prevented. The cannula fits into a reservoir containing cardioplegic solution and flow into the heart and the upward displacement of air bubbles is facilitated by a side arm on the cannula to which is attached a manometer tube. The apparatus is then fitted into a vacuum-walled flask from which a duct allows the equilibration of the pressure across the fitting. Gradations on the outflow container allow the flow rate to be calculated. The reservoir contains sufficient fluid to last for 24 hours of perfusion, dependent on the formulation of the solution and the infusion pressure head, which can be adjusted by raising or lowering the Mariotte tube.

2. Results

The 30 minute period of normothermic ischaemia was more than sufficient to ensure that all 12 hearts so treated were arrested, although all retained some electrical activity composed of P waves with occasional ventricular complexes.

a. Induction and Maintenance of Cardiac Arrest

In Group A (hearts left without perfusion for 30 minutes at 37°C and then 6 hours at 4°C) mechanical arrest, with the exception of occasional escape beats, occurred within the 30 minute period although some electrical activity remained and was composed almost exclusively of P waves.

In Group B (hearts left without perfusion for 30 minutes before cardioplegic infusion) the pattern of arrest was the same as for Group A. Coronary flow at the end of the initial 2 minute cardioplegic infusion was 11.7ml/min (s.e.m. = 1.1ml/min) and the mean volume of cardioplegic solution infused over the next 6 hours was 85.2ml (s.e.m. = 10.7 ml), approximately 1.05 per cent of the volume expected had coronary flow remained at the rate it was after the first 30 minute settling period.

In Group C (hearts infused with cardioplegic solution whilst still beating), arrest occurred after a mean of 24 seconds (s.e.m. = 3.8s) and coronary flow at the end of the initial 2 minute cardioplegic infusion was

7.4ml/min (s.e.m. = 1 ml/min). The mean volume of cardioplegic solution infused at low pressure over 24 hours was 139.6ml (s.e.m. = 12.5ml), of which a mean of 78.5ml (s.e.m. = 9.3ml) was infused over the first six hours. Over the full 24 hours the mean volume of low pressure cardioplegic infusion was 0.5 per cent of what could have been expected had perfusion remained at the same rate as it was 30 minutes after setting up the preparation.

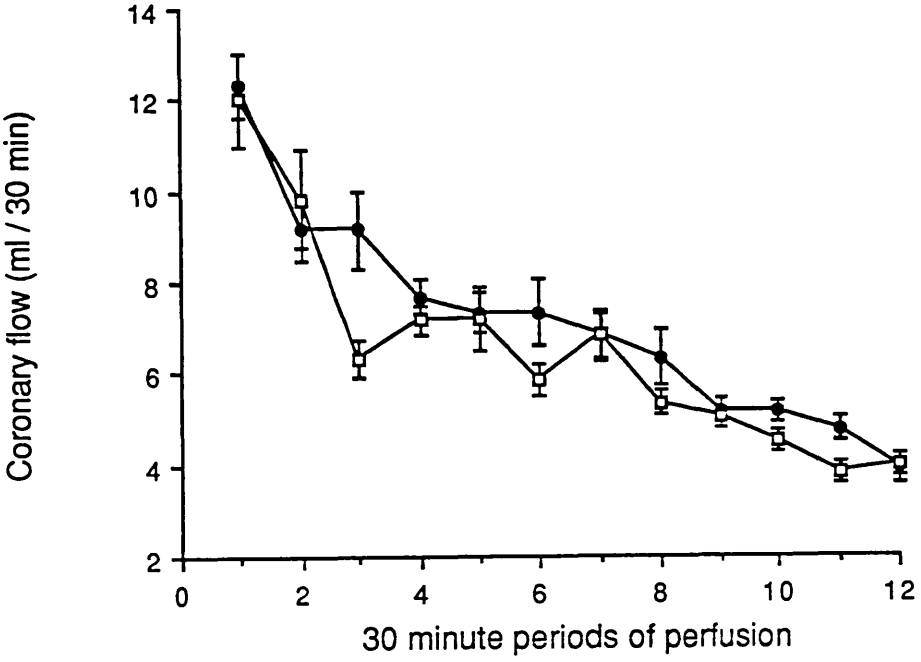


Figure 4D.2

Coronary flow during low pressure infusion of cardioplegic solution. The total flow (ml) passing through the heart in each of twelve sequential 30min periods is shown for Group A (circles) and Group C (squares). Each point is the mean and the vertical bars the s.e.m. of 6 observations, n = 12 in total. (note for Group C only the first 6h of the 24h infusion period is shown).

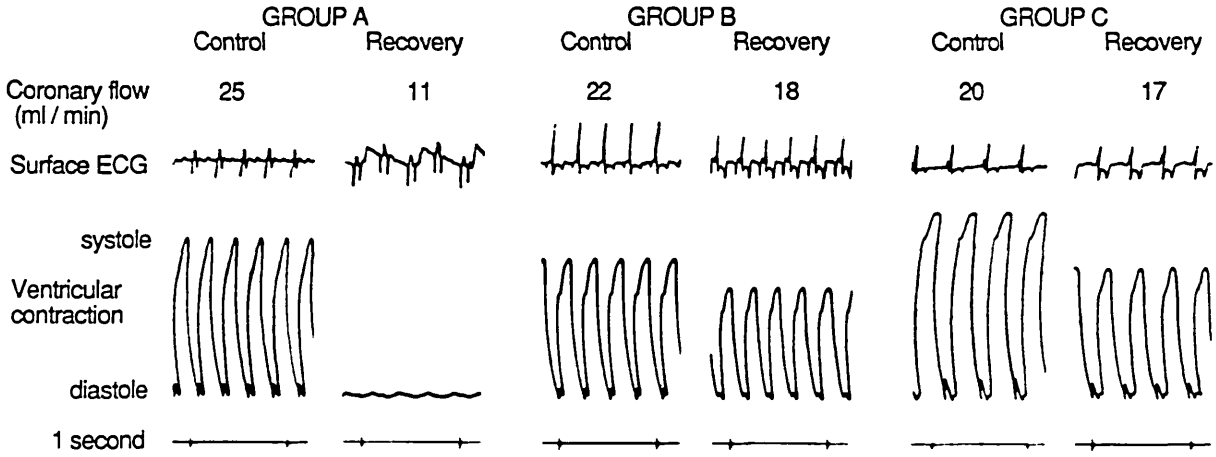


Figure 4D.3

Typical tracings of rabbit isolated heart activity before and after prolonged arrest. Control tracings were taken 30 minutes after setting up each heart and recovery tracings 30 minutes after the beginning of reperfusion. Group A, no perfusion (without prior arrest) for 30 min at 37°C and then at 6°C for 6 h. Group B, no perfusion for 30 min at 37°C (without prior arrest) and cardioplegic infusion for 2 min (40 cmH_2O, 22°C) and 6 h (5cm H_2O pressure and 4 - 6 °C). Group C, cardioplegic infusion for 2 min (40 cmH_2O, 22°C) and then for 24 h (5cm H_2O pressure and 4-6°C)

b. Rate and Extent of Recovery

The extent of recovery was assessed at 6 time points during reperfusion. Differences between the groups at these points were compared using grouped "t" tests with a pooled variance.

For amplitude of contraction, the recovery of Group B was significantly better (P<0.001) than that of Group A at all times, being approximately 8 times as great.

There were no significant differences (P<0.05) between Group B and Group C.

For coronary flow, the recovery of Group B was also significantly better (P<0.01) than for Group A at all times and was approximately twice as gooc. There were no significant (P<0.05) differences between Group B and Group C

For heart rate, the recovery of Group B was significantly better (P<0.05) than for Group A at 5, 15 and 120 minutes after reperfusion. The recovery of Group C was significantly better (P<0.05) than for Group B at 60 and 90 minutes of reperfusion.

These results may be summarised as follows, 30 minutes of normothermic ischaemia followed by 6 hours of hypothermic ischaemia reduced the functional activity of the hearts enormously. However, if cardioplegic solution was infused during the six hour period, subsequent recovery was significantly better. The recovery after 30 minutes of normothermic arrest and six

hours cardioplegia was not significantly different from that after 24 hours of cardioplegic infusion. It appears, therefore, that an initial 30 minute period of normothermic ischaemia, resulting in arrest, was approximately equivalent in detrimental effect to 18 hours of cardioplegic infusion in hearts originally arrested by that infusion.

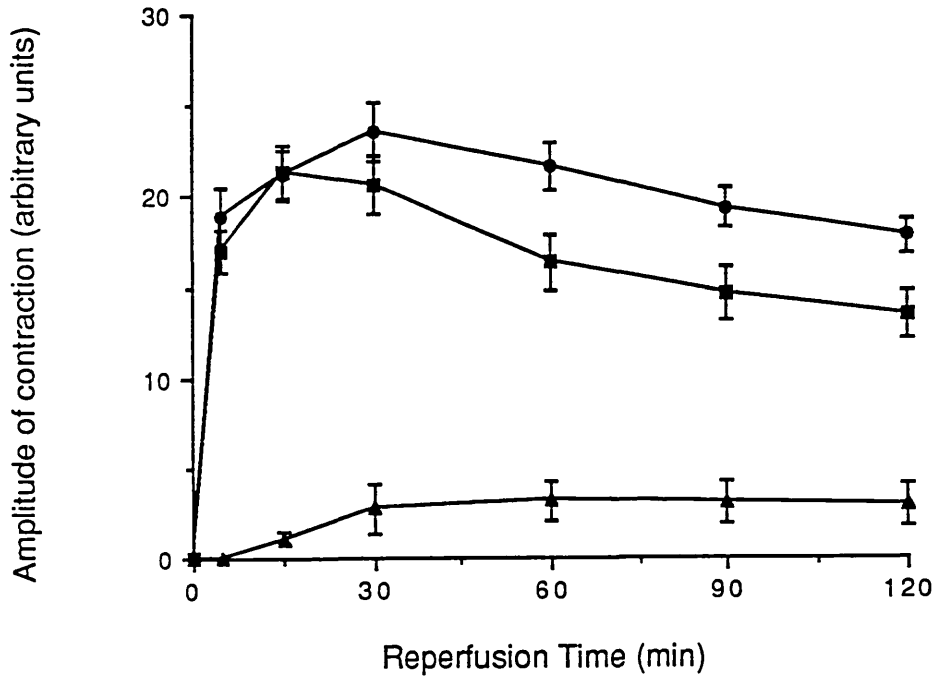


Figure 4D.4

Recovery of amplitude of contraction after periods of cardioplegia. The amplitude of contraction in arbitary units during reperfusion after the procedures described for Groups A (triangles), B (circles) and C (squares). Each point is the mean and the vertical bars the s.e.m of 6 observations in each group, n = 18 in total.

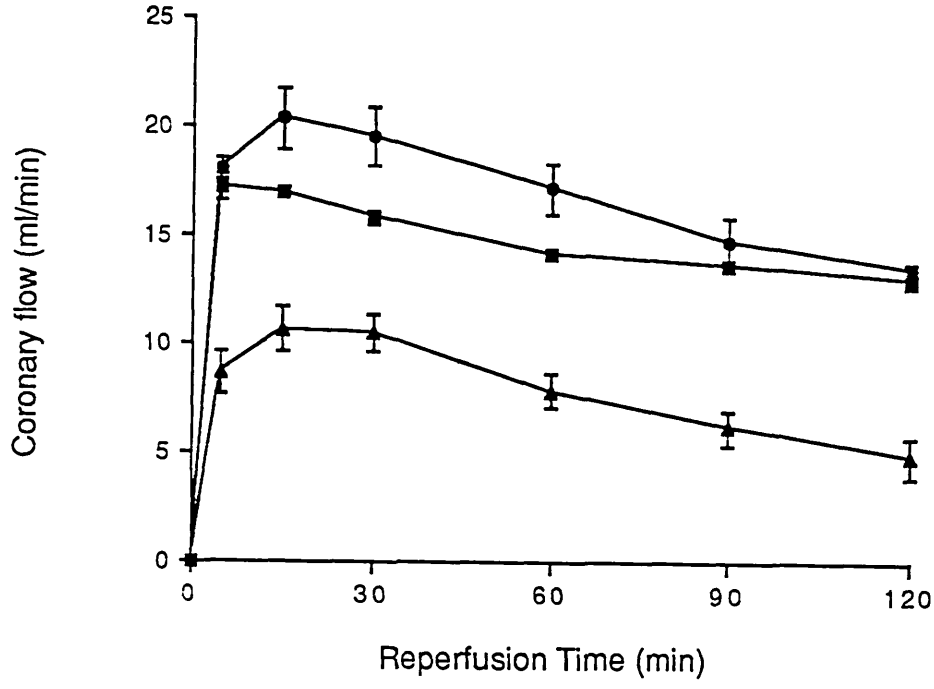


Figure 4D.5

Recovery of coronary flow after periods of cardioplegia. The coronary flow in ml/min during reperfusion after the procedures already described in text and fig. 4D.3. Groups A (triangles), B (circles) and C (squares). Each point is the mean and the vertical bars the s.e.m of 6 observations in each group, n = 18 in total.

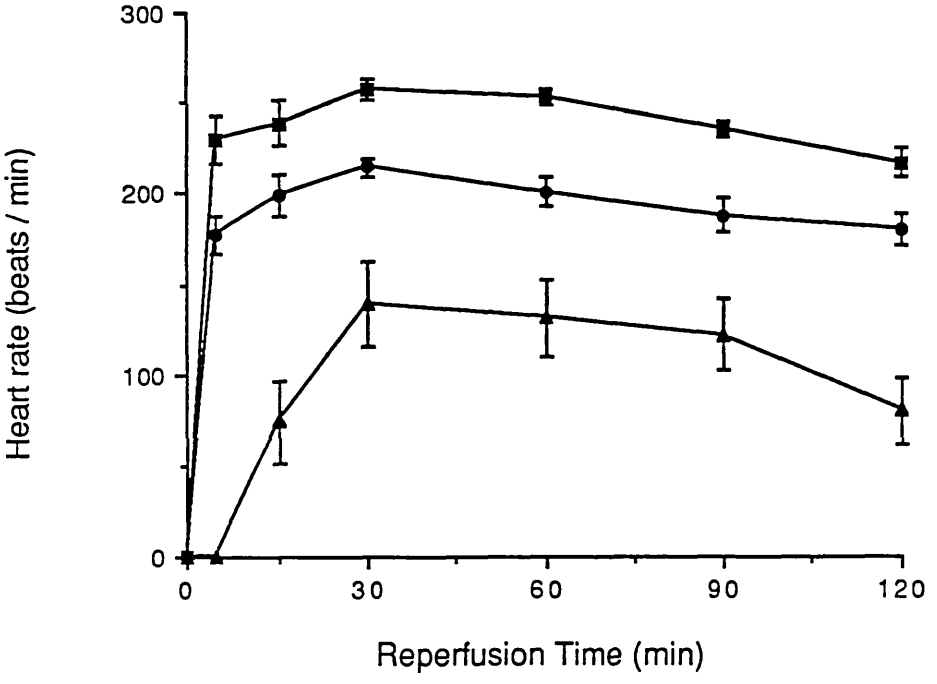


Figure 4.D6

Recovery of heart rate after periods of cardioplegia. The heart rate during reperfusion after the procedures already described in text and fig. 4D.3. Groups A (triangles), B (circles) and C (squares). Each point is the mean and the vertical bars the s.e.m of 6 observations in each group, n = 18 in total.

3. Discussion of Results Presented in Section 4D

In Groups A and C, the twelve hearts which received the new cardioprotective solution for donor hearts, recovery was spontaneous on restoration of coronary flow under normal conditions. There were no instances of ventricular fibrillation and no need for any inotropic support or other pharmacological intervention. The maximum recovery was achieved rapidly, being completed within 15 to 30 minutes of re-perfusion. The mean maximum recoveries of Group A were heart rate 93%, coronary flow 89% and amplitude of contraction 76%. These results, coupled with the rhythmicity of the heart beat, surface ECG patterns, shape of systolic contraction and general appearance made this group of hearts almost indistinguishable from hearts freshly set up. This impression was strengthened by the pattern of function after recovery, as can be seen from comparing these recovery curves with the curves presented in Section One (fig. 1B.6) the stability of the hearts after recovery was as good as for the freshly set up hearts. It must be remembered that the hearts described above had been subjected to 30 minutes without coronary perfusion at 37^{o}C and had arrested as a consequence, cardioplegic solution was then infused for a further six hours, before the hearts were returned to the perfusion apparatus. On the other hand, hearts treated in the same way, Group B, but not perfused with cardioplegic

solution, hardly recovered at all and did so much more slowly. Hearts subjected to 30 minutes without perfusion (at 37°C) and reperfused immediately afterwards, recoverd only 57 (s.e.m. 13) per cent of amplitude of contraction (fig. 2B.2). These results show, apparently for the first time, that with a specifically designed solution the protective effects of cardioplegia need not be confined to the rapid induction of arrest. A period of 30 minutes asphyxiation was chosen because this was considered by Cooper (1975) to have "significant clinical application" to the acquistion of donor hearts for transplantation. These results thus demonstrate that it may well be possible to use donor hearts which have stopped beating, which would increase the number available for transplant needs.

The recoveries after 24 hours of arrest and cardioplegic infusion (Group C) were also very good.

These results are in dramatic contrast to those obtained in a very similar rabbit isolated perfused heart model by Foreman, Pegg and Armitage (1985). They tested the protective effects of St. Thomas' Solution Number Two, and several new solutions of their own devising, by infusing 150 ml of each into isolated beating hearts at 0°C before storage in the same solutions for three hours at 0°C. The mean recoveries of hearts which had received St. Thomas' Solution Number 2 were 9% for amplitude of contraction, 46% for heart rate and 58% for coronary flow. Foreman et al. (1985) report also the

effects of a solution of their own, which they suggested should be investigated further in a transplantation model. After only 3 hours of arrest (at 0°C), amplitude of contraction, recovered to 26 per cent, heart rate 100 per cent and coronary flow 73 per cent after an hour of reperfusion, but after 18 hours of arrest there was no recovery at all. This latter result is similar to that obtained when a solution (containing KCl 24mM, $CaCl_2$ 0.73mM, sodium nitroprusside 10^{-4}M, lignocaine 1mM and sodium salts as present in McEwen's solution) described in Section Three was given as a low volume infusion at 4-6°C for 20 hours. Of the 6 rabbit hearts so treated, none recovered co-ordinated electro-mechanical activity (see section 4A.5). It is possible that a more complete recovery would have been achieved had the infusion protocol been different. It was this consideration which lead to no direct comparison being made between the new solution designed in this study (Section Four) and any established solutions. To impose on any other solution the new infusion protocol designed specifically for use with the new solution used in this study, would have yielded invalid results. The results presented in this section are in marked contrast to the clinical situation where it is at present rare for human donor hearts to be used after more than 4 hours of arrest, even though they are beating when acquired.

It was calculated in Section 4C that a flow rate between 3 and 6ml/30min would provide the oxygen requirement of the tissue under the chosen experimental conditions. After 6 hours of infusion the mean flow rate in Group B was 3.9ml/30min (s.e.m. 0.61ml/30min) and Group C was 4ml/30min (s.e.m. 0.3ml/30min), but by 21 hours of infusion the flow in Group C had fallen to 1.66ml/30min (s.e.m. 0.2ml/30min) and by 24 hours flow had stopped in three of the six hearts. This might account for the slight differences between recovery of the two groups as it is reasonable to assume that for several hours the hearts in Group C were receiving less than their full oxygen requirement. Experiments in which the flow rate is maintained by alterations in the infusion pressure might yield even better results.

The reasons for the inclusion of the various components of this cardioplegic solution at the chosen concentrations were discussed in section 4.C. It is not possible to attribute the apparent success of this solution to an individual aspect of the solution or infusion protocol because of the complexity of the possible interactions between them.

E. CONCLUSIONS DRAWN FROM SECTION FOUR

In rabbit isolated hearts, the new type of cardioplegic solution and infusion protocol described here enabled significant functional recovery after long periods of cardioplegia (24h) and after moderate periods of cardioplegic infusion (6h) preceded immediately by arrestive normothermic asphyxiation (30 min).

The solution and infusion protocol should be investigated in a full experimental model of cardiac transplantation, possibly in a pig, involving detailed haemodynamic and enzymatic investigations of cardiac preservation.

SECTION FIVE

GENERAL CONCLUSIONS AND SUGGESTIONS FOR FURTHER EXPERIMENTAL WORK

A. GENERAL CONCLUSIONS

The feasibility of performing potentially clinically relevant experiments incorporating multiple periods of arrest, long recovery periods (5 hours) and continuous cardioplegic infusions for up to 24 hours, in the convenient and economical rabbit isolated heart model has been established.

The basic experiments have elucidated further the mechanisms by which components of cardioplegic solutions exert their effects. In particular, the safety of carefully limited reductions in the extracellular calcium concentration has been confirmed under a wide variety of conditions and such reductions have formed the basis of 5 new cardioplegic solutions and one which is specially designed for the protection of potential donor hearts over long periods of transport even if arrest was originally asphyxial. The induction of arrest by potassium-rich solutions has been shown to be highly dependent on the calcium concentration and appears, initially at least, to be a consequence of A-V conduction block. Evidence has been gathered suggesting that sodium nitroprusside acts on myocardium as well as on coronary vascular smooth muscle. The effects of cardioplegic solutions on coronary vasculature have emphasised this important though often forgotten aspect of cardioprotection and led to the observation that complete interruptions in coronary flow can actually

correct ventricular fibrillation. This may possibly have significance for the clinical management of the condition. An increased understanding, gained during this study of the mechanisms responsible for poor viability after prolonged conventional cardioplegia, has enabled a highly promising cardioprotecive solution and infusion protocol to be designed and tested experimentally. The formulation of this solution and the infusion protocol are presented for clinical appraisal and in the hope that they lead to a significant increase in the number of viable donor hearts.

There remains a substantial body of work to be completed in order to evaluate the benefits of these findings, both in terms of clinical application and in relation to further laboratory progress. Accordingly, suggestions are now presented on further experimental work for which it would have been a great pleasure to be in a position to carry out immediately.

B. SUGGESTIONS FOR FURTHER EXPERIMENTAL WORK

1. The Mechanisms of Action of Cardioprotective Compounds and Procedures

a. Protection Against the Calcium Paradox

In its most severe form cardiac glycoside toxicity may result in unco-ordinated electromechanical activity,

calcium overload and a pattern of cardiac arrest which finally leaves the heart in a hypercontracted condition. The appearance of the heart can be very similar to that after the calcium paradox. It is possible that both conditions share the common aetiology of an intracellular overload of calcium secondary to an increase in intracellular sodium (In the case of the glycosides, caused by inhibition of the sodium-potassium pump). Two potentially useful interventions are suggested. It is well known that phenytoin is particularly effective against cardiac glycoside-induced arrhythmias, thus it is possible that it might be of use in cardioplegic solutions which contain low calcium concentrations and thereby result in calcium paradox type of changes in some circumstances.

There is great potential for an agent which inhibits the Na/Ca exchanger whilst the cells are depolarised or sodium-overloaded. Such an action has been proposed for amiloride and its analogues (Siegl, Cragoe, Trumble and Kaczorowiski 1984) but more recently it has been suggested that a direct effect on sodium-calcium exchange is unlikely to be the mode of action of amiloride (Kaila and Vaughan-Jones 1987). Prevention of exchanger reversal during depolarisation or sodium overload might actually reduce cytosolic calcium concentration and be of enormous therapeutic value during cardioplegia and cardiac ischaemia generally.

b. Escape Beats and the Anti-fibrillatory Paradox

Observations on the effects of cardioplegic solutions in different regions of the myocardium, particularly the tissues of the A-V node and the SAN and those adjacent to them, may help to determine the nature and possible consequences of the electrical activity seen frequently during periods of mechanical arrest. The possible involvement of catecholamines released from nerve tissue within the myocardium and the loss of intracellular potassium in the generation of the anti-fibrillatory paradox could be investigated using High Performance Liquid Chromatography and mass spectrography to identify the components of the effluent displaced from the heart immediately afterwards.

c. Mechanisms of Action of Sodium Nitroprusside

The experiments with sodium nitroprusside so far have vindicated the idea behind the suggestion that it could be cardioprotective. Further investigations would seem to be justifiable, for instance to determine the effects of temperature on the actions of this substance, associated with detailed electrophysiological studies under a variety of conditions to characterise its effects on the myocardium.

2. Cardioplegia For Use During Reparative Surgery

Further experiments in this area should centre on testing the solutions in different species and at different temperatures. With regard to formulation, the possible inclusion of magnesium should also be further considered. Many potentially beneficial effects have been proposed for magnesium, but these are to some extent species specific, rat hearts appearing to benefit greatly from its inclusion at optimum concentrations, but harmful effects can be revealed if they are exceeded. Rabbit hearts appear not to benefit and the actions on human hearts are largely unresolved. The effects depend also on variables of solution design and infusion and inclusion in a solution to be infused continuously is warranted, as has been discussed in Section 4, but the effects of magnesium, and the mechanisms by which it may exert them during reparative surgery remain unclear.

Although the solution described in Section Four was designed to be used during the transportation of donor hearts, its effects in a model of reparative surgery should be investigated also, especially if similar conditions of perfusion could be applied.

3. Cardioplegia For The Acquisition and Storage of Hearts Donated for Transplantation

a. Ionic Redistribution During Prolonged Cardioplegia

As the cardioplegic solution passes through the heart, loss or accumulation of ions will become detectable as a change in their concentration in the effluent compared with that in the solution entering. This will enable a simple assessment of the extent to which the solution is maintaining the desired ionic balance. Analysis of the effluent will also allow various enzymes to be assayed and indicate the extent of cell breakdown. Use of ion-sensitive microelectrodes will enable accurate estimations of the intracellular activities of K^+ and Na^+ and their relationship with membrane potential, but techniques such as fluorescent dyes would be appropriate to the measurement of intracellular Ca^{++} activity. These observations should yield information which might enable further effective fine-tuning of the formulation and infusion parameters of cardioprotective solutions designed to control specific ion movements.

b. Addition of Metabolic Substrates

Experiments should be undertaken to examine the possible benefits of adding a metabolic substrate, such as glucose or a high energy phosphate, to the cardioplegic solution.

c. Maintenance of Contractile Element Arrangement During Transportation

The relationship between diastolic load and systolic tension is well known and has been studied extensively. On the other hand, there appears to be less known about the consequences of leaving the myocardium without a load, as for instance during transit. It is possible that over long periods and under hypothermic and ischaemic conditions, rearrangement of the contractile elements occurs, resulting in a permanent reduction in the number of cross-bridge sites available. This would be consistent with the results so far obtained, which suggest that the main abnormality of post cardioplegia contraction is the amplitude. Recovery might be enhanced if, during cardioplegia, hearts remained under some tension, possibly by inflating balloons in each chamber. The optimum load would have to be established as an excess could increase myocardial contracture during cardioplegia and accelerate the utilisation of ATP to a point at which it would be a serious drawback.

d. Pharmacological Response After Cardioplegia

The extent to which the mechanisms responsible for mediating respones to pharmacological agents have survived periods of cardioplegia could be investigated by comparing concentration-response characteristics before and after periods of experimental cardioplegia.

The information derived from such a study might also be of benefit in the post-operative management of patients who have undergone reparative surgery or cardiac transplantation.

e. Predictors of the Viability of Arrested Hearts

Cardiac transplantation is both costly and dangerous and the outcome is very dependent on the condition of the donor heart when it reaches the transplant centre. The absence of a simple objective method to assess the damage suffered by a heart during transit may lead to the erroneous rejection of a viable heart or the acceptance of one which is unsuitable. Two promising parameters which could perhaps be used to assess damage are changes in the rate of perfusion during the arrest period and the development of tension within the myocardium, which results in a loss of tissue compliance. Changes in the coronary flow rate at a given infusion pressure or changes in the pressure required to maintain a given flow rate could be monitored easily with an apparatus similar to that used for Langendorff perfusion. Solution could be infused directly into each coronary artery, a modification which would allow for greater accuracy and separate analysis of each coronary artery distribution. Both parameters have the advantage that they could be used during the arrest period.

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